
SMB
IV Congreso
Especies Reactivas del Oxígeno
en Biología y Medicina de la Sociedad
Mexicana de Bioquímica
19 al 22 de marzo del 2013
Queretaro, Queretaro, México
V TALLER INTERNACIONAL DE ASPECTOS COMPARATIVOS DEL
ESTRÉS OXIDATIVO EN SISTEMAS BIOLÓGICOS

MEMORIA

SOCIEDAD MEXICANA DE BIOQUÍMICA

IV CONGRESO DE ESPECIES REACTIVAS DEL OXÍGENO EN BIOLOGÍA Y MEDICINA

HACIENDA JURICA, QUERÉTARO, 19 AL 22 DE MARZO DE 2013

PROGRAM

TUESDAY, MARCH 19	
14:00 – 18:00	Registration
18:30 – 18:45	Opening Ceremony
19:00 – 20:00	*Plenary Opening Lecture* **Dr. Henry Forman** *Redox and electrophilic signaling in cancer, air pollution, and aging* President Elect Society for Free Radical Biology and Medicine, University of Southern California, Los Angeles
20:00 – 21:00	Welcome Cocktail

WEDNESDAY, MARCH 20	
7:30 – 8:30	*Early Morning Course day one* Oxidative stress: fundamental aspects and physicochemical insights **Dr. Annia Galano Jiménez** Departamento de Química, UAM, Iztapalapa
9:00 – 10:00	*Plenary Lecture* **Dr. Enrique Cadenas** *Mitochondrial energy-redox signaling in brain aging and neurodegeneration* University of Southern California, Los Angeles
10:00 – 10:30	Coffee break
10:30 – 12:30	*Symposium. Nutrition and natural antioxidants* **Dr. Nimbe Torres y Torres** *Dieta, estrés oxidante y diabetes* Instituto Nacional de Ciencias Médicas y Nutrición “Salvador Zubirán” **Dr. Jorge Luis Rosado Loria** *Efecto de la deficiencia de antioxidantes en la obesidad* Universidad Autónoma de Querétaro

	Dr. Héctor Issac Rocha González *Natural antioxidants in the neuropathic pain treatment* Escuela Superior de Medicina del IPN, Unidad de Posgrado **Dr. Juan Raúl Álvarez Idaboy** *Glutathione endogenous antioxidant: radical scavenger and damaged DNA repairer* Facultad de Química, UNAM
12:30 – 13:30	Business session
13:00 – 15:00	Lunch
15:00 – 16:00	Oral Presentations **Oxidative stress in cell death and disease** **Dr. Christian Cortés Rojo** *Short-term supplementation with avocado oil ameliorates liver mitochondrial dysfunction and oxidative stress in streptozotocin-induced diabetic rats* IIQB, Universidad Michoacana **Mario Negrette Guzmán** *Sulforaphane attenuates gentamicin-induced nephrotoxicity: role of mitochondrial protection* Facultad de Química, UNAM **Adriana Guadalupe Pérez Ruiz** *Effect antiproliferative of (-) epicatechin and its relationship with reactive oxygen species in breast cancer cell lines* Escuela Superior de Medicina, IPN **María de Jesús Rincón Víquez** *Detection of insulin polymers in plasma from obese patients and its relationship with insulin resistance* Escuela Superior de Medicina, IPN
16:00 – 17:00	*Plenary Lecture* **Dr. Arturo Keller** *Nanoparticles in the environment: life-cycle, fate & transport, and reactivity* University of California, Santa Bárbara
17:00 – 17:15	Coffee Break

17:15 – 19:00	*Symposium. Nanoparticles and oxidative stress* **Dr. Andrea de Vizcaya** *Challenges associated with the toxicity evaluation and oxidative stress of engineered nanomaterials* CINVESTAV-IPN Unidad Zacatenco **Dr. Carmen González Castillo** *Study of silver nanoparticles on biological experimental models. Role of nitric oxide* Facultad de Ciencias Químicas, UASLP **Dr. Aracely Angulo Molina** *Functional nanofoods: synthesis, characterization and nanotoxic effects in cancer cells* Universidad de las Américas Puebla **Dr. Patricia Ramírez Noguera** *Efectos citotóxicos y genotóxicos asociados a nanopartículas fabricadas a base de polietilcianoacrilato, SiO2 y quitosan* Facultad de Estudios Superiores – Cuautitlán, UNAM
19:00 – 21:00	Poster Session. Odd numbers

THURSDAY, MARCH 21

7:30 – 8:30	*Early Morning Course day two* *Cell signaling by oxidative stress* **Dr. María Elena Ibarra Rubio** Facultad de Química, UNAM
9:00 – 10:00	*Plenary Lecture* **Dr. Michael Hitchler** *Reprogramming the epigenome through oxidative stress and metabolism* Kaiser Permanente Los Angeles Medical Center
10:00 – 10:30	Coffee break
10:30 – 12:30	*Symposium. Oxidative stress and cell signaling* **Dr. Wilhelm Hansberg Torres** *ROS, RAS-1, growth and development* Instituto de Fisiología Celular, UNAM **Dr. Concepción Gutiérrez-Ruiz** *Reactive oxygen species and the survival response in the liver* UAM Iztapalapa

	Dr. Rolando Hernández Muñoz *Role of lipid peroxidation and of oxidative stress in the rat liver regeneration induced by partial hepatectomy* Instituto de Fisiología Celular, UNAM **Dr. Ma. Lourdes Rodríguez Fragoso** *EGF receptor phosphorylation in ethanol-induced oxidative stress is associated with CYP2E1 overexpression in MCF-10A cells* Facultad de Farmacia, UAEM
13:00-15:00	Lunch
15:00-16:00	Oral Presentations **Reactive species and signaling** **Mabel Buelna Chontal** *Postconditioning increases NRF2 nuclear translocation via PKC activation and protects against reperfusion injury in heart* Instituto Nacional de Cardiología "Ignacio Chávez" **Ahiezer Rodríguez Tobón** *Reactive oxygen species and tyrosine phosphorylation in the epididymal sperm maturation of the mexican big-eared (Corynorhinus mexicanus) bat* Departamento de Biología de la Reproducción. UAM-I **Ricardo Alberto Santana Martinez** *Treatment of curcumin on alterations induced in a model of striatal degeneration in rats: protective effect related to its ability as enhancer of phase II enzymes.* Instituto Nacional de Neurología y Neurocirugía MVS. **Ricardo Santillán Mendoza** *Insulin stimulates autophagy in INT-1 tobacco cell cultures throught H_2O_2* IIQB, Universidad Michoacana
16:00-17:00	*Plenary Lecture* **Dr. Tania Zenteno Savín** *Ischemia-reperfusion: lessons from marine mammals* Centro de Investigaciones Biológicas del Noreste, Baja California
17:00 – 17:15	Coffee break
17:15 – 18:45	*Symposium. Oxidative stress in biolgy and medicine* **Dr. Laura Roxana Torres Avilés** *Oxidative stress as a possible mediator of life-history tradeoffs* Instituto de Ecología, UNAM

	Dr. Verónica Mireya Rodríguez Córdova *The implications of oxidative stress in the development of neurodegenerative disorders caused by the environmental contaminants arsenic and atrazine* Instituto de Neurobiología, UNAM **Dr. Brenda Anguiano** *Iodine and redox balance: from physiology to cancer* Instituto de Neurobiología, UNAM
18:45 – 21:00	Poster Session. Even Numbers
	FRIDAY, MARCH 22
7:30-8:30	*Early Morning Course day three* *Evaluation of oxidative stress* **Dr. José Pedraza Chaverrí** Facultad de Química, UNAM
9:00-10:00	*Plenary Lecture* **Dr. Mauricio Díaz Muñoz** *Pro-oxidant reactions and reticular response in the liver are modulated by a protocol of restricted food access at daytime* Instituto de Neurobiología, UNAM
10:00 – 10:30	Coffee break
10:30 – 12:00	Oral Presentations **Emerging topics in the field of oxidative stress** **Rodrigo Martínez Espinosa** *Oxidative stress is involved in albendazole damage to Giardia duodenalis* CINVESTAV, IPN **Dr. Igor Pottosin** *Ros-induced changes in K^+ and Ca^{2+} conductance across the root cell membrane in higher plants and their modulation by natural polyamines* CUIB. Universidad de Colima **Jorge Alejandro Sosa Gutiérrez** *Ascorbate-glutathione system participates in dormancy breakage in Vitis vinifera L. induced by chemical agents* Facultad de Ciencias Químicas, UJED **Dr. Wilhelm Hansberg** *How catalase recognizes H_2O_2 in a sea of water* Instituto de Fisiología Celular, UNAM

	Dr. Marco A. Liñán Cabello *Physiological responses to oxidative stress associated with Ph variations in the hermatypic coral Pocillopora capitata* FACIMAR, Universidad de Colima. **Cristo Omar Puente Valenzuela** *Arsenite effect on GSH/GSSG ratio in hepatocytes (HEPG2)* Facultad de Ciencias Biológicas, UJED
12:00-13:00	*Plenary Lecture* **Dr. Rafael Vázquez Duhalt** *Intramolecular delocalization of free radicals: hemoproteins, a matter of life or death* Instituto de Biotecnología, UNAM
13:00 – 13:30	Final announcements and closing ceremony
13:00 – 15:00	Lunch

Organizing Committee

Dr. Lourdes Massieu Trigo
Instituto de Fisiología Celular, UNAM

Dr. Mahara Valverde Ramírez
Instituto de Investigaciones Biomédicas, UNAM

Dr. María E. Gonsebatt Bonaparte
Instituto de Investigaciones Biomédicas, UNAM

Dr. Alejandro de las Peñas Nava
Instituto Potosino de Investigación Científica (IPICYT)

Local Committee
Dr. Teresa García Gasca
Facultad de Ciencias Naturales, UAQ

Lic. Elsa Fernanda Chávez Alabat
Facultad de Ciencias Naturales, UAQ

POSTER SESSION. ODD NUMBERS
WEDNESDAY 18:45 – 21:00

ESPECIES REACTIVAS Y SEÑALIZACIÓN

1. NORDIHYDROGUAIARETIC ACID ATTENUATES THE OXIDATIVE STRESS-INDUCED DECREASE OF CD33 EXPRESSION IN HUMAN MONOCYTES. **Silvia Guzman-Beltran**, José Pedraza-Chaverri, Susana González-Reyes, Yolanda González, Karen Bobadilla, Fernando Hernández-Sánchez, Martha Torres
3 ETHANOL AND RESVERATROL REGULATE NR1 AND NR2A NMDA RECEPTOR SUBUNIT EXPRESSION IN CEREBRAL ISCHEMIA. **Cerón Silva AL,** Millán Vega A, Ortiz-Plata A, Salazar MI, Espinoza-Rojo M, Aguilera P.
5 OXIDATIVE DAMAGE DURING FRAGILITY AND ITS RELATIONSHIP WITH THE ANTI-INFLAMATORY RESPONSE AND NADPH OXIDASE ACTIVITY. **González Puertos Viridiana Yazmín**, Alarcón Aguilar Adriana, Conde-Pérezprina Juan Cristóbal, Juárez Cedillo Teresa, Rosas Carrasco Oscar, Königsberg Mina. Luna-López Armando.
7 NF-KB P50 SUBUNIT REGULATES BCL-2 OVEREXPRESSION DURING OXIDATIVE CONDITIONING HORMESIS RESPONSE. **Luna-López Armando,** González-Puertos Viridiana Yazmín, Romero-Ontiveros Jaquelinne, Ventura-Gallegos Jose Luis, Zentella Alejandro, Gómez-Quiroz Luis Enrique, Königsberg Mina.
9 CHARACTERIZATION OF SECONDARY METABOLITES FROM OPPORTUNISTIC FUNGAL PATHOGEN *Candida glabrata*. **Mancilla Montelongo MG**, Gutiérrez Escobedo MG, De Las Peñas Nava A.
11 COMPARATIVE ANALYSIS OF ANTIOXIDANT CAPACITY OF MELATONIN (MLT) AND SEROTONIN (5-HT) ON THE ACTIVITY OF HUMAN LEUCOCYTES. **Mendieta Irasema,** Moreno K, Rodríguez A, García-Alcocer G, Escobar J, Berumen LC.
13 SILVER NANOPARTICLES INDUCED BIOCHEMICAL AND PHYSIOLOGICAL CHANGES IN RESPIRATORY PRIMARY CULTURE CELLS AND ISOLATED RAT TRACHEAL SEGMENTS. ROLE OF NITRIC OXIDE. **Salazar-García S**, Ramírez-Lee MA, Espinosa-Tanguma R, Ali S, González C.
15 BENEFIT OF PHYSICAL ACTIVATION ON OXIDATIVE STRESS. **Eneida Camarillo Romero,** Montenegro Morales LP, Cerecero Aguirre P, Vázquez de Anda FG, Camarillo Romero MS, Huitrón Bravo GG
17 SIGNAL TRANSDUCTION PATHWAYS INDUCED BY FCγ RECEPTORS IN THP-1 AND U937 MONOCYTIC CELL LINES. **Rodríguez Cruz A**, Mora N, Rosales C
19 DYNAMIC OF REACTIVE OXYGEN SPECIES IN ROOT HAIR CELLS AND POLLEN TUBES ARE ESSENTIAL FOR POLAR GROWTH. **Luis Cárdenas**, Alejandra Hernández-Barrera, Rosana Sánchez, Jesús Montiel, Eric Johnson, Federico Sánchez, Carmen Quinto, Hen-ming Wu, Alice Cheung

ESTRÉS OXIDANTE EN LA DIFERENCIACIÓN Y EL DESARROLLO

21 ACTIVITY OF GLUCOSE 6-PHOSPHATE DEHYDROGENASE DURING SEXUAL DIFFERENTIATION IN THE MALE RAT HYPOTHALAMIC PERINATAL TREATED WITH TAMOXIFEN. **Chávez García R**, Ortega Camarillo C, Ávalos Rodríguez A, Borderas Tordecillas F, Vergara Onofre M.

ESTRÉS OXIDANTE EN LA MUERTE CELULAR Y LA ENFERMEDAD

23 OXIDATIVE DAMAGE BY AIR ENVIRONMENTAL EXPUSURE IN URBAN CHILDREN. **Alvarado-Cruz I.**, Sánchez-Guerra M., Hernández-Cadena L., Espinosa-Juárez L., Monroy Pérez V, Solís-Heredia M.J., Pelallo-Martínez N., Quintanilla-Vega B.

25 ANTIOXIDANT-MEDIATED PROTECTIVE EFFECT OF HAWTHORN (*Crataegus mexicana*) SKIN EXTRACT IN ERYTHROCYTES AGAINST OXIDATIVE DAMAGE. **Banderas-Tarabay José Antonio,** Grada-Sánchez M, Pérez-Cholula JC, Rodríguez-Torres L, and Méndez-Iturbide D.

27 EFFECT OF POLYPHENOL EXTRACT FROM GREEN TEA [P60] ON GENOTOXIC AND CYTOTOXIC DAMAGE INDUCED BY Cr (IV) IN MICE CD-1 STRAIN. **García-Rodríguez MC**, Carvente-Juárez MM, Montaño-Rodríguez AR, Altamirano-Lozano MA

29 SUBCRHONIC ADMINISTRATION OF S-ALLYCYSTEINE (SAC) ACTIVATES Nrf2 FACTOR IN CEREBRAL CORTEX. **González García María Cecilia,** Chávez-Domínguez R, Silva-Islas C, Chánez-Cárdenas ME, Barrera-Oviedo D, Maldonado PD.

31 ROLE OF NADPH-OXIDASE IN THE DEATH OF CULTURED CEREBELLAR ASTROCYTES. **Guadalupe Domínguez,** Julio Morán.

33 OXIDATIVE STRESS EFFECT OF A SPORTS MEDICAL PROGRAM ON OLDER ADULTS WITH AND WITHOUT METABOLIC SYNDROME. **Gutiérrez López Liliana,** García Galindo Miguel Ángel, Hernández Hernández Héctor Daniel, García Sánchez José Rubén, Rincón Víquez Ma de Jesús, Olivares Corichi Ivonne María

35 THE NATIVE FLORA OF YUCATAN PENINSULA AS A SOURCE OF BIOACTIVE METABOLITES WITH ANTIOXIDANT, ANTI-INFLAMMATORY AND/OR ANALGESIC ACTIVITY. **Hiatzy Eislin Zapata Estrella,** Mijangos-Ramos IF, Escalante-Erosa F, García-Sosa K, Valdir Cechinel Filho, Meira-Quintão NL, Peña-Rodríguez LM

37 HUMAN RENAL EPITHELIUM AND OXIDATIVE STRESS. **Martínez Alcaraz Edith Ruth,** Myrna Sabanero López, Gloria Barbosa Sabanero.

39 EFFECT OF 3-HYDROXYKYNURENINE AND 3-HYDROXYANTHRANILIC IN OXIDATIVE DAMAGE MARKERS INDUCED BY FeSO4 AND ONOO. **Ramírez Ortega Daniela.** Vázquez Cervantes GI, Ugalde-Muñiz P, Pineda Olvera B, Pedraza-Chaverrí J, Santamaría Del Ángel A y Pérez De la Cruz V.

41 PROTECTIVE EFFECT OF CURCUMIN AGAINST HEMIN-INDUCED NEUROTOXICITY IN PRIMARY CULTURES OF CEREBELLAR GRANULE NEURONS OF RATS. **Susana González-Reyes,** Silvia Guzmán-Beltrán José Pedraza-Chaverri.

43 ESTROGENIC MODULATION ON THE OXIDATIVE STRESS AND AGING IN THE DORSAL HIPPOCAMPUS. **Vicente Beltrán Campos,** Medina-Aguirre IG, Díaz-Ruiz A, Padilla-Gómez E, Alcaraz-Zubeldia M, Aguilar-Zavala H, Rios C, Díaz Cintra S.

45 EVALUATION OF THE PROTECTIVE EFFECT OF ETHANOL & RESVERATROL IN A MODEL OF CEREBRAL ISCHEMIA IN RAT. **Yaseck_Trejo,** Montes de Oca Balderas P. Ortiz-Plata A. Pedraza-Chaverri J. Aguilera P.

47 RELATIONSHIP BETWEEN OXIDATIVE STRESS AND PERIODONTAL DISEASE IN OLDER PEOPLE. **Beatriz Hernández Monjaraz**, Santiago-Osorio E, Betancourt-Rule JM, Mendoza-Núñez VM

ESTRÉS OXIDANTE EN MICROORGANISMOS

49 ANTIOXIDANT EFFECT OF PHYCOBILIPROTEINS EXTRACTED FROM *Spirulina maxima* ON PLASMID DNA FROM *Escherichia coli*. **Alan Rubén Estrada Pérez,** M Patricia Cervantes Cervantes, Nora B Medina Jaritz, Roxana Olvera Ramírez, José Luis Muñoz Sánchez JL.

51 CARBONILATION OF HSP70 PROTEIN FROM *Trypanosoma cruzi* DURING IT *in vitro* CULTURE. **Ignacio Martínez Martinez,** Bertha Espinoza Gutiérrez

53 IRON-SULFUR PROTEIN BIOGENESIS AND OXIDATIVE STRESS IN *Saccharomyces cerevisiae*. **Pérez-Gallardo Rocío Viridiana,** Díaz-Pérez Alma Laura Campos-García Jesús.

55 REGULATION OF *Candida glabrata* EPA2 EXPRESSION BY OXIDATIVE STRESS. **Jacqueline Juárez Cepeda**, Orta Zavalza E, De las Peñas Nava A

57 THE SINGLE CATALASE OF *Candida glabrata* PROTECTS *Saccharomyces cerevisiae* FROM HYDROGEN PEROXIDE. **Israel Cañas-Villamar**, Castaño I, De Las Peñas A

ESTRÉS OXIDANTE EN PLANTAS

59 QUANTIFICATION OF PHENOLIC COMPOUNDS AND ASSESSMENT OF ANTIOXIDANT CAPACITY IN COLORIN (*Erythrina americana* miller). **Laura Lisset Bata García,** Ana Angélica Feregrino Pérez, Andrés Cruz-Hernández, Ramón Guevara González, Adriana Jheny Rodríguez Méndez

61 ASSESSMENT OF THE EFFECT OF DETERGENTS IN THE DUCKWEED (*Lemna gibba* L.) **Alma Sobrino**, Morales-Torres J

MÉTODOS Y MODELOS PARA EL ESTUDIO DE LAS ESPECIES REACTIVAS

63 ANTIOXIDANT ACTIVITY OF PHYCOCYANIN. **Fernández-Rojas Berenice,** Medina-Campos Omar Noel, Pedraza-Chaverri José.

65 COMPUTATIONAL STUDY OF DIHYDROXYBENZENES RELATED WITH THE ENZYMATIC BROWNING. **Ortega Moo María Cristina,** Vargas Fosada Rubicelia

67 TOXIC EFFECT OF ANTI-FLU DRUGS IN *Daphnia magna*. **Alma Sobrino Figueroa**

TÓPICOS EMERGENTES EN EL CAMPO DE LAS ESPECIES REACTIVAS

69 SYNTHESIS, CHARACTERIZATION AND TOXICOLOGICAL EVALUATION OF MALTODEXTRIN CAPPED CADMIUM SULFIDE NANOPARTICLES IN HUMAN CELL LINES AND CHICKEN EMBRYOS **León-Buitimea A**, Rodríguez-Fragoso P, Reyes-Esparza J, Rodríguez-Fragoso L.

71 MANIPULATION OF XANTHINE OXIDASE-DERIVED REACTIVE SPECIES REDUCES OBESITY-INDUCED INFLAMMATION AND IMPAIRMENT OF GLUCOSE TOLERANCE. **Cantu-Medellin N,** Weidert ER, Shoenborn CJ, Champion HC, Baust J, Tarpey MM and Kelley EE.

73 SEMICONDUCTOR NANOPARTICLES EXHIBIT CYTOTOXICITY AND MITOCHONDRIAL IMPAIRMENT THROUGH ROS STIMULATION IN A459 CELLS. **Vicente Escamilla Rivera**, Marisela Uribe-Ramírez, Velumani Subramaniam, Andrea De Vizcaya-Ruiz.

75 ANTIOXIDANT DEFENSES IN *Perna viridis* (BIVALVIA: MITILIDAE) UNDER ANOXIA, ANHIDROBIOSIS Y REOXYGENATION, IN COPPER AND CADMIUM PREEXPOSURE ORGANISMS. **Edgar Zapata Vívenes,** Sánchez G. & Nusetti O

77 L-CARNITINE ANALOGS MODIFies THE PROLIFERATION AND INDUCE PHAGOCYTOSIS AND RESPIRATORY BURST IN THP-1. **Moctezuma-Ocampo AA**, León-Buitimea A, De la Cruz Cordero R, Reyes-Esparza J y Rodríguez-Fragoso L.

POSTER SESSION. EVEN NUMBERS
THURSDAY **18:45 – 21:00**

ESPECIES REACTIVAS Y SEÑALIZACIÓN

2. MODULATION OF NERVE GROWTH FACTOR EXPRESSION BY IONIZING RADIATION IN MOUSE TISSUES. **Albarrán-Ponce LÁ**, Fajardo-Miranda RM, Gamboa de Buen I, Valdovinos-Flores C, Vázquez-Vázquez MA and Gonsebatt ME
4 POSTFATIGUE TENSION IS REDUCED BY SODIUM ASCORBATE AND NICORANDIL. **Elizabeth Sánchez Duarte,** Trujillo Trujillo Xochitl, Saavedra-Molina Alfredo, Huerta-Viera Miguel, Cortés-Rojo Christian, Calderón-Cortés Elizabeth, Montoya-Pérez Rocío
6 EFFECT OF ARSENIC EXPOSURE ON THE CYSTINE/GLUTAMATE TRANSPORT IN NEONATAL MOUSE BRAIN. **Lucio A. Ramos Chávez,** Pavel Petrosyan y María E Gonsebatt
8 EFFECT OF REDOX STATE DURING PREMATURE SENESCENCE INDUCED DUE PROTEOSTASIS LOSS, IN PRIMARY MICE LUNG FIBROBLASTS. **Maciel Barón L,** Gónzalez Puertos VY, Galván-Arzate S, Tello-Solís S, Castro-Obregón S, Konigsberg Fainstein Mina
10 ELEVATED CONCENTRATIONS OF GLUCOSE SELECTIVELY BLOCK THE NITRIC OXIDE – DEPENDENT VASORELAXATION INDUCED BY PROLACTIN IN RAT CORONARY VESSELS. **Martínez-Cuevas PP,** Rubio R, González C.
12 ROLE OF OXIDATIVE STRESS IN THE ACTIVATION OF THE MAPK PATHWAY DURING THE APOPTOTIC DEATH OF NEURONS. **Morán J**, Zaragoza-Campillo MA
14 SYSTEMIC NERVE GROWTH FACTOR MODULATES THE TRANSCRIPTION OF AMINO ACID TRANSPORTERS AND GLUTATHIONE (GSH) SYNTHESIS IN MICE STRIATUM. **Valdovinos-Flores, C,** Petrosyan P y Gonsebatt ME
16 EFFECT OF RESVERATROL AND ETHANOL ON THE mRNA EXPRESSION OF GLUT3 IN CEREBRAL ISCHEMIA. **Anahí Tornes Reyes,** Ortíz-Plata A, Medina-Campos ON, Salazar MI, Pedraza-Chaverri J, Aguilera P and Espinoza-Rojo M

ESTRÉS OXIDANTE EN LA DIFERENCIACIÓN Y EL DESARROLLO

18 INSULIN PROMOTES ARABIDOPSIS ROOT HAIR GROWTH IN A ROS-DEPENDENT MANNER. **Edgar José Pascual Morales,** García Pineda Ernesto, Mellado Rojas María Elena, Beltrán Peña Elda María
20 CHANGES IN THE ACTIVITY OF THE GLUCOSE-6-PHOSPHATE DEHYDROGENASE DURING HYPOTHALAMIC SEXUAL DIFFERENTIATION IN RATS TREATED WITH TESTOSTERONE PROPIONATE PERINATALLY FEMALES. **González González Alicia,** Chávez García R, Ortega Camarillo C, Ávalos Rodríguez A, Borderas Tordecillas R,Vergara Onofre M.

ESTRÉS OXIDANTE EN LA MUERTE CELULAR Y LA ENFERMEDAD

22 ROLE OF OXIDATIVE STRESS ON HYPOGLYCEMIC CEREBRAL DAMAGE AND ITS POSSIBLE PROTECTION BY D-β-HYDROXYBUTYRATE. **Alberto Julio-Amilpas**, Teresa Montiel y Lourdes Massieu
24 EFFECT OF HYPOXIA ON A549 EPITHELIAL CELLS AND LUNG F-13 FIBROBLASTS VIABILITY. A PRELIMINARY STUDY. **Arnoldo Aquino Gálvez,** González-Ávila G, Gutiérrez-González LH, Delgado Tello J, Sommer B.
26 DIALLYLDISULPHIDE ACTIVATES Nrf2 FACTOR AND DECREASES THE CEREBRAL ISCHEMIC INJURY. **Chávez Domínguez Rodolfo**, González García Cecilia Ortíz, Plata Alma Maldonado Jiménez Perla

28 IN VIVO EFFECT OF GREEN TEA FLAVONOIDS (EPIGALLOCATECHIN-3-GALLATE AND QUERCETIN) ON THE GENOTOXICITY OF HEXAVALENT CHROMIUM: ANTIOXIDANT AND PROOXIDANT. **García-Rodríguez MC,** Montaño-Rodríguez AR, Nicolás-Méndez T, Altamirano-Lozano MA.

30 OXIDATIVE DNA DAMAGE ASSOCIATED WITH DECREASED ENZIMATIC ACTIVITY AND GENE EXPRESSION IN THYMUS OF MALNOURISHED RATS. **GRACIELA GAVIA GARCIA**, González Martínez H, Nájera Medina O, Miliar García A, Koninsberg Fainsten M, Luna López A, Conde Pérez-Prina C, Bonilla González E, González Torres MC

32 SCAVENGING CAPACITY OF TRYPTOPHAN METABOLITES: 3-HYDROXYANTHRANILIC ACID AND 3-HYDROXYKYNURENINE. **Gustavo Ignacio Vázquez Cervantes**, Ramírez-Ortega D, Lugo-Huitrón R, Rangel-López E, Pedraza-Chaverrí J, Santamaría A, Pérez de la Cruz V.

34 EFFECTS OF A PROGRAM OF PHYSICAL ACTIVITY INSTITUTIONAL ABOUT OXIDATIVE STRESS ON ELDERLY ADULTS WITH AND WITHOUT METABOLIC SYNDROME. **Hernández Hernández Héctor Daniel,** Gutiérrez López Liliana, García Galindo Miguel Ángel, García Sánchez José Rubén, Rincón Víquez Ma de Jesús, Olivares Corichi Ivonne María

36 ASSOCIATION BETWEEN TOTAL ANTIOXIDANT AND BIOMARKERS OF ENDOTHELIAL DYSFUNCTION. **María del Socorro Camarillo Romero,** Eneida Camarillo Romero, Hugo Mendieta Zeron, Ma. Victoria Dominguez Garcia, Miriam Flores Merino, Araceli Amaya Chavez, G. Gerardo Huitron Bravo

38 MECHANISMS OF CELL DAMAGE DURING OXIDATIVE STRESS IN LYMPHOCYTES. **Oxana Dobrovinskaya**, Edgar Bonales-Alatorre, Ulises Díaz-Llerenas, Igor Pottosin

40 CHANGES IN PANCREATIC REDOX BALANCE DURING CHRONIC EXPOSURE TO CADMIUM IN WISTAR RATS. **Samuel Treviño Mora**, Patricia Aguilar Alonso Alfonso, Daniel Díaz Fonseca Eduardo Miguel Brambila Colombres.

42 OXIDATIVE STRESS CONTRIBUTES TO CALPAIN ACTIVATION AND NEURONAL DEATH INDUCED BY GLUCOSE DEPRIVATION (GD). **Teresa Montiel,** Paramo B, Rivera-Martínez M, MORAN J, MASSIEU L.

44 ANTIDIABETIC ACTIVITY OF TIMBE (*Acaciella angustissima*) PODS METHANOLIC EXTRACT ON STREPTOZOTOCIN-INDUCED DIABETIC RATS. **Wendy Monserrat Carmen Sandoval,** Guevara-González RG, Reynoso-Camacho R, Salazar- Olivo L, Torres-Pacheco I, Feregrino-Pérez AA, and Lomas-Soria C

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PLENARY

Redox and electrophilic signaling in cancer, air pollution, and aging

Henry Jay Forman

Environmental exposure to air pollution and cigarette smoke results in multiple pathological events. Surprisingly, although the lung is the principal organ through which these agents enter, the effects are systemic. The mechanisms underlying the pathology are varied and incompletely understood. Nonetheless, much of the initial events appear to be mediated by signaling caused by hydroperoxides and electrophiles and the reaction with specific cysteine residues in critical signaling proteins but may also involve altered membrane structure. Cigarette smoke contains thousands of compounds; however, we have shown that two of them, H_2O_2 and acrolein are able to activate epithelial mesenchymal transition in small cell lung cancer epithelial cells. This occurs through modification of cysteines in regulatory regions of c-Src. Nanoparticles present in air pollution were previously shown to activate inflammatory cytokine production systemically. We have shown the nanoparticulate silica can induce cytokine production in macrophages and that this is largely dependent upon iron-induced lipid peroxidation in the plasma membrane and disruption of lipid rafts. We have shown that air pollution nanoparticles induce protective genes systemically; however, that occurs in young adult but not middle-aged mice. The protective enzyme induction is mediated by the Keap1/Nrf2 system, which becomes refractory with age.

OXIDATIVE STRESS: FUNDAMENTAL ASPECTS AND PHYSICOCHEMICAL INSIGHTS

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The endogenous and exogenous factors contributing to the increase of oxidative stress (OS) will be discussed, as well as the damaging effects of such stress on living organisms, and especially on humans. Different strategies aiming to ameliorate the deleterious effects of OS will be also discussed. Particular attention would be paid to chemical processes involved in both the molecular damages caused by OS, and the prevention (and/or repairing) strategies. A detailed discussion on the related data that can be obtained using different methods of the Computational Chemistry will be provided, as well as on the attainable reliability that can be achieved. Strategies based on kinetic considerations will be presented. The relative importance of different reaction mechanisms will be discussed as well as the influence of the polarity of the environment and of the acid-base equilibria. Specific examples of particular interest will be analyzed.

Mitochondrial Energy-Redox Signaling in Brain Aging and Neurodegeneration

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Alterations in mitochondrial energy metabolism (maintenance of the energy – redox axis) impinge on all aspects of cell function and are involved in the development of age-related pathologies. Mitochondria –viewed as a 'nexus for aging, calorie restriction, and sirtuins'– are involved in critical steps for cell function. Hence, defects in mitochondrial function that encompass alterations of the energy–redox axis seem early events in brain aging and neurodegeneration. The maintenance of glucose homeostasis is vital for brain function, for glucose is the primary fuel meeting the metabolic demands of neurons and glial cells. Aging is associated with decreases in the levels of both insulin/IGF-1 and their receptor. Dynamic microPET scanning demonstrated a significant decline in glucose uptake and metabolism as a function of age. This was associated with a decreased expression of neuronal glucose transporters GLUT3 and GLUT4 and microvascular endothelium GLUT1 (55 kDa), an increase in IRS phosphorylation at Ser^{307} (inactivation of IRS) and a decrease in IRS phosphorylation at Tyr^{612} (activation of IRS) as well as decreased phosphorylation (activation) of Akt at Ser^{473}. These changes translated as a decrease in synaptic transmission, expressed as decreases in input/output (measured as by measuring field excitatory postsynaptic potential) and long-term potentiation (LTP), which is widely believed to be a form of plasticity responsible for learning and memory. The dependence of primary cortical neuron bioenergetics on the PI3K/Akt pathway of insulin signaling was evidenced by the reduced ATP turnover and maximal respiratory capacity upon inhibition of PI3K. Decreased glucose metabolism appears as a coordinated response to JNK signaling (that results in inhibition of pyruvate dehydrogenase and cellular bioenergetics) and PI3K/Akt signaling, processes regulated by mitochondrial H_2O_2. H_2O_2 homeostasis and its modulation of redox-sensitive signaling pathways (IIS and JNK) may be viewed as a coordinated regulatory control of brain aging rate. The decline in mitochondrial bioenergetics is linked to impaired mitochondrial biogenesis (measured as the COX3/ 18SrDNA ratio), augmented levels of acetylated PGC1α, and loss of balance mitochondrial dynamic remodeling.

Regresar

Dieta, estrés oxidante y diabetes.

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La nutrigenómica ha permitido estudiar los mecanismos de acción de los nutrimentos y conocer su efecto en la salud. Uno de las aplicaciones de la nutrigenómica es el desarrollo de portafolios dietarios (combinación de dos o mas alimentos funcionales diseñados para corregir las anormalidades bioquímicas de un padecimiento específico). Debido a que la diabetes es un grave problema de salud en México, se han desarrollado diferentes tipos de estrategias para controlar este problema de salud. Una de estas estrategias son las dietarías en las cuales se han tratado de introducir alimentos mexicanos y de fácil acceso para el control de esta enfermedad. El nopal se ha utilizado en la medicina tradicional para controlar la diabetes, sin embargo no se conocía su mecanismo de acción. El nopal tiene un índice glucémico e índice insulinémico bajo por lo cual se ha recomendado en pacientes con diabetes tipo (PDT2) para un mejor control de los picos postprandiales de glucosa. Un hallazgo interesante fue que el consumo de nopal disminuye significativamente el péptido gastrointestinal GIP (glucose-insulinotropic peptide) cuya función es la de regular las concentraciones de insulina. Por otra parte, la presencia de compuestos bioactivos como los polifenoles quercetina, kaemferol e isorahmnetina, además del contenido de vitamina C y β carotenos le dan al nopal actividad antioxidante, siendo capaz de atrapar 3 especies reactivas de oxígeno. La capacidad antioxidante del nopal es similar a la del café que se considera uno de los alimentos con mayor actividad antioxidante. Interesantemente después del consumo de nopal hay un aumento en la actividad antioxidante en el plasma, eritrocitos y sangre total. Los resultados del presente trabajo muestran que el consumo de nopal disminuye los picos postprandiales de glucosa en PDT2 que están asociados con la formación de especies reactivas de oxígeno. También se encontró que la combinación de proteína de soya con nopal evita por completo los picos postprandiales de glucosa, disminuye los picos postprandiales de GIP que son evidentes en los PDT2 y están asociados con un aumento en la lipogenesis ene el tejido adiposo. Estudios en animales genéticamente dispuestos a desarrollar diabetes (rata Zucker fa/fa) mostraron que el consumo de nopal a largo plazo disminuye significativamente el desarrollo de esteatosis hepática, disminuye la formación de especies reactivas de oxígeno y la formación de malondialdehido. Por lo que el portafolio dietario a base de nopal y proteína de soya diseñado para diabetes reduce los picos postprandiales de glucosa y GIP, tiene un índice glucémico bajo, disminuye al esteatosis hepática, aumenta la actividad antioxidante y que trae como consecuencia un mejor control de las anormalidades bioquímicas desarrolladas durante la diabetes.

Regresar

NATURAL ANTIOXIDANTS IN THE NEUROPATHIC PAIN TREATMENT

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Neuropathy is the most common and debilitating complication of diabetes and results in pain, decreased motility, and amputation. The prevalence of neuropathy is estimated to be about 8% in newly diagnosed diabetic patients and greater than 50% in patients with long-standing disease. Hyperglycemia clearly plays a key role in the development and progression of diabetic neuropathy since glucose metabolic pathways cause an imbalance in the mitochondrial redox state of the cell, and lead to excess formation of reactive oxygen species, which in turn, promote inflammatory reactions and neuronal dysfunction. To date, currently accepted medical approaches are only partially successful and they are only thought to alleviate the symptoms of diabetic neuropathy; however, they are not eliminate the root causes and have serious side effects. Alternative to these drugs are natural product-derived compounds, which represent great structural diversity that is not commonly seen in synthetic compounds, and some of them offer combined antioxidant, anti-inflammatory and antineuropathic properties. In this regard, our data showed that 7-hydroxy-3,4-dihydrocadalin, an active compound of Mexican arnica with antioxidant, anti-inflammatory and antinociceptive activities, is a natural drug with therapeutic potential for diabetic neuropathy treatment, since acute and chronic oral administration of this compound decreased hyperalgesia and allodynia in a similar way than pregabalin, but did not affect motor coordination. The effect seems to involve activation of 5-HT1 receptors and inhibition of nitric oxide synthesis, as well as antioxidant properties. On the other hand, epicatechin, a flavonoid present in cacao, is another promising compound due acute or chronic treatment with this compound, reduces nociceptive hypersensitivity in diabetic rats through the involvement of the nitric oxide-cyclic-GMP-K^+ channels pathway as well as activation of 5-HT1A and 5HT1B, and at a lesser extent 5-HT1D, but not opioid, receptors.

GLUTATHIONE ENDOGENOUS ANTIOXIDANT: RADICAL SCAVENGER AND DAMAGED DNA REPAIRER.

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Glutathione, which is the most abundant cytosolic thiol, plays important roles in the non-enzymatic antioxidant defense system. Its free radical scavenging activity towards radicals of different nature (•OH, •OOH, •OCH3, •OOCH3, •OOCHCH2 and •OOCCl3) have been studied[1] using the Density Functional Theory. It was found that the rate constants range from 2 x 10^4 M^{-1} s^{-1} to diffusion limit. Therefore it can be stated that glutathione is an excellent free radical scavenger. It reacts exclusively by H transfer, and with the exception of its reaction with -OH there is only one important channel of reaction, yielding to the S-centered radical. For the reaction with •OH, on the other hand, a wide product distribution is expected. Glutathione was found to be exceptionally good as a •OOH radical scavenger. Also the chemical repair of radical-damaged DNA by glutathione in aqueous solution has been studied[2]. Two main mechanisms were investigated: the single electron transfer (SET) and the hydrogen transfer (HT). Glutathione was found to repair radical damaged DNA by HT from the thiol group with rate constants that are close to the diffusion-limited regime, which means that the process is fast enough for repairing the damage before replication and therefore for preventing permanent DNA damage. The SET mechanism was found to be of minor importance for the repairing activity of glutathione.

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Regresar

Nanoparticles in the environment: life-cycle, fate & transport, and reactivity

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Abstract

Nanotechnology, and in particular engineered nanomaterials (ENMs), is now becoming a significant fraction of the material flows in the global economy. We are already reaping the benefits of improved energy efficiency, material use reduction, better performance in many applications, and many new applications that have been enabled by this technological advance. However, it is important to estimate the potential for human and ecological receptor exposure to ENMs. This involves bringing together market information and a life-cycle based understanding of the potential releases of ENMs and thus likely exposure concentrations. In addition, their distribution and eventual fate in the environment will determine the dose that humans and ecological receptors may be exposed to. Emerging information on their reactivity and toxicity will allow us to better understand the potential risks of different ENMs.

Regresar

CHALLENGES ASSOCIATED WITH THE TOXICITY EVALUATION AND OXIDATIVE STRESS OF ENGINEERED NANOMATERIALS

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The use of engineered nanoparticles (ENM) in a variety of applications, such as electronic devices, paints, cosmetics and as drug-delivery or therapeutic agents, has increased exponentially. Their unique physicochemical (mechanical, chemical and electrical) properties, which depend directly on their size, surface, structure and functionalization, are likely to increase their reactivity in contact with biological systems, and thus their hazardous potential. Amongst the main toxic mechanisms described for NP toxicity are oxidative stress and protein interaction which are known to lead to cell death or inflammation responses. We have carried out comparative studies of different engineered nanoparticles, which are being used in several applications: copper indium gallium diselenide (CIGS), amorphous silica (SiO_2), iron oxide (Fe_3O_4), zinc oxide (ZnO) and titanium dioxide (TiO_2), to determine their *in vitro* toxicity, cell death induction, observation of morphology, reactive oxygen species (ROS) generation and mitochondrial or lysosomal destabilization, as markers of oxidative stress and cell homeostasis alteration.
To ensure nanometer fractionized suspensions and reduce agglomeration, NP were resuspended in culture media containing 2-5 mg/mL of BSA. Stable average sizes bellow 350 nm and zeta potential of ~-15 mV, in culture medium, were measured using dynamic light scattering (DLS) and confirmed by scanning electron microscopy (SEM). Cytotoxicity was determined using two different techniques (MTT assay and crystal violet) in human alveolar epithelial A549 cells, exposed to 6.25 - 100 µg/ml for 6 - 72 h; ZnO was the most cytotoxic NP, followed by CIGS>Fe_3O_4>SiO_2>TiO_2 NP. Intracellular ROS generation using DCFH-DA, showed that CIGS NP induce a significant dose-dependent increase, while Fe_3O_4 NP induced a moderate generation, and no significant differences were observed with ZnO, SiO_2 and TiO_2 NP. A significant reduction in mitochondrial membrane potential was observed with ZnO, CIGS, Fe_3O_4 and SiO_2 NP, although lysosomal integrity at early time-points changed slightly. Our results demonstrated that ZnO, CIGS, Fe_3O_4, SiO_2 and TiO_2 NP were readily incorporated into cells, inducing different cellular responses depending on the type of ENM; higher cytotoxicity (cell death, mitochondrial and lysosomal destabilization) did not always correlate with higher induction of ROS generation, ie. ZnO. Hence, specific properties of size, chemical composition and shape by which ENM are designed for influence their cytotoxicity, in addition conventional *in vitro* systems to test cytotoxicity and oxidative stress should be adequated according to each type of ENM to accurately assess their toxicity.

(Acknowledgement: ICyTDF 396/10 for partial financial support).

Regresar

STUDY OF SILVER NANOPARTICLES ON BIOLOGICAL EXPERIMENTAL MODELS.

ROLE OF NITRIC OXIDE

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The convergence of nanotechnology and medicine has generated a new expectation in the field of pharmaceutical therapy. The silver nanoparticles (AgNPs) are widely used in medicine due to their antimicrobial properties. However there is a lack of information about their new biophysical properties, functions and effects at different levels of biological organization, and their impact on human health. The aim of the current presentation is show the recent advances that our laboratory has been investigate, related with the effects that confer the AgNPs at different biological targets, and their potential toxic or beneficial implications in the cardiovascular (CVS) and respiratory (RS) system. Physical characterization of AgNPs by transmission electron microscopy (TEM) demonstrated that nanoparticles ranging in size from 8 to 80 nm confer selective biological effects. We observed in coronary endothelial cells and aortic blood vessels, that AgNPs induced dual effects, at low concentrations, induced vasoconstriction endothelium dependent, anti-proliferative and cytotoxic effects; at high concentrations, stimulated vasodilation endothelium dependent, proliferation and no cytotoxicity mediated by the activation of endothelial nitric oxide synthase (eNOS), which produces low concentrations of nitric oxide (NO), an important vasodilator and antihypertensive agent. However in the RS, in respiratory primary culture cells and isolated rat trachea rings, AgNPs induced toxic effects, modifying the contractile action in presence of the endogenous contractile molecule, acetylcholine (ACh), inducing hyper-reactivity mediated by the inducible nitric oxide synthase (iNOS), which promotes large amounts of NO related with allergic mechanisms. These data suggest a specific and selective mechanism of action induced by AgNPS depending on the biological target. Further studies are warranted to elucidate the signaling pathways responsible to promote their toxic or beneficial effects in the CVS and RS.

Regresar

Functional Nanofoods: Synthesis, Characterization and Nanotoxic Effects in Cancer Cells

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Key words: nanofood, nanoparticles, magnetite, vitamin E, cancer

BACKGROUND: Nanofoods include nanocarriers for food and delivery of nutrients as vitamins. Magnetite nanoparticles (Nps) can be used as nanocarriers to enhance and improve the efficiency of delivery of vitamin E analogues. Although the best understood function of vitamin E is its antioxidant activity, numerous studies have shown that certain vitamin E analogues exhibit antitumour and citotoxic properties. Herein we report the effect of nanofoods synthesized with magnetite Nps functionalized with alpha tocopheryl succinate (α-TOS), the most effective anticancer analogue of vitamin E. One problem with α-TOS is its vulnerability in cervical cancer cells with high levels of esterases. **OBJETIVE**: To evaluate the nanocytotoxicity and antitumor effect of magnetite Nps functionalized with α-TOS (Nps-α-TOS). **METHODS**: Magnetite Nps-α-TOS were prepared by a coprecipitation method and functionalized with α-TOS. The synthesized magnetite Nps-α-TOS were characterized by FTIR, TGA, EDS, TEM, SEM. Then a human cervix cancer cell line high in esterases was treated with Nps-α-TOS by 24, 48 and 72 h. The nanocytotoxicity and antitumor effect were evaluated using Confocal Microscopy and MTT assay. **RESULTS**: TEM and SEM studies revealed an average Nps size of 15 nm and irregular spherical in shape. EDS and FTIR results support the formation of Nps detecting mineral and organics constituents respectively. The *in vitro* tests shows by first time the Nps-α-TOS are more cytotoxic and effective that α-TOS alone in resistant cervix cancer cell. We also observed the Nps-α-TOS is selective to cancer cells and it can be internalized and produce dramatic morphological changes in treated cells associated to apoptosis. **CONCLUSION**: In this study we synthesized a nanofood with magnetite nanoparticles and α-TOS with enhanced anticancer activity in resistant cancer cells.

Regresar

Efectos citotóxicos y genotóxicos asociados a nanopartículas fabricadas a base de polietilcianoacrilato, SiO_2 y quitosan.

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En relación a las Nanociencias y Nanotecnología, se han creado expectativas amplias con impacto y progresos considerables en diferentes en áreas como la medicina, energía y medio ambiente. Los esfuerzos al respecto han orientado estudios de investigación en el área de las Ciencias de la Salud. Al respecto, existen evidencias que sugieren que los sistemas nanoestructurados pueden comprometer la función celular ejerciendo efectos tóxicos asociados a diferentes eventos celulares. Aunado a esto, las evidencias muestran diferencias fisicoquímicas específicas de los materiales que conforman a las nanopartículas comparativamente con su estado inicial así como en la capacidad para inducir toxicidad *in vitro* e *in vivo*. Se presentaran algunos resultados acerca de la capacidad tóxica *in vitro* e *in vivo* de sistemas de nanopartículas únicas e híbridas preparadas a base de polietilcianoacrilato, SiO_2 amorfo y quitosan que caracterizamos mediante microscopia electrónica de transmisión, tamaño de partícula y potencial z.

Los resultados muestran diferencias significativas respecto al control negativo en la inducción de especies reactivas al ácido tiobarbitúrico, la concentración de GSH, la viabilidad celular, el índice mitótico y la cinética de proliferación celular *in vitro* para algunos de los sistemas de nanopartículas obtenidos.

Cabe mencionar que no se mostraron en todos los casos estudiados, efectos dosis-respuesta en los parámetros evaluados. Para algunos de los sistemas en estudio se encontraron evidencias de daño genotóxico estimado mediante electroforesis unicelular (ensayo cometa) y en algunos de estos casos no existió citotoxicidad significativa.

En general podemos decir que los sistemas de nanopartículas obtenidos, optimizados y caracterizados mostraron una susceptibilidad diferencial en la

inducción de los efectos citotóxicos y genotóxicos estudiados. Estos resultados son importantes y deben considerarse durante la fabricación, manejo y estudio de los sistemas nanoparticulados capaces de ser considerados a usarse en diferentes áreas del conocimiento.

Regresar

CELL SIGNALING BY OXIDATIVE STRESS

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Reactive oxygen species (ROS) had been traditionally implicated in causing cell damage, but nowadays it is clear that they also play a major physiological role in several aspects of cellular signaling and regulation. However, many evidences indicate that ROS are implicated in several pathologies when they exceed their physiological concentrations, such as cancer, diabetes, atherosclerosis and neurodegeneration. This participation may be via direct or indirect oxidation of proteins, lipids or DNA. Particularly, ROS may contribute to disease by affecting cellular signaling molecules, resulting in alterations of different cellular events. Some redox sensitive signaling molecules (which may gain, loss or even switch to new functions), are transcription factors, protein kinases, protein phosphatases, phospholipids, fatty acids, ion channels, small GTPases and contractile elements. Oxidative alterations in signaling cascades or molecules like MAPKs, PI3K, phospholipase C and/or PKC, for example, lead to enhanced cell proliferation and/or survival, while ROS mediated ASK1 or caspase 9 activation leads to induction and inhibition of apoptosis, respectively. Also, it has been shown that oxidative stress may induce the activation of several b-zip transcription factors as some of the AP-1 or Nf-kB family members involved in different cellular processes. Despite the great advances in this area of oxidative stress-cell signaling-pathology relationship, we are still far away from understanding it clearly. There are experimental models of human pathologies which may be powerful tools to continue studying this.

Regresar

Reprogramming the epigenome through oxidative stress and metabolism

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Cancer has been speculated to arise from a clonogenic progenitor that acquires a series of molecular alterations to progress into malignancy. Likewise, the development of multicellular organisms follows a similar path in which a fertilized ovum obtains molecular changes as it matures into a complete organism with diverse cell types. The free radical theories of cancer and development suggest that changes in oxidants and metabolism create an environment that manifests the gross phenotypic changes of these biological processes; however a linkage between oxidants and gene expression changes remains obscure. In the past decade, epigenetics has been proposed as a means to drive phenotypic changes in cancer and development by altering gene expression. With the recent discovery that the enzymes responsible for initiating and perpetuating epigenetic events are linked to metabolism by their cofactors, a new paradigm linking oxidative stress and gene expression can be forged. Here, we summarize the foundation of such a paradigm by on the origins of cancer, in which metabolic alterations create an epigenetic progenitor that clonally expands to become cancer. We suggest that metabolic alterations and oxygen gradients reprogram the epigenomeof cells by disrupt the availability of cofactors such as S-adenosylmethionine, α-ketoglutarate, $NAD(^{+})$, and acetyl-CoA. We further speculate that redox biology can change epigenetic events through oxidation of enzymes and alterations in metabolic cofactors that affect epigenetic events such as DNA methylation. Combined, metabolic and redox changes serve as a flywheel for creating the epigenetic complexity observed during cancerand development.

ROS, RAS-1, GROWTH AND DEVELOPMENT.

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In *Neurospora crassa*, asexual spore formation (conidiation) is started when an aerated liquid culture is filtered and the resulting mycelial mat is exposed to air. Three morphogenetic transitions take place: filament (hyphae) adhesion, aerial hyphae growth and asexual spores (conidia) development [1]. Each transition is started by an unstable hyperoxidant state (HO) and results in growth arrest, autophagy, antioxidant response and an insulation process from dioxygen [2-4]. These responses stabilize the system and growth can restart in the differentiated state. ROS production is required for development: the NADPH oxidase NOX-1 for sexual and asexual reproduction and the NOX-2 for sexual spore (ascospore) germination. Both phenotypes are observed in the Δ*nor-1* (p67 regulatory subunit) mutant strain [5].

A point mutation in *ras-1*, *ras-1*^{bd}, results in increased ROS formation during conidiation and the strain produces more aerial mycelium and increased conidiation. Different *ras-1* point mutations were generated that affected growth and conidiation under submerged conditions. Only three proteins have a predicted RAS association domain: NRC-1 (MAPKKK), the yeast STE50p orthologue (STE50) (a scaffold protein associated with NRC-1) and adenylate cyclase (AC). The Δ*nrc-1* strain was female sterile and produced non-viable ascospores. It was more resistant than Wt to H_2O_2 and osmotic stress. Δ*ste50* strain was also female sterile, grows slowly and was sensitive to H_2O_2 and osmotic stress. The AC mutant strain *cr-1* affects vegetative growth and aerial hyphae formation, but not conidiation. Thus the NRC-1, MEK-1 and MAK-2 kinases cascade is involved in both sexual and asexual sporulation.

Oxidative stress and RAS-1 determined partially cAMP levels during the first two HOs of the conidiation process. Higher cAMP levels than Wt were observed in *ras-1*^{bd}. In both strains, [cAMP] decreased within minutes at the start of the first two HOs and thereafter, almost as rapidly, levels recover to initial value. *N. crassa* has a high (PDE_H) and a low affinity (PDE_L) phosphodiesterase. The Δ*pde*$_H$ strain grows slowly and does not conidiate; no evident phenotype was reported for Δ*pde*$_L$. We found that PDE_L was mainly responsible for the cAMP decrease during the first HO and that hyphal adhesion was retarded in Δ*pde*$_L$. Both PDE_H and PDE_L were responsible for cAMP decrease during the second HO. H_2O_2 and low Ca^{++} activated PDE_L and inhibited PDE_H. This opposite regulation can explain the cAMP decrease during the HOs of the *N. crassa* conidiation process.

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This work was supported by grants CONACyT 132687Q and PAPIIT-UNAM IN213811, N209313 to WH.

REACTIVE OXYGEN SPECIES AND THE SURVIVAL RESPONSE IN THE LIVER

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NADPH oxidase has a dedicated function of generating reactive oxygen species (ROS). A particular feature of NADPH oxidase/ROS-mediated signaling is the heterogeneity of its activating stimuli. In this way, it could be consider as an important component of the cellular stress signal transduction network. Activation of the cell survival pathway by NADPH oxidase (Nox) family may have a critical role in coordinating the responses of the cells to deal with the adverse effects, either by activating stress kinases and promoting cell adaptation, whereas Nox may also convey signals toward apoptosis in irreversibly injured cells. A role for the NADPH oxidases Nox1, Nox2 and Nox4 have been implicated in different liver pathologies. A role for Nox1, Nox2 and Nox4 has been implicated in liver fibrosis, while Nox4 contributes to ROS production y may be related to hepatitis C virus-induced liver disease. On the other hand, the hepatocyte growth factor (HGF) and its receptor c-Met are involved in the antioxidant response and protect against oxidative stress-induced cellular damage. Data from our lab show that HGF induced a biphasic mechanism of NADPH oxidase regulation in order to improve the protective response and cell viability. The first phase employed the rapid increase in ROS production as signaling effectors to activate the Nrf2-mediated protective response resulting in up-regulation of the antioxidant proteins, such as NAD(P)H quinone oxidoreductase (NQO-1) and gamma- glutamyl cysteine synthetase (gamma-GCS). The second phase operated under a prolonged HGF exposure, caused a suppression of the NADPH oxidase components, including Nox2, Nox4, p22 and p67, and was able to abrogate the TGF-beta induced ROS production and improve cell viability. A differential regulation of the NADPH oxidase isoforms is observed in HGF treated hepatocytes. HGF induces p47 phox phosphorylation by a mechanism mediated by PKC-delta resulting in NADPH oxidase-mediated cell survival. In conclusion, we provide evidence that HGF regulates NADPH oxidase in the hepatocytes by a dual mechanism. HGF treatment leads to NADPH oxidase activation via p47 phox activation and ROS generation, which in turn results in PKC-delta Nrf2 activation and expression of survival proteins. NADPH oxidase regulation is also a transcriptional repression of the expression of p22, Nox2, Nox4 and p67. We propose that this outcome constitutes the central mechanism by which HGF/c-Met signaling pathways counteracts the cytotoxic effects of growth factors such as TGF-beta (CONACYT # 131707).

El papel de la lipoperoxidación y el estrés oxidante en la regeneración hepática inducida por hepatectomía parcial en ratas.

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En la década de los 80's comenzó el estudio de la participación de los eventos lipoperoxidativos durante la progresión del ciclo celular, principalmente en aquellas células provenientes de tejidos con una tasa alta de proliferación, como es el caso de la regeneración hepática en ratas, a las que se someten a una remoción hasta del 70% de la masa del tejido a través de cirugía. Con antecedentes previos de que las membranas del retículo endoplásmico (microsomas) obtenidas de tejidos tumorales, presentan una lipoperoxidación disminuida cuando se comparan con tejidos sanos de lento recambio proliferativo, se encontró que los microsomas obtenidos de hígados proliferantes, también producían una menor cantidad de radicales libres, lo que llevó a concluir que existe una relación inversa entre proliferación celular y estrés oxidante. Sin embargo, otros autores usando otras fracciones subcelulares, encontraron lo contrario; esto es que, la regeneración hepática inducida por hepatectomia si cursa con un aumento significativo en la tasa de lipoperoxidación. A partir de esta discrepancia, nosotros encontramos que en este mismo modelo de estudio de la proliferación hepática y usando diferentes técnicas para cuantificar la formación de radicales libres y de productos de la lipoperoxidación, que existe una lipoperoxidación selectiva: disminuye en microsomas y existe un aumento confinado a las membranas plasmáticas y al citosol (citoplasma) del hígado en regeneración. Además, estos cambios son órgano-específicos, transitorios y dependientes de un umbral, es decir, que solo ocurren con hepatectomias mayores del 40% de la masa hepática. Estos datos nos llevaron a proponer que la lipoperoxidación es un evento fisiológicamente importante que puede regular la progresión de la proliferación hepática. De aquí, y a través de manipular el estado redox celular in vivo, en particular de las especies reactivas de oxígeno, con la administración de un pro-oxidante, como el etanol, o de un antioxidante, como la vitamina E (α-tocoferol), hemos demostrado que la regeneración hepática, inducida quirúrgicamente en ratas, sí depende de una lipoperoxidación cuya magnitud se mantiene en un rango muy estrecho, que solo ocurre en fracciones celulares claves, y que tiene una temporalidad muy definida. Los blancos de esta lipoperoxidación selectiva, hasta ahora identificados, son principalmente la expresión de las STATs 1 y 3 (Signal transducer and activator of transcription), de ciclina D1 y de PCNA (Proliferating celular nuclear antigen), así como el aumento en la actividad de la timidina cinasa. Estos efectos están íntimamente ligados a un aumento en las defensas antioxidantes de la célula, un tasa baja de peroxidación nuclear, cambios de la producción de retinoides y de la actividad de la alcohol deshidrogenasa, que participa en el metabolismo de dichos retinoides. Estos eventos parecen estar regulados por el redox de la pareja NAD/NADH, tanto en citoplasma como el mitocondrial. Sin embargo, aún no hemos identificado la fuente principal de estas especies reactivas de oxígeno, en las diferentes estructuras subcelulares.

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EGF Receptor phosphorylation in ethanol-induced oxidative stress is associated with CYP2E1 overexpression in MCF-10A cells.

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Ethanol (EtOH) consumption is a well-established risk factor for breast cancer in women. However, the mechanism by which EtOH exerts its carcinogenic activity in breast tissue remains known. In the present work, we investigated the hypothesis that EtOH increases reactive oxygen species (ROS) and oxidative stress and, as a consequence, transactivates the epidermal growth factor receptor (EGFR) in human mammary epithelial cells. Cells were exposed at ethanol concentrations relevant to blood levels in humans (10, 30 and 100mM) for 18 h or every 12 h for 3 days. Expression of ethanol-metabolizing enzymes was determined by RT-PCR and Western blot analysis, production of reactive oxygen species was examined using dichlorofluorescein fluorescence, and pY1086 EGFR phosphorylation was determined by immunoprecipitation and Western blot analysis. Our results show that EtOH decreased CYP2E1 protein expression at 10, 30 and 100 mM when cells were treated every 12 h for 3 days. Also, the mRNA expression of EtOH-metabolizing enzymes in primary human mammary epithelial cells (HMEC) from different donors was found to be highly variable, with HMEC expressing different levels of ADH1B, ADH1C, ALDH2 and CYP2E1 as compared to MCF-10A breast cells. We also found that 30 and 100 mM EtOH increased ROS levels after 2 h treatment in CYP2E1over-expressing 4.1.2E1 cells. Additionally, we found that in CYP2E1over-expressing 4.1.2E1 cells increased the level of pY1086 EGFR phosphorylation after 18 h ethanol treatment (at 30 and 100 mM EtOH). These studies suggest that ethanol can transactivate EGFR and activate downstream cell signaling in MCF10A cells, mediated by oxidative stress.

Regresar

ISCHEMIA-REPERFUSION: LESSONS FROM MARINE MAMMALS

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In humans and other mammals, if blood flow to an organ or tissue is interrupted or decreased (ischemia) and later returned to normal (reperfusion), an excessive amount of free radicals and other reactive oxygen species (ROS) is produced. A number of liver, kidney and heart diseases, such as myocardial infarction or stroke, are associated to ischemia and reperfusion. However, marine mammals, including seals and dolphins, experience repeated cycles of ischemia and reperfusion without signs of the cellular damage due to excessive ROS production. As part of their natural history, marine mammals dive holding their breath for periods that can range from 3 to 190 minutes. The ability of marine mammals to make frequent long dives depends on oxygen conserving mechanisms, including reduction and redistribution of cardiac output. The result is vasoconstriction in organs and tissues, some of which are deprived of blood throughout much or all of the dive. Blood flow is restored when the animal surfaces and resumes breathing. The mechanisms that allow marine mammals to tolerate repetitive cycles of ischemia and reperfusion accompanying frequent dives and to avoid oxidative stress will be discussed.

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OXIDATIVE STRESS AS A POSSIBLE MEDIATOR OF LIFE-HISTORY TRADE-OFFS

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Life history theory is concern on how natural selection shape organisms to optimize survival and reproduction in the face of ecological challenges. Central to our understanding of life-history evolution is the concept of trade-offs among life history components. Oxidative stress has been suggested as a mediator of these trade-offs, and hence a determinant of the evolution of life history strategies. In this talk I will show data from our studies in the blue-footed booby, a long-lived marine bird, that supports the idea that sexually selected traits might advertise oxidative stress levels, particularly in older males.

THE IMPLICATIONS OF OXIDATIVE STRESS IN THE DEVELOPMENT OF NEURODEGENERATIVE DISORDERS CAUSED BY THE ENVIRONMENTAL CONTAMINANTS ARSENIC AND ATRAZINE.

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The herbicide atrazine (ATR) and the metalloid arsenic (As) are two substances widely present in the environment. Recent studies in rodents showed that exposure to these substances cause dysfunctions in the central nervous system, their main targets being the nigrostriatal and mesolimbic DAergic systems. It is known that the main mechanism of toxicity for As is oxidative stress, but for ATR the mechanism has not been described yet. In order to evaluate the integrity of the DAergic systems after As or ATR exposure, we evaluated locomotor activity, monoamine levels and antioxidants mRNA expression of exposed rodents as shown in the three experiments detailed below. Experiment I. Male and female C57Bl/6J mice were treated with 0.05, 0.5, 5.0, or 50 mg As/L of drinking water (D.W.) for 4 months, and locomotor activity was evaluated every month. Female mice treated with 0.05, 0.5, and 5.0 mg As/L showed hyperactivity in every monthly test, whilst at 4 months of As exposure hyperactivity and hypoactivity were present in male mice groups exposed to 0.5 mg As/L and 50 mg As/L, respectively. Striatal dopamine (DA) content was decreased only in female mice; mRNA expression of tyrosine hydroxylase (TH) and cytosolic thioredoxin (Trx-1) decreased in striatum (STR) of male and nucleus accumbens (NAcc) of female mice, respectively. Experiment II. Male Sprague-Dawley rats were exposed to 0.05, 0.5 or 50 mg As/L of D.W. for one year, and locomotor activity was evaluated monthly. The group treated with 50 mg iAs/L showed hypoactivity at 12 months of As exposure and increases in striatal DA content. We observed a dose-dependent up-regulation of mRNA for Mn-Superoxide Dismutase and Trx-1 and a down-regulation of DA receptor 2 (DAR-D_2) mRNA expression in the NAcc. In addition mRNA expression of DA receptor 1 (DAR-D_1) and nuclear factor erythroid 2-related factor 2 (Nrf2) were down-regulated in NAcc of the group exposed to 50 mg iAs/L. Increased DAR-D_1 mRNA expression was found in the STR of the 0.5 mg iAs/L group. Experiment III. Adult male Sprague-Dawley rats were I.P. injected thrice a week with 100 mg ATR/kg BW or vehicle over two weeks. Hypoactivity was found after every ATR administration and it lasted up to five days. We observed decreases in striatal DA, DOPAC, and HVA levels without any alteration in the striatal expression of the mRNA for Mn-SOD, Trx-1, DAR-D_1, or DAR-D_2. In contrast, in the NAcc no changes in monoamine markers were observed, but a down-regulation of Trx-1 expression was detected shortly after ending the ATR treatment. We can conclude that chronic exposure to As or the repeated injection of ATR causes behavioral and neurochemical alterations related to DAergic systems, that it involves changes in mRNA expression of antioxidants, and that the most affected region was the NAcc.

IODINE AND REDOX BALANCE: FROM PHYSIOLOGY TO CANCER

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Iodine is a micronutriment that is essential for life. In all vertebrates, the uptake of iodine by thyroid gland is crucial for thyroid hormone synthesis and to maintain the integrity of the thyroid epithelium. Iodine is also taken up by extra-thyroid tissues such as the gastric mucosa, intestine, and salivary, mammary, and prostate glands. The uptake of iodide (I^-) and molecular iodine (I_2) is mediated by the Na+/I- symporter (NIS) and by facilitated diffusion, respectively. In thyroid and mammary glands, there is evidence that I^- can be oxidized into more reactive species, such as I_2, by specific peroxidases (thyro-, lacto-, and myeloperoxidase). In vivo and/or in vitro studies on cancer (thyroid, mammary, and prostate) have shown that I_2-treatment exerts antitumoral effects (induces cell arrest and apoptosis, and reduces the expression of invasion genes) through direct or indirect mechanisms. In the first case, I_2 could acts as an antioxidant, neutralizing reactive oxygen species. The second case involves the formation of an iodolipid (6-iodolactone, 6-IL) and the activation of gamma type peroxisome-activated receptors (PPARγ). PPARγ regulates gene expression associated with the cell cycle (increases p53, p21), apoptosis (increases Bax-caspase pathway), cell invasion (decreases VEGF, µPA). In a cell-free system we have shown that 6-IL can directly inhibit the activity of 15-lipooxigenase-1 (15-LOX-1, pro-tumoral oxidase) independently of PPARγ. Currently, studies are analyzing I_2 effects on the inflammatory response in prostate pathologies. Studies supported by UNAM/DGAPA (202513, 200813) and CONACYT (127368, 176911).

EVALUATION OF OXIDANT STRESS.

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We have used in our laboratory several approaches to evaluate oxidant stress and oxidant damage including (a) activity of the antioxidant enzymes catalase, superoxide dismutase, glutathione peroxidase, glutathione reductase, glutathione-S-transferase, gamma glutamylcysteine ligase, and heme oxygenase, (b) endogenous antioxidants such as glutathione, (c) hydrogen peroxide levels, (d) NADPH oxidase activity, (e) aconitase in isolated mitochondria, (f) reactive oxygen species production in cells in culture using fluorescent probes such as dihydroethidium and 5-(and 6-) carboxy-2,7-dichlorodihydrofluorescein diacetate and (g) protein carbonyl content, malondialdehyde, 4-hydroxy-2-nonenal and 3-nitrotyrosine.

PRO-OXIDANT REACTIONS AND RETICULAR RESPONSE IN THE LIVER ARE MODULATED BY A PROTOCOL OF RESTRICTED FOOD ACCESS AT DAYTIME

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Physiological activity is influenced by the circadian system, hence, most of the behavioral, hormonal and metabolic responses show diurnal variations related to the light-dark cycles associated to the rotational movement of the Earth. The principal pacemaker in vertebrates is located in the hypothalamus within the suprachiasmatic nuclei. However, an alternative clock has been recognized that is independent of light synchronization but is entrained by food access. The anatomical location of this oscillator is not determined, and is known as the Food entrained oscillator (FEO). The FEO expression implicates a restricted feeding schedule (2 h of food access per day) during 3 weeks. This feeding protocol involves a long fasting time (22 h) and a short mealtime (2 h). Hence, the FEO expression consists in a repetitive cycles of long fasting and brief period of intense and abundant feeding.

Pro-oxidant reactions were measured by the malone dialdehyde (MDA) and conjugated dienes techniques. The first method is proportional to the sample oxidative potential whereas the second one is an estimation of the *in vivo* lipid peroxidation. The techniques were done in subcellular fractions of the liver (mitochondria, microsomes, plasma membrane, nuclei, etc.). The results indicated a reduction in the peroxidative activity in most of the liver subcellular fractions in the group under restricted feeding schedules.

The reticular response was evaluated by estimating the expression of IRE, GRP94, BIP and CHOP. The data showed a generalized increase in the presence of these markers in the liver of the rats with restricted food access. The results were more evident with GRP94, BIP and CHOP.

In addition, the presence and the nuclear location of NF-κB (a marker of metabolic cellular stress) were also more abundant in the hepatocytes of the group with restricted mealtime.

The results will be discussed in terms of a reosthatic and chronostatic adaptation shown by the hepatic physiology and metabolism during the expression of the FEO.

INTRAMOLECULAR DELOCALIZATION OF FREE RADICALS: HEMOPROTEINS, A MATTER OF LIFE OR DEATH.

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All hemeproteins, including peroxidases, are inactivated in the presence of catalytic concentrations of hydrogen peroxide. The inactivation involves the radical generation that is essential for the enzyme activity, followed by an intramolecular electron transfer that ends in the unspecific oxidation of some protein residues. This inactivation process is especially important in the absence of reducing substrates, limiting the potential application of peroxidases [1, 2].

The electron flows during the peroxidase self-inactivation of the variant CYPBM3 “21B3” from *Bacillus megaterium* were studied [3]. Hybrid computational chemistry tools, such as quantum mechanics and molecular mechanics (QM/MM), were used to calculate the probability of electron transfer from Compound I (high redox catalytic intermediate) to near amino acid residues. The selected residues were those of low redox potential. The atoms in the QM region were studied by Density Functional Theory, and the atoms in MM region were calculated by Optimized Potentials for Liquid Simulations. Because the spin multiplicity of iron, doublet (S=1/2), quartet (S=3/2) and sextet (S=5/2) states were studied.

In order to validate the computational chemistry simulation, site-directed mutations were designed. Two CYPBM3 “21B3” variants (W96A and F405L) showed better stability than the parental enzyme. These variants are consistent with the computational calculations and the spin delocalization. A double mutant showed to be 260 times more stable than the original protein. In conclusion, the computational chemistry seems to be a powerful tool to predict intramolecular radical delocalization and then to design peroxidases and heme-proteins with increased stability.

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SHORT-TERM SUPPLEMENTATION WITH AVOCADO OIL AMELIORATES LIVER MITOCHONDRIAL DYSFUNCTION AND OXIDATIVE STRESS IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

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Increased mitochondrial oxidative stress has been implicated in the etiology and pathology of diabetic complications. Liver is one of the organs in which diabetes exert deleterious effects in mitochondrial function, which has been associated with defective respiration due to deficiencies in electron transfer at the complex III-complex IV segment of electron transport chain and increased levels of both lipid peroxidation and reactive oxygen species (ROS) generation. This has sparked a growing interest for the search of nutritional approaches to decrease mitochondrial oxidative stress for the amelioration of diabetic complications. Avocado (*Persea Americana* Mill) oil may be a potential candidate to achieve this objective due to its high (up to 70%) content of oleic acid (C18;1) and a variety of carotenoids antioxidants. In order to test whether avocado oil may improve liver mitochondrial dysfunction along a decrease in oxidative stress, we have tested the effects of short-term dietary avocado oil supplementation on some parameters of mitochondrial function and oxidative stress in streptozotocin-induced diabetic rats. Mitochondria from diabetic rats exhibited decrements up to 60% in oxygen consumption rate (OCR) in both states 4 and 3, which was attributed to impaired activity of complex III. An alteration in complex III activity was also revealed by an increase in ROS production after the addition of antimycin A. 15-days supplementation of avocado oil caused a 1.42-fold increase in OCR in diabetic rats, which was associated with a prevention of the complex III impairment induced by diabetes and up-regulation of the activity of complex II. With regard to the development of oxidative stress, a 3.65-fold increment in the levels of lipoperoxidation was observed in mitochondria from diabetic rats, which was fully prevented by avocado oil. Furthermore, when mitochondria were challenged against *in vitro* oxidative stress induced by exposure to 25µM Fe^{2+}, it was revealed a hypersensitivity of the complex III activity from diabetic rats and increased ROS production; these effects were also prevented by avocado oil administration. Together, these results suggest that avocado oil decrease mitochondrial oxidative stress in liver mitochondria from diabetic rats by improving ETC function, which in turn could be due to a better preservation from peroxidative damage of mitochondrial membrane lipids where ETC complexes are embedded. Acknowledgments: the authors appreciate the financial support of grants from Consejo Nacional de Ciencia y Tecnología (CONACYT México) (130638 to CCR) and Programa de Investigación 2013 de la Coordinación de la Investigación Científica de la Universidad Michoacana de San Nicolás de Hidalgo (CIC-UMSNH to CCR),

SULFORAPHANE ATTENUATES GENTAMICIN-INDUCED NEPHROTOXICITY: ROLE OF MITOCHONDRIAL PROTECTION

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Sulforaphane (SFN) is a potent isothiocyanate naturally occurring in cruciferae, which has been tested in several models of toxic renal injury. Its protective effect has been associated with its ability to induce phase II detoxifying enzymes through an Nrf2-dependent pathway. It has been also demonstrated that SFN exerts its protective role by preservation of mitochondrial function and integrity. Gentamicin (GM) is a widely used antibiotic in spite to its high intra-hospital incidence of nephrotoxicity. Nephrotoxic effects of GM are localized in proximal tubules. Its pathophisiological mechanism involves lysosomal phospholipidosis, reticular stress, mitochondrial damage, and therefore reactive oxygen and nitrogen species generation. Ultimately, GM toxicity can be expressed as necrosis or apoptosis, leading to alterations at the renal cortical structure and a fall in renal functions. In this study, it was investigated if SFN is able to induce protection against GM-induced nephropathy both in renal epithelial LLC-PK1 cells in culture and in rats. SFN prevented death and loss of mitochondrial membrane potential in LLC-PK1 cells treated with 8 mM GM for 72 h. In addition, SFN (intraperitoneally 1 mg/Kg/24 h for 4 days) attenuated GM-induced renal injury and dysfunction (proteinuria, increases in serum creatinine, in blood urea nitrogen and in urinary excretion on N-acetyl-ß-D-glucosaminidase and decrease in creatinine clearance and in plasma glutathione peroxidase activity), necrosis and apoptosis in rats injected with GM (subcutaneously 70 mg/Kg/12 h for 4 days). The apoptotic death (mainly seen in proximal tubules) was associated with enhanced active caspase-9, an element of mitochondria-dependent apoptosis pathway. Caspase 8 was unchanged in all the studies groups. Finally, SFN was also able to prevent GM-induced protein nitration and decrease in the activity of antioxidant enzymes catalase and glutathione peroxidase in renal cortex. In conclusion, the protective effect of SFN against GM-induced acute kidney injury could be associated with the preservation in mitochondrial function that would prevent the intrinsic apoptosis and nitrosative stress.

Regresar

IEFFECT ANTIPROLIFERATIVE OF (-)-EPICATECHIN AND ITS RELATIONSHIP WITH REACTIVE OXYGEN SPECIES IN BREAST CANCER CELL LINES.

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Introduction: Breast cancer is the neoplasia of increased morbidity and mortality in women from Mexico and the world. The search of new therapeutic strategies against this neoplasia has focused on natural products such as polyphenols. Ours research group had shown that (-)-epicatechin (a polyphenol) present an antiproliferative effect in breast cancer cell lines. It is well documented that cancer cells have an increased metabolism, which induce higher production of reactive oxygen species (ROS). However cancerous cells are able to evade the damage by ROS, process that it has been related with the overexpression of a protein called uncoupling protein 2 (UCP2). This protein has been found overexpressed in several cell lines and biopsies from breast cancer**. Aim:** To determine if antiproliferative effect of (-)-epicatechin is related with a downregulation in UCP2 expression, an increase in ROS production and apoptosis induction. **Material and methods:** (-)-epicatechin, MCF-7 and MDA-MB-231 breast cancer cells and endothelial cells (non-transformed cells) were used. Inhibitory concentration fifty (IC_{50}) of (-)-epicatechin was determined in breast cancer cells by 1-(4, 5-Dimethylthiazol-2-yl)-3,5-diphenylformazan (MTT) assays. UCP2 expression was established by semi-quantitative RT-PCR. ROS production was analyzed by the values of biomarkers of oxidative damage (carbonyl groups and malondialdehyde (MDA)) in absence and presence of (-)-epicatechin; also the activity of glutathione peroxidase (GSH-mPx) was analyzed. Finally, DNA fragmentation assay was performed to determine the induction of apoptosis. **Results:** The data obtained showed higher UCP2 expression in MCF-7 and MDA-MB-231 than endothelial cells (non-transformed cells). Interestingly, the antiproliferative effect of (-)-epicatechin showed an IC_{50}=350 µM. Furthermore, cell growth inhibition by (-)-epicatechin was coordinated with increase in the values of biomarker of oxidative damage; carbonyl group without and with (-)-epicatechin (21.08 vs 36.62 nmol/mg protein, respectively), MDA without and with (-)-epicatechin (0.686 vs 1.029 nmol/mg protein, respectively) and decreasing in GSH-Px activity with (-)-epicatechin (0.97 mU/mg protein) in comparison without (-)-epicatechin (2.15 mU/mg protein). Finally, DNA fragmentation assay showed that all these data were related with an induction of apoptosis. **Conclusions:** The data obtained in this work, suggest that the effect antiproliferative of (-)-epicatechin is mediated by an induction of apoptosis and that is related with an increase in the production of ROS, decreasing of antioxidant defenses and UCP2 expression.

Regresar

Detection of insulin polymers in plasma from obese patients and its relationship with insulin resistance.

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Introduction: Several studies have showed that obesity is tightly associated with insulin resistance, therefore is considerate a risk factor to develop Diabetes mellitus type 2 (DM2). Nowadays, it is not known if a modification in the insulin could be related with the mechanism of insulin resistance in these patients. Recently, we showed that the incubation of human recombinant insulin (HRI) in whole blood from obese patients induces a polymerization and loss of biological activity in the hormone. Indeed these effects were related with high levels of oxidative stress in these patients. In this study, our **aim** was to detect the presence of insulin polymers in the plasma from obese patients and to establish its relationship with the presence of insulin resistance**. Methods:** Women of 20 to 40 years old were included in the study. One group with Body Mass Index (BMI) of 30 to 34.9 (O1), and other one with BMI > 40 (O3). Healthy subjects with BMI of 20 to 24.9 (NW) was included as control group. A polyclonal antibody against insulin polymers was generated in mouse and coupled to magnetic particles. Briefly, 50µL of magnetic beads were incubated overnight at 25°C with two milliliters of plasma from NW, O1 and O3. Magnetic beads were recovered with a magnet, washed with PBS-0.5% SDS (ten times) and treated with loading SDS-buffer. SDS-polyacrylamide gel (10%) was performed and western blot was used to evidence the presence of insulin polymers. Finally, densitometry analysis of the insulin polymers was performed and correlated with HOMA values. **Results:** We corroborated the existence of an increase in markers of oxidative stress in the plasma from obese patients. Western blot revealed a protein with molecular weight of 70 kDa (insulin polymer). Interestingly, we found a positive correlation between levels insulin polymers and HOMA values (Pearson $r = 0.5274$; $p < 0.01$) **Conclusion:** These data show for first time, the existence of insulin polymers in plasma from obese patients and its relationship with the insulin resistance. These results suggest the existence of a new mechanism of insulin resistance, where insulin inactivation by polymerizations and oxidative stress play an important role.

Regresar

POSTCONDITIONING INCREASES NRF2 NUCLEAR TRANSLOCATION VIA PKC ACTIVATION AND PROTECTS AGAINST REPERFUSION INJURY IN HEARTS.

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Ischemic heart disease, the leading cause of death worldwide, is generated by coronary obstruction which reduces oxygen supply to the cardiac tissue. This condition can be reverted by re-establishing the blood flow through the occluded artery by means of reperfusion. Paradoxically, in several cases this procedure may generate additional damage to the heart, situation known as reperfusion injury which has been related with overproduction of reactive oxygen species (ROS) and with calcium overload, producing cell death through complex mechanisms. Therefore, in the last years several therapeutic strategies have been studied and applied to protect the myocardium against reperfusion injury. In this regard, it has been observed that reperfusion protocol modification produce beneficial effects limiting the number of dead cells. This mechanical procedure has been termed postconditioning (PC) and consists in the re-establishment of blood flow to ischemic myocardium by intermittent cycles of ischemia and reperfusion. Our main goal is to elucidate the endogenous protective mechanisms activated by PC in the myocardium. Particularly, we studied the participation of the transcription factor Nrf2 in this response and, the role of some cardioprotective kinases in its activation. Our preliminary results showed that Nrf2 phosphorylation was increased early after postconditioning in rat hearts using an *in vivo* model. Phosphorylated amino acids in Nrf2 were Ser40, threonine and tyrosine residues; these amino acids are potential phosphorylation sites of PKC, PI3K and ERK1/2 whose activation has been associated with PC application. To determine the role of PKC in Nrf2 activation, we assayed the effect of chelerytrine, a specific PKC inhibitor in Nrf2 phosphorylation. We found that PKC has a critical role in Nrf2 activation and that participates in the endogenous mechanisms activated by PC in myocardium, since its inhibition altered the cardioprotection conferred by PC and diminished Nrf2 phosphorylation. On the other hand we determined the catalase and glutathione-S-transferase (GST) activities, antioxidant enzymes whose expression are modulated by Nrf2. Our results showed that at both early and long lasting reperfusion, the activity of both antioxidant enzymes were increased in the postconditioned hearts. Interestingly we observed that PKC inhibition diminished catalase and GST activities and reduced glutathione (GSH) content in the postconditioned hearts when reperfusion was extended until 60 minutes.

According to the obtained results, we propose that Nrf2 is phosphorylated by PKC in postconditioned hearts, contributing to the endogenous protective mechanisms activated in the myocardium, since PKC inhibition significantly compromises Nrf2 activation, avoiding the cardioprotective response.

REACTIVE OXYGEN SPECIES AND TYROSINE PHOSPHORYLATION IN THE EPIDIDYMAL SPERM MATURATION OF THE MEXICAN BIG-EARED (CORYNORHINUS MEXICANUS) BAT.

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For most mammalian species studied, the epididymal sperm maturation ends in the distal region of the body. During this process occur several biochemical and structural changes in sperm, involving distinct signaling pathways, in some of them, involve reactive oxygen species (ROS), that allow the activation of second messengers, among which are involved in the tyrosine phosphorylation. This in turn allows mainly several events phosphorylation / dephosphorylation sperm flagellar protein, inter alia involved in the acquisition of the mobility and the ability of fertilization of the oocyte. A long period of storage of spermatozoa in the epididymis exists in the *Vespertilionidae* seasonal bat *Corynorhinus mexicanus*, up to 5 months in the caudal region, and it has been suggested that, associated with this phenomenon, sperm maturation ends in the caudal region. The aim of this study was to determine if epididymal sperm maturation process in *C. mexicanus* is performed in the caudal region and what is its relationship with the production of ROS. To verify, 32 adult males bats were captured during the breeding months (August-October), to which were extracted sperm from different regions of the epididymis, from the moment of occurrence of sperm entry, and during storage in the caudal region. By the evaluation of the capacitation with staining chlortetracycline (CTC), induction of the acrosome reaction and the amount of tyrosine phosphorylation, was determined the status maturity of sperm also determined ROS production. In the sperm obtained from the caudal region showed the most significant changes, since in the second half of September was an increase in tyrosine phosphorylation, followed by an increase of 40% of sperm that were capacitate in the month of October, although there were no changes in the percentage of acrosome reaction. In the last month there was also a significant increase in ROS production. The results allow us to consider that in the signaling pathways involved in epididymal sperm maturation, the participation of ROS is important and independent of tyrosine phosphorylation in sperm *C. mexicanus*. Besides confirming that, the sperm of *C. mexicanus* finish their maturation in the cauda epididymis unlike those reported for the majority of mammals studied. Supported by CONACYT (0105961/I0110/194/09) and UAM, MEXICO. A. Rodríguez-Tobón is supported by a CONACYT scholarship, number: 332837.

TREATMENT OF CURCUMIN ON ALTERATIONS INDUCED IN A MODEL OF STRIATAL DEGENERATION IN RATS: PROTECTIVE EFFECT RELATED TO ITS ABILITY AS ENHANCER OF PHASE II ENZYMES.

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Quinolinic acid (QUIN), an endogenous competitive agonist of NMDAr, is considered an excitotoxin, and its intrastriatal administration to rats has been used to reproduce some biochemical, behavioral and morphological alterations similar to those observed in some neurodegenerative disorders. QUIN induces selective neuronal death in the striatum due to overactivation of NMDAr (excitotoxicity), which has been related to acute oxidative stress. Curcumin (CUR) is a major polyphenol derived from the rhizome *Curcuma longa* and its biological properties as direct (free radical scavenger) and indirect (Nrf2 inducer) antioxidant are well known. Transcriptional factor Nrf2 plays a critical role in the cellular protection against oxidative stress. It modulates the expression of several genes involved in the detoxification of reactive oxygen species (ROS) and electrophile spices, including glutathione-S-transferase, hemeoxygenase-1, glutathione reductase and glutathione peroxidase. Additionally, CUR is highly lipophilic and is able to reach the brain. In this context, we studied the protective effects of CUR in a neurodegenerative model produced by QUIN in rats.

Animals intrastriatally infused with QUIN (240 nmol/μl) and after 24 h, received CUR (400 mg/kg, i.g.) daily during 6 consecutive days, and on day seven, animals were euthanized to evaluate histological changes by hematoxylin & eosin staining. The behavioral study was assessed by the limb-use asymmetry test at day -1 (one day before QUIN microinjection), as well as on days 2 and 6 after QUIN injection. In this test, we estimated the number of contacts the animals on the walls using the ipsilesional (affected limb), the contralesional (unaffected limb), and both limbs simultaneously. Additionally, six days after QUIN microinjection, the number of ipsilateral rotations to the lesioned side was recorded for 1 h following apomorphine (1 mg/kg, *s.c.*) administration. CUR treatment significantly decreased the QUIN-induced striatal morphological alterations by reducing the number of lesioned cells. CUR administration also partially recovered the ability of QUIN-lesioned animals to use both limbs, while decreased the single use of the ipsilesional limb (20 % below the QUIN condition) and reduced the number of ipsilateral turns. This impairment in behavioral test could be related to ability of treatment CUR in significantly increase Nrf2 levels in striatal homogenate and with its capacity to increase the glutathione metabolism-related activity of enzymes (GST, GR and GPx), suggesting that the induction of Nrf2 may be a key element in the antioxidant defense mechanism exhibited by CUR. Our results suggest that CUR treatment may constitute an effective therapeutic tool to ameliorate some features of neurodegenerative events in which the optimal maintenance of the redox status is crucial for neuronal survival. Further studies are needed to elucidate the precise mechanism of CUR actions and to explain the protective effect observed in the toxic paradigm induced by QUIN.

This work was supported by CONACYT (Grant 103527).

Regresar

INSULIN STIMULATES AUTOPHAGY IN NT-1 TOBACCO CELL CULTURES THROUGHT AT H_2O_2

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ABSTRACT

In all eukaryotic organisms, reactive oxygen species (ROS) and autophagy have been associated with cell death. However, recent evidence indicates that ROS, specifically hydrogen peroxide (H_2O_2) and autophagy play important roles in signaling and cellular adaptation to stress. As a catabolic process autophagy allows eukaryotic cells to recycle intracellular components, including organelles during development or under unfavorable conditions such as nutrient limitation, providing amino acids, lipids and sugars that allow temporal adaptation to adverse conditions. Furthermore to recycling, autophagy is required for the degradation of damaged or toxic materials that may be generated as a result of H_2O_2 accumulation during oxidative stress. These findings suggested a strong link between autophagy and H_2O_2 levels (Foyer and Noctor, 2009; Perez-Perez et al., 2012).

On the other hand, the phytohormone auxin is a mayor regulator of plant growth and development (Zazímalová, et al., 2010). While, the insulin in metazoan is under the tight control of blood glucose levels, it addition activated two signaling pathways: PI3K/TOR and MAPK that regulates cellular growth, proliferation, metabolism and survival (Baumann and Saltiel, 2001). In the first pathway, TOR is a negative regulator of autophagy. In our working group was determined in NT-1 tobacco cell cultures that the insulin stimulates cell proliferation and such effect requires the presence of auxins (Fierros-Romero, 2012). Our preliminary results indicated that insulin addition promotes an increased from 24 and up to 48 h of H_2O_2 compared to control, which correlates with a gradual increase in autophagy from 24 h. As there are no reports of the effect of auxin and insulin on autophagy process in plants, would be important determine whether the availability of auxin and/or the addition of insulin induce autophagy in NT-1 tobacco cell cultures throught at H_2O_2 synthesis.

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OXIDATIVE STRESS IS INVOLVED IN ALBENDAZOLE DAMAGE TO Giardia duodenalis

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Keywords: *Giardia duodenalis*, albendazole, oxidative stress, drug resistance.

Giardia duodenalis, a protozoan parasite that causes giardiasis, is a highly prevalent diarrheal infection that has a worldwide distribution. Treatment of this infection is manly based on drugs which include albendazole (ABZ). Cytotoxic mechanism of ABZ involves its binding to tubulin and it has also been suggested that oxidative stress may play a role in this mechanism, possibly mediated by ABZ sulfoxide (ABZ-SO) and ABZ sulfone (ABZ-SOO). In our research group we have obtained *G. duodenalis* clones resistant to different concentrations (1.35, 8, 250 uM) of ABZ. Transcriptomic and proteomic analysis of these clones showed an over-expression of some antioxidant enzymes. To further analyze the effect of ABZ in *Giardia* we characterized the oxidative effect of this drug in *G. duodenalis* trophozoites. When reactive oxygen species such as ROS, ABZ-SO and-ABZ-SOO were determined by Dichlorofluorescein diacetate it was observed that these species were higher in the ABZ susceptible strain as compared to the resistant clones. Likewise the exposure of trophozoites to ABZ resulted in the formation of **8**-hydroxy (dideoxy) guanosine groups and DNA degradation (determined by electrophoretic analysis) which are indicative of nucleic acid oxidative damage. When cysteine, a major anti-oxidant molecule in *Giardia*, was added to trophozoite cultures exposed to ABZ, a protective effect was observed in parasite growth. On the other hand, lipoperoxidation damage and protein carbonylation showed no significant differences among susceptible and resistant clones. These data suggest that ABZ affects *G. duodenalis* genetic material by oxidative stress mechanisms, possibly mediated by its metabolites ABZ-SO and / or ABZ-SOO.

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ROS-INDUCED CHANGES IN K^+ AND CA^{2+} CONDUCTANCE ACROSS THE ROOT CELL MEMBRANE IN HIGHER PLANTS AND THEIR MODULATION BY NATURAL POLYAMINES

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Plant subjected to abiotic stresses respond with increases in levels of reactive oxygen species (ROS) and polyamines (PAs). In this work, applying non-invasive MIFE technique to monitor ion fluxes *in vivo* and conventional patch-clamp in whole cell mode, we have studied responses of intact roots and isolated root protoplasts to artificially generated hydroxyl radicals (OH*). Our results on the dicotyledonous species, pea, may be summarized as following: 1) OH* but not H_2O_2 caused rapid inactivation of constitutively expressed non-selective channels and K^+ outward rectifiers; 2) following a lag of 5-10 minutes a new passive conductance, mediating K^+ efflux and Ca^{2+} influx was activated and reached a stable state within 30-40 min; 3) this conductance was permeable also for anions (Cl^-) and was sensitive to non-specific blockers of either non-selective cation (Gd^{3+}) or anion (niflumate) channels; 4) OH* induced active Ca^{2+} pumping, sensitive to fluorescein derivates (eosine yellow); 5) externally applied PAs strongly potentiated OH*-induced K^+ efflux in intact roots and respective currents in whole cell mode; 6) PAs (Spm^{4+}> Spd^{3+}> Put^{2+}) switched the balance between OH* induced passive Ca^{2+} influx and active Ca^{2+} efflux in favor of the latter. Interestingly, a modulation of OH*-induced K^+ and Ca^{2+} fluxes by PAs was pertinent only for the root mature zone but hardly can be detected in the elongation region. Qualitatively, results obtained on pea roots, were reproduced on a different plant model, barley, a monocot species. An important result was that potentiation of OH*-induced K^+ fluxes by PAs was different in barley varieties, contrasting in their salt-sensitivity, with a several-fold larger effect in a salt-sensitive one. Barley salt resistance is strongly correlated with K^+ retention; salt stress causes K^+ leak from roots due to Na^+-induced membrane depolarization, but, as it can be deduced from our data, also due to the interaction between stress-related factors, ROS and PAs, with membrane transport components. Overall, increases of PAs and ROS under stress result in a substantial remodeling of the plasma membrane ionic conductance, with important consequences for cation homeostasis and Ca^{2+}-signalling. Supported by CONACyT grant 82913 to PI and ARC grant DP1094663 to SS.

Regresar

ASCORBATE-GLUTATHIONE SYSTEM PARTICIPATES IN DORMANCY BREAKAGE IN *Vitis vinifera* L. INDUCED BY CHEMICAL AGENTS

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Ascorbate is one of the most important antioxidants in plants and animals. This hydro soluble vitamin detoxifies radical oxygen species either by itself, or by its participation in the ascorbate-glutathione system. Besides, ascorbate is also involved in REDOX signaling and regulation of enzymatic activity. However, there is a lack of knowledge regarding the role of the peroxide scavenging system enzymes during bud dormancy breakage in caducifolius plants. Bud dormancy breakage is a well-studied phenomenon at a physiological level; but molecular and genetic components of the signaling networks regulating dormancy are yet poorly understood. Many studies have been performed regarding the effects of the application of chemical agents such as mineral oils, hydrogen Cyanamid (H_2CN_2), and plant growth regulators over the dormancy breakage in grape vines. Indeed, such treatments have also been used to elucidate the biochemical processes involved in bud breakage. There is evidence that application of chemicals or thermal treatments, increase the activity of peroxide scavenging system enzymes in apple bud breakage. The current study aimed to examine the effects of two chemical agents (H_2CN_2 and Bro-T), over the ascorbate-glutathione (Asc-GSH) system during bud breakage of grapevine (*Vitis vinifera* L.). Highlighting that both agents have been proven in field experiments as effective to induce bud dormancy termination in grapevine. Experimental work consisted in evaluating the enzymatic activity of glutathione reductase (GHR), dehydroascorbate reductase (DHAR), and ascorbate peroxidase (APX) at different time periods (before treatments, and after 1 and 24, h) from exposure of grapevine buds to the H_2CN_2 and Bro-T. Results in samples evaluated before treatments, and after 1 and 24 h from application of agents show that Bro-T did not cause changes in enzymatic activity. On the other hand, application of H_2CN_2 resulted in lower DHAR and APX activity than untreated samples or control group, while GR activity did not change in any treatment. These preliminary results suggest that DHAR and APX activity could play a central role in dormancy bud breakage in grapevine, possibly by regulating ascorbate and hydrogen peroxide levels. In addition, it is also possible that the applied bud-breaking agents could affect the enzymatic activity in the Asc-GSH system at different velocity. Hence, it is required to extend the time of analytical evaluations in order to elucidate the role of the chemical agents tested over the ascorbate-glutathione system during bud dormancy breakage.

Regresar

HOW CATALASE RECOGNIZES H_2O_2 IN A SEA OF WATER.

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Monofunctional heme-catalases dismutate H_2O_2 at a high rate even though water is expected to compete with H_2O_2. Using molecular dynamic simulations and the crystal structure of *N. crassa* catalase-1 (CAT-1) we addressed the question as how the enzyme selects H_2O_2 in water.

Catalases are structured as homo-tetramers formed by two active dimers. In all catalase crystal structures a long channel (major channel) is observed that leads to the active site. In CAT-1 there are different entrances to the major channel and the passage is longer due to an extra loop of four amino acid residues that forms a constriction (gate), separating the entrances from the final section (FS). The different entrances all lead to the gate that is functionally connected to the FS. The FS is a straight and narrow, ~15Å long, passage that lays perpendicular to the heme. The gate of two contiguous subunits (R-related) is connected by the interconnecting channel.

Selection of H_2O_2 starts at the protein surface and is accomplished along the protein channels and at the active site. One important selection mechanisms is the higher residence time of H_2O_2 in the vicinity of certain amino acid residues that are present at the protein surface, the entrances to the major channel [1]. The effect is an increase in the H_2O_2/water ratio. Movements of amino acid residues of the entrances, the gate and the final section of the channel are increased in the presence of H_2O_2 and rotamers movements of several residues are coordinated. In the final section of the channel, solvent molecules do not freely diffuse to the active site. A gate valve mechanism, consisting of movement of two contiguous phenylalanine residues, drives water molecules out of the final section of the channel. These residues can close and open the access to the active site. Only when both gate valves are open a mean of 5 solvent molecules enter the emptied region. In H_2O_2, both gate valves are open only 35% of the time (mean of the 4 subunits). However, for the tetrameric enzyme, there is a high probability of having one channel open at any time. The same two phenylalanines, together with other amino acid residues, form a hydrophobic barrier before the active site that is crossed easier by H_2O_2 which, unlike water, keeps most of its hydrogen bonds while passing. Only two molecules reach the region of the active site. Because H92 and N165 of the active site have an increased residence time for H_2O_2, H_2O_2 can displace water from it. The displaced water molecule can coordinate with heme Fe, a site where H_2O_2 does not go. Taken together, selection of H_2O_2 over water is exerted from the protein surface up to the active site by various mechanisms that involve the physical differences between H_2O_2 and water, giving an explanation of why such a large tetrameric protein with a deep buried active site is required to efficiently accomplish H_2O_2 dismutation.

[1] Domínguez L; Sosa A; Hansberg W* (2010) Catalase evolved to concentrate H_2O_2 at its active site**.** Arch Biochem Biophys **500**: 82-91.

This work was supported by grants CONACyT 132687Q to WH.

Regresar

PHYSIOLOGICAL RESPONSES TO OXIDATIVE STRESS ASSOCIATED WITH PH VARIATIONS IN THE HERMATYPIC CORAL *Pocillopora capitata.*

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Abstract

As a result of increased ocean temperature and pH decreased coral reefs face the challenge of adaptation and/or acclimatization. To evaluate the response mechanisms in oxidative stress indicators of the cnidarian symbiotic coral *Pocillopora capitata* to reduced seawater pH in an in vitro system, 112 branches with have no apparent damage from bleaching were collected from the coral community La Boquita (LB). Two pH treatments were evaluated: a) pH 8.00/8.40 control treatment b) rank 7.85/7.95 (Treatment C_2) and c) 7.60/7.70 rank (Treatment C_3). The coral branches were randomly assigned to experimental units (n=38 per treatment). Specimens were collected at the beginning of the experiment (T_0) and at different times of experiment: T_1 (5-h), T2 (12-h), T3 (48-h) and T_4 (168-h). We examined lipid peroxidation (MDA), antioxidant enzyme activities (SOD, CAT), antioxidant capacity (CA), chlorophyll a (Chl *a*). The results of the different specimens from each treatment showed a different response between zooxanthellae and cnidaria. In SOD-zooxanthellae we observed an apparent response in the C_3 treatment in the first few hours, but this response did not prevent cell damage. In SOD-cnidaria, C_3 treatment showed greater activity from the middle to the end of the experiment, also in cnidarians, it was greater synchrony antioxidant enzyme activities which resulted in less cell damage during the experiment. We discuss possible relationships between CAT activity and Chl *a* and its possible relationship to environmental history that are subject to the holobiont.

Temática:

Topicos emergentes en el campo de las especies reactivas

Regresar

ARSENITE EFFECT ON GSH/GSSG RATIO IN HEPATOCYTES (HEPG2)

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Arsenic is a metalloid, which has different human health effects, such as damage and repair of DNA, the development of lung, bladder and liver cancer. Many of its effects have been associated with the development of reactive oxygen species (ROS), caused by its metabolic intermediates as well as their accumulation in different tissues; this accumulation occurs when its metabolism is inefficient and fails its efflux. The metabolism of arsenic is not elucidated; it is mediated by the enzyme AS3MT, which employs glutathione (GSH) as a reducing agent and S-adenosylmethionine as methyl group donor for the synthesis of methylated species of arsenic (arsenic monomethylated and dimethylated, MMA and DMA respectively). Glutathione may be the limiting factor in the metabolism of arsenic, because AS3MT used as substrate to arsenite, likewise the glutathione is used to convert arsenic species pentavalent to trivalent species, also the glutathione is used to form complexes with arsenic species for efflux to through MRP1, MRP2 and MRP3. That said, it is clear that the determination of GSH/GSSG ratio, is key to efficient metabolism. In our working group, the hydride generation - cryogenic tramp - Atomic absorption spectrometry (HG-CT-AAS) technique was implemented for quantification of arsenic species, obtaining detection limits of 0.7, 0.3 and 0.6 µg/L for inorganic arsenic (iAs), MMA and DMA, respectively. For the GSH and GSSG quantification was used an HPLC with diode detector, obtaining detection limits of 1.0 µM for both GSH and GSSG at 210 nm. The samples for arsenic quantification were made in PBS and for GSH and GSSG quantification in 2% Metaphosphoric acid. Our results show that the low dose arsenite in the range of 0 to 0.5 µM, the intracellular GSH concentrations correlate positively with an increase of 225% compared with the control. Likewise at the concentration of 0.5 µM begins the decrease in metabolic efficiency for arsenite; with the profile of species 23.1% of iAs, 34.1% of MMA and 42.0% DMA and 80 % of cells viability (essayed by MTT). The same behavior has been observed in cell lines keratocytes, fibroblasts and breast cancer; this could be because arsenic species are able to trigger the antioxidant response through activation of Nrf2 and subsequent expression of gamma glutamylcysteine synthase, as were reported in other studies.

POSTER

NORDIHYDROGUAIARETIC ACID ATTENUATES THE OXIDATIVE STRESS-INDUCED DECREASE OF CD33 EXPRESSION IN HUMAN MONOCYTES.

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Nordihydroguaiaretic acid (NDGA) is a natural lignan isolated from *Larrea tridenta.* In this study, we evaluated the effect of NDGA on the down-regulation of oxidant stress-induced CD33 in human monocytes (MNs). CD33 is a constitutive receptor involved in immune response, is a syalic acid-binding immunoglobulin Ig-like lectin [SIGLECS]. Ooxidant stress was induced by iodoacetate (IAA) or hydrogen peroxide (H_2O_2) and was evaluated using reactive oxygen species (ROS) production and cell viability. NDGA (<25 µM) did not caused cell toxicity during 120 h of incubation. In addition, NDGA attenuated toxicity, ROS production and the oxidative stress-induced decrease of CD33 expression induced by IAA or H_2O_2 in human MNs. These results suggest that NDGA has a protective effect on CD33 expression, which is associated with its antioxidant activity in human MNs.

MODULATION OF NERVE GROWTH FACTOR EXPRESSION BY IONIZING RADIATION IN MOUSE TISSUES.

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Absorption of ionizing radiation by cells produces alterations in structure and function of biomolecules. Damage can be created by direct (disruption of the atomical structure of macromolecules) or indirect action (production of free radicals as a consequence of water radiolysis). Given that water is the major constituent of the cells, the indirect damage of ionizing radiation is more relevant since it generates reactive species of oxygen that can change the redox state of the cell inducing cell damage. The nerve growth factor (NGF) is a neurotrophin involved in development, survival, differentiation and plasticity of sensory and sympathetic neurons. In addition to these functions, there are evidences that show that NGF is involved in antioxidant response in the CNS and other tissues such as the heart or the liver. These responses are achieved when NGF binds to TrkA, a tropomyosin-related kinase receptor that is specific for this NT. We investigated the modulation of *ngfb* transcription and downstream phosporilation of TrkA in male mouse tissues, 1 h after whole body irradiation with 0.5, 2.5 and 4 Gy of gamma rays. Our results show that ionizing radiation upregulates the transcription of *ngfb* in those animals exposed to the lowest and intermediate doses. The treatment also modulated the phosporilation of TrkA in brain and spleen. Thus, our initial results suggest that NGF-signalling cascade is being activated in response to the free radicals generated as a consequence to the exposure to ionizing radiation.

Supported by PAPIIT IN207611.

Regresar

ETHANOL AND RESVERATROL REGULATE NR1 AND NR2A NMDA RECEPTOR SUBUNIT EXPRESSION IN CEREBRAL ISCHEMIA

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Stroke causes brain injury in millions of people worldwide each year; however, there is no approved therapy to reduce the infarct size and counteract the neurological disability. The goal of therapy in stroke is to prevent neuronal death in the penumbra area, where the cells still preserve their metabolism. Antioxidants have a neuroprotective effect and may limit the spread of oxidative damage induced by ischemia through regulation of mRNA synthesis. On the other hand, the expression of the glutamate receptor type N-methyl-D-aspartate (NMDA) subunits is modulated by antioxidants. **Objective.** The aim of the present study was to evaluate the effect of resveratrol (RSV) in NR1 and NR2A mRNA expression after cerebral ischemia. **Methods.** Male rats (250 to 350 g) were subjected to transient middle cerebral artery occlusion (MCAO). RSV was administered (1 mg/kg; i.v; diluted in 50% ethanol) 2 h post-occlusion. Frontoparietal cortex was dissected from hemisphere ipsilateral to lesion, 2 and 4 h after blood flow recovery (reperfusion, R). Expression of NR1 and NR2A mRNA was analyzed by quantitative real-time PCR. **Results**. MCAO/R induced an increment in mRNA expression of NR1 (6.49 ± 5.96; 5.9 ± 2.56) and NR2A (9.28 ± 8.14; 11.1 ± 3.2) after 2 and 4 h of reperfusion, respectively. Meanwhile, administration of RSV and vehicle (50% ethanol) induced a decrease in the mRNA expression of NR1 (0.28 ± 0.16; 1.91 ± 1.41) and NR2A (0.47 ± 0.25; 1.8 ± 1.1) after ischemia, respectively. **Conclusions.** Previous epidemiological and experimental reports showed that ethanol inhibits the release of excitotoxic amino acids, depress glutamate receptor function, and scavenge free radicals. In accord, we observed that resveratrol (diluted in ethanol) and ethanol (50%) prevented the increase in NR1 and NR2A mRNA expression induced by MCAO/R. This effect may alter the functionality of the NMDA receptor, probably decreasing its activity which is elevated in cerebral ischemia. However, to establish the actual effect of RSV on NR1 and N2A expression using innocuous vehicles must be tested.

POSTFATIGUE TENSION IS REDUCED BY SODIUM ASCORBATE AND NICORANDIL

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The decrease in the ATP has an important role in the development of muscle fatigue and any event that alters the production of this can determine the tendency of muscle fatigue. At the peripheral level, it has been proposed that the reactive oxygen species (ROS) play an important role in the regulation of several signaling pathways involved in the proper functioning of muscles, particularly during fatigue. Moreover, it has also suggested the involvement of channels mitochondrial K_{ATP} channels (mitoK_{ATP}) plays an important role in protecting the muscle during fatigue. So, the aim of this study was to determine the participation of ROS in correlation with the mitoK_{ATP} in the muscle fatigue process induced by repetitive electrical stimulation, exploring the effect of Nicorandil (mitoK_{ATP} channel opener) and an antioxidant (sodium ascorbate), on the tension of slow skeletal muscle of chicken in a model of fatigue *in vitro*.
Anterior Latissimus Dorsi (ALD) muscle of chicken was dissected and mounted on an experimental recording chamber by placing the proximal end to the bottom of the chamber and the distal end hook mechanic-electric transducer (Grass FT03), which through an amplifier and a 320 CyberAmp analog-digital interface (Digidata 1322A) allowed to acquire the muscle tension generated by a computer (Pentium 4) and a "software" of data acquisition (AXOTAPE, pCLAMP 9.2). We performed a fatigue protocol by twitches, which consisted of repetitive electrical stimulation (pulses of 100 Volts, 300 ms duration, frequency of 0.2 Hz). The bundle was stimulated until the force decreased by 60 %, then was applied the study drug for 6 min to observe its effect. We used concentrations of Nicorandil and sodium ascorbate (10, 30 and 100 uM).
In a dose-response curve the mayor effect observed by Nicorandil was 10 uM and 10 uM by sodium ascorbate that is an antioxidant. In combination of Nicorandil and sodium ascorbate the tension post-fatigue increased to 29.05% ± 6.90% in peak tension, while in total tension was an increase by 9.90% ± 28.80% compared regarding fatigue. So far, we have seen a mayor increased with the application of Nicorandil alone that in the combination with the sodium ascorbate, the observed effect it might be to the necessity of the presence of enough reactive species for the activation of mitoK_{ATP} pathway that are removed by the antioxidant.

Regresar

OXIDATIVE DAMAGE DURING FRAGILITY AND ITS RELATIONSHIP WITH THE ANTI-INFLAMATORY RESPONSE AND NADPH OXIDASE ACTIVITY

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It is now known that during aging, organisms inevitable loss their physiological biochemical and structural capacities leading them to health detriment and eventual death. However, not all individuals deteriorate equally. This phenomenon depends on their personal characteristics (genetic background), as well as their environment and life quality. Therefore Linda Fried developed a fragility scale to evaluate human wear and tear during aging. The fragility syndrome during old age is characterized by loss of weight, feeling of exhaustion, strength loss, and physical activity decrease. These entire features contribute to the incapability of maintaining a healthy homesostais and resistance to oxidative stress during aging. Coupled with these, oxidative stress generated by inflammation and diminished aerobic capacity. Here we used the blood form a cohort of 157 Mexican major citizens, classified as: Non fragile (NF), pre-fragile (PF) and fragile (F). Lipid peroxidation, protein oxidation, as well as their index was determined. Along with the antioxidant system represented by the enzymatic activity of catalase (CAT), superoxide dismutase (SOD), gamma-glutamine-cysteine-synthetase (γ-GCS), and total glutathione (GSH); in order to evaluate the inflammatory state, interleukin 6 (IL-6) and tumoral necrosis factor alpha (TNF-α) expression were determined. Finally NADPH oxidase activity (NOX) was also evaluated to determine ROS generation. Our results did not show a significant difference between gender, however when the fragility groups were compared, we found an increase in oxidative markers in the F and PF groups in relation to the NF. The enzymatic activities did not show differences, but the levels of pro-inflammatory cytokines were higher in F and PF groups.

This work was supported by CONACyT's grants CB-2006-1-59659 and CB-2012-1-178349. As well as the "Red Temática de Investigación en Salud y Desarrollo Social" from CONACYT. Conde-Pérezprina was a CONACYT scholarship holder.

Title: "Effect of arsenic exposure on the cystine/glutamate transport in neonatal mouse brain"

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Abstract

Millions of people worldwide are chronically exposed to inorganic arsenic (iAs), mainly through drinking water. The exposure to iAs is associated with cancer, neurotoxicity, and endocrine and cardiovascular disorders. The neurotoxicity caused by lifelong iAs exposure were associated with learning and memory alterations. Once absorved, iAs undergoes oxidative methylation consuming glutathione (GSH) and S-adenosyl methionine (SAM). iAs and its metabolites are differentially accumulated in mouse brain. The damage caused by arsenic is thought to occur through oxidative stress due to the decreased of the levels of GSH and related proteins. The synthesis of GSH is limited by the availability of cystine, which is transported to the brain by X_c^- transporter. Different compounds that have been found to generate oxidative stress also overexpress the transporter, so X_c^- transporter is consider as a part of the cellular antioxidant system.

Gestational exposure in mice can explain clinical aspects of hydroarsenicism. The iAs crosses the placenta and its metabolites have been found in the embryonic brain. However, the alterations these metabolites might cause in hippocampus, cortex and cerebellum are largely unknown. This study assessed changes in the GSH/GSSG levels and X_c^- transporter expression in 15 days old CD1 mice gestationally exposed to 20 ppm of iAs through drinking water. The results show that exposure to 20 ppm of iAs causes an increment of GSH synthesis, and that GSSG levels significantly increase in both males and females in all three brain regions. On the other hand, we observed a significantly increased expression of the X_c^- transporter in the cortex of male and female and in the hippocampus of the female offsprings. These changes correlate with the alterations of the behavior in adult mice, which presented decreased recognition index after gestational iAs exposure.

Keywords: Arsenic, gestational exposure, neurotoxicity, X_c^- transporter, GSH.

Acknowledgments
This work was supported by grant from PAPIIT (UNAM) IN 207611 to MEG.

NF-KB P50 SUBUNIT REGULATES BCL-2 OVEREXPRESSION DURING OXIDATIVE CONDITIONING HORMESIS RESPONSE.

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Mammalian cells can respond to damage and stress by activating different repair and survival pathways. Pre-conditioning the cells to sub-lethal stress is known to induce a pro-survival response that prevents damage and death. Hence, as a consented terminology to unify the main mechanism that preconditioning and adaptive responses have in common, the term hormesis has been proposed, suggesting that the exposure to low levels of stress will activate existing cellular and molecular pathways that will enhance the ability of the cell and organism to withstand to more severe stress.
Bcl-2, an antiapoptotic protein recognized by it antioxidant and pro-survival functions, has been documented to play an important a role during the oxidative conditioning hormesis. Hence, using an oxidative hormetic model, which was previously established in L929 cell line by subjecting the cells to a mild oxidative stress of 50μM H2O2 for 9 h, we were able to identify two different transductional mechanisms that essentially participate in the regulation of Bcl-2 expression during the hormetic response. These mechanisms converge in activating the nuclear transcription factor NF-κB.
Interestingly, the non canonical p50 subunit of the NF-κB family is apparently the one that participates during the oxidative hormetic response.

The authors would like to thank Dr. A. Hernández from CINVESTAV for generously donating us the actin antibody and M. Sc. R. Lazzarini and the CBS-UAMI Confocal Core for confocal images acquisition and analysis. This work was supported by CONACyT's grants CB-2006-1-59659 and CB-2012-1-178349. As well as the "Red Temática de Investigación en Salud y Desarrollo Social" from CONACYT.

Regresar

"Effect of redox state during premature senescence induced due proteostasis loss, in primary mice lung fibroblasts"

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Senescence is recognized as a cellular state in which cells lose their ability to replicate. However, it is known that cells can achieve this state as a response to different stimuli, such as telomere shortening (Replicative Senescence, RS) or oxidative stress (Stress Induced Premature Senescence, SIPS). Recent studies have suggested that the loss of protein homeostasis (proteostasis) is also capable of inducing premature senescence.

In this work, we induced premature senescence in primary mice lung fibroblasts by inhibiting proteasome activity, using sub lethal doses of the proteasome inhibitor epoxomicin. We evaluated the classic parameters of senescence like cellular proliferation, DNA synthesis and β-Galactosidase activity assay (SA-β-Gal). Likewise, we evaluated the proteasome activity and the GSSG/GSH ratio in a comparative study between RS, SISP and senescence induced due proteostasis loss, at three different culture stages: at the beginning, in the pre-senescent state and during the established senescence, in order to elucidate the proteasome inhibition effect, and its relationship with the changes in cellular redox state during the senescence induction by proteostasis loss.

The authors would like to thank MVZ Rocío González Vieira for providing the animals used in this study. Maciel-Barón L is a CONACYT scholarship holder.

This work is supported by CONACYT. No. CB-2012-1-178349

Regresar

CHARACTERIZATION OF SECONDARY METABOLITES FROM OPPORTUNISTIC FUNGAL PATHOGEN *Candida glabrata*

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Candida glabrata, an opportunistic fungal pathogen, is highly resistant to oxidative stress, mainly due to the efficiency of its only catalase, Cta1. However, a *C. glabrata* strain without catalase is still virulent in *in vivo* assays. This means there are additional factors that may compensate for the lack of Cta1 *in vivo*. One of them could be secondary metabolites excreted by the pathogen.

There are not many chemical reports about production and role of *C. glabrata* secondary metabolites, and an appropriate identification could lead us to the design of brand new therapies for its control. For example, this knowledge would lead to surface treatments, like in catheters, with analogue products to their own metabolites and biosynthesis intermediates as possible fungistatic agents, by blocking the biofilms development.

On the other hand, it is described that *Candida albicans* conditioned medium protects to its exponential phase cells from oxidative stress. It is suggested that this protection could be mediated by the expression of *CAT1, SOD1, SOD2* and *SOD4*, induced all of them by the exposition of the cells to the spent medium. Besides, it is described that farnesol, a quorum sensing molecule (QSM), is partially responsible of this response.

In this communication, we reported *C. glabrata* BG14 metabolic profile, by GC-SM, being phenylethanol, the compound with the highest concentration, a described QSM for *Saccharomyces cerevisiae*. Also, we characterized that protection provided by phenylethanol to exponential cells from oxidative stress is almost the same to that of *C. glabrata* conditioned medium. And finally we corroborated the results by testing conditioned medium from the corresponding null-mutants, involved in the biosynthesis of phenylethanol. These experiments point out that phenylethanol is also a QSM for *C. glabrata* and is involved in the signaling of oxidative stress response.

ELEVATED CONCENTRATIONS OF GLUCOSE SELECTIVELY BLOCK THE NITRIC OXIDE –DEPENDENT VASORELAXATION INDUCED BY PROLACTIN IN RAT CORONARY VESSELS.

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Cardiovascular disorders represent the main cause of the worldwide death, and are also closely related to the development and progression of diabetes, which severely compromise the functioning of blood vessels and body homeostasis, this condition can be solved in part through stimulating vasodilation. Recent studies have shown that hormones such as prolactin (PRL), induce vascular dilation dependent of endothelial nitric oxide (NO), a potent vasodilator agent. However, the role of PRL in cardiovascular diseases and its relationship with diabetes is controversial and has not been fully studied. The purpose of this work was to evaluate whether elevated concentrations of glucose could modify the NO-dependent vasodilation induced by PRL in isolated and perfused rat hearts using the Langendorff system. Preliminary data show that increasing concentrations of glucose block the vasodilation induced by the hormone, being glucose 20 and 30 mM, the concentrations which completely blocked this response, as well as decreased the release of NO in the venous effluent, in comparison with the control, in presence of glucose 5 mM. Moreover this blockage, under that, conditions was partially reverted in presence of the antioxidant enzymes superoxide dismutase and catalase, coupled with the fact that vascular actions promoted in presence of elevated glucose, were selective for the PRL vasodilation, since the cardiac administration of other NO-dependent vasodilators like acetylcholine (ACh), or negative controls for vasodilation (vasoconstrictors) as noradrenaline, were not blocked. These data suggest that probably glycosylated PRL receptor located in the inner layer of coronary vessels or, endothelium is interacting specifically and finely with high concentration of glucose though specific sequences of carbohydrates promoting oxidative stress. These findings could clarify the role of PRL in the control of vascular function during normal and under pathophysiological stages. This work was supported by CONACyT, grant 134595.

COMPARATIVE ANALYSIS OF ANTIOXIDANT CAPACITY OF MELATONIN (MLT) AND SEROTONIN (5-HT) ON THE ACTIVITY OF HUMAN LEUCOCYTES.

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The concentration of free-radicals, reactive oxygen species (ROS) or reactive nitrogen species (RNS) that occur *in vivo*, can be determinant to how harmful or beneficial those are to living organisms. When the delicate balance of the low concentration for these species is kept, they might have beneficial effects, considering that they are necessary for maturation process of cellular structures and mitogenic responses, and they are involved in the defense system, where ROS are produced for example by cytotoxic T lymphocytes and monocytes to destroy recognized cells. There are numerous studies about the regulation for these cell activities and it is well recognized the influence of melatonin and serotonin, as endocrine and paracrine factors.

Melatonin has been proven to scavenge some radicals and has been stated that is a good antioxidant; the effect of this scavenger can be direct, but it also has immune-enhancing properties, as well as other important receptor-related functions, as activating monocytes by increasing the production of ROS and nitric oxid. Melatonin activates T helper cells by increasing IL-2 production and enhances antibody-dependent cellular cytotoxicity. It also enhances IFN gamma production by splenocytes. Serotonin antioxidant activity has been studied, and the participation of this indolamine can be adressed to the stimulation of immune system as well. It has been related with the glutathione antioxidant and detoxification system, but not recognized as a good antioxidant.

We studied the antioxidant capacity of melatonin and serotonin in presence of circulating leucocytes. By itself, serotonin had greater antioxidant capacity than the well-known scavenger melatonin measured with different techniques. When the antioxidant capacity was tested for and in presence of circulating leucocytes, the results were not so different, showing the remarkable functional versatility of melatonin exhibiting its antioxidant function. Furthermore, it has been shown the melatonin synthesis in cultured human lymphocytes with a possible role as intracrine signal, implicating a special regulation of melatonin activity in these cells. In conclusion, the antioxidant capacity of melatonin and serotonin were different when tested in the presence of leucocytes.

ROLE OF OXIDATIVE STRESS IN THE ACTIVATION OF THE MAPK PATHWAY DURING THE APOPTOTIC DEATH OF NEURONS.

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Reactive oxygen species (ROS) modulate apoptosis of cerebellar granule cells (CGC), but the mechanisms implicated have not been clarified. According to a previous study, the mitogen-activated protein kinases (MAPK) JNK and p38 are activated by oxidative stress in CGC and promote apoptosis. Both, JNK and p38 participate in THE apoptotic death of CGC. The mechanism by which these MAPK are activated by ROS in CGC is still unknown. In non-neuronal cells, it has been suggested that MAPK could be activated by the interaction of ROS with ASK1 that is upstream of the JNK and p38. It has been demonstrated that the reduced form of thioredoxin (Trx1) binds to ASK1 in basal conditions, keeping ASK1 in an inactive state. Under oxidizing conditions, Trx1 dissociates from ASK1 allowing the activation of ASK1 and then the activation of JNK and p38, which could lead to the programmed cell death. Based on these studies, one possible scenario in the CGC is that ROS generated early by apoptotic conditions induce the dissociation of Trx1 from ASK1, which would lead to the activation of p38 and JNK that and apoptotic death. In this study, we evaluated this possibility by using a model of apoptotic death of cultured CGC induced by high potassium deprivation (K5) and staurosporine (Sts). Under these conditions, we found an early increase in the generation of ROS induced by K5 and Sts treatment. In addition, we found that the death of CGC stimulated by K5 and Sts was also dependent on time, being slower in the GCC treated with Sts. On the other hand, using Western blot assays we found that under basal conditions CGC express Trx1 and ASK1. Based on assays of co-immunoprecipitation we observed that K5 and Sts significantly reduced the interaction between Trx1 and ASK1 after 30 minutes of treatment, suggesting that ROS generated by these two conditions could be modulating, at least partially, the complex Trx1-ASK1. The binding of Trx1 to ASK1 decreased after 30 minutes of K5 treatment, a time when, according to previous studies, a peak of ROS generated by K5 was detected. In the case of Sts, the Trx1-ASK1 interaction also decreased after 30 minutes, but it did not change with time. Finally, we evaluated the effect of an antioxidant to corroborate that ROS generated by K5 and Sts are responsible for modulate binding between Trx1 and ASK1. Under these conditions we found that the antioxidant induced a partial reversion in the effect of K5 and Sts on the interaction ASK1-Trx1. These data suggest that ROS generated by K5 and Sts could regulate ASK1-Trx1 interaction and thus activate the JNK/p38 signaling pathways involved in the activation of the apoptotic machinery of CGC.

This work was partially supported by CONACYT grant 179234 and PAPIIT IN218310

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SILVER NANOPARTICLES INDUCED BIOCHEMICAL AND PHYSIOLOGICAL CHANGES IN RESPIRATORY PRIMARY CULTURE CELLS AND ISOLATED RAT TRACHEAL SEGMENTS. ROLE OF NITRIC OXIDE

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Silver nanoparticles (AgNPs) have been used to manufacture materials with new biophysical properties and functions. However, few experimental approaches have been performed to assess their toxic potential or beneficial effects on human health in association with the size, concentration and biological target, especially in the respiratory system. The aim of this work was to evaluate the effects of the AgNPs (29±5.3 nm) on airway physiology and cell processes. We used respiratory primary culture cells and isolated rat trachea rings. A short-time exposure of airway cells to AgNPs (0.1-10 µg/mL), induced in a dose dependent manner the release of nitric oxide (NO), a free radical involved in oxidative stress and hyperreactivity However, a treatment in presence of AgNPs and acetylcholine (ACh), a contractile agent in airways, potentiated the release of NO without induction of citotoxicity. In order to evaluate whether these cellular effects could unbalance the airway smooth muscle tone. We used the isolated rat tracheal rings model, where single or cumulative administrations of AgNPs did not modify the basal smooth muscle tone. However, when the tracheal rings were pre-treated with acetylcholine (ACh), the following exposure to AgNPs, resulted in a contractile effect. Similar to cell culture, and only in presence of ACh, the contractile AgNPs-induced effect was associated with an excessive NO production, generated by the inducible nitric oxide synthase (iNOS), enzyme that releases high levels of NO during hyper-reactivity, since the contractile response to the AgNPs was completely blocked when the tracheal rings were incubated with 1400 W, specific iNOS blocker, and moreover, we identifiy through western blot analysis, the expression of iNOS, when the treatments in presence of ACh and AgNPs were done. In addition, a pretreatment in presence of EGTA (an extracellular calcium chelant) or atropine (muscarinc blocker), the the contractile AgNPs-induced effect was completely inhibited in presence of ACh, suggesting that AgNPs can alter ACh muscarinic receptor signaling, through the activation of iNOS expression, and the consequent contractile effect depends on extracellular calcium, resulting in a tracheal smooth muscle hyper-reactivity.

SYSTEMIC NERVE GROWTH FACTOR MODULATES THE TRANSCRIPTION OF AMINO ACID TRANSPORTERS AND GLUTATHIONE (GSH) SYNTHESIS IN MICE STRIATUM

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Nerve growth factor (NGF) is a member of structurally related proteins, named neurotrophins (NTs), that regulate neuronal survival, development , function, and plasticit y. Moreover, NGF is an important activator of antioxidant mechanisms. These functions of NGF are mediated by the tropomyosin-related kinase receptor A (TrkA). There is evidence that NTs and their receptors are expressed also in visceral tissues. Physical exercise and stress increase levels of NGF in plasma. Using a murine model we have shown that systemic inhibition of GSH synthesis with L-buthionine-S-R-sulfoximine (BSO) increased brain GSH content and induced the transcription of ngfb in liver. Murine striatum cholinergic neurons express TrkA receptors thus, we investigated if an i.p. injection of BSO or of sodium arsenite (iAs) modulate the transcription of ngfb and trka as well TrkA phosphorilation in mice striatum. Both agents induced the activation transcription of ngfb and trka as well TrkA phosphorilation in mice striatum. Both agents induced the activation of the NGF/TrkA pathway which correlated with an increased transcription of *xCT, LAT1, EAAC1* amino acid transporters system genes that provide L-cys/L-cys_2 to central nervous system and of *GCLm* which participates in the de novo synthesis of GSH. The inhibition of TrkA phosphorylation by K252a or anti-NGF neutralizing antibody abrogated the BSO and iAs induced transcription of *xCT, LAT1, EAAC1* and *GCLm* suggesting the participation of t his pathway in the in vivo antioxidant response at least in striatum. Furthermore, since anti-NGF neutralizing antibodies would not cross the blood-brain barrier, our results suggest that NGF functions as a systemic redox-sensor in both CNS and peripheral tissues and that the NGF/TrkA pathway plays a critical role in the antioxidant response in the striatum in our murine model.

Supported by CONACYT 02287

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BENEFIT OF PHYSICAL ACTIVATION ON OXIDATIVE STRESS

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Área del conocimiento: Estrés oxidante en la muerte celular y la enfermedad

Aims. To evaluate the effect of a physical activity (PA) program on the oxidative stress markers (lypoperoxidation, superoxide dismutase, catalase and glutathione peroxidase), in teenagers with metabolic syndrome (MS).

Materials and Methods. In this cohort study we included 38 teenagers (18 women and 20 men) with and without MS. Both, men and women, received a PA program during 3 months. Before and after the PA program, we evaluated all clinical variables of the MS and oxidative stress markers.

Results. While women with and without MS were significantly different for almost all components of the MS, men were significantly different in half of the components. After a PA program, the MS components were not different from basal values except for HDL-C levels. In case of the oxidative stress markers, only superoxide dismutase was significantly different between teenagers with and without MS at the beginning of the study. After the PA program, there was a significant decrease in the degree of lypoperoxidation in women and men, unlike, superoxide dismutase, that only decreased in women.

Conclusions. A PA program effectively reduces some markers of oxidative stress in teenagers with MS, although these desirable effects of PA were not associated with changes in all markers of oxidative stress. Perhaps the benefits of PA in these markers would appear with a longer PA program.

EFFECT OF RESVERATROL AND ETHANOL ON THE mRNA EXPRESSION OF GLUT3 IN CEREBRAL ISCHEMIA

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Ischemic stroke results from occlusion of a major cerebral artery and involves a cascade of metabolic alterations following the event, this continues until the blood flow recovery period (reperfusion). Impairment of cerebral blood flow restricts the delivery of important substrates, particularly oxygen and glucose with the consequent decline in the energy metabolism and the induction of glutamate excitotoxicity. Antioxidants as resveratrol prevent the ischemic-induced damage by increasing mitochondrial function through mechanisms that impact glucose metabolism. In neurons, glucose is up taken by the facilitative glucose transporter GLUT3, which expression is up-regulated in neurons tolerant to excitotoxicity. In this study, we investigated whether or not resveratrol up-regulates GLUT3 mRNA expression after cerebral ischemia. Male Wistar rats (250-350 gr.) were divided in six groups: Control, (CT); Vehicle, ethanol 50%, (VH); Resveratrol, 1 mg/kg, i.v., (Tx); ischemia/reperfusion, middle cerebral artery occlusion (2 h) followed by reperfusion (2 and 4 hrs.), (I/R); I/R-VH; and I/R-Tx (administrated at the onset of reperfusion). Real time RT-PCR experiments showed that GLUT3 mRNA was significantly increased after ischemia (4.9 ± 2.9), and it remarkably decreased after 2 hrs (I/2R-Tx, 0.19 ± 0.14) and 4 hrs (I/4R-Tx, 0.34 ± 0.25) of reperfusion. We observed that ethanol also affects GLUT3 mRNA expression after ischemia and reperfusion (I/2R-VH, 0.1 ± 0.05; I/4R-VH, 0.21± 0.16). To understand the effect of ethanol, we evaluated its antioxidant capacity. We found that ethanol was able to scavenge peroxyl radical from 96 to 13% (163 - 36 mM) and $O_2^{\cdot}$ radical from 96 to 33% (163 - 57 mM). Thus, the mentioned results clearly showed that ethanol decrease GLUT3 mRNA expression, probably through its antioxidant activity. Although resveratrol still could modify expression of GLUT3 mRNA, it will be necessary to perform additional experiments using other vehicles to unmask its actual effect. This change might positively alter the glucose transport and decrease the damage caused by an excess of glucose uptake during reperfusion.

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SIGNAL TRANSDUCTION PATHWAYS INDUCED BY FCγ RECEPTORS IN THP-1 AND U937 MONOCYTIC CELL LINES

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Human monocytes constitutively express the family of cell-surface receptors that bind the Fc portion of immunoglobulin G (FcγRs), this family includes three classes FcγI, FcγII y FcγIII. The activation of FcγRs is a critical in linking humoral and cellular immune response. The signal transduction pathways mediated through FcγRs have been partially described. In general it is known that in leukocytes FcγRs activate several intracellular responses through signaling cascades including: phagocytosis, respiratory burst, degranulation, antibody-dependent-cell-mediated cytotoxicity (ADCC), and fluxes in the intracellular calcium concentration $[Ca^{2+}]_i$.

Simultaneous activation of the three receptors expressed in monocytes occurs under physiological conditions. However, the cellular response that triggers the increase of $[Ca^{2+}]_i$ by each of the FcγRs is still poorly defined. Ca^{2+} is considered an important reporter of signal transduction pathways. The elevation of this ion is important during phagocytosis. Therefore, in this project we examine if the increase of $[Ca^{2+}]_i$ induced by each of the FcγRs expressed in two monocytic cell lines, THP-1 and U937, is different.

The expression of the FcγRs family in monocytes was detected by flow cytometry. Both cell lines expressed FcγRI and FcγRII but neither of them showed FcγRIII. In order to evaluate if each receptor was capable of inducing different Ca^{2+} response; monocytes were stimulated by cross-linking selectively each type of FcγR with specific mAbs, and $[Ca^{2+}]_i$ elevation was then analyzed with fluorescent calcium measurements.

THP-1 monocytes are capable of inducing a $[Ca^{2+}]_i$ elevation by both receptors FcγRI and FcγRII. However, when FcγRII is cross-linked, $[Ca^{2+}]_i$ elevation is 2.7 folds higher than when FcγRI is cross-linked. In contrast, U937 monocytes are only capable of increasing the level of $[Ca^{2+}]_i$ when FcγRII is activated. These data clearly suggest that each FcγR, expressed in both monocytic cell lines, is capable of inducing a different intracellular response.

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Dynamic of reactive oxygen species in root hair cells and pollen tubes are essential for polar growth.

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Many responses in animal and plant cells depend from reactive oxugen species (ROS). These ROS can activate calcium channels and receptors involved in signaling processes and metabolism. In plant cells ROS accumulation have been involved in several processes such as: development, hypersensitive response, hormonal perception, gravitropism and stress response. (Mittler and Berkowitz, 2001). In guard cells from *Vicia faba* regulates the opening of stomata and more recently in root hair cells from *Arabidopsis* ROS levels generate and maintain an apical calcium gradient. This ROS accumulation plays a key role in root hair tip growth and suggested to play a similar role in pollen tubes (Pei et al., 2000; Foreman et al., 2003).
Herein we report a new molecular probe to depict the ROS dynamic during root hair cell and pollen tube apical growth. Hyper is a new generated GFP fused to the OxyR domain that result in a hydrogen peroxide specific probe. With this probe in root hair cells an apical gradient of H2O2 is observed and support the polar growth, furthermore we were able to visualize the ROS oscillation, which are couple to growth oscillations. In pollen tubes we found a different ROS distribution, however their oscillations were clearer and couple to growth oscillations. In both tip growing cells, the apical domain result the site with the more dynamic ROS changes.

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INSULIN PROMOTES ARABIDOPSIS ROOT HAIR GROWTH IN A ROS-DEPENDENT MANNER

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ABSTRACT

Insulin in mammalian regulates blood glucose levels and activates the mitosis (via MAPK) and metabolic branches of insulin signaling PI3K/TOR (Olivares-Reyes y Arellano-Plancarte 2008), which has been shown to be conserved in plants. Recently, in maize a peptide named ZmIGF has been found in actively growing tissues. It targets the maize TOR pathway at the same extent as insulin and, by doing so it induces growth, as well as ribosomal proteins and DNA synthesis (Garrocho-Villegas and Sánchez de Jiménez, 2012). Our working group have observed that insulin stimulates root hairs growth of *Arabidopsis* of a dose dependent manner (Pascual-Morales et al., 2012). Has been widely reported that root hairs growth involves activation of PI3K and that its inhibition reduced such growth and ROS levels (Lee et al., 2008). Has also been reported that activation of PI3K and its product PI3P are required for auxin- induced production of ROS and root gravitropism (Joo et al., 2005). PI3K is thought to modulate these processes by regulating endocytosis and ROS production. This last is reduced by PI3K inhibitors in various cell types of plants including root hair. Lee et al., 2010 have proposed a model in which this effect of the inhibitors is due to the inhibition of PI3K-mediated activation/delivery of NADPH oxidase, a mayor source of ROS. Would be important determine whether root hairs growth stimulated by insulin involved PI3K activation and increased ROS levels.

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Changes in the activity of the glucose-6-phosphate dehydrogenase during hypothalamic sexual differentiation in rats treated with testosterone propionate perinatally females.

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Sexual dimorphism is a fundamental process for reproduction and preservation of species. Sexual differentiation in mammals involves various aspects anatomical, physiological and behavioral that includes primary and secondary sexual different characteristics morphological, reproductive and non-reproductive behaviors, as well as structural and ultrastructural differences at the level of the central nervous system (CNS). Sexual differentiation is a process regulated by multiple factors, including the hormones secreted by the gonads during critical developmental periods that determine neuronal population nuclei specific dimorphic in the hypothalamus as the ventromedial nucleus so it is important to determine the role of the antioxidant system during the sexual differentiation of the hypothalamus. The biological material was obtained from the vivarium of the UAM-Xochimilco (UPEAL, UAM-X). Pregnant female rats of the Wistar strain were used. He was the monitoring of pregnancy and at the time of delivery I regard as zero hour of birth is (Krinke, 2000) identifying the young females that were used for this project. An hour after birth, the treatment of the female rats was conducted and administered 30 µg of testorerone in 20 µl, with their respective control with sesame oil and were sacrificed to the 1, 3, 6, 12, 24, 48 and in adulthood to 90 days post treatment, obtaining immediately the hypothalamus (Vangala 1999). The activity of the glucose 6-phosphate dehydrogenase and GSH (Ortega et al. 2009) were measured. To compare the results between the groups, and differences among these was conducted an analysis of variance (ANOVA). They were considered significant differences when reached a value of $p < 0.05$ in all details. It was performed using SAS/STAT software. The results shows the activity of G6PDH in controls decrease significantly from 6 hrs, and in adulthood increases above initial values, however in the treaties decrease after 6. While the GH, acquires the highest values at 12 and remains so until the 48 hrs., not in adulthood. GSH is in higher concentrations at 12 hrs, which is not only associated to the synthesis of glial cells but also neuronal, indicating a neuroprotective effect during sexual differentiation hypothalamic important event for the people of nuclei dimorphic reproductive, G6PD activity from 6 hrs post-treatment differences clearly indicate its role in decrease in cell proliferation both in female controls as masculinized females chords with reset them populations in the dimorphic nuclei during sexual differentiation of rat hypothalamic. Activity of the glucose-6- phosphate dehydrogenase during sexual differentiation in the male rat hypothalamic perinatally treated with Tamoxifen.

Activity of glucose 6-phosphate dehydrogenase during sexual differentiation in the male rat hypothalamic perinatalmente treated with Tamoxifen.

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The determination of the sexual dimorphism of the CNS and the genital internal and external depends on the action of several hormones by the male fetal gonad. (Audi, 2001;) Herrera et al., 2005; Morales, 2002). There are morphological, physiological, biochemical and differences of behavior which are, as a whole, sexual dimorphism in the central nervous system (CNS) of males and females, this refers mainly to differences in the size of the nucleus or brain circuit, therefore, the oxidizing environment plays an important role in proliferation and cell death during the process of neuronal differentiation. The biological material was obtained from the vivarium of the UAM-Xochimilco (UPEAL, UAM-X). Female rats of the Wistar strain were used. He was the monitoring of pregnancy and at the time of delivery I regard as zero hour of birth is (Krinke, 2000) identifying the young females that were used for this project. An hour after birth, male rats treatment was conducted and administered 200 µg of Tamoxifen in 20 µl of vehicle, with its respective control with sesame oil and were sacrificed to the 1, 3, 6, 12, 24, 48 and in adulthood to 90 days post treatment, obtaining immediately the hypothalamus (Vangala 1999). The activity of the glucose 6-phosphate dehydrogenase and GSH (Ortega et al. 2009) were measured. To compare the results between the groups, and differences among these was conducted an analysis of variance (ANOVA). They were considered significant differences when reached a value of $p < 0.05$ in all details. He was carried out using the software SAS / STAT ®. The biological material was obtained from the vivarium of the UAM-Xochimilco (UPEAL, UAM-X). Female rats of the Wistar strain were used. He was the monitoring of pregnancy and at the time of delivery I regard as zero hour of birth is (Krinke, 2000) identifying the young females that were used for this project. An hour after birth, male rats treatment was conducted and administered 200 µg of Tamoxifen in 20 µl of vehicle, with its respective control with sesame oil and were sacrificed to the 1, 3, 6, 12, 24, 48 and in adulthood to 90 days post treatment, obtaining immediately the hypothalamus (Vangala 1999). The activity of the glucose 6-phosphate dehydrogenase and GSH (Ortega et al. 2009) were measured. To compare the results between the groups, and differences among these was conducted an analysis of variance (ANOVA). They were considered significant differences when reached a value of $p < 0.05$ in all details. He was carried out using the software SAS / STAT®. Within the values obtained from the G6PDH activity seen until 3 ha, and reduces significantly even up to adulthood, with regards the GSH, higher values were presented at 12 hrs and diminish after 24 and 48 hrs, in the control rats, and values in the treated rats do not present significant differences at any time or in adulthood. Apparently low GSH concentrations both produce better effect in the synthesis of glial cells neurons to rear at 3 o'clock, causing a defeminization of nuclei dimorphic, not in the feminized males showing a response counter during the hypothalamic sexual differentiation, the G6PD antioxidant protection is diminished by what the neuronal survival changes significantly during sexual differentiation of the hypothalamus of the male even with the males feminised causing irreversible changes in adulthood.

ROLE OF OXIDATIVE STRESS ON HYPOGLYCEMIC CEREBRAL DAMAGE AND ITS POSSIBLE PROTECTION BY D-β-HYDROXYBUTYRATE.

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Glucose is the main energy source in brain and it is essential for correct brain functioning. Whenever blood glucose (BG) levels decrease below the normal range, 70 to 110 mg/dl, hypoglycemia takes place. Diabetic patients frequently experience periods of moderate hypoglycemia (BG between 60-40 mg/dl), and even of severe hypoglycemia (BG below 40 mg/dl) as a consequence of insulin treatment. The first is usually a transient condition while the second involves a serious risk of neuronal damage. In rodent models of severe insulin-induced hypoglycemia accompanied by 30-60 min coma, it has been observed a selective neuronal death in brain regions such as the cerebral cortex (CC), hippocampus (HP) and striatum. Recent evidence supports the presence of oxidative stress markers in the hypoglycemic brain, such as elevated levels of nitrosylated proteins, thiobarbituric acid reactive species (TBARS), and 4-hidroxynonenal. However, the distribution of ROS production in discrete brain regions has not been investigated. The ketone bodies (KB) acetoacetate and D-β-hydroxybutyrate (DBHB), can be used in brain as alternative energy source in certain conditions such as the suckling period and prolonged starvation, or whenever their blood concentration is increased by the ketogenic diet or KB administration. Recent evidence shows that KB can prevent neuronal damage induced by hypoxia/ischemia and in models of Parkinson's and Alzheimer's disease. We have previously reported that KB reduce neuronal death and the production of reactive oxygen species (ROS) induced during energy-limiting conditions in cultured cells (*Neurosci.* 2003, 120: 365-378). Furthermore, we have observed that KB show an antioxidant action scavenging ROS (*Exp. Neurol.* 2008, 211:85-96). In the present study we have investigated the distribution of ROS using the fluorescent oxidation-sensitive marker dihydroethidine, which oxidizes to ethidium (Et) and cell death using Fluro-Jade B (FJ-B), in discrete regions of the rat brain after severe hypoglycemia. Rats were injected with insulin and BG levels declined to 20 mg/dl or less. A group of animals was rescued by glucose infusion after 3 h of hypoglycemia, while a second group was rescued after showing a short period of coma (3-8 min). The protective effect of DBHB against cell death and ROS production was tested in animals receiving two i.p. DBHB administrations, the first 1 h after insulin administration and the second when animals lost their righting reflex (before reaching the coma). Brains were extracted 24 h later and analyzed. The number of FJ-B-positive cells was larger in animals showing coma and were located mainly in the CC, particularly in the 2-4 superficial layers of the frontal and parietal cortices. Animals treated with DBHB did not reach the coma state and showed a reduced number of FJ-B-positive cells. ROS production was detected in the CC and the HP. Among the CC, the frontal, temporal and parietal cortices showed the largest increases, while in the HP, the crest of dentate gyrus and CA1 show the largest increases in Et fluorescence. DBHB significantly reduced the Et signal in all the regions tested. These results demonstrate that DBHB efficiently prevents neuronal death and reduces ROS production associated with severe hypoglycemia, supporting the potentiality of KB for the treatment of cerebral ischemia.

This work was supported by S-112179 CONACYT grant to LM.

OXIDATIVE DAMAGE BY AIR ENVIRONMENTAL EXPUSURE IN URBAN CHILDREN

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Ecatepec County, located in the north of Metropolitan area of Mexico City, is one of the most polluted and populated urban areas in Mexico. It has an important industrial activity and heavy traffic (1,500,000 trips/day); therefore it has high levels of air contaminants, like particulate matter (PM), polycyclic aromatic hydrocarbons (PAHs), benzene (BZ) and lead (Pb). PAHs and benzene are metabolized by enzymes such as the cytochromes P450 (CYP) generating reactive oxidative species, while Pb can produce oxidative stress by an indirect way. The oxidative stress can produce genetic damage, such as purine oxidation (8-OHdG), which is highly mutagenic that can contribute to cancer development such as leukemia, the third mortality cause in Ecatepec. We evaluated the genetic and oxidative damage in children from two areas of this County: Xalostoc (highly industrial with heavy vehicular traffic) and Jardines de Morelos (low vehicular traffic area). A cross-sectional study was conducted in 179 school children (7-10 years old) from Xalostoc (N=94) and Jardines de Morelos (N=85). Air samples were collected from both areas and environmental $PM_{2.5}$ and $PM1_0$ levels were determined by gravimetry. Blood and urine samples were obtained, a medical examination was done and parents answered a structure questionnaire. DNA damage in mononuclear cells was evaluated by the comet assay (OTM parameter), 8-OHdG levels with an specific antibody by flow citometry and lipoperoxidation (plasmatic malondialdehyde-MDA) by colorimetry. Exposure biomarkers of PAHs (1-hydroxypirene; 1-OHP) and benzene (*t,t*-muconic acid; *t,t*-MA) were determined by HPLC and blood lead (BPb) levels by atomic absorption spectroscopy. The geometric mean (GM) of PM_{10} in Xalostoc was higher than in Jardines de Morelos (135.7 vs 107.3 µg/m^3, $p<0.05$), while the GMs of $PM_{2.5}$ weren´t different in Xalostoc (28.9 µg/m^3) and Jardines de Morelos (20.2 µg/m^3). The GM of PbB in Xalostoc was 5.3 µg/dL with 62% of children with > 5 µg/dL (maximum tolerable limit), and 3.1 µg/dL in Jardines de Morelos with 21% of children with > 5 µg/dL. The GM of *t,t*-MA in Xalostoc was 88.2 µg/g creatinine and 146.7 µg/g creatinine in Jardines de Morelos ($p<0.05$); while the GM of 1-OHP in Xalostoc was 16.9 nmol/mol creatinine and 28.5 nmol/mol creatinine in Jardines de Morelos ($p<0.05$). The higher oxidative damage (MDA and 8-OHdG levels) was observed in Xalostoc ($p<0.05$), while the DNA damage (OTM) was similar in both areas. A positive association was observed between the oxidative damage (8-OHdG) with PM_{10} and $PM_{2.5}$ and between BPb and MDA levels in Xalostoc children ($p<0.05$), while the genetic damage (OTM) was associated with PM_{10} and $PM_{2.5}$ ($p<0.05$) in children from Jardines de Morelos. These results suggest that the scenario of air contaminants is complex and different in both areas, but is contributing to the genetic and oxidative damage observed in children. Therefore, children from Ecatepec County are exposed to oxidative pollutants and are at risk of developing genetic damage associated-diseases.
Supported by CONACYT (#155179) and ICyTDF (#396/10) grants.

EFFECT OF HYPOXIA ON A549 EPITHELIAL CELLS AND LUNG F-13 FIBROBLASTS VIABILITY. A PRELIMINARY STUDY.

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Hypoxia is a stress condition that in some circumstances can compromise cell viability by promoting apoptosis, inducing senescence, cytotoxicity or the epithelial-mesenchymal transition process. It has been observed that human fibroblasts that are resistant to hypoxia can increase their proliferative rate. The aim of the present work was to evaluate the viability of two lung cell lines (A549 and fibroblast F-13) under hypoxic conditions. We cultured cells at a concentration of 1% oxygen for 3, 6, 9, 12, 24, 48 and 72 hrs and compared them with the cells growing in normoxia (5% CO_2/95% air). A549 cells were cultured in DMEM and F-13 fibroblasts in Ham F-12 culture medium enriched with 10% fetal bovine serum, 200 U/ml penicillin and 200 U/ml streptomycin at 37 ° C. To culture cells in hypoxic conditions a special chamber (Billups-Rothenberg MIC-101, California, USA) was used. A mixture of 95% nitrogen/5% CO_2 gas that displaces the oxygen into the chamber was injected. The oxygen concentration was measured by an oxygen sensor. Fifteen thousand cells were cultured in 48 well plates during the periods mentioned above. After the culture time, cells were counted by the erythrosine B exclusion method. The results showed a progressive decrease in A549 cell viability except for the 6 and 12 hrs periods, in which cells grow seems to stabilize and then decrease to a 30% of viability at 72 hrs. Lung fibroblasts viability also decreased at 3 hrs, however at 6 hrs cells began to proliferate with an increase up to100% viability at 24 hrs; at 48 hrs, viability was above 100%.

We found significant differences among A549 cells viability cultures with 1% oxygen and cells cultured in normoxia at 48 hrs ($p = 0.027$) and 72 hrs ($p = 0.009$). There were no significant differences between lung fibroblast viability cultured in normoxic and hypoxic conditions. We also observed that A549 cells are more sensitive to hypoxia than F-13 lung fibroblasts.

ANTIOXIDANT-MEDIATED PROTECTIVE EFFECT OF HAWTHORN (*Crataegus mexicana*) SKIN EXTRACT IN ERYTHROCYTES AGAINST OXIDATIVE DAMAGE.

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ABSTRACT: Hawthorn *Crataegus mexicana* is a traditional fruit in mexican gastronomy. They are reportedly used to treat many ailments. Our earlier studies have shown that extracts derived from hawthorn skin (HSE) possess strong antioxidant activity in chemical and biological model systems *in vitro*, attributable to its polyphenolic content. The main objective of this study was to investigate the ability of acetone HSE to protect erythrocytes against oxidative damage, *in vitro and ex vivo*. The protection rendered by HSE in erythrocytes was studied in terms of resistance to oxidative damage by TBARS assays, morphological alterations by light microscopy as well as kinetic alterations in a flow cell system *ex vivo*. The total polyphenolic content in HSE was found to be 2.65 ± 0.23 mg as the equivalent of gallic acid per gram, and the carotenoids content was 26.4 ± 0.02 µg/g in HSE powder. The results for scaveniging DPPH free radicals and inhibiting TBARS formation by HSE was 21.9 ± 0.15% and 13.27 ± 0.70% at 10 mg/L respectively. We chose the experimental prooxidant system: FeSO4 to induce lipid peroxidation in human RBC membranes. HSE was found to inhibit lipid peroxidation (37.23% inhibition by HSE at 2.5 mg/ml). While HSE *per se* retard the morphological alteration in the erythrocytes-eryptosis, under the experimental conditions, HSE inhibited the FeSO4-induced morphological alterations in human RBCs as revealed by light microscopy *in vitro* and *ex vivo*. Further, HSE was found to offer significant protection to human membrane erythrocyte in up to 28 days from oxidative damage induced by ferrous sulfate. In conclusion, our results indicate that HSE is capable of protecting erythrocytes against oxidative damage probably by acting as a strong antioxidant.

TEMÁTICA: Estrés oxidante en la muerte celular y la enfermedad

DIALLYLDISULPHIDE ACTIVATES Nrf2FACTOR AND DECREASES THE CEREBRAL ISCHEMIC INJURY.

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The Cerebrovascular Accident (CVA) is the sixth cause of death in the world and the first of disability in the people of economical active age. One of the most important mechanisms involved in the neuronal damage is reactive oxygen species (ROS) production during the event of ischemia/reperfusion (IR). Due to this, is important to find therapeutic alternatives based in the administration of antioxidant drugs. It has been shown that the organosulfur compounds present in the garlic oil have a potent antioxidant effect through the activation of the Nuclear Factor (erytrhoid-derived 2)-like (Nrf2). This transcription factor is the master regulator of the antioxidant machinery in the cell by the upregulation of antioxidant enzymes. In this work we studied the effect of the diallyldisulphide (DADS) -an organosulfur compound of the garlic oil – on the neurological deficit, the morphological alterations, the activation of the Nrf2 factor, and the activity of principal phase 2 detoxifying enzymes in a cerebral ischemia model. Rats were subjected to 1 h of ischemia plus 24 h of reperfusion using the middle cerebral artery occlusion model. DADS (25 mg/kg weight, *i.p.*) was administered at onset of reperfusion. The treatment with DADS decreased neurological deficit of the animals submitted to IR (IR+DADS group) in comparison to the animals of IR group. DADS decreased the signs of neuronal damage induced by IR in the striatum. On the other hand, DADS alone increased Nrf2 factor activation (at 2 h of reperfusion), and glutathione peroxidase (GPx) and glutathione reductase (GR) activities (at 24 h of reperfusion) in the ipsilateral cortex. Moreover, DADS treatment prevented the decrease in GPx activity observed in IR group. In conclusion, DADS reduced the neurological deficit and cellular damage caused by the IR and increased Nrf2 factor activation andGPx and GR activities in the cortex. These data suggest that the protective effect of DADS could be related with its ability to regenerate glutathione levels and support that DADS treatment could be an important therapeutic alternative in the stroke model.

This work was supported by **CONACYT** (Grant **103527** to PDM).

Regresar

EFFECT OF POLYPHENOL EXTRACT FROM GREEN TEA [P60] ON GENOTOXIC AND CYTOTOXIC DAMAGE INDUCED BY Cr (IV) IN MICE CD-1 STRAIN

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Green tea’s polyphenols have been associated with beneficial effects against the development of diseases related to oxidative stress. These effects include the capture of free radicals and reactive oxygen species (ROS), the regulation of cell growth and the induction of detoxifying enzymes. On the other hand, compounds of Cr (VI) within the cell generate ROS during its reduction to Cr (III), inducing DNA damage. In this study we evaluated the effect of an extract of green tea polyphenols [P60] on the genotoxic and cytotoxic damage induced by Cr (VI). DNA damage was analyzed with the MN assay by scoring the total MN found in 2000 polychromatic erythrocytes (PCE); while cytotoxicity was evaluated by scoring the PCE ratio respect to the normochromatic erythrocytes (NCE) in a total of 1000 cells. We used the acridine orange technique in peripheral blood of mice CD-1 strain. Groups of 5 mice were treated as follows: 1) control group (vehicle administered orally by gavage); 2) P60 group (30 mg/kg P60 orally by gavage); 3) CrO_3 group (20 mg/kg CrO_3 by i.p. route); 4) P60 + CrO_3 (30 mg/kg P60 by gavage 4 hours before the administration of 20 mg/kg CrO_3 by i.p. route). Blood samples were obtained from the tail vain at 0, 24, 48 and 72 hours after each treatment. The results showed that the sole administration of P60 does not increase the frequencies of MN, while the CrO_3 treatment shows a statistically significant increase in the frequencies of MN at 48 and 72 hours after the treatment. The combined treatment (P60 + CrO_3) showed a decrease in MN frequencies at 24, 48, and 72 hours after the treatment with CrO3, but the MN reduction at 48 hour was statistically significant compared with the control group. These results indicate that the increase of MN by CrO3 is partially blocked by the P60 extract of green tea in the protocol used. However it requires to perform more experiments using different protocols to have more supported results. On the other hand, the frequency of PCE was not-modified at any of the treatments evaluated, concluding that they do not have a cytotoxic effect.

Financial support was obtained from DGAPA-UNAM IN217712.

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IN VIVO EFFECT OF GREEN TEA FLAVONOIDS (EPIGALLOCATECHIN-3-GALLATE AND QUERCETIN) ON THE GENOTOXICITY OF HEXAVALENT CHROMIUM: ANTIOXIDANT AND PROOXIDANT

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Inhibition of oxidative damage constitutes the first line of defense against DNA damage, and can be considered as the most effective way to prevent some forms of cancer. Flavonoids like quercetin and epigallocatechin-3-gallate (EGCG), present in green tea, have shown strong antioxidant properties. Quercetin is considered to be the most potent reactive oxygen species (ROS) scavenger, it has also shown *ex vivo* protection against H_2O_2-induced DNA damage, and has been suggested to empower the plasma endogenous antioxidant capacity[1,2]. EGCG, the major green tea catechin, besides acting as a ROS scavenger, it has also shown antiproliferative and pro-apoptotic *in vitro* effects[2]. On the other hand, hexavalent chromium [Cr (VI)] generates ROS during its reduction to Cr (III), leading to DNA damage. As part of our ongoing research program to evaluate chemopreventive or chemoprotective potential components of diet, the ability of quercetin and EGCG to reduce genotoxic damage induction, by Cr (VI) in peripheral blood polychromatic erythrocytes (PCE) of mice, was evaluated in the present study. Mice were treated with flavonoids (quercetin 100 mg/kg and EGCG 10 mg/kg); CrO_3 (20 mg/kg); or the flavonoid prior to CrO_3 administration. DNA damage was evaluated by analysis of micronucleus (MN), using the acridine orange technique. Blood samples were obtained from the tail vein at 0, 24, 48 and 72 h after each treatment. The results showed that the treatments with quercetin and EGCG alone did not modify MN frequency. CrO_3 treatment significantly increased MN frequency after the injection. However the group treated with quercetin and CrO_3 showed a partial decrease MN frequency 24, 48 and 72 after the administration of the treatments, but mainly at 24 h (64% reduction MN); suggesting a limited protection against chromium-induced-genotoxicity; whereas the treatment with EGCG, prior to CrO_3, showed a higher increase in MN frequency at 24 h (30% increase MN) compared with the group treated only with CrO_3 suggesting a pro-oxidant effect. These results indicate that quercetin is capable to prevent DNA damage possibly by ROS scavenging, but also that the increase of MN by CrO_3, is not blocked by the EGCG.

Financial support was obtained from DGAPA-UNAM IN217712.

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Keywords: EGCG, quercetin, chromium (VI), micronucleus, flavonoids.

SUBCRHONIC ADMINISTRATION OF *S*-ALLYCYSTEINE (SAC) ACTIVATES Nrf2 FACTOR IN CEREBRAL CORTEX

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S-allylcysteine (SAC) is the most abundant compound in aged garlic extract, an odorless garlic preparation. A large number of studies have demonstrated the antioxidant activity of SAC in both *in vivo* (in diverse experimental animal models associated to oxidative stress) and *in vitro* conditions (using several methods to scavenge reactive oxygen species or to induce oxidative damage). Derived from these experiments, the protective effect of SAC has been associated with the prevention or amelioration of oxidative stress, mainly as direct antioxidant.
In this work, we evaluated the ability of SAC (indirect antioxidant) to activate Nrf2 factor -a master regulator of the cellular redox state - in different cerebral regions (striatum and cortex).
Male Wistar rats (90-100 g) were administered with SAC (25, 50, 150, 300 and 600 mg/kg- body weight each 24 h, *i.g.*) by 30 days (this time was used as a model of a long administration useful in several chronic diseases, such as stroke or neurodegenerative diseases). Striatum and cortex were obtained to evaluate Nrf2 activation using a ELISA Kit.
SAC treatment induced a transitory activation of Nrf2 factor in cerebral cortex, from the dose of 25 mg/Kg (184% compared to control group), and increasing gradually until 300 mg/Kg dose, when the highest level of activation (326%) was observed. At 600 mg/Kg the Nrf2 activation decrease but not returned to basal levels (224%). On the other hand, any dose evaluated have a significantly effect on Nrf2 activation in striatum.
In conclusion, the protective effect of SAC observed in other studies has been associated mainly with its direct antioxidant properties (scavenging of free radicals and prooxidant species); however, other mechanisms have been reported (inhibition of prooxidant enzymes, or chelating effects) and our data shown for the first time that SAC is able to activate the Nrf2 factor en cerebral cortex, emphasizing its potential use as therapeutic agent.

This work was supported by ICyTDF (Grant PICSA 12-171 to PDM).

OXIDATIVE DNA DAMAGE ASSOCIATED WITH DECREASED ENZIMATIC ACTIVITY AND GENE EXPRESSION IN THYMUS OF MALNOURISHED RATS

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Introduction. Malnutrition is an important public health problem affecting millions of children worldwide. The nutritional privation has an important impact on lymphoid tissues size, particularly the thymus. Malnutrition has been associated with DNA damage and with alterations in redox metabolism due to decreased levels of molecules involved in the antioxidant defense. Some of these molecules are enzymes necessary to protect the cells against DNA oxidative damage and their decreased activity and/or expression could be one of the related factors with the poor condition of the antioxidant defense observed in malnourished organisms. **Objective.** The aim of the present work was to elucidate thymus oxidative DNA damage and its association with enzymatic activity and mRNA expression of genes involved in antioxidant protection, in malnourished [first (DN1, n=5); second (DN2, n=5); third (DN3, n=5) degree malnourished] and well-nourished, 21 day old rats. **Experimental desing.** Malnutrition was induced in the rats during the lactation period, by the food competition method. Oxidative DNA damage was determined quantifying 8-hidroxydeoxyguanosine adduct (8-OHdG) by HPLC. Antioxidant enzyme activities were measured spectrophotometrically, and the relative mRNA expression of p53 and Nrf2 by qPCR technique. These transcription factors are important because they regulate the transcription of genes involved in antioxidant protection such as SOD, GPx, CAT, which were also determined. **Results.** The results showed significantly higher levels of oxidative DNA damage in the thymus obtained from malnourished animals in comparison with well-nourished. This finding was related to a decreased in the relative mRNA expression of SOD, GPx, CAT, p53 and Nrf2 genes; all were affected in greater extent as the degree of malnutrition and thymus weight deficit was more evident. Additionally, we found an association between CAT enzymatic activity and its mRNA expression levels. **Conclusion**. The results obtained in the present study led us to conclude that malnutrition induced during lactation in rats, is associated with higher oxidative DNA damage, which is correlated with a low enzymatic activity (CAT) and a decreased in mRNA levels of genes transcribed by p53 and Nrf2. Additionally, our results showed that a higher body weight deficit was related to a more severe alteration in the thymus redox state, which leads to increased levels of oxidative DNA damage.

The authors thank to MVZ Rocío González Vieira for providing the animals used in this study. Gavia-García and Conde-PérezPrina are CONACYT scholarship holders.

ROLE OF NADPH-OXIDASE IN THE DEATH OF CULTURED CEREBELLAR ASTROCYTES.

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In the nervous system, cell death can occur through a genetically programmed mechanism in response to pathological and physiological conditions, including the elimination of neural cells during development. In different models of neuronal death, there is a generation of reactive oxygen species (ROS). During the cerebellar granule neurons (CGN) death also occurs ROS generation and cell death is prevented by antioxidants. This condition seems to be mediated by the activation of the enzyme NADPH-oxidase. On the other hand, there is not enough information about the death of glial cells and there is no information about the role of ROS and NADPH-oxidase involved in this process. In the present study we assessed the participation of the ROS in the death of cultured astrocytes of rat cerebellum using the general inhibitor of protein kinases staurosporine (St) as death inductor. Here, we found that cells die in a concentration- and time-dependent manner. Treatment with St provoked an increase in the generation of ROS after 15 min of exposure, which reached a maximum at 2 h. In addition, the use of antioxidants significantly reduced the production of ROS as well as cell death. By using Western blot and immunocytochemistry assays, we identified the expression of some subunits of the NADPH-oxidase, including $p67^{phox}$ and $p22^{phox}$ and the catalytic subunits Nox1 and Nox4. Treatment with a variety of inhibitors of NADPH-oxidase induced a significant decrease in the production of ROS as well as cell death evoked by St. These data suggest that call death of astrocytes could be mediated by ROS produced by NADPH-oxidase.

This work was partially supported by CONACYT grant 179234 and PAPIIT grant IN218310/23

Regresar

SCAVENGING CAPACITY OF TRYPTOPHAN METABOLITES: 3-HYDROXYANTHRANILIC ACID AND 3-HYDROXYKYNURENINE

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3-Hydroxyanthranilic acid (3-HAA) and 3-hydroxykynurenine (3-HK) are molecules derived from the tryptophan oxidative metabolism. There has been controversy about their role in inflammatory processes as well as the different neurodegenerative pathologies in which they are related through oxidative stress. On the one hand, it has been reported that both 3-HAA and 3-HK exert a pro-oxidant role, and in high concentrations these molecules have been related with cellular death (Okuda *et al.*, 1998; Sun-Mi *et al.*, 2010). On the other hand, evidence has shown that 3-HAA as well as 3-HK possess anti-oxidant properties and play an important role in anti-inflammatory processes (Christen *et al.*, 1990; Krause *et al.*, 2011; Leipnitz *et al.*, 2007; Quagliarello *et al.*, 1966).The aim of this work is to characterize the scavenging capacity of 3-HAA and 3-HK in synthetic systems specific for anion superoxide ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), peroxynitrite ($ONOO^-$) and hydroxyl radical ($OH^{\cdot}$), as well as to determine their effect on protein and DNA degradation. The concentration range used for these molecules was 5 to 500 µM. Results show that 3-HK presents anion superoxide, hydroxyl radical and peroxynitrite scavenging capacity while it protects against protein and DNA degradation. To a lesser extent, these markers were also prevented by 3-HAA. Results suggest that while under experimental conditions 3-HK maintains scavenging properties, the protective effect of 3-HAA does not seem to be related to its radical scavenging capacity, but to a chelant effect that needs to be further explored.

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OXIDATIVE STRESS EFFECT OF A SPORTS MEDICAL PROGRAM ON OLDER ADULTS WITH AND WITHOUT METABOLIC SYNDROME

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Introduction: Aging is a natural process that involves every living genetic and environmental factors, which involves a number of degenerative changes such as reduction in physiological function and increased likelihood of disease and death. One of the most widely accepted theory is the shortening of telomeres, one theory is that the formation of free radicals lead to aging, and other effects of free radicals is the damage to biomolecules such as lipids, proteins and nucleic acids, well that could be related to aging and disease is often related as obesity, hypertension, metabolic syndrome and type 2 diabetes mellitus, the aim of our study was the plasma analizaren seniors between 60 and 70 years of healthy and diseased levels of oxidative stress, and modification of these levels with a sports medical program.

Methods: Women form three groups: a control healthy individuals between 25 and 40 years (CTJ) n = (30), another of seniors between 60 and 70 clinically healthy (CT3) n = (30), and other older adults with metabolic syndrome between 60 and 70 years (P) n = (30). The two groups of healthy older adults and patients was applied sports medicine program for 3 months, plasma was taken before and three months after, the intervention to determine oxidative stress markers 1. MPI MDA technique as damage to lipid marker 2. Carbonyl groups as marker protein damage 3. SH groups as antioxidant defense, as well as anthropometric and clinical biochemistry variables.

Results: we establish the differences of redox status in healthy older adults compared with young healthy adults. Older adults with metabolic syndrome are in a state of oxidative stress, increases in markers like MDA, carbonyls and decreased antioxidant defense SH groups. Three months after the sports doctor oxidative stress levels decreased in healthy elderly and in those with metabolic syndrome, as well as the anthropometric and clinical biochemistry variables.

Conclusions: the healthy older adult is in a redox state, and sports medicine program is a meaningful strategy to reduce oxidative stress levels in individuals with healthy elderly with metabolic syndrome and anthropometric parameters and clinical biochemical variables.

EFFECTS OF A PROGRAM OF PHYSICAL ACTIVITY INSTITUTIONAL ABOUT OXIDATIVE STRESS ON ELDERLY ADULTS WITH AND WITHOUT METABOLIC SYNDROME

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In the world literature there is no article that relates to aging, oxidative stress, and morbid states to exercise in people over 60 years, and this work a step for the study of these interactions.

Aging is a natural process that involves every living genetic and environmental factors, which involves a number of degenerative changes such as reduction in physiological function and increased likelihood of disease and death. One of the most accepted theories is the shortening of telomeres, structures that protect the ends of eukaryotic chromosomes, preventing mergers occurring between them or that genetic material can recombine stored improperly.

Likewise, the formation of free radicals lead to aging, since they alter the replication and production of mitochondria, causing damage to biomolecules such as lipids, proteins and nucleic acids, which leads to aging and diseases such as obesity, hypertension, metabolic syndrome and type 2 diabetes mellitus. This increase of free radicals during exercise is studied, with the ability to generate a state of oxidative stress preconditioning, ie increase free radicals increases similarly but the antioxidant.

The aim of our study was to analyze the plasma of elderly between 60 and 70 years, the levels of oxidative stress with MDA, SH and carbolilos, plus biochemical variables such as cholesterol, triglycerides, and glucose, and also anthropometric parameters BMI, percentage of fat mass, muscle percentage, weight, waist circumference and blood pressure before and after application of a program ISEM Institutional

Our intervention was to 3 groups: control healthy individuals aged 25 to 40 years n = (30), another older adults between 60 and 70 relatively healthy n = (30), and other older adults with underlying conditions between 60 and 70 years n = (30). Both groups of older adults was applied ISEM program, was taken for plasma oxidative stress markers, Medical records and anthropometric measurements at day 0 and day 90.

After 3 months we establish the differences of redox status of healthy older adults compared with young healthy adults.

We also established the differences between the redox status of healthy older adults and oxidative stress.

Similarly demonstrated the ability to reduce ISEM program with statistical significance, markers of oxidative stress in diseased individuals not so in healthy individuals, increasing the markers in this group.

THE NATIVE FLORA OF YUCATAN PENINSULA AS A SOURCE OF BIOACTIVE METABOLITES WITH ANTIOXIDANT, ANTI-INFLAMMATORY AND/OR ANALGESIC ACTIVITY

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Introduction: Reactive oxygen species (ROS) contribute to maintain conditions of pain and inflammation. Accordingly, antioxidants represent an important option for the development of new analgesics and anti-inflammatories. The plant kingdom, including plants used in Mexican traditional medicine, represents one of the most important sources of secondary metabolites with biological activity, including antioxidant. Objective: To evaluate the antioxidant, anti-inflammatory and analgesic activities of crude extracts, semipurified fractions and pure metabolites obtained from native plants of the Yucatan peninsula traditionally used to treat pain and inflammatory problems. Materials and Methods: The DPPH radical-reduction assay and the models of acetic acid-induced writhing, carrageenan-induced edema and formalin in mice were used to evaluate the antioxidant, anti-inflammatory and analgesic activities, respectively, of six native medicinal plants of the Yucatan peninsula: *Acacia gaumeri* Blake(Fabaceae), *Acmella pilosa* RK Jansen (Arteraceae), *Calea urticifolia* (Miller) DC. (Arteraceae) *Cnidoscolus souzae* McVaughn (Euphorbiaceae), *Eupatorium hemipteropodum* Robinson (Arteraceae) and *Scutellaria gaumeri* Epling (Arteraceae). Results: Crude extracts from the roots of *Calea urticifolia* and *Cnidoscolus souzae* showed the best results in terms of antioxidant, analgesic and anti-inflammatory activities. Metabolites CUR-4 and CUR-5, obtained from the purification of the medium polarity fraction of the root extract of *C. urticifolia*, showed the strongest antioxidant and anti-inflammatory activities, while metabolites CSR-1 and CSR-2, obtained from the medium polarity fraction of the roots of *C. souzae* showed good analgesic activity. Conclusions: The results obtained coincide with the traditional use of the plants used in the practice of Yucatecan traditional medicine.

ASSOCIATION BETWEEN TOTAL ANTIOXIDANT AND BIOMARKERS OF ENDOTHELIAL DYSFUNCTION

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The aim of this study was to measure the association between serum total antioxidant (TAS) and adhesion molecules and inflammation in subjects with high and low cardiovascular risk and determine the most sensitive biomarker for use as a diagnostic test of endothelial dysfunction.

The study was conducted at the Center for Research in Medical Sciences (CICMED) of UAEMex, from the database Cohort UAEMex Workers. We conducted a case-control study, which included 63 subjects classified by the Framingham equation and two high cardiovascular risk group (31 subjects) and low (32 subjects). The serum level of total antioxidant and biochemical parameters were determined with automated equipment and Selectra 2 ® Total Antioxidant kit for total cholesterol, HDL cholesterol and glucose Randox ®, in the case of the biomarkers were determined by the ELISA method through the corresponding kit.

The average age of the population was 45 ± 10.9 years with a range of 23 to 68 years, 66.7% had decreased HDL cholesterol, hypercholesterolemia 57.1%, 39.6% hypertension, 25.4% diabetes and 25.4% smoked. There was a positive correlation between total antioxidant and IL-6 in subjects at high cardiovascular risk ($r = 0.256$, $p = 0.049$), same as dividing by quartiles Q4 antioxidant levels = 1.5-1.7 mmol / L increased the association with IL-6 ($r = 0.721$, $p = 0.002$), IL-1B ($r = 0.590$, $p = 0.021$) and VCAM-1 ($r = 0.579$, $p = 0.024$). ROC curve was performed and found that the more sensitive biomarker for endothelial dysfunction, IL-6 was followed by VCAM-1, IL-1β and TNF-α.

The presence of cardiovascular risk releases a series of signals that result in an increased expression of cytokines and adhesion molecules due to the presence of reactive species acting on the endothelium, thereby stimulating serum antioxidant capacity

HUMAN RENAL EPITHELIUM AND OXIDATIVE STRESS

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Diabetic nephropathy is a microvascular complication with higher rates of morbidity and mortality in patients with type 2 diabetes mellitus, is also the leading cause of end-stage renal disease. A key role in the pathogenesis of diabetic nephropathy has been attributed hyperglycemia as it leads to the formation of advance glycation end products (AGEs) and the production of reactive oxygen species (ROS), subsequently to cell death and kidney dysfunction. There is increasing evidence that reactive oxygen species play a major role in the development of diabetic complications. The hydrogen peroxide (H_2O_2) is one of the most important reactive oxygen species. It is involved in multitude of intracellular process such as cell survival and changes in cell morphology. The identification of the mediators that determines the progression of morphological changes of diabetic nephropathy expressed may lead to new therapeutic approaches. In the present study, we examined the effect that the H_2O_2 on cell viability and morphological changes of human renal epithelial cells. For that purpose, the human kidney epithelial cells (HEK-293T, ATCC) were exposed to hydrogen peroxide concentration: 50 µM, 100 µM y 250 µM for a time of 1, 4, 12 y 24 h, subsequently analyzed changes in cell morphology and cell viability was tested with trypan blue staining. After 4 h exposure to a concentration of 250 µM of hydrogen peroxide, we observed significant changes in cell morphology associated with cellular stress, generally observed an excessive vacuolation, presenting a granular appearance suggesting a decrease in cell volume compared to control, at 24 h showed significant changes associate with cell death, compromising the integrity of the monolayer. In addition, we observed a loss of cell viability in a concentration- and time-dependent manner, observing to concentration of 250 µM of H_2O_2 and 4 h exposure, 76% of viable cells and only 30% of viability after 24 h exposure. Exposure of a renal epithelium to a highly oxidizing agent that produces ROS alters the integrity of the cell monolayer and it is possible that similar changes can occur by exposing a epithelium to high concentrations of glucose. At present, experiments in this direction are underway.

MECHANISMS OF CELL DAMAGE DURING OXIDATIVE STRESS IN LYMPHOCYTES

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Reactive oxygen species (ROS) are important regulators of normal cellular processes, but high ROS levels may cause cell damage and different types of cell death. Autophagy is one of the first lines of defense against oxidative stress damage. On the other hand, extensive autophagy under oxidative stress conditions can eventually lead to the cell death. K^+ is the more abundant intracellular cation. The loss of the K^+ is considered as a characteristic feature of the apoptosis and as important factor in regulation of caspases activity. Nothing is known about K^+ balance during autophagy and necrosis.

In the present work we used Jurkat cells as a model. Treatment with equimolar mixture of copper ($CuCl_2$) and Na-ascorbate, Cu/A, was applied to generate ROS (mostly hydroxyl radicals via Fenton reaction). Balance between autophagy, apoptosis, and necrosis was monitored. Monodansylcadaverine (MDC), fluorescent compound that is incorporated into the multilamellar bodies, was used as a probe for autophagic vacuoles detection. Phosphatidylserine externalization and caspase 3 activities were determined to reveal apoptosis. Membrane damage and necrosis were detected by trypan blue exclusion test and staining of nuclei with propidium iodide. MIFE (Microelectrode Ion Flux Estimation) technique was applied to carry out non-invasive measurements of K^+ and Ca^{2+} fluxes during early phases of oxidative stress.

It was shown that the oxidative stress, induced by Cu/A (1 - 10 μM) during the first hour of incubation, caused K^+ efflux in a concentration-dependent manner. Yet, no changes in Ca^{2+} conductance across the plasma membrane were observed. MDC accumulation, characteristic for autophagy, was more pronounced at low Cu/A concentrations (1, 2.5 μM). High concentrations of Cu/A (10 μM) caused large K^+ efflux, followed by changes in membrane integrity and necrosis, without previous activation of autophagy. At moderate oxidative damage (2.5-5 μM Cu/A) both autophagy and apoptosis were detected. It appears that autophagy and apoptotic pathways interact each with other. But specific factors that determine the choice between autophagy and apoptosis during oxidative stress in lymphocytes remain to be elucidated.

Supported by CONACyT grant 128971 to OD.

Regresar

EFFECT OF 3-HYDROXYKYNURENINE AND 3-HYDROXYANTHRANILIC IN OXIDATIVE DAMAGE MARKERS INDUCED BY $FeSO_4$ AND $ONOO^-$

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3-hydroxykynurenine (3-HK) and 3-hydroxyanthranilic acid (3-HAA) are two molecules of the kynurenine pathway, which leads to the degradation of tryptophan that has been associated with diverse neurodegenerative disorders. There is evidence that 3-HK causes cellular death in both cellular lines and neuronal cultures and that 3-HAA only does so in the latter (Okuda et al., 1998; Clifford et al., 1989). In contrast, there are reports that these compounds show anti-inflammatory, antioxidant and cytoprotective character (Leipnitz et al., 2007). These opposite effects are related with the concentration and the pH used (Vazquez, 2001).

The aim of this work is to study the effect of these metabolites in brain, liver, and kidney homogenates exposed to iron sulfate ($FeSO_4$) and peroxynitrite ($ONOO^-$). The oxidative damage markers employed were lipid peroxidation (TBA-RS), ROS and cellular viability (MTT reduction).

Our results show that 3-HK (0-100 μM) reduces in all homogenates basal levels of ROS and lipid peroxidation; while 3-HAA keeps these levels steady. In addition, 3-HK (50 μM) reduces ROS and PL induced by $FeSO_4$ (5 μM) and $ONOO^-$ (25 μM) and it is capable of recovering cell viability associated with these toxins. Meanwhile, 3-HAA *per se* increased ROS levels in liver and kidney homogenates and decreases cell viability in all homogenates. Furthermore, no effect was observed in the presence of $FeSO_4$ y $ONOO^-$. These results suggest that, at least under these conditions, 3-HK is an anti-oxidant agent and 3-HAA is a pro-oxidant agent.

CHANGES IN PANCREATIC REDOX BALANCE DURING CHRONIC EXPOSURE TO CADMIUM IN WISTAR RATS

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Cadmium (Cd) is an element that is found naturally in the earth's crust and is attached usually in combination with other elements. During the last two centuries, human and industrial activities have led to higher emissions of Cd in the environment exceeding the amount of Cd originated from natural sources. The Cd induces both cell damage and induce the mechanisms responsible for maintain the cellular redox state. Although the Cd is not able to directly generate reactive oxygen species (ROS), in combination with other elements favor the appearance of ROS. The present study was designed to evaluated the redox balance of the pancreas in Wistar male rats after a chronic exposure to 65.5 mg Cd /L in drinking water for 2, 3 and 4 months. The quantification of pancreatic Cd concentration in exposed rats shows a significant increase, which was dependent on exposure time. Also, as an indication of nitric oxide production, was quantified the amount of nitrites, which did not differ between exposed and unexposed animals. In order to assess the existence of lipid peroxidation, was determined the amount of malondialdehyde and 4 hydroxyalkenals, which were increased compared to control animals. For evaluation of the pancreatic antioxidant status in response to exposure to Cd, the concentration of total metalothionein (MT) and the activity of the enzymes catalase and superoxide dismutase were determined. The results showed an increase in the amount of MT and enzymatic activity for the two enzymes studied. To assess the viability of pancreatic cells, hystochemical studies were performed with acrydine orange, results showed a significant decrease in the stain in rats exposed by 4 months; however viability remained above 75%. In conclusion, Cd chronic exposure can increase oxidative stress in pancreas; however, pancreatic anti-oxidant protection mechanisms are capable to control the pro-oxidative status induced by Cd, maintaining cellular survival and therefore pancreatic function.

PROTECTIVE EFFECT OF CURCUMIN AGAINST HEMIN-INDUCED NEUROTOXICITY IN PRIMARY CULTURES OF CEREBELLAR GRANULE NEURONS OF RATS

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Curcumin, a yellow pigment derived from *Curcuma longa Linn*, has attracted great interest during the past decades. Extensive studies documented that curcumin has diverse biological activities as anti-inflammatory, anticarcinogenic and antioxidant, among others. This work was designed to determinate the potential protective effect of curcumin on neurons in culture on oxidative stress.

Primary cultures of cerebellar granule neurons (CGNs) from rats were treated with curcumin (10-50 μM) to evaluate its cytotoxicity by MTT reduction and FDA methods. Then, we evaluated if this compound has protective effect. CGNs were pretreated with curcumin and subsequently exposed to hemin as a generator of oxidative stress and cell viability was monitored.

It was found that curcumin (1-30 μM) was unable to modify cell viability significantly. In contrast, hemin alone (30 μM) decreased viability to 50% but when CGNs were pretreated with curcumin (5, 10 and 15 μM) before the addition of hemin, the viability diminished 12% compared with curcumin or control. In addition, it was found that curcumin induced heme oxygenase-1 (HO-1) in a concentration and time-dependent way. Furthermore, HO activity was directly correlated with the amount of intracellular protein. Curcumin protection was blocked by the HO inhibitor tin mesoporphyrin. Moreover, the content of reduced glutathione (GSH) was increased when the cells were incubated with curcumin alone and this increase was diminished by buthionine sulfoximine, an inhibitor of GSH synthesis. Hemin decreased GSH content and this decrease was blocked when CGNs were exposed to curcumin and hemin simultaneously,

It is concluded that curcumin has a protective role against hemin toxicity and that HO activity, HO-1 expression and GSH are involved in this protective effect.

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OXIDATIVE STRESS CONTRIBUTES TO CALPAIN ACTIVATION AND NEURONAL DEATH INDUCED BY GLUCOSE DEPRIVATION (GD).

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Brain function depends on the continuous supply of glucose from blood. When blood glucose levels decrease below 20 mg/dl for 30 min or more, the hypoglycemic coma takes place and massive neuronal death is observed several hours after glucose reintroduction in vulnerable brain regions. Excitotoxicity triggered by the release of excitatory amino acids and oxidative stress are implicated in hypoglycemic neuronal damage. Hence, we studied the contribution of reactive oxygen species (ROS) to the activation of the calcium-dependent cystein protease, calpain, a well-known mediator of excitotoxic damage, during glucose deprivation (GD) in hippocampal cultured neurons, used as an in vitro model of hypoglycemia. Calpain activity as monitored by the cleavage of the cytoskeletal protein, α-spectrin, increased 2- and 3-fold after 15 min and 2 h of GD, respectively. Neuronal viability, as assessed by the MTT reduction assay, was 50% reduced 24 h after depriving cells from glucose during 2 h. Neuronal death and calpain activity were prevented by the calpain inhibitor MDL-28170 and the glutamate receptor antagonist, MK-801. We have previously demonstrated that ROS are rapidly produced during GD (Neuroscience 2010, vol. 167:1057-69), through the activity of superoxide producing enzymes such as xanthine oxidase (XaO), phospholipase A2 (PLA2) and NADPH oxidase (NOX). ROS contribute to neuronal death since it is reduced in the presence of antioxidants (such as trolox) and inhibitors of XaO, PLA2 and NOX. In the present study we observed that inhibitors of these ROS-producing systems reduce by 30-40 % the activation of calpain induced by 1 h of GD. Furthermore, hippocampal slices obtained from NOX2-deficient mice, showed attenuated calpain activity when subjected to GD. These results suggest that ROS production during GD modulates calpain activity contributing to neuronal death. The present results help to understand the mechanisms by which hypoglycemia causes neuronal damage.

This work was supported by CONACYT grant S-112179 and PAPIIT grant IN211710 to LM.

Regresar

ESTROGENIC MODULATION ON THE OXIDATIVE STRESS AND AGING IN THE DORSAL HIPPOCAMPUS

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Estrogens (E) are hormones with an antioxidant action and on the maintenance of synaptic plasticity in young and senile animals. The interaction between the systems of antioxidant defense and sex steroids induces the activity of antioxidant elements such as glutathione (GSH). In the hippocampus, receptors for E make it vulnerable during aging when it loses its capacity for antioxidant response and synaptic plasticity, phenomena required to preserve the cognitive function. Dendritic spines have a high plastic capacity and can modify their structure as a result of hormonal influences. This study raised the question of the protective effect of estrogens to oxidative stress and the post synaptic plasticity (dendritic spines) of the hippocampus. Five groups of adult Sprague-Dawley rats were formed: three young in proestrus (Pro), two of them ovariectomized (Ovx) with the vehicle alone (Ovx+V) or supplemented with 17-β-estradiol (Ovx+E); and two senile groups (Sen+V and Sen+E). The results show that supplementation with 17β-estradiol increases the GSH redox ratio (reduced/oxidized GSH/GSSG), and decreased the levels of lipid peroxidation, both have the same effect in OVX+E and Sen+E. The Ovx and age produce spines pruning and the supplementation with 17β-estradiol returns the spine density in the same proportion. There are a differential distribution of the type of spines on the 3 segments studied, which is affected in a different way depending on the type and analyzed segments. Thus, the total density of the spines in the distal segment is greater than in the medial and proximal, which did not differ between them, and the treatments used do not modify this distribution, with the exception of the proximal segment in the senile animals where supplementation does not retain the same distribution pattern. This study highlights the neuroprotective and antioxidant action of hormonal supplementation on the plasticity of dendritic spines of hippocampal CA1.
Acknowledgements: For the technical support to MVZ Martin García Servín, M. en C. Águilar Vazquez Azucena Rhut and for the financing of CONACyT (173299) and DGAPA-PAPIIT-UNAM (IN202809).

ANTIDIABETIC ACTIVITY OF TIMBE (*Acaciella angustissima*) PODS METHANOLIC EXTRACT ON STREPTOZOTOCIN-INDUCED DIABETIC RATS.

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In Mexico the genus Acacia is distributed where we can find the species known as Timbe (*Acaciella angustissima*) having a high content of bioactive compounds called phenols, which act as chain terminators of free radical reaction, transforming reactive species radicals to more stable products, conferring antioxidant activity that helps protect cells from oxidative stress and improve capillary resistance and inhibit inflammatory processes in diseases like diabetes. This study evaluated the antioxidant activity of extract Timbe sheaths (*Acaciella angustissima*) and the effect on the oral administration of said extract in serum, urine and kidney of diabetic rats induced by streptozotocin.

The antioxidant activity was performed from methanolic extract Timbe by the ABTS and DPPH method described by Van Den Berg et al. (1999) and Fukumoto et al. (2000) respectively, showing that this species has a strong antioxidant activity with 70.34 ± 13.96 values mg eq. Trolox / g. for ABTS and 349.1 ± 1.03 mg / ml. for DPPH, this high content of phenols is related to the ability of dissociation of bioactive components to polar solvents such as methanol, which explains its high antioxidant activity. Moreover we found that the extract applied at high concentrations (100 mg/kg B.W.) significantly reduced the blood glucose levels, accompanied by an increase in serum insulin concentration and an improvement in renal damage parameters and hypolipidemic effect after treatment with the aforementioned extract. This findings suggest that this specie could exert antidiabetic activity by two mechanisms of action such as increasing the secretion of insulin by the β-pancreatic cells existing or regeneration thereof.

EVALUATION OF THE PROTECTIVE EFFECT OF ETHANOL & RESVERATROL IN A MODEL OF CEREBRAL ISCHEMIA IN RAT

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Stroke is the second most common cause of death worldwide. At present, intravenous administration of recombinant tissue plaminogen activator (t-PA) is the only clinically approved treatment for acute ischemic stroke. However, t-PA treatment has been shown to have secondary risk associated with blood flow recovery (reperfusion). Thus, additional strategies are required to prevent this damage. Resveratrol is a polyphenol with antioxidant properties that has been tested in animal models of ischemic brain damage. Resveratrol induces cerebral protection when it is administered before an ischemic insult, with a single or multiple doses given during ischemia and during reperfusion. However, its protective effect has not been characterized after a unique dose given early after reperfusion. This is an important fact because reactive oxygen species (ROS) are generated in the first 30 minutes of reperfusion. When ROS production exceeds cellular antioxidant capacity, it induces cellular damage. In this study, we investigated the effect of resveratrol in rats subjected to 2 hours of middle cerebral artery occlusion (MCAO) followed by 24 hours of reperfusion. Resveratrol (1 mg/kg; i. v. in 100 µL of 50% ethanol) was given at the beginning of the reperfusión. Male Wistar rats (250-320 g) were randomly assigned into four experimental groups: control, (CT); MCAO and reperfusion, (MCAO/R); MCAO/R + Resveratrol, (MCAO/R+RES); and MCAO/R + vehicle (MCAO/R+VH). The number of fluoro-jade positive cells was determined by using ImageJ software (image.nih.gov) and was normalized with the number of cells stained with Hoechst (100%). It was found that in the ipsilateral hemisphere to lesion, cerebral ischemia induced cellular damage. The density of fluoro-jade positive cells increased to 43% (635.65 cell/mm^2) in the MCAO/R group. Treatment with resveratrol prevented the damage induced by ischemia: MCAO/R+RES group (3.5%, 4.79 cell/mm^2) showed no significant difference with the CT group (1.8%; 1.86 cell/mm^2). We also found that MCAO/R+VH group, in which 50% ethanol was administered, the number of degenerated neurons decline (59.21 cell/mm^2). This result revealed a substantial protective effect from vehicle. In conclusion, we found a decrease of 92% of damaged cells in the ipsilateral side to the lesion in the MCAO/R+RES group and 30.3% in the MCAO/R+VH indicating neuroprotection with resveratrol in 50% ethanol and with ethanol per se administered at the onset of reperfusion. The result indicates that resveratrol administration potentially provide protection in stroke and might be successful in clinical trials.

EFFECT OF (-)-EPICATECHIN ON THE FORMATION OF INSULIN POLYMERS

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Introduction: It has been reported that the blood from diabetic and obese (grade 1) patients have the ability of to oxidize human recombinant insulin (HRI). This ability is due to the existence of oxidative stress (OS) in blood of these patients, which induce the formation of insulin polymers (18 kDa), and a loss in the function of the hormone. **Aim:** In this study, we investigated whether the antioxidant capacity of a flavonoid ((-)-epicatechin), it is able to prevent the polymerization and inactivation of insulin observed under oxidative stress. **Material and methods**: Ten women (20 to 40 years old, morbidly obese (BMI ≥ 40 kg/m2) with insulin resistance) and 10 healthy volunteers were included in the study. Fifteen milliliters of peripheral venous blood were obtained and used as is mentioned. Six milliliters of blood was placed in a tube and mixed with (-)-epicatechin until 6 mM of final concentration. Then dialysis bag containing 30 IU of HRI was introduced and incubated at 37 ° C for 3 hrs. Same volume of blood without (-)-epicatechin was used as control. Plasma was obtained before and after of the incubation with (-)-epicatechin to determine oxidative stress biomarkers (Malondialdehyde (MDA), thiobarbituric acid reactive compounds (TBARs), Nitroblue tetrazolium (NBT) and carbonyl groups). Total sulfhydryl (SH) and GPx activity was performed as markers of antioxidant capacity. Finally, HRI was analyzed by nondenaturing gel electrophoresis after its blood incubation. **Results:** Plasma biomarkers no showed statistical difference between treatments with and without epicatechin. However, insulin incubated in blood with (-)-epicatechin showed a decrease in the formation of insulin polymer, versus insulin incubated in blood without (-)-epicatechin. These data suggest that the capacity of (-)-epicatechin to inhibit the formation of insulin polymers could be relating with a direct interaction between these two molecules. **Conclusions:** (-)-epicatechin decrease the formation of insulin polymers.

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RELATIONSHIP BETWEEN OXIDATIVE STRESS AND PERIODONTAL DISEASE IN OLDER PEOPLE

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Background: Periodontal disease (PD) is a chronic infection of the tooth supporting tissues, characterized by gingival bleeding, periodontal pocket formation and destruction of connective tissue attachment and alveolar bone loss. If left untreated, the disease continues with progressive bone destruction, leading to tooth mobility and subsequent tooth loss. In this sense, it has been pointed out that the periodontal lesions can increase the levels of reactive oxygen species (ROS) as a result of chronic inflammatory responses; however this is not conclusive.

Objective: To determine the relationship between oxidative stress and periodontal disease in older adults.

Materials and Methods: A cross-sectional and comparative study was carried out in a sample of 23 older adult subjects, (i) 11 periodontal disease subjects (PDS) and (ii) 12 healthy subjects (HS). After the clinical measurements, saliva samples were collected. Superoxide dismutase (SOD), thiobarbituric acid reactive substances (TBARS) and total antioxidant capacity (TAC) were spectrophotometrically assayed in saliva.

Results: We observed TBARS levels higher in PDS than HS (0.12±0.06 µmol/L versus 0.10±0.04 µmol/L, p=0.06). Likewise, it was observed SOD enzymatic activity significantly higher in PDS than HS (1.577±0.65 U/L versus 1.170±0.25 U/L, $p< 0.01$). On the other hand, TAC activity showed not significant difference between both groups ($p>0.05$).

Conclusions: Our findings suggest that older subjects with periodontal disease present an increased of TBARS linked to efficient response of SOD enzymatic activity.

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CATALASE T FROM *Debaryomyces hansenii* CLONED INTO *Saccharomyces cerevisiae* SCAVENGES MORE HYDROGEN PEROXIDE THAN THE NATIVE CATALASES AND IMPROVES THE BIOMASS REACHED IN RICH MEDIA CULTURES.

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Debaryomyces hansenii have two catalase genes. Catalases from *D. hansenii* have more catalytic activity than those from *S. cerevisiae*. Catalase drives the decomposition of hydrogen peroxide to water and oxygen. By previous experiments made on this laboratory, we known that catalase T expression occurs on rich media (YPD) only in late stationary phase.

With this in mind, we had constructed a plasmid with the catalase T gene including 1000 bp upstream to conserve the 5'UTR and the promoter region and 400 bp downstream to conserve the 3'UTR. The gene was amplified by PCR using a high fidelity polymerase and using primers containing restriction sites for further cloning into the vector pRS316. We have cloned catalase T gene into a double mutant of *S. cerevisiae* that lacks both catalases.

We confirmed that the catalase activity was restored to the mutant by spectrophotometry activity assay and by separating them with PAGE and then by making them react in the native gel. The growing cultures shows that this gene improves the maximum biomass reached by *S. cerevisiae* when it is grown on rich medium (YPD), this does not occurred when the transformed cells are grown on YPD+NaCl 0.6 M. *D. hansenii* is a salt loving yeast and is highly adapted to NaCl and for this reason we tested the performance of this enzyme on this condition, it is known that dehydration causes malfunction of many enzymes in organism that are not well adapted as it is the case of *S. cerevisiae*.

This improved growth on YPD may be due to an enhanced catalase activity and by lower levels of hydrogen peroxide, that are known to produce reactive oxygen species (ROS) that damage a wide variety of molecules into the cell. Furthermore by a bioinformatics analysis of the putative transcription factors that may be regulating this promoter in *S. cerevisiae,* we found that it may be tightly regulated by the carbon source and this correlates good with the fact that we only found catalase when the carbon source is non fermentable and leads to the idea that the ROS comes from the respiration.

YPD medium contains glucose, which is a fermentable carbon source. The ethanol, which is the product of the fermentation (and non fermentable), is redirected to the TCA cycle with the aid of the alcohol dehydrogenase II, thus making active use of the mitochondria for the respiration process.

ANTIOXIDANT EFFECT OF PHYCOBILIPROTEINS EXTRACTED FROM *Spirulina maxima* ON PLASMID DNA FROM *Escherichia coli*

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It is well known that cyanobacteria has been used as antioxidants in some toxicological models, as well as fluorescent markers and as natural pigments in food industry, but little is known about its role in oxidative stress protection. Although it has been demonstrated that phycocyanin from *Spirulina maxima* and phycoerythrin from *Pseudanabaena tenuis* can protect from damage caused to liver by exposure to Hg, the aim of this study is to assess the possible protective effect of phycobiliproteins (PBP's) on plasmid DNA obtained from *Escherichia coli* and exposed to a reactive oxygen species (ROS) generating system. A raw extract of PBP's was obtained by two methods. The first involved the use of streptomycin as a precipitating agent, while the second one was performed using ammonium sulfate to precipitate proteins; the yield was higher when using the inorganic salt. PBP's was characterized by spectrophotometry. Then a series of tubes, each containing 20 µL of plasmid DNA, were added with different amounts of a ROS generating system and PBPs, after an incubation period of 24 h at room temperature, absorption spectra were obtained, and an electrophoretic characterization was performed. Controls without PBPs and PBPs-ROS were also incubated at the same time. The results showed shifts in the DNA absorption spectra for samples exposed to ROS system, tending to restore in the presence of PBPs. The extent of recovery depended on the amount of PBPs added to the incubation media. Similar results were obtained in the electrophoretic analysis. These results suggest that PBPs exert a protective effect on plasmid DNA subjected to oxidative stress.

We are looking forward to elucidate if PBP's can individually exhibit some protective effect against oxidative damage to plasmid DNA.

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THEORETICAL STUDY OF MOLECULAR EVOLUTION OF A DUPLICATE THIOREDOXIN PEROXIDASE IN *SACCHAROMYCES CEREVISIAE*

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In classical evolutionary genetic studies, Ohno model (1970) explains functional innovation and increased phenotypic complexity by gene and genome duplications. This hypothesis/model identifies three fundamental predictions about the genetics origins, molecular evolution and functional destination of paralogous genes. These are: first, that descendant copies acquires new functions after a duplication event; second, that neofunctionalization occurs after a period initial relaxation of the pressure of natural selection; and third, that both copies undergo asymmetric rates of molecular evolution, were the copy that maintains the original function evolves under stronger purifying selection, and the redundant copy evolving at an accelerated rate under positive selection towards a new function.

TSA1 and *TSA2* genes of *Saccharomyces cerevisiae* are of interest for their antoxidant activity reported under oxidative stress. On the basis of data generated by functional genetics analysis we performed a bioinformatic study to: 1) understand its evolutionary history; and 2) test the Ohno model. There is experimental evidence of non-full redundancy between the phenotypic effects of null mutants for either paralogs (Ascencio, unpublished), which is indicative of functional divergence of proteins encoded by the two genes.

For this research we implemented analysis of natural selection at post-duplication branches in the phylogeny of these genes, and analysis by sites in the multiple alignment. The index of natural selection in both cases was estimated as the ratio between non-synonymous nucleotide substitutions relative to synonymous substitutions (dN/dS). In this poster will present evolutionary and functional inferences obtained from our bioinformatic analysis. We also present a theoretical interpretation of molecular regions that evolved under one of the three regimes of molecular evolution (negative selection, positive selection and neutral evolution) in relation to its functional importance in the structure of the thioredoxin peroxidase protein encoded by the *TSA1* gene.

CARBONILATION OF HSP70 PROTEIN FROM *Trypanosoma cruzi* DURING IT *in vitro* CULTURE.

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The carbonylation of proteins is a natural phenomenon generated by reactive oxygen species. It is an irreversible process and results from stressful events in the cell, such as fasting and cellular aging. Some important proteins are the main targets of carbonylation, one of them is the heat shock protein 70 (HSP70) family. *Trypanosoma cruzi* is the protozoan that causes Chagas disease. Little is known about the carbonylation of proteins in this parasite. Therefore the aim of this work was to determine whether HSP70 carbonylation increases during cellular culture *in vitro*. *T. cruzi* Silvio strain ($5x10^6$ cells/ml) was cultured in medium LIT with 10% fetal bovine serum and 25 μg/ml of hemin (Sigma, H5533). Aliquots of the cultures were taken at day 4 (log phase of growth) and 10 (stationary phase of growth). Total proteins were extracted by lysis buffer (SDS 6%, HEPES 10 mM) and 10 μg of them were separated by 12% SDS-PAGE, transferred to nitrocellulose and blotted to determine carbonylation using a commercial kit (Oxiblot Kit, Chemicon,). Expression of HSP70 was evaluated using a specific antibody (Biosciences, 610607). This protein was more expressed in *T. cruzi* than in other eukaryotic cells. It was also, showed higher amount of carbonylated HSP70 in *T. cruzi* stationary phase than in log phase of culture. Additionally, there was a wide range of proteins carbonylated in *T. cruzi*, which number was higher than observed in other cells as *Leishmania mexicana*, *Escherichia coli* (strain MC4507) and a mouse lymphoma cell line (Jurkat). It will be interesting also to characterize these other carbonylated proteins.

The authors acknowledge the financial support of DGAPA-UNAM grant IN206512.

THE EFFECT OF RAW PHICOBILIPROTEIN EXTRACT OF *Spirulina maxima* IN THE SURVIVAL OF *Saccharomyces cerevisiae* PREVIOUSLY SUBMITTED TO OXIDATIVE STRESS.

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Oxidative stress is caused by an imbalance between the production of species reactive to oxygen (ROS) and the capacity of a biological system to either rapidly detox the precursor reagents, or to repair the resulting damage. All ROS are associated to several illnesses and health disorders like cancer, aterosclerosis, Alzheimer´s disease, diabetes and age related deterioration amongst others. The reason for that relies in the fact that ROS react with biomolecules such as lipids, proteins and DNA, and neutralise their function.

Previous reports have proven that the protein extract of some species of *Spirulina*(i.e. *S. platensis*) acts as a free radical scavenger. The aim of this project was to observe the antioxidant effects of a raw phycobiliprotein extract of *Spirulina maxima* in the survival of *Saccharomyces cerevisiae* previously submitted to oxidative stress through the formation of –OH radicals as result of the Fenton reaction.

S. cerevisiae strain was grown in agar Sabouraud, and then transfired to an oxidative stress treatments in presence (I) and absence (II) of raw phycobiliprotein extract of *Spirulina maxima.* The results of eight replications show that the survival of *S. cerevisiae* in dilutions of 10^{-2} and 10^{-3}, go from 7 X $10^{5}$to zero in the presence or absence of the mentioned raw extract. Therefore, applying a non-parametric statistical test, a meaningful difference between treatments I and II was obtained. All this led us to conclude that the presence of phycobiliproteins enhances the growth of *Saccharomyces cerevisiae* in an oxidative media.

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IRON-SULFUR PROTEIN BIOGENESIS AND OXIDATIVE STRESS IN *Saccharomyces cerevisiae*

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Biogenesis of Fe-S proteins is conserved among organisms. In yeast is carried out by proteins encoded in a set of genes called ISC (iron-sulfur-cluster). The proteins that contain Fe-S clusters are involved in essential oxide-reduction cellular processes; however these are susceptible to reactive oxygen species (ROS). The aim of this study was to evaluate in *S. cerevisiae* the involvement of proteins belonging to ISC system with the oxidative stress tolerance. Susceptibility to H_2O_2 and menadione (ROS generators) was tested in the *ISC* yeast mutants (*ssq1*Δ, *isu1*Δ, *isa1*Δ, and *iba57*Δ). Results showed that under H_2O_2 (6.25 mM), the growth rate was diminished in the *ssq1*Δ, *isa1*Δ, and *iba57*Δ mutants comparing to that wild type; whereas that it was abolished totally in the mutants at H_2O_2 12.5 mM, except for the *isu1*Δ mutant. Similar behavior was showed with menadione at 80 µM. Also was determined the inhibitory concentration 50 (IC_{50}) for H_2O_2 (mM): *ssq1*Δ (7.1±0.96), *isu1*Δ (19.1±0.81), *isa1*Δ (10.1±0.18), and *iba57*Δ (8.8±0.27), while for the wild type (20.1±0.91 mM). IC_{50} for menadione were (µM): *ssq1*Δ (99.6±0.12), *isu1*Δ (148.7±0.21), *isa1*Δ (88.1±0.11), *iba57*Δ (76±0.27), and for WT (158.1±0.17). In addition, the ROS generation in the yeast strain was determined by flow cytometry using fluorescent ROS-indicators as probes. Findings indicate that the *ssq1*Δ, *isa1*Δ, and *iba57*Δ mutants showed significant increase ROS determined as mean fluorescence intensity, respect to WT or *isu1*Δ strains. These results indicate that the tolerance of *S. cerevisiae* to different ROS generators was clearly diminished in the *ssq1*Δ, *isa1*Δ, and *iba57*Δ mutants, and that these mutations provoked a synergistically increased ROS generation in the yeast.

Candida glabrata increases its fluconazole resistance when L-proline biosynthesis is diverted to glutathione biosynthesis

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Candida spp. cause serious infections in immunocompromised patients. Their prevalence has increased in the last decade. The common treatment for fungal infections is based in azolic agents. *Candida glabrata* has innate resistance to fluconazole, and this is dependant of the Pleiotropic Drugs Resistance (PDR) system, which is regulated by the transcriptional controller Pdr1 and two main transporters, Cdr1 and Cdr2. When fluconazole is present, Pdr1 conformation changes and promotes transcription of the efflux fluconazole pumps. Fluconazole inhibits the lanosterol 14 α-demethylase (encoded by *ERG11*), a key enzyme in the biosynthesis of ergosterol. As a consequence of accumulation of toxic 14-methyl sterols, cell membranes destabilize. Moreover, fluconazole induces oxidative and nitrosative stress. Glutathione (GSH) is a pivotal molecule to detoxify xenobiotics such as fluconazole and to remove reactive species. GSH is synthesized by sequential action of Gsh1 and Gsh2, encoded by *GSH1* and *GSH2*, respectively. Therefore, we wanted to evaluate the fluconazole resistance of a *gsh1*Δ null mutant. Suprisingly we found that a suppressor mutation in *PRO2* (which encodes a key enzyme of the proline biosynthesis pathway) is necessary for the construction of a *gsh*Δ*1* null mutant, thus generating a *gsh1*Δ*pro2-4* mutant. In this work, we evaluated the fluconazole resistance of a *gsh1*Δ*pro2-4* mutant. We found *gsh1*Δ*pro2-4* mutant to be more resistant to fluconazole than the wild type strain. In order to know if the resistance depends of the PDR system, we deleted the *PDR1*, *CDR1* and *CDR2* genes in the *gsh1*Δ*pro2-4* mutant background. We found that the double mutants are more resistant to fluconazole than single mutants. When we reconstituted the *GSH1* gene to the *gsh1*Δ*pro2-4* mutant in its chromosomic context, we also found it to be more fluconazole resistant. However, a *pro2-4* mutant maintains wild type fluconazole resistance levels. We suggest a link between the glutathione and proline biosynthesis pathways. This connection could help *C. glabrata* to export the fluconazole through unknown transporters, not Cdr1 or Cdr2.

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REGULATION OF *Candida glabrata EPA2* EXPRESSION BY OXIDATIVE STRESS

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Candida glabrata has emerged as an important opportunistic pathogen in both mucosal and bloodstream infections. Some virulence traits of this yeast have been identified in the last three decades. One of these aspects is the high resistance of *C. glabrata* to oxidative stress which confers the ability to proliferate inside macrophages. In addition, *C. glabrata* is able to adhere avidly to epithelial cells *in vitro*, an interaction that depends mainly on Epa1. *EPA1* gene is part of a family of epithelial adhesins, and most of these genes are localized in subtelomeric loci where they are subject to chromatin-based transcriptional silencing. *EPA1* forms part of a gene cluster near to the telomere that contains *EPA1*, *EPA2* and *EPA3*. So far, it has been shown that *EPA1* is the only gene that is expressed in vitro, however we found that *EPA2* gene is induced in presence of oxidative stress. The induction by oxidative stress of these adhesins is specific for *EPA2*, because *EPA3, EPA4* and *EPA5* are not induced under these conditions. These data suggest a relation between the silencing-regulation of adhesins and the response to oxidative stress. We found that the absence of subtelomeric silencing increased basal expression of *EPA2* and its induction was higher in presence of oxidative stress. Also, we found that the expression of *EPA2* required at least three oxidative stress responsive transcription factors: Yap1, Skn7 and Msn2 or Msn4. However, in absence of subtelomeric silencing, only Yap1 and Skn7 were required.
To understand the role of chromatin silencing in the regulation of *C. glabrata* adherence and virulence, we asked if *EPA2* is normally transcribed during the course of an infection. To evaluate *EPA2* expression in vitro and in vivo, we will use a recombinational IVET approach. The use of this system will lead us to understand how environmental signals produce an adaptation of transcriptional profile during stress conditions.

INHIBITION OF LOCAL SILENCING INCREASES THE RESISTANCE OF *Candida glabrata* TO FLUCONAZOLE AND OXIDATIVE STRESS

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In *Candida glabrata*, the sirtuins Sir2 and Hst1 control the expression of a wide number of genes including adhesins required for host colonization and niacin transporters needed for growth. Given that these sirtuins can be inactivated during infection, we asked if their inhibition could modify the response of *C. glabrata* to other stressful conditions. Here, we found that deletion of *HST1* increased resistance of *C. glabrata* to fluconazole and hydrogen peroxide. The transcription factor Pdr1 and the ABC transporter Cdr1 mediated the fluconazole resistance phenotype of the *hst1*Δ cells, whereas the transcriptional activator Msn4 and the catalase Cta1 were necessary to provide oxidative stress resistance. Also, we show that the transcription factor Sum1 interacts with Hst1, indicating that Sum1 participates in the regulation of these genes. Interestingly, even when *C. glabrata* and *Saccharomyces cerevisiae* are closely related phylogenetically, deletion of *HST1* led to resistance to fluconazole and hydrogen peroxide only in *C. glabrata* but not in *S. cerevisiae*, indicating a different transcriptional control by two similar sirtuins. Our findings suggest that Hst1 acts as a regulator of stress resistance associated-genes and we propose that this epigenetic regulation might be important for stochastic generation of resistant cells within a population during infection.

THE SINGLE CATALASE OF *Candida glabrata* PROTECTS *Saccharomyces cerevisiae* FROM HYDROGEN PEROXIDE

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It has been shown that the opportunistic fungal pathogen *Candida glabrata* can replicate inside phagosomes. The phagocytes are the first line of defence against pathogens. Phagocytes attack engulfed pathogens with Reactive Oxygen Species (ROS), such as hydrogen peroxide (H_2O_2) to cause oxidative damage in biomolecules. However, pathogens like *C. glabrata* have adapted the conserved Oxidative Stress Response (OSR) to counteract these effects. The OSR maintains redox balance and controls the oxidative damage of self-generated ROS by metabolism, as well as external ROS.

One important component of the OSR is the ubiquitous enzyme catalase. Catalases are tetrameric heme-containing enzymes that decompose H_2O_2 into water and oxygen. We are interested in characterisation of the single catalase *Cg*Cta1 of *C. glabrata*, encoded by *CgCTA1*. Phylogenetically, *C. glabrata* is closely related to the non-pathogenic yeast *Saccharomyces cerevisiae*, but the latter has two catalases: a cytoplasmic catalase *Sc*Ctt1, encoded by *ScCTT1*; and a peroxisomal catalase *Sc*Cta1 encoded by *ScCTA1*. *Cg*Cta1 is homologous to *Sc*Cta1, but their promoter regions do not share recognizable elements.

Our group previously established that *Cg*Cta1 is absolutely required for the high resistance of *C. glabrata* to H_2O_2. When compared to that of *C. glabrata*, *S. cerevisiae* resistance to H_2O_2 of is lower regardless of its two catalases. *Candida albicans*, another important fungal pathogen with one catalase (*Ca*Cat1) also has lower resistance to H_2O_2.

We wanted to know if the higher resistance conferred by *Cg*Cta1 is due to properties inherent to the enzyme. Preliminary data with episomal expression of *Cg*Cta1 indicate that *S. cerevisiae* improves resistance to H_2O_2. We have now replaced the peroxisomal *Sc*Cta1 with *Cg*Cta1 in the chromosome. We confirmed higher levels of H_2O_2 resistance granted by *Cg*Cta1 to different strains *S. cerevisiae*, wild type or acatalasemic. This indicates that the scavenging capacity of *Cg*Cta1 is superior to that of *Sc*Cta1, regardless of its genetic location.

Also, we subcloned *Cg*Cta1, *Sc*Cta1 and *Ca*Cat1 for expression in *Escherichia coli* to evaluate its activity. This allows *in vitro* verification of the different biochemical activity of the enzymes.

Additionally, we want to define the essential elements for transcription in the unusually long 4.5Kbp promoter region of *Cg*Cta1. For this purpose, we transcriptionally fused portions of different sizes from the upstream region of *Cg*Cta1 to the green fluorescent protein as a reporter to determine their promoter capacity.

With the sum of these results, we are able to establish the distinctiveness of the catalase of *C. glabrata* in its regulation and activity, when compared with other similar catalases from related fungi.

VOLATILE ORGANIC COMPUNDS (VOCs) IN *Candida Glabrata.*

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Candida glabrata is an opportunistic fungal pathogen that is a commensal in human gastrointestinal and genitourinary tracts and might lead to severe invasive infections. *Candida* species are responsible for about 8% of all hospital-acquired bloodstream infections, and among these species, the two most frequently isolated are *Candida albicans* and *C. glabrata.* Phylogenetically, *C. glabrata* is distinct from *C. albicans* but is closely related to *Saccharomyces cerevisiae*. In the other hand, Pathogens like C. glabrata adapted the pathways that regulates the oxidative stress response (OSR) produced by phagocytic cells.

Volatile organic compounds, VOCs, are secreted by many species from microorganisms to humans. Microorganisms signal information through VOCs. It has been shown that *S. cerevisiae* colonies grown on complex agar form a turbid path in the vicinity of another colony and this reaction is induced by ammonia, a volatile compound. In *C. albicans* it is known that elevated concentration of $CO_{2(g)}$ in the environment induces hyphae formation, a morphological switch. In the laboratory, we have evidence that *C. glabrata* secretes 2-phenyl ethanol in the medium, and this molecule is important for the oxidative stress response (OSR). We are interested to determine whether VOCs are important for OSR in *C. glabrata*. To identify VOCs produced by *C. glabrata* we used a headspace solid phase micro extraction (HS-SPME) coupled to gas chromatography with mass spectrometric detection (GC–MS). This technique has been extensively used in the extraction and pre-concentration steps for analytical procedures applied to a wide range of matrixes compounds. Preliminary results show that *C.glabrata* in stationary phase at 42º secretes two VOCs, 2-phenyl ethanol and acetic acid,. Previously these molecules have been implicated in OSR and in Chronological life span.

QUANTIFICATION OF PHENOLIC COMPOUNDS AND ASSESSMENT OF ANTIOXIDANT CAPACITY IN COLORIN (*Erythrina americana* miller)

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The phytochemicals, such as phenolic compounds, are considered secondary metabolites of plants with a chemical structure suitable to exert an antioxidant action as free radical captors. The colorin flower (*Erythrinaamericana* Miller) is native to Mexico. Traditionally they are prized for their flavor and are used as food and for medicinal purposes dates back to prehispanic times.

This study identified specific phenolic compounds and total flavonoids, condensed tannins and antioxidant capacity was evaluated by $ABTS^+$ and DPPH methods in colorin flower. Flower collection was conducted in public communities in Queretaro, after cleaning and separation of calyx and pistil samples, they were lyophilized. Methanolic extracts were used and Catechin was considered a s reference for condensed tannins. Samples were read with Multiskan Ascent ® team, resulting 25.24 ± 0.06 (+)catechin /g of colorin flower. For flavonoids use routine as reference was obtained 26.65 ± 0.13 mg routine / go colorin flower. In assessing antioxidant capacity was used Trolox as antioxidant reference. For the $ABTS^+$ method yielded a % inhibition of 83.71 ± 1.25 and 813.87 ± 4.78 mMTrolox equivalents / g of colorin flower. To DPPH method the results were expressed as antiradical activity (ARA%) 67.08 ± 0.05 and 103.78 ± 1.24 mMTrolox equivalents /g of colorin flower. With this, the phenolic compounds involved as natural food antioxidant, so obtaining and preparing food with a high content of these compounds is a reduction in the use of antioxidant additives, while healthier foods obtained.

ANTIOXIDANTS ENZYMES PRESENT IN *AZADIRACHTA INDICA* LEAVES

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Although oxidation reactions are crucial for life, they can also be damaging; plants and animals maintain complex systems of multiple types of antioxidants, such as glutathione, flavonoids, phenolic compounds, vitamin A, and vitamin E as well as enzymes such as catalase, polyphenoloxidase and various peroxidases. Insufficient levels of antioxidants, or inhibition of the antioxidant enzymes, cause oxidative stress and may damage or kill cells.
This work focuses on the identification and characterization of the antioxidant enzyme content existing in leaf extracts of the neem tree. As part of the expectation to explain and spread some of the properties of this tree scientifically recognized, leads us to study it in depth from a biochemical viewpoint.
The presence of total phenolic compounds in neem leaves (0.08%), and flavonoids (0.04%), and the presence of antioxidative enzymes catalase, peroxidase, poliphenoloxidase, indicate potential health benefits. They may reduce the risk of cardiovascular disease and cancer, characteristics that have been proved in recent years.
For the extraction of enzymes of the neem tree leaves extractants were used since different ionic strength (distilled H_2O, NaCl 1%, 5% NaCl), which yielded different extraction efficiencies.
The activity assay showed solutions the effect of NaCl concentration on each of the extractants. I was found the moderate increases in ionic strength promotes enzymes removal without interfering with their activity. The results showed that polyphenoloxidase were more active when it was extracted with a NaCl 5% solution (1010 UPFO). By other hand the peroxidase were better extracted with NaCl 5% (72 UPER). Catalase demonstrated high activity with distilled H_2O (298 UCAT).
The presence of high activity of antioxidative enzymes suggest that the azadirachta indica leaves contribute by far to their antioxidants properties.

Regresar

ASSESSMENT OF THE EFFECT OF DETERGENTS IN THE DUCKWEED (*Lemna gibba* L.)

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The duckweed is a aquatic macrophyte that lives in the lacustrine zone of Xochimilco. Because in the last 40 years the deterioration process in the zones where they live has accelerated, the objective of this study was to determine the effects of detergents (LAS, Triton X and 3 commercial formulations (biological and detergents with enzymes) in 4 biomarkers: chlorophyll concentration, carotenes production, biomass production and lipoperoxidación. Bioassays with 96 hours duration were carried out to determine the EC_{50} and in sublethal tests (CL_{10}), during 10 days, the effect in the biomarkers were evaluated. Statistically significant differences were found between the control group and the aquatic macrophytes exposed to detergents. High toxicity was observed in the test with triton x and detergents containing enzymes, in these experiments a decrement in the chlorophyll levels and an increase in the lipoperoxidation degree were observed. No differences were found between duckweed exposed to biological detergent and those exposed to LAS. In Mexico only 14% of residual waters receive some sort of treatment and 86% directly reach the aquatic ecosystems. Due to this fact, it is important to know the effect of the detergents with the purpose of proposing adequate measures to reduce the risk posed by the presence of detergents in aquatic ecosystems.

EVALUATION OF THE EFFECT OF METALS Cd, Cu, Cr, Ni AND Pb IN THE GERMINATION OF LENTIL SEEDS *Lens esculenta* L.

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Because the seeds are important in the food web, the aim of this study was to evaluate the efficiency of germination, growth inhibition of the radicle and lipoperoxidation degree of lentil seeds (*Lens esculenta*) exposed to the metals Cd, Cr, Cu, Ni and Pb. Bioassays were carried out where 5 concentrations (0.01, 0.1, 1.0, 5 and 10 mg/L) of each metal plus a control without toxic in quintuplicate were tested. After 5 days of incubation, we evaluated the percentage of seed germination, was measured radicle length (cm) and assessed the concentration of MDA (malondialdehide). Significant difference was observed between the organisms exposed to metals and controls. The toxicity of metals based on the LC_{50} calculated was: Cd> Ni> Cr> Cu> Pb. The germination percentage inhibition was 24%, 36%, 28%, 20% and 10% for the tests with metals Cd, Cu, Cr, Ni and Pb respectively. In tests with metals Cd, Cu and Cr the degree of lipid peroxidation was high. The results obtained in this study show that the more toxic metals were Cd and Cu. The tests with lentil seeds could be an alternative method for assessing the toxicity of water and sediment from water systems.

ANTIOXIDANT ACTIVITY OF PHYCOCYANIN

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Phycocyanin (C-PC) is a blue biliprotein water soluble found in organelles called phycobilosomes in cyanobacteria (Samsonoff and MacColl, 2001). C-PC is involved in the light-harvesting and energy-transfer processes in the photosynthetic system. Based on the fact that C-PC is non-toxic and non-carcinogenic, it has gained importance in food industry such as alcoholic drinks, desserts, sweet cake decoration, milk shakes, and in cosmetics, thus gaining commercial importance especially in pharmaceutical application (Laksi *et al*., 2008; Subhashini *et al*., 2003). In fact, it is considerate a nutraceutical compound due to its therapeutic value as hepatoprotective, anticoagulant, neuroprotective, renoprotective, anti-inflammatory, anti-carcinogenic, anti-diabetic and others illnesses. These properties have been associated with the antioxidant activity of pure compound. In this way, the aim of this study was to evaluate the *in vitro* antioxidant activity of lyophilized C-PC for several reactive oxygen species (ROS). **Methods**: Scavenging activity was defined as the capacity of C-PC to inhibit the interaction of ROS with specific probes. Xanthine–xanthine oxidase system was used for superoxide anion radical ($O_2^{\cdot-}$) generation and nitroblue tetrazolium was used as target compound. Hydroxyl radical ($OH^{\cdot}$) were generated by Fe^{3+}–EDTA–H_2O_2 reaction system and terephtalic acid was employed as fluorescent indicator. Peroxynitrite anion ($ONOO^-$) scavenging activity was measured by the oxidation of dihydrorhodamine-123. For peroxyl radical ($ROO^{\cdot}$) scavenging activity 2,2-azobis(2-amidinopropane) dihydrochloride and fluorescein were used as radical generator and probe, respectively. The production of singlet oxygen ($^1O_2$) by sodium hypochlorite and H_2O_2 reaction was determined by using a fluorometric method with 1,3-diphenyl-isobenzofuran. Hydrogen peroxide (H_2O_2) scavenging capacity was evaluated by using Amplex red reagent and horseradish peroxidase. Besides we used reference compounds for $O_2^{\cdot-}$, $OH^{\cdot}$, $ONOO^-$, $ROO^{\cdot}$, $^1O_2$, H_2O_2 scavenging: nordihydroguaiaretic acid (NDGA), dimethylthiourea, DL-penicillamine, Trolox, NDGA and pyruvate, respectively and scavenging capacity was expressed as IC_{50} value. **Results:** IC_{50} for C-PC (μg/mL) for $ONOO^-$, $^1O_2$, $ROO^{\cdot}$, $OH^{\cdot}$, $O_2^{\cdot-}$, H_2O_2 were: 5.00, 12.88, 21.00, 28.33, 334.00, 450.00, 757.72, respectively**.** **Conclusion**: C-PC is an effective ROS scavenger, with more efficiency for $ONOO^-$.

Analysis of reactive oxygen species (ROS) in *Debaryomyces hansenii*.

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The accumulation of oxygen in the Earth's atmosphere and ancestral oceans began since the emergence of photosynthetic organisms; environment ceased being reducing to be oxidizing. Reactive oxygen species are the result of the metabolism of organisms that have adapted to the new conditions of life.

Oxidative stress is a term used to denote the stress associated with the sensitivity of the cells, the response to, and the protection against reactive oxygen species that themselves produce (ROS) (Michan *et al.* 2012). In general, cells have different enzymatic and non-enzymatic antioxidant defense mechanisms; however the study and specific detection of free radicals is quite problematic, due to the coexistence of more than one form of reactive oxygen within a cell.

For their study, it's often used techniques with different markers from dihydro derivatives of fluorescent compounds; the oxidation of these compounds allows to differentiate diverse ROS. It has been described that neither the dichlorodihydrofluorescein nor the dihydrorhodamine 123 are able to react directly with the superoxide radical but it can with hydrogen peroxide, in the presence of peroxidases, cytochrome C or Fe^{2+} ions (Gossen et al. 1995; Pawley et al. to the. 1995). The dihydroethidium, unlike the previous two, is able to react with superoxide radicals (Henderson et al. 1993; Bindokas et al. 1996). These markers can be detected using flow cytometry (FC).

In this work the non-conventional yeast *Debaryomyces hansenii* was used as a model. *D. hansenii* is an halophilic and euryhaline yeast, able to tolerate concentrations from 0 up to 4 M of NaCl. It is reported that growing it in high concentrations of NaCl, the intracellular Na^+ is up to 800 mM without being toxic for it (Prista, et al. 1997). On the other hand, in 2011 it was reported that when *D. hansenii* is grown with different carbon sources, a differential activity of enzymes occurs for catalase A and T, while the transcript coding for these proteins is affected by the presence or absence of salt (Segal, et al. 2011).

To complement this study, it was carried out a quantification in real time (qPCR) of coding transcripts for catalases A and T, and additionally an analysis using FC in cultures of *D. hansenii* on different carbon sources, in the presence or absence of NaCl in the growth medium and comparing them with *Saccharomyces cerevisiae* in the same conditions.

COMPUTATIONAL STUDY OF DIHYDROXYBENZENES RELATED WITH THE ENZYMATIC BROWNING

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Enzymatic browning is a mechanism that explains the phenomenon observed in fruits or vegetables that have suffered physical damage and expose their plant tissues to the atmospheric oxygen. This mechanism involved the polyphenol oxidase, an enzyme that oxidizes phenolic compounds contained in the tissue, the oxidation reaction produces quinones, which polymerize in melanin and give the dark color to the tissues. It is common to use food additives to prevent the enzymatic browning that avoids the oxidation of the phenolic compounds.

In this work, we study the capacity of certain molecules, related to enzymatic browning phenomenon, to donate electrons. We present a computational study of dihydroxybenzenes as catechol, hexylresorcinol, hydroquinone, 4-hexylresorcinol and chlorogenic acid.

To study the ability of these compounds to donate charge, as an advance in the exploration of their antioxidant capacity, we presented the analysis of chemical reactivity indices as their ionization energies, electron affinities and electrodonating power defined within the Density Functional Theory (DFT). All these indices are calculated in both, gas phase and in solvent (dimethyl sulfoxide and water). All calculations were performed using DFT with the exchange-correlation functional B3LYP and PBE combined with DZVP, 6-31+G**, 6-31++G** and 6-311++G** basis sets.

RELATIONSHIP OF THE PROTECTIVE EFFECT OF METALLOTHIONEINS OVER THE OXIDANT STRESS CAUSED BY OBESITY TROUGH LIFETIME IN OBESE MICE MSG

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The obesity is a multifactorial etiology syndrome characterized for an increase in the fat tissue that induces an inflammatory process of low grade, which generates oxidative damage. This ailment elevates the risk of acquiring diabetes up 10 times, and it increases when the level of obesity is higher. It is known that the metallothioneins (MT) are likely required for cell repair and regeneration after certain types of stress conditions such as oxidative stress and toxic agents, however the function that MT plays in obesity and aging it is not known.
Objective: This research aims to understand the relationship of the protective effect of metallothioneins over the oxidant stress caused by obesity trough lifetime, by quantification of lipoperoxidation. The model used was neurointoxicated mice with monosodium glutamate (MSG), generating obese animals.
Method: This research was carried out using mice CD-1 neurointoxicated neonatally with MSG, of 4, 8, 12 and 16 months old. Four experimental groups were studied: female control mice (C-F), male control mice (C-M), female mice (MSG-F) and male mice (MSG-M). The presence of metallothionein and the lipid peroxidation in the liver, lungs, heart and kidney was determined.
Results: The levels of lipid peroxidation were similar in the analyzed organs, in which from the eighth month starts to increase the amount of oxidized lipids, being significantly major in the obese males group. The results show that obese mice-MSG, such females and males presented a decreasing value in the metallothionein in the lung, heart and kidney in comparison with their controls over the time. However, in the liver occurred a contrary behavior, the levels of metallothionein increased according to the age, such as in females and in males.
Conclusion: The MSG-F presented a less lipid oxidation in comparison with the MSG-M throught the experiment. Also, the acute phase protein metallothionein just increased in the liver, probably because the damage caused by oxidative stress in obesity through the time induced the MT expression that could activate various metalloenzymes and transcription factors required for cell regeneration and tissue repair in the injured liver.
Acknowledgements: Mtra Rocío González Vieyra for animal supply. René Hernández Bautista is scholarship holder.

TOXIC EFFECT OF ANTI-FLU DRUGS IN *Daphnia magna*

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Anti-flu drugs are mixtures of two or more drugs with different effects: in order to decrease the allergic reaction, respiratory congestion and as analgesic-anti-inflammatory. They are products of free sale and the sustances that more frequently are eliminated aquatic systems. In countries like USA, Canada, Sweden, France have been detected in wastewaters at concentrations of ppb. These xenobiotics can cause deleterious effects on aquatic organisms, because these drugs are designed to have a physiological effect in very low concentrations. The aim of this paper is to evaluate the toxicity and lipoperoxidation degree of 6 products antiflu in *Daphnia magna*. Static bioassays were conducted with duration of 48 hours, where 5 concentrations of the drugs were tested to determine the LC_{50}. The survivors organisms were changed to recipients without xenobiotics to assess their recovery.The LC_{50} obtained ranged from 0.106 to 0.178 mg/L. The most toxic compounds that cause elevated levels of lipid peroxidation were the formulations presented in higher concentrations of anti-allergic and decongestants drugs. The organisms exposed to sublethal concentrations (less than the CL_{10}) did not reproduce and died between the second to fourth day of recovery period.

cAMP regulation in *Neurospora crassa* conidiation

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In *N. crassa*, conidiation is started when an aerated liquid culture is filtered and the resulting mycelial mat is exposed to air. Three morphogenetic transitions take place: hyphae adhesion, aerial hyphae growth and conidia development [1]. Each transition is started by an unstable hyperoxidant state (HO) and results in growth arrest, autophagy, antioxidant response and an insulation process from dioxygen [2,3]. These responses stabilize the system and growth can restart in the differentiated state. We found that *ras-1*^{bd} has increased ROS formation during conidiation resulting in increased aerial mycelium growth and increased submerged conidiation. Different *ras-1* point mutations were generated that affected growth and conidiation. Only three proteins have a predicted RAS association domain: NRC-1, the STE50p orthologue (STE50) and adenylate cyclase (AC). The Δ*ncr-1* was more resistant whereas the Δ*ste50* more sensitive to added H_2O_2. The AC mutant strain *cr-1* affects vegetative growth and aerial hyphae formation. Oxidative stress and RAS-1 determined partially cAMP levels during the first two HOs of the conidiation process. Higher cAMP levels than Wt were observed in *ras-1*^{bd}. In both strains, [cAMP] decreased within minutes at the start of the first two HOs and thereafter, as rapidly, levels recover to initial values. *N. crassa* has a high (PDE_H) and a low affinity (PDE_L) phosphodiesterases. The Δ*pde*$_H$ strain grows slow and does not conidiate; no evident phenotype was reported for Δ*pde*$_L$. We found that PDE_L was mainly responsible for the cAMP decrease during the first HO and that hyphal adhesion was retarded in Δ*pde*$_L$. Both PDE_H and PDE_L were responsible for cAMP decrease during the second HO. H_2O_2 and low Ca^{++} activated PDE_L and inhibited PDE_H. This opposite regulation can explain the cAMP decrease during the HOs of the *N. crassa* conidiation process. [1] Toledo I *et al.* (1986) Aerial growth in Neurospora crassa: characterization of an experimental model system. Exp Mycol. **10**:114-125. [2] Hansberg W; Aguirre J (1990) Hyperoxidant states cause microbial cell differentiation by cell isolation from dioxygen. J Theoret Biol **142**:201-221. [3] Hansberg W *et al.* (2008) Cell differentiation as a response to oxidative stress. In: Stress in Yeasts & Filamentous Fungi (Ed. Avery *et al.*) Elsevier IBSN 978-0-12-374184-4.

SYNTHESIS, CHARACTERIZATION AND TOXICOLOGICAL EVALUATION OF MALTODEXTRIN CAPPED CADMIUM SULFIDE NANOPARTICLES IN HUMAN CELL LINES AND CHICKEN EMBRYOS

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Our aim was to synthesize, characterize and evaluate maltodextrin coated cadmium sulfide (CdS-MD) semiconductor nanoparticles in human cell lines and chicken embryos. The particle size and morphology of the CdS-MD nanoparticles were characterized using TEM. Toxicological evaluation was carried out by using human tumor cell lines. Fertile chicken eggs were used for stereoscopic evaluation of growth defects in CdS-MD nanoparticles exposed embryos. CdS-MD nanoparticles at 5µg/mL induced cell death by apoptosis and necrosis in MDA-MD-231 cells in a dose response manner. The exposure of these cells to 7-9µg/mL of CdS-MD nanoparticles induced ROS production. CdS-MD nanoparticles-treated MDA-MB-231 cells at >3µg/mL increased cell proliferation in a dose response manner at 7 days. Exposures of chicken embryos to 6µg/mL CdS-MD nanoparticles resulted in a dose-dependent increase in anomalies centered on the heart, central nervous system, placodes, neural tube and somites. Our results indicate that CdS-MD nanoparticles induce cell death and alter cell proliferation in human cell lines at concentrations higher than 3µg/mL, and they are embryotoxic at doses of 6µg/mL and higher.

Regresar

The mixture ibuprofen-diclofenac induces oxidative stress on the brain of *Cyprinus carpio*

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Modalidad: Presentación oral (X)

- Temática: Métodos y modelos para el estudio de las especies reactivas

Nonsteroidal anti-inflammatory drugs (NSAIDs) are chemicals with anti-inflammatory, analgesic, and antipyretic effects. The most common members of this group of are acetylsalicylic acid, paracetamol, diclofenac (DCF), ibuprofen (IBP), and naproxen. These pharmaceutical compounds are widely used globally and have been recurrently found in water bodies and is report that trace concentrations of NSAIDs may induce toxic effects on different aquatic organisms; however, its potential ability to induce oxidative stress in species which are economically valuable due to their high consumption by humans, such *Cyprinus carpio*, remains unknown. This study aimed to evaluate potential mixture IBP-DCF induced oxidative stress in brain of *Cyprinus carpio*. The median lethal concentration of DCF and IBP at 96 h (96-h LC_{50}) was determined and used to establish the concentration equivalent to the lowest observed adverse effect level. The following oxidative stress biomarkers were evaluated: lipid peroxidation (LPX), and the activity of the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX). LPX ,SOD and GPX increased significantly ($p<0.05$) while the GPX decreased significantly ($p<0.05$) with respect to control. The mixture ibuprofen-diclofenac induces oxidative stress on *Cyprinus carpio* with the highest incidence of oxidative damage occurring at 12 and 72 h, furthermore, the biomarkers employed in this study are useful in the assessment of the environmental impact of this agent on aquatic species.

MANIPULATION OF XANTHINE OXIDASE-DERIVED REACTIVE SPECIES REDUCES OBESITY-INDUCED INFLAMMATION AND IMPAIRMENT OF GLUCOSE TOLERANCE

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Obesity is associated with elevated xanthine oxidase (XO) levels and allied enhancement of reactive species formation contributory to systemic inflammation. Despite a long standing association between increased XO activity and negative clinical outcomes, recent reports describe a paradigm shift where XO demonstrates beneficial actions by reducing NO_2^- to $^\bullet$NO. While provocative, these observations contradict both reports of improved outcomes in similar models upon XO inhibition and reports revealing anoxia as a requisite for XO-mediated $^\bullet$NO formation. To garner a clearer understanding of conditions necessary for XO-catalyzed $^\bullet$NO production, we identify a vascular microenvironment where NO_2^- reductase activity of XO is operative in the presence of O_2 as well as examine effects of XO inhibition vs. NO_2^- supplementation in a high-fat diet (HFD, 60% fat, 20 wks) model of obesity. Concurrent measurement of O_2 and $^\bullet$NO during reaction of purified XO with xanthine and NO_2^- revealed that $^\bullet$NO generation does not occur until all O_2 is consumed; suggesting O_2-mediated e^- withdrawal at the FAD inhibits NO_2^- reduction. However, sequestration of XO by endothelial cell glycosaminoglycans (GAGs) confers capacity for $^\bullet$NO formation in the presence of O_2 (1-2% or ~13-26 μM O_2) and concomitantly diminishes (40%) XO-derived ROS production. Treatment of HFD mice (final 6 wks) with the XO-specific inhibitor febuxostat (0.5 mg/L, drinking H_2O) reduced fasting blood glucose (223 vs. 181 mg/dL), improved (39%) impaired glucose tolerance, diminished oxidative stress and improved indices related to obesity-mediated right ventricular (RV) dysfunction and the onset of pulmonary arterial hypertension including RV end systolic pressure (RVESP) (41.2 ± 7.3 vs. 27.2 ± 5.2 mmHg), RV contractility index, pulmonary vascular resistance (2.51 ± 0.47 vs. 1.96 ± 0.24 mmHg/mL/min), mean pulmonary artery pressure (25.2 ± 3.7 vs. 18.5 ± 3.0 mmHg) and Tau (diastolic function) (6.8 ± 1.3 vs. 4.5 ± 0.9). Febuxostat significantly reduced RV hypertrophy, pulmonary arteriole smooth muscle (SM) remodeling and pulmonary tissue macrophage infiltration. Treatment of HFD mice with NO_2^- produced similar yet more pronounced beneficial effects than febuxostat in all the parameters listed above. However, combined treatment with NO_2^- + febuxostat completely abolished these protective effects, suggesting the salutary actions of NO_2^- were mediated by XO. Combined, these data demonstrate that under hypoxic/inflammatory conditions XO-catalyzed NO_2^- reduction increases $^\bullet$NO generation, decreases ROS production and serves to diminish indices of obesity-mediated pathology.

DETECTION OF OXIDATIVE DNA DAMAGE IN CHILDREN LIVING NEAR THE FRAILE MINE TAILINGS IN NORTH OF GUERRERO STATE, MEXICO.

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A biomonitoring study was conducted with children of the Fraile suburban community, which is located beside the Fraile mine tailings and near the abandoned Fraile mine, 15 km from Taxco of Alarcon City, north of Guerrero State, México. In this community, people occasionally use leachates as an alternative source of domestic water during the dry (winter/spring) seasons. Furthermore, edible plants like tomatoes, peppers, and corn for human consumption are grown in the nearby soil, and animals also consume plants that grow near the Fraile tailings.Thus, increasing the health risk in the trophic chain (Armienta et al., 2008). Many studies in the area have indicated that water, soil, and plants contain heavy metals such as Pb, As, Cd and other elements like Fe, Mn, Cu (e.g., Talavera-Mendoza et al., 2005; Romero et al., 2008). High heavy metals levels in human urine, vegetal tissues, soil and water clearly indicate high exposure of the general population (Moreno et al 2010). However, the developing child is the most sensitive population group for adverse effects. Heavy metals exert their toxicity directly or indirectly by producing a large quantity of reactive oxygen species (ROS), such as hydroxyl radical and superoxide radical as well as non-radical species like H_2O_2. Of these, hydroxyl radical is the most reactive species; thus, it can chemically attack bases in DNA and give rise to a range of oxidative DNA damage products including 8-hydroxyadenine, 5,6-dihydroxyuracil, 5-hydroxycytosine, and several others. One lesion of this type is the 8-hydroxyl-2'-deoxyguanosine (8-OHdG), which has been extensively studied and proposed as a key biomarker of oxidative DNA damage. Oxidative DNA damage was evaluated in 100 children (48 girls and 52 boys) exposed to heavy metals, with a range of 6 to12 years of exposure. We also had a comparison group consisting of 100 non-exposed children (51 girls and 47 boys) in the same age range, from the City of Chilpancingo, Guerrero, Mexico. The oxidative DNA damage was determined via the detection of 8-OHdG urinary levels by ELISA. Significant differences between the exposed group and the comparison group were observed in urinary levels of 8-OHdG (13.05 ± 0.23 µg/creatinine vs. 4.02 ± 0.24 µg/creatinine). Analysis of variance revealed that age and sex did not have a significant effect on genetic damage. However, there was a positive correlation (MANOVA, $P<0.5$) between time of exposure to heavy metals and oxidative DNA damage. These results might be due to the exposure of children to a heavy metal mix. This study provides with valuable data to estimate children's health risks associated with heavy metals exposure.

TEMATICA: Tópicos emergentes en el campo de las especies reactivas. Modalidad oral.

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SEMICONDUCTOR NANOPARTICLES EXHIBIT CYTOTOXICITY AND MITOCHONDRIAL IMPAIRMENT THROUGH ROS STIMULATION IN A459 CELLS

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Copper indium gallium diselenide (CIGS) and cadmium sulfide (CdS) nanoparticles (NP), are specifically designed to improve the efficiency of photovoltaic solar cells (PV), by increasing the capture of photons from the sunlight and converting them into free electrons, these NP are considered as the most promising materials for PV devices for its low manufacture cost and high efficiency. Due to the growing concern about the risks associated to the use of engineered NP, and the need of information for safe handling and disposal, we assessed the redox ability of CIGS and CdS and the induction of cytotoxicity mediated by reactive oxygen species (ROS) in human alveolar epithelial A549 cells. In order to determine the intrinsic oxidative properties of the NP, we performed the acellular dithiothreitol (DTT) oxidation assay, results showed a greater oxidant activity of CIGS *vs*. CdS, 41.21 and 1.2 pmol DTT*μg^{-1}*min^{-1} respectively. To guarantee the stability of NP dispersion, we suspended NP in 2 mg/mL of BSA solution before the addition to culture media, this led to a reduction in the size and number of agglomerates and the overall hydrodynamic diameter compared with the NP-water dispersions, this was confirmed by Dynamic Light Scattering (DLS) and Scanning Electron Microscopy (SEM). Average NP size and zeta potential were 357 nm with -14.7mV for CIGS and 533 nm with -17.2 mV for CdS in a stable dispersion. Induction of cytotoxicity was evaluated using the crystal violet and MTT reduction assays, CIGS induced more cytotoxicity than CdS in all the concentrations and time points evaluated. The evaluation of intracellular ROS levels using DCFH-DA, showed that CIGS NP induce a significant dose-dependent increase in intracellular ROS after 6h, with a maximum of 1.92-fold versus control after exposure to 25 µg/mL, while CdS NP induced a moderate ROS increase of 1.5-fold, which was not concentration-dependent. With the purpose to determine if CIGS and CdS NP have mitochondria as a potential target, we evaluated the changes of mitochondrial membrane potential (Δψm), using the ratio between tetramethylrhodamine ethyl ester perchlorate (TMRE) and Mitotracker Green fluorescence. After 6h, both CIGS and CdS NP caused a decrease of Δψm in a similar extent (28 and 30% Δψm) indicating depolarization of the mitochondrial inner membrane. Antioxidant pre-treatment with Trolox prevented the loss of Δψm and cytotoxicity induced by both NP, suggesting that the induction of ROS is the main mechanism by which these NP are cytotoxic. CdS NP showed a lower, yet steady, ability to induce ROS compared to CIGS, thus long-term effects should be further investigated considering the possible deposition of Cd, a known toxic metal. Our results support the hypothesis that intrinsic properties of NP are underlying factors that determine cellular toxicity of engineered NP. For semiconductors engineered NP such as CIGS and CdS bandgap energy is the property of electrons transfer from an energy level to another, that in an intracellular environment may favor the formation of oxidizing molecules leading to cellular toxicity, such as organelle impairment and cell death. (Acknowledgment: ICyT-DF 396/10 for partial financial support).

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Modulation of the transcription factor Nrf2 by arsenic in the mouse brain.

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INTRODUCTION. Arsenic (As) contaminates well water in several countries, including Mexico. Toxic effects of this metalloid include neurochemical, memory and learning alterations, which may be related to its differential accumulation in the central nervous system (CNS). The CNS is vulnerable to oxidative stress due to their high oxygen consumption and high lipid composition. Glutathione (GSH) and thioredoxin (Trx) are two CNS antioxidants involved in metabolism of As. It has been documented that these two systems are modulated by exposure to As, mainly due to oxidative stress generated by this metalloid. Nrf2 is a transcription factor that modulates the expression of proteins of GSH and Trx systems. **APPROACH.** In this work we investigate the expression of Nrf2 and γ –glutamyl cysteine synthetase (γ-GCS), thioredoxin reductase 1 (TrxR1) in murine CNS regions exposed to As. **EXPERIMENTAL DESIGN**. CD-1 male mice (28-30g). be treated with 2.5 mg / kg NaAsO2 and 5.0 mg / kg orally $NaAsO_2$. Animals were sacrificed 24 h after As exposure. Immunohistochemistry and immunofluorescence techniques in sagittal cuts were used to compare the expression of γ-GCS, TrxR1 and transcription factor Nrf-2. **RESULTS.** Preliminary results show a differential expression γ-GCS and TrxR1 in CNS regions in basal conditions. We also observed changes in γ-GCS and TrxR1 expression in cerebellum and hippocampus in animals exposed to $NaAsO_2$. In $NaAsO_2$ exposed animals, Nrf2 expression changes were also observed in striatum and hippocampus. **CONCLUSION.** There is a distinctive modulation of γ-GCS, TrxR1 and Nrf2 expression by $NaAsO_2$ exposure in different regions of the mouse CNS.. **BIBLIOGRAPHY**. 1) Rodriguez et al. 2003. Toxicol Lett. 145: 1-18. 2) Thomas D. 2007.Toxicol Appl Pharmacol. 222: 365-373. 3) Limón et al. 2007. Free Radic Biol Med 43: 1335 to 1347. 4) Pena et al. 2010. Environ Res 110: 428 - 434.

ANTIOXIDANT DEFENSES IN *Perna viridis* (BIVALVIA: MITILIDAE) UNDER ANOXIA, ANHIDROBIOSIS Y REOXYGENATION, IN COPPER AND CADMIUM PREEXPOSURE ORGANISMS

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P. viridis can occupy muddy and poorly oxygenated bottoms, or habitats that are periodically exposed at low tide. In this specie, the information about antioxidant defense system is poor, especially when they are exposed to anhydrobiosis or anoxia, and how heavy metal contaminated organisms can be affected. Here, we analyzed some antioxidant responses: catalase (CAT), total thiol (-SH), metallothionein (MT), glutathione-S-tranferase (GST) and thiobarbituric acid reactive substances (TBARS) in digestive glands of green-mussels (60–80 mm) collected from Guayacán, Sucre state (Venezuela). The animals were exposed to 200 µg copper/L y 100 µg cadmium/L during 7 d, and translated to air (24 h) and anoxic water (48 h); then re-submersed for 5 h in sea-water aerated. An increment in MT levels was observed during exposure period to metals; these values were kept under anhydrobiosis and anoxia conditions. Similarly, a significant increase was observed in –SH rich compounds and TBARS during anhydrobiosis, demonstrating an oxidative stress condition both in control as Cu-preexposure organisms. GST activity showed a falling in the Cd-exposure organisms and all experimental conditions. CAT activity was incremented under anoxia, anhydrobiosis and reduces the activity in condition of reoxygenation in control and Cd-preexposure organisms. This study evidenced adjusts and competence of antioxidant responses in digestive gland in *P. viridis* exposed to oxygenation variations, particularly in anhydrobiosis; conditions affected for heavy metals exposure.

Key words: Anoxia, anhydrobiosis, green-mussel, heavy metals, oxygenation.

GENOTOXICITY IN HUMAN PERIPHERAL LYMPHOCYTES EXPOSED *IN VITRO* TO HEAVY METALS ASSOCIATED TO PM_{10} OF THE AIRBORNE OF ECATEPEC ZONE (STATE OF MEXICO)

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Urban air pollution threatens human health in Mexico, especially the industrials zones near of the Mexico City (Villalobos-Pietrini et al., 2011). The township of Ecatepec is to Northeast of the Zone Metropolitan of the Valley of Mexico (ZMVC), it has the fourth place of the townships more industrialized in the country. There are almost 1550 factories, mainly of iron, chemicals manufactures, furniture, textiles and thermoelectric plant, among others. The pollution produced for these industries and the vehicular exhaust emissions is increased by the orography of the ZMVC which makes difficult the dispersion of the pollutants and it cause cycles of sedimentation-re-suspension. In this zone, there are high concentrations of Zn, Pb, and Cr (Hernández, 2002; Mofett et al., 2008). Humans are exposed to heavy metals (HM) by direction inhalation of polluted air, dietary intake and dermal contact. Inhalation of polluted air is the dominant exposure pathway and outdoor air pollution is associated to respiratory illness. HM exert their toxicity direct or indirectly by production a large quantity of reactive oxygen species (ROS), such as superoxide anion (O2), hydrogen peroxide (H2O2) and hydroxyl radicals (OH-), which can interact with proteins, lipids and nucleic acids to induce oxidative damage that produce DNA strand breaks or bases modifications or DNA adduct (Mena et al., 2008). They can be human mutagens and carcinogens, commonly found food, water, soil, and adhering to airborne particulate matter. Oxidative stress is thought to contribute to DNA damage that leads to aging and cancer. The comet assay is a useful biomarker for DNA damage to assess human exposure to genotoxic compounds (Moller, 2005). In the present study, human peripheral blood lymphocytes were exposed *in vitro* to different concentrations of HM of airborne particulate matter of four different areas of Ecatepec zone were examined with an alkaline comet assay. The DNA damage was evaluated with three genotoxicity parameters: the frequency of comets, tail moment, and the comet tail length. The results showed significant increase three genotoxic parameters compared with the control values with a concentration-effect relationship. The HM identified of atmospheric particles were Al, Cd, Co, Cu, Cr, Fe, Mn, Ni, Pb, and V. The results of this study demonstrate that the comet is rapid, suitable and sensitive method to detect *in vitro* heavy metals-induced DNA damage in human peripheral lymphocytes.

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L-CARNITINE ANALOGS MODIFies THE PROLIFERATION AND INDUCE PHAGOCYTOSIS AND RESPIRATORY BURST IN THP-1

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Several data indicate that the deficiency of carnitine is a factor of contribution to the progression of human immunodeficiency virus infection. Since then, the carnitine treatment in these patients could counteract the process of regulating apoptosis of lymphocytes and improve CD4 counts. These results, combined with the safety profile of L-carnitine, emphasize the potential therapeutic value of l-carnitine. Therefore, its analogs or derivatives in patients, sufferS from inflammatory processes. The aim of this work was to study the effects of analogs of L-carnitine on cell proliferation, phagocytosis and respiratory burst in THP-1 cells. THP-1 cells were grown in RPMI-1640 supplemented with 10% FCS, were incubated at 37°C with 5% CO_2 and humidity. To study the effect on cell proliferation, cells were treated with three analogs of L-carnitine (A1, A2 and A3) at concentrations of 0.01, 0.1, 1.10, 100 and 1000 mM; an MTS assay was carried out to evaluate cell proliferation in non treated (7 days), hydrocortisone-treated cells (10 ng / mL, for 48 hours) and LPS co-stimulated cells (10 ug / mL). Moreover, we also evaluated the effect of three analogs on phagocytosis and respiratory burst by flow cytometry. Our results shown that all analogs induced cell proliferation, reaching its maximum effect at 7 days after treatment in normal cells. Same effect on cell proliferation was observed (≈ 20%) in hydrocortisone-pretreated cells and treated with three analogs in a biphasic manner. Furthermore, it was observed that analogs were able to inhibit cell proliferation in LPS-co-stimulated cells, the effect was greater at high concentrations (0.01-1 mM). When we evaluated the effect of analogs on phagocytic capacity, it was observed that there was a high phagocytic ability in the cells treated with carnitine and analogs. It was noted that there were significant differences between carnitine and its analogs, which varied depending on the concentration used. By quantifying the production of free radicals (respiratory burst) we found that there was a significant increase in free radical production in cells treated with carnitine and its analogs. These results indicate that the analogs of L-carnitine have the potential for using as immunomodulators.

CONSEQUENCES OF OXIDATIVE STRESS IN DIABETES FOLLOWED BY EPR

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The prevalence of diabetes has dramatically increased worldwide due to the vast enhancement in the obesity rate. Diabetic nephropathy and cerebrovascular complications are two of the major complications of type 1 and type 2 diabetes and they are currently the leading cause of end-stage renal disease and stroke respectively. Hyperglycemia is the driving force for the development of diabetic complications. It is well known that hyperglycemia increases the production of free radicals resulting in oxidative stress.

Reactive oxygen species (ROS) production in experimental and clinical diabetes have been linked to vasoconstriction, vascular smooth muscle cell growth and migration, endothelial dysfunction, and increased renal sodium reabsorption. But also reactive nitrogen species (RNS) production has been discovered in this pathology, species like Nitric Oxide (NO) is one of the sources of cerebrovascular complications in diabetes.

In the present work, diabetes was induced in rats and later on a quantitative study of the production of NO was made by Electron Paramagnetic Resonance (EPR), EPR is the only analytical approach that enables direct detection of free radicals such as NO, superoxide, and hydroxyl radical.

The EPR signal observed from the NO adduct is significantly greater in the blood of diabetic rats than in control rats, but the signal of NO in brain of diabetic rats is lower than in control rats.

Because of the concentration of NO present in brain of diabetic rats it is concluded that the brain is being protected by other organs, but in turn is being inhibited blood supply to that vital organ, causing cerebrovascular complications in this pathology.