VI Congreso de Especies Reactivas del Oxígeno en Biología y Medicina y VII Taller Internacional de Aspectos Comparativos del Estrés Oxidante en Sistemas Biológicos

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PLENARY SESSIONS

Redox changes in liver diseases

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Reactive oxygen species (ROS) have gained considerable attention in recent years because of their direct involvement in the regulation of multiple physiological and pathological processes. Under normal conditions, ROS have an important role in cell signaling and function as essential mediators of cell homeostasis. However, imbalance between ROS and antioxidant systems induces oxidative stress, which leads to cell and tissue damage. The cellular redox modulation by HGF and its receptor c-Met in the liver has been studied extensively in our team. The generation of liver-specific c-Met knockout mouse has allowed to demonstrate the fundamental importance of HGF/c-Met in the control of cellular redox state by regulating the expression of antioxidant proteins and a parallel inhibition of pro-oxidants systems. Regulation of cellular redox status by HGF/c-Met is a key determinant for liver function and the lost in the balance is strongly related to the initiation and progression in liver diseases, such as fibrosis, steatohepatitis, alcoholic liver diseases and hepatocellular carcinoma. We have found that HGF elicits regulation by increasing antioxidant response and decreasing the activity or expression od pro oxidant systems, such as NADPH oxidase. Our data indicate that HGF/c-Met is a master regulator of the cellular redox status, and the imbalance of the systems leads to cellular dysfunction and liver disease. Conacyt: CB-252942, and Fronteras de la Ciencia 1320.

CELL DIFFERENTIATION: DIOXYGEN AVOIDANCE THEORY

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Key words: Cell differentiation, response to oxidative stress, dioxygen avoidance theory

The talk will emphasize ideas over experimental results. We will present experimental data from others and ours. The objective is to critically analyze the current concept on cell differentiation and its experimental approaches and what we think, based on some experiments and theoretical considerations, could be done to understand cell differentiation. I have divided my talk into three parts 1) a critical review on the current dominant model of cell differentiation and a possible alternative view, 2) cell differentiation as a response to oxidative stress and 3) towards formalization of dioxygen avoidance theory and its possible use to model a cell differentiation system.

In the first part, I will analyze how a program of gene expression became the dominant model of cell differentiation and why, based on experimental and theoretical considerations, this model is untenable. We will, present an alternative model.

In the second part, I will mention some experiments that gave support to our notion of cell differentiation as a response to oxidative stress. We use the formation of asexual spores (conidia) in *Neurospora crassa* as a model system. A hyperoxidant state develops at the start of each morphogenetic transition that leads to conidia development. As a consequence of the hyperoxidant state, cells of the fungus become increasingly insulated from environmental dioxygen.

In the third part of my talk, I will present our theory and, based on it, will give definitions for the critical terms used in microbial cell differentiation. Than, I will analyze what we think are the most important parameters that have to be taken into account to formalize our theory and will present a first mathematical approach. We think that, linking differential equations for the changes in concentration of intracellular dioxygen, reducing power and reactive oxygen species, we could attain a general description of a cell differentiation process. The dynamical system should reach a bifurcation from which a stable branch should lead to a differentiation state by adjusting parameters that limit oxygen permeability. The dynamic system should also describe the return to the growth state from any differentiated (insulated) state.

A MULTI-HOLISTIC APPROACH TO THE UNDERSTANDING OF THE (EPI)GENETICS AND MOLECULAR BASES DETERMINING NUTRITIONAL QUALITY AND BIOLOGICAL ACTIVITY IN FRUITS

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The thorough understanding of the relationships between the factors that determine productivity and quality in crop species requires holistic approaches based on the collection and analysis of data exposing changes in these variables at different organizational levels: molecular, metabolic and phenotypic. Tomato, in addition to its nutritional and economic importance, is an excellent model for the application of systemic approaches to the understanding of the problems of productivity and quality, since it has a wide genetic variability (natural and induced), sequenced genomes of different accessions and related species, along with a battery of high processivity phenotyping techniques set for the species. By using a germplasm collection from along the Argentine Andean valleys and cultivated ex-situ during several growing seasons an extensive set of data about morpho-agronomic and biochemical characters; including transcriptomics, metabolomics and sensory attributes has been obtained. The application multi-modal clustering algorithms that can fuse such diverse data set without normalization reveals unknown associations between the assayed variables and allow selecting eliteaccessions that can be used as input for breeding programs of the species.

REDOX STATE´S ROLE IN THE MECHANISMS AND SIGNALING PATHWAYS OF SENESCENCE INDUCTION

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Cellular senescence is a multifactorial phenomenon of growth arrest and distorted function, which has been recognized as an important feature during tumor suppression mechanisms and a contributor to aging. Various pathways for senescence induction have been proposed; the most studied is replicative senescence due to telomere attrition called replicative senescence (RS) along with premature senescence, that is achieved when cells are exposed to diverse stimuli such as oxidative stress (Stress-Induced Premature Senescence, SIPS).
Recent data support the concept that cellular senescence does not equate to aging, although senescent cells have an impact on organismal health, particularly during old age due to their singular secretion pattern called Senescent Associated Secretory Phenotype (SASP). The SASP comprises a complex mix of factors including cytokines, growth factors, chemokines and matrix metalloproteinases.
Alterations in redox state certainly play a significant role in the mechanisms and signaling pathways of senescence induction and SASP secretion, in relationship to aging deterioration and diseases development. Moreover, variations in glutathione-redox balance and SASP components were also observed between SIPS and RS.
The understanding of the signaling pathways during senescence and the factors involved in SASP induction will expose new targets to intervene age-associated pathology, including neurodegenerative diseases and cancer, by modulation of the senescence program.

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ROLE OF OXYGEN REACTIVE SPECIES AND NOX IN THE DEVELOPMENT OF THE NERVOUS SYSTEM

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Reactive oxygen species (ROS) generated by NADPH-oxidases (NOX) are involved in numerous physiological processes, including several events of the nervous system development. For instance, in neurogenic regions the levels of ROS are increased and the ROS produced by NOX modulate the differentiation of neural precursor cells and neuronal migration. Other studies have also shown that ROS modulate the cytoskeletal dynamics in growth cones. Furthermore, it has been reported that many neurotrophic factor actions are mediated by ROS. We have previously found that programmed death of cerebellar granule neurons (CGN) is dependent on ROS produced by a NOX. Little is known about the mechanisms involved in the action of ROS and NOX in neuronal development.

Regarding programmed neuronal death, we demonstrated that the mitogen-activated protein kinases (MAPK) pathway is activated by ROS in CGN death. We suggest that the MAPKs JNK and p38 are activated by ROS probably by regulating ASK1, a member of the MAPK. It is known that ROS may act on different regulators of ASK1, particularly thioredoxin (Trx), Akt, and thioredoxin-interacting protein (TXNIP). By using a model of apoptotic death of cultured CGN (K5), we proposed that ROS produced by apoptotic conditions induce the dissociation of Trx1 from ASK1 and regulate the activation of Akt. In addition, ROS might regulate the activity of Trx1 (negative regulator of ASK1) through its binding to TXNIP. All these events would guarantee ASK1 activation and thus the downstream activation of the signaling pathways (JNK and p38) involved in the control of the apoptotic machinery of CGN.

We have also explored the mechanisms by which ROS and NOX are regulated during neuronal development and the implications of these molecules in this process. Our results show that during the first 3 days of CGN development in vitro (DIV), the levels of ROS increased, reaching a peak at 2 and 3 DIV. Besides, the mRNA levels of NOX1 and NOX4 reach their highest expression level at 1 DIIV, while for NOX2 is at 3 DIV. Concomitantly with the increment of ROS levels, the expression of microtubule associated proteins MAP2 and TAU increase and when CGN are treated with antioxidants, both the levels of ROS and the levels of MAP2 and TAU are diminished. Furthermore, we found that during the first 3 DIV, NOX2 was expressed in filopodia and growth cones, which correlated with the H2O2 distribution in the cell. Finally, NOX2 KO CGN showed shorter neurites than wild type CGN. Taken together, these results suggest that H_2O_2 produced by a NOX regulate the maturation of CGN. Particularly, ROS could be involved in the axonal development by controlling axonal growth cone dynamics and filopodia formation.

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Key words: NADPH-oxidase; neuronal development, redox signaling

ORAL SESSIONS

MOLECULAR MECHANISMS OF ALPHA MANGOSTIN IN COMBINATION WITH CISPLATIN IN CERVICAL CANCER.

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Introduction. Cervical cancer is a cellular alteration that originates in the epithelium of cervix, is manifested initially through low and high grade precursor intraepithelial lesions, progressing slowly and progressively towards invasive cancer. Cervical cancer is the second leading cause of the adult female population of Mexico, the first line treatment for this neoplasm is chemotherapy with cisplatin (CDDP). However, it has serious side effects. Alpha-mangostin (α-M) is a xanthone derived from the ethanolic extract of *Garcinia mangostana Linn* (GML) pericarp, which has antioxidant and anticancer properties. Previously, in the laboratory we demonstrated that α-M increases the cytotoxicity of CDDP by increasing reactive oxygen species (ROS), apoptosis and changes in the cell cycle in both *in vitro* and *in vivo*. **Objective**. Examinated the effect of antioxidant and apoptotic pathway in tumor of cervical cancer treated with the combination of α-M+CDDP. **Methods**. Expression of antioxidant proteins (superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx)) and expression of apoptotic proteins (Bax, Bcl2 and caspase 3) in tumors of cervical cancer. Actin protein was measured as a load control. Protein was isolated using RIPA solution with protease inhibitor cocktail. Protein concentration was determined by the Bradford method and Western Blot was performed on SDS-PAGE gels, and then transferred into polyvinylidene fluoride (PVDF) membranes. The membrane was then blocked with 5% (w/v) nonfat dry skim milk in TBS-T (20 mM Tris base, 150 mM NaCl, 0.1% (v/v) Tween-20, pH 7.4) for 1 h at room temperature, followed by overnight incubation with primary antibody at 4°C on a shaker and subsequently with secondary antibodies for 1h at 25° C, transfer bands were detected using chemiluminescence reagents, band density was measured using the ImageJ program. Values are expressed as the mean ± standard deviation of at least three independent experiments, analyzed by one-way ANOVA followed by the Student Newman Keuls post-test for multiple comparisons. Differences were considered significant at $p \leq 0.05$. **Results**. We show that the combination of α-M+CDDP increases SOD expression, while decreasing catalase expression, without significant changes in GPx expression in compared with the control group, whereas in the apoptotic pathway, we found an increase in a pro-apoptotic protein. **Conclusion**. Our results indicate that α-M increase CDDP cytotoxicity due to increase ROS production which in turn increase cell death by apoptosis.

EVALUATION OF OXIDATIVE STRESS IN HDL AND LDL LIPOPROTEINS IN NEWBORNS OF WOMEN WITH PREECLAMPSIA

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KEY WORDS: Lipoproteins, oxidative stress, neonates, preeclampsia.

INTRODUCTION. Foetopathies by pre-eclampsia are the set of alterations observed in newborns of women with preeclampsia (PE) and whose etiology is unknown; however, this has been associated with oxidative stress (OS) and placental ischemia. These alterations can cause adverse effects in the growth, development and homeostasis from the fetal stage until the adult life. OBJECTIVE. To evaluate the oxidative stress in HDL and LDL of newborns of women with preeclampsia. MATERIAL AND METHODS. We included 30 newborns of women without PE (control group) and 30 newborns of women with PE. From plasma, ischemia-modified albumin (IMA) was quantified and HDL and LDL were isolated. In these lipoproteins were determined biomarkers of lipid damage: conjugated dienes (CD), malondialdehyde (MDA) and lipohydroperoxides (LHP), and biomarkers of protein damage: reduction of nitroblue tetrazolium (NBT), protein carbonylation (CP) and dityrosines (DT). The antioxidant activity of PON-I was evaluated in HDL. RESULTS. Newborns of women with PE presented an increase of 24.08% of IMA, compared with the control group. In HDL and LDL of the newborns of the problem group, there was an increase in the concentrations of CD (11.7%) (23.3%), LHP (21.2%) (82.4%) and MDA (51.5%) (103.8%); unlike the biomarkers of protein damage, which did not show significant differences. The PON-I activity decreased by 35%, compared to the control group. CONCLUSIONS. Newborn of women with PE had elevated levels of IMA, which indicates the presence of ischemia caused by inadequate placentation. This leads to an increase in reactive oxygen species (ROS), causing oxidative lipid damage in lipoproteins (LPs) from neonates of women with PE, associated with a decrease in PON-I activity, these oxidative modifications compromise vascular endothelial function. Because the LPs in the fetus do not cross the uterus-placental barrier, their synthesis is mainly produced by the fatty acids from the mother, which have been shown to have elevated levels of oxidation. Therefore, in future research, it is imperative to propose the use of therapeutic adjuvants such as the use of antioxidants to reduce OS in the mother-newborn binomial.

EFFECT OF F1 AND F3 SUBFRACTIONS OBTAINED FROM THE CS-AQ TO CONTROL ENDOTHELIAL DYSFUNCTION: *in vitro STUDIES*

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Keywords. Endothelial dysfunction, inflammation, ROS

Introduction. Endothelial dysfunction is a marker of vascular damage, characterized by deteriorated vasodilatation and prothrombotic, proinflammatory and prooxidant profile, the two latter being the main inducers of this pathology. **Objective.** Evaluate *in vitro* the subfractions F1, F2 and F3 obtained from the standardized AQ-Cs to control endothelial dysfunction. **Materials and methods.** HMEC-1 cells were cultured with 0, 8, 40, 200, 1000 and 5000 nM of AGII and two different concentrations of F1, F2 and F3 fractions (0.08 and 10 µg/mL) or 50 µg/mL of losartan or silymarin as controls, for 12 hours at 37 ° C with 5% CO2. At the end of the culture time, the medium was harvested and the IL6 concentration was quantified by ELISA, which allowed discarding the F2 subfraction. Thereafter, cells were cultured under these same conditions but in presence of 4 different combinations of F1 and F3, named C1, C2, C3 and C4 to evaluate their antiinflammatory effect (decrease of IL6 and ICAM-1 and CD62E expression in HMEC-1 cells). The C4 combination resulted as the most efficient. Afterwards the production of nitric oxide was evaluated by the Griess reaction. Finally, to examine the effects of C4 on oxidative stress, intracellular ROS production was examined by DHE. **Results.** F1 and F3 subfractions prevented the production of IL6 in endothelial dysfunction conditions, and the C4 combination was the best to avoid the production of IL6 and the expression of adhesion molecules in HMEC-1 cells. Moreover, C4 avoided the decrease of the availability of nitric oxide and decreased the ROS production. **Conclusion**. C4 is anti-inflammatory, promotes the availability of nitric oxide and decreases ROS production.

NADPH OXIDASE SUBUNITS EXPRESSION CORRELATES WITH MALIGNANCY IN HEPATOCELLULAR CARCINOMA

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KEYWORDS. p22phox, HCC, c-Met. **INTRODUCTION.** Primary liver cancer is one of the most rapidly evolving malignant tumors worldwide. NADPH oxidases are a major source of reactive oxygen species (ROS) production in cells. In non-phagocytic cells the to producing ROS by NADPH oxidases can regulate diverse physiological processes including cell proliferation, differentiation and death. The non-phagocytic homologs constitute the NOX family NADPH oxidases with a total of seven members in human. Activation of most NOX enzymes and generation of ROS require the assembly with numerous regulatory proteins, functioning as multicomponent enzymatic complexes. p22phox is one of the regulatory proteins whose major function is to stabilize the NOX enzymes to which it binds. This is supported by the studies showing that p22phox down-regulation results in decreased activity of several NOX enzymes. Despite being a key modulator for NOX enzymatic activity, the role of p22phox in cancer progression is poorly unknown. **AIM.** Determine the role of c-Met in the regulation of NADPH oxidase in hepatic cancer. **METHODS.** We performed a microarray analysis of 53 patients with different grade of HCC. Patients were classified according to their diagnosis (good and bad). With the relevant genes of the analysis of the microarrays proceeded to measure characteristics of tumorogenicity, by means of genetic engineering manipulated to the subunits of the NADPH oxidase more relevant in this pathology. **RESULTS.** The analysis of the microarrays showed that only the regulatory subunits present a significant change, likewise Nox4 presents a change in the patients with bad diagnosis. A previous analysis of our work group carried out a correlation analysis between p22phox and c-Met, revealing that there is a correlation between both proteins. By means of genetic engineering it is silent to Nox4 and we realized a test of formation of spheroids, where it is seen that the cells where Nox4 is silenced the tumor is not able to form. In **conclusion** we can observe that there is an ample relation in the expression of p22phox and Nox4 with the aggressiveness of the HCC, this can locate p22phox as a promising therapeutic target in liver cancer. **Conacyt:** Fronteras de la Ciencia 1320, CB 252942.

MODULATION OF HUMAN NEONATAL CD8$^+$ T LYMPHOCYTES ACTIVATION AND DIFFERENTIATION BY ROS SIGNALING

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RESUME

Newborn mortality represents a serious health problem in human population worldwide. Better strategies and therapeutic approaches for promoting neonatal immunity are greatly needed. Recent studies in our lab aiming to depict the differences and similarities between human newborn and adult immune cells, showed that CD8+ T lymphocytes from newborns have a very distinct transcriptional and epigenetic profile, making them prone to innate immune responses. The same studies showed that cells from newborns produce more reactive oxygen species (ROS), as compared to adults' cells, but the functional consequences of this feature remain to be elucidated. Following a computational approach, we constructed a mathematical model developed on the GINsim platform, aimed to study the influence of ROS on CD8+ T cell signaling. The model uses logical formalisms and is composed of nodes and arcs, representing different regulatory components, such as signaling proteins and transcriptional factors, of the CD8+ T lymphocytes and its regulatory influences. There is a set of rules and parameters that define the functional level for each node as part of the whole regulatory network. The statics and dynamical analysis of this regulatory network helped us to determine stable states, to make predictions on key signaling cascades influenced by ROS, and to make predictions on the functional outcomes of the CD8+ T lymphocytes from newborns and adults upon activation. Further, on an experimental approach, we measured by flow cytometry the differences between the cytosolic and mitochondrial ROS levels on CD8+ T cells from human newborns and adults. Using non-toxic concentration of antioxidants and ROS inhibitors we assessed the influence of ROS signaling on the activation state of key signaling proteins related to T cell activation. Our results suggest fundamental differences in the ROS signaling and redox status between CD8+ T lymphocytes from newborns and adults, these differences influence the functional outcome of these cells upon activation.

Keywords: newborns, CD8 T lymphocytes, ROS.

ROLE OF REACTIVE OXYGEN SPECIES IN THE REGULATION OF THE AKT-TXNIP SIGNALING UNDER APOPTOTIC CONDITIONS IN CEREBELLAR GRANULE NEURONS

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PALABRAS CLAVE: TXNIP, Akt, neurons

INTRODUCTION: The reactive oxygen species (ROS) play a critical role in neuronal apoptosis; however, the mechanisms are not well understood. Some proteins regulate the redox state of other proteins that are involved in the control of the oxidative levels in the cell, as well as the activation/inactivation of multiple signaling pathways, that is the case of the thioredoxin-interacting protein (TXNIP), whose overexpression renders cells more susceptible to oxidative stress and promotes apoptosis. It has been shown that the expression of TXNIP is regulated by the PI3K/Akt pathway.

OBJECTIVE: TXNIP is a pro-apoptotic protein whose function is important for the regulation of redox signaling and cell apoptosis. Because Akt is critical for cell survival, we explored the role in Akt-TXNIP signaling in ROS-induced neuronal death.

METHODS: Cultured cerebellar granule neurons. Cell viability assays. ROS levels assays. Western Blot

RESULTS: We evaluated the role of ROS in the regulation of Akt activity and the subsequent regulation of the TXNIP expression in a model of apoptotic death of cerebellar granule neurons (CGN). We observed that two apoptotic conditions that generate ROS at short times led to an increase in the expression of TXNIP in a time-dependent manner; antioxidants significantly reduced this expression. Also, caused an increase in TXNIP expression. Moreover, apoptotic conditions induced inactivation of Akt in a time-dependent manner similar to TXNIP expression and treatment led to Akt inactivation. Besides, the pharmacological inhibition of Akt increases TXNIP expression and induces CGN cell death.

CONCLUSIONS: Our results suggest that ROS promote neuronal apoptosis through the Akt-TXNIP signaling pathway, supporting the idea that the PI3K/Akt pathway regulates the TXNIP expression. This study highlights the potential importance of this mechanism in neuronal death.

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In vitro AND *In vivo* ANTIOXIDANT ACTIVITY OF PHENOLIC COMPOUNDS FROM COMMERCIAL AND LANDRACE PINTO BEAN SEEDS

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Key words: Oxidative stress, phenolic compounds, antioxidants.

Oxidative stress is a consequence of an imbalance between the production of free radicals and the antioxidant capacity of an organism. Antioxidants are molecules that deactivate free radicals and inhibits the initiation or propagation of free radicals chain reactions. It has been shown that common bean (Phaseolus vulgaris L.) is a rich source of antioxidant substances, mainly phenolic compounds. These compounds have been shown to prevent chronic-degenerative diseases in which oxidative stress is generated. The objective of this work was to evaluate the *in vivo* antioxidant activity of phenolic compounds of comercial and landrace pinto bean, which have been reported could prevent cancer and diabetes. The methanolic extraction of the seeds of commercial and landrace (Escumite) bean varieties was performed. The phenolic compounds were quantified by the Folin-Ciocalteu method. *In vitro* antioxidant activity was determined by the DPPH-, ABTS + and FRAP techniques. For the *in vivo* tests, the nematode *Caenorhabditis elegans* N2 (WT) was used as a biological model. *C. elegans* were placed in 96 well plates with M9 medium added with 25 and 50 µg mL^{-1} of total phenols, and incubated for 48 hours in the dark at 18 °C. Subsequently, the nematodes were washed with M9 medium and subjected to oxidative stress by the addition of H_2O_2 at concentrations of 5 to 10 mM. Nematodes were counted every hour for 6 h and the lethal concentration 50 (LC_{50}) of H_2O_2 was determined. Additionally, oxidative stress was generated by the addition of glucose (80 mM) in the medium, and the nematodes survival percent was recorded for 25 days. The results showed that the extract of commercial bean had a total phenol concentration of 3.8 mg EAG g^{-1}, while the Escumite bean had 1.1 mg EAG g^{-1}. *In vitro* antioxidant tests showed higher activity of commercial pinto extract in comparison to Escumite. For example in the $DPPH^{\cdot}$ test, commercial bean showed an activity of 436 µM TROLOX mL^{-1} compared to 187 µM TROLOX mL^{-1} of the Escumite. Interestingly, *in vivo* antioxidant assays showed that Escumite bean extract had the best protective effect compared to commercial. The Escumite extract had an H_2O_2 LD_{50} of 8 mM compared to 7.2 mM of commercial bean and 6.2 mM of the control. In the glucose stress assay, the extract of Escumite increased the survival of the nematode by 20% in comparison to the control. These results suggest that the Escumite bean may provide greater protection to oxidative stress in biological systems, demonstrating that antioxidant activity measured *in vitro* will not reflect the antioxidant activity *in vivo*.

AN ALTERNATIVE POLYAMINE PATHWAY MANTAINS LYSINE PLASTICITY IN RESPONSE TO OXIDATIVE STRESS IN *Saccharomyces cerevisiae*

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Key words: lysine, cadaverine, H_2O_2

The polyamines (PAs) are metabolites derived from amino acids that are involved in different processes in the cells such as gene expression, cell proliferation and stress response (1). Previously, it was determined that in *Saccharomyces cerevisiae*, the polyamine transporter TPO1 was involved in the antioxidant response to H_2O_2 by controlling the spermine and spermidine intracellular concentrations; and the induction of some proteins related to oxidative stress response (2). Moreover, in the same work, a proteomic analysis also revealed that during incubations with H_2O_2, the enzymes of the lysine biosynthesis pathway via aminoadipate, were overexpressed during the time of exposure in the TPO1 knockout strain (*Δtpo1)*. These data suggested a possible role of lysine in the response to oxidative stress in which a connection with the PA metabolism could be involved. Thus the aim of this work was to elucidate the role of lysine and its connection with the PA metabolism in the response to H_2O_2.

S. cerevisiae lacks a lysine decarboxylase gene however, we could identify by isotopic labeling and LC/MS-MS that it was able to synthesize cadaverine by an alternative reaction of ornithine decarboxylase (ODC), the first enzyme involved in the synthesis of the PA spermidine. Then we cloned, over-expressed, kinetically characterized and crystallized the recombinant enzyme. We determined that ODC could synthesize cadaverine with a *Km* 20 fold higher compared to the one for ornithine. Despite this, the *Km* for lysine was physiologically relevant when the yeast was grown in media supplemented with lysine. Moreover, a docking analysis with lysine and ornithine in the crystal structure of the apoenzyme at 3.2Å resolution, confirmed that lysine was binding to the same site as ornithine. All these data confirmed that indeed lysine had a connection to PA metabolism via cadaverine in yeast.

Besides, cells grown in media supplemented with lysine accumulated this amino acid after the incubation with 1.5 mM of H_2O_2 in mid-log phase, and had a shorter lag phase after the addition of the oxidant. Moreover, the supplementation of lysine decreased ROS in cells exposed to H_2O_2 and increased the reduced glutathione concentrations, probably by re-directing the NADPH pools to these reactions.

Thus, we conclude that lysine can protect the cells from the stress generated by H_2O_2. And that the alternative reaction of ODC can contribute in mantaining the balance of lysine intracelular concentrations.

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Candida glabrata RESISTANCE TO OXIDATIVE STRESS THROUGH *CTA1* GENE REGULATION

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Abstract

Candida glabrata is a haploid yeast found as a commensal in healthy individuals but causes serious infections in immunosuppressed patients. It is the second most common cause of candidiasis. *C. glabrata* has a well-defined oxidative stress response (OSR), which include enzymatic and non-enzymatic mechanisms. *C. glabrata* has one catalase, *CTA1*, which functions as a scavenger of H_2O_2 to maintain the redox balance in the cell. *CTA1* expression is induced in the presence of oxidative stress and by carbon source deprivation. *CTA1* has an upstream intergenic region of 4.5 kb. The resistance to oxidative stress generated by H_2O_2 is mediated by *CTA1*, and is much higher than *S. cerevisiae* or *C. albicans*. In fact, in a heterologous complementation assay in *S. cerevisiae*, *CgCTA1* expressed in *S. cerevisiae* confers a higher resistance to H_2O_2. Furthermore, we measured the enzymatic activity of Cta1. Consistent with the increased resistance to H_2O_2, we found that CgCta1 conferred an increased enzymatic activity to *S. cerevisiae*. In order to understand how *CTA1* expression is regulated, we fused in a plasmid the *CTA1* intergenic region with GFP and generated 5'-3'. We then analyzed the activity of the *CTA1* promoter in different background strains (WT, *yap1Δ*, *skn7Δ*, *yap1 skn7Δ*, *msn2Δ*, *msn4Δ*, *msn2Δ msn4Δ*) in the presence of H_2O_2 by FACS. We determined that the minimal promoter is located at -1 kb from the ATG. We identified three positive regulatory *cis* acting elements located between -4 kb and -3.3 kb, -1.34 kb and -1 kb, and -1 kb and -0.75 kb. Furthermore, we found that both, Yap1 and Skn7, are required to induce the promoter, whereas Msn2 and Msn4 are not involved for induction. *CTA1* regulation is mediated by the presence of *cis* acting regulatory elements.

Keywords: gene regulation, catalase, hydrogen peroxide (H_2O_2).

EVALUATION OF THE ACTIVITY ANTITUMORAL OF NANOPARTICLES (-)-EPICATECHIN-LOADED CHITOSAN AND ITS RELATIONSHIP WITH REACTIVE OXYGEN SPECIES

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Key words: (-)-epicatechin, nanoparticles, reactive oxygen species

Introduction: The search of new therapeutic strategies against cancer has been focused on natural products, such as the flavonoids. In this context, our studies have shown that (-)-epicatechin (a flavonoid) present an antiproliferative effect in breast cancer cell lines. In addition, in vitro, we have proposed a probable mechanism of action of this flavonoid, which it was characterized by an induction of apoptosis, an increase in the production of reactive oxygen species (ROS), decreasing of antioxidant defenses and downregulation in UCP2 expression. Although the action of this flavonoid in cancerous cells shows its therapeutic potential, the flavonoids are molecules with a high sensitivity to physical, chemical and biological factors that limits its activity *in vivo*. **Aim:** In this study, (-)-epicatechin-loaded chitosan nanoparticles were generated, characterized and antitumor activity was studied. The effects of these nanoparticles on ROS production, UCP2 expression and apoptosis induction in the tumour were investigated.
Material and methods: Nanoparticles were prepared by the supramolecular self-assemble. The nanoparticles obtained were characterized by size and polydispersity index using dynamic light scattering. The Z potential was determined by particle electrophoresis and the morphology was established with transmission electron microscopy. The load of (-)-epicatechin in the nanoparticles was determined by Folin-Ciocalteau assay. Antitumoral effect was evaluate in a syngeneic transplant model on BALB/c mice. Twenty-four mice were randomly divided into four groups and were intraperitoneally administered with different treatments (control, (-)-epicatechin, nanoparticles of chitosan and nanoparticles of (-)-epicatechin-loaded chitosan). The tumours size was measured on alternate days, at the end of the experiments the animals were sacrificed. Tumors were collected and stored to -80°C. ROS production in tumours was determined by the values of biomarkers of oxidative damage such as carbonyl groups, quantification of formazan by NBT's reduction (NBT), malondialdehyde (MDA), thiobarbituric reactive substance (TBARS), also the activity of glutathione peroxidase (GSH-Px) was analyzed. ANOVA was used to determine differences statistically significant. P-values < 0.05 were considered statistically significant.
Results: Nanoparticles with size less to 200 nm were obtained, with a polydispersity index of 0.2, a zeta potential between -18 mV and -33 mV and spherical morphology. The efficiency of encapsulation of (-)-epicatechin and loading in the nanoparticles were 56.10 ± 3.9 % and .42 ± 0.85 respectively. Interestingly, nanoparticles of (-)-epicatechin-loaded chitosan showed a high antitumor activity in comparison to the others study groups. Finally, the differences in biomarkers of oxidative damage (MDA, TBARS and NBT) and decreasing of GSH-Px activity were observed in tumours of mice treated with nanoparticles of (-)-epicatechin-loaded chitosan.
Conclusions: The data obtained in this study, suggest that the antitumor activity of nanoparticles (-)-epicatechin-loaded chitosan is mediated by a similar mechanism of oxidative damage as it is observed for (-)-epicatechin *in vitro*.

RELATIONSHIP BETWEEN OXIDATIVE STRESS MARKERS AND GLUCEMIA IN DIABETIC ELDERLY

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Palabras clave: Diabetes mellitus, oxidative stress, elderly.

Introduction

Diabetes mellitus is a chronic disease characterized by chronic hyperglycemia due to deficient production or action of insulin. This hyperglycemia favors alterations such as oxidative stress, which predisposes the body to a state of constant damage associated with the development of complications, hence the importance of control in patients. However, the studies in our country about this are scarce, hence the importance of this research.

Objective

Evaluate the relationship between glycemia and markers of oxidative stress in elderly diabetics.

Methodology

A cross-sectional and analytical study was performed on a sample of 129 patients diagnosed with type 2 diabetes mellitus. Prior authorization (informed consent) blood samples were taken and clinical markers were determined, markers of oxidative stress were also quantified: F2-isoprostanes, (ELISA, Cayman Chemical), Total antioxidant status (TAS) glutathione peroxidase (GPx) and superoxide dismutase (SOD) (Randox Lab. Kits); glycosylated hemoglobin (HbA1c) by turbidimetry and the concentration of the receptor for advanced end glycosylation products (RAGE) by ELISA (R & D). The data were analyzed using the SPSS IBM V. 20 program, using ANOVA and multiple linear regression analysis (95% confidence level).

Results

Patients were grouped according to the percentage of HbA1c; a tendency to decrease of the activity or concentration of the antioxidant markers SOD, TAS, GAP, uric acid and albumin was observed, as well as an increase in the oxidation markers F2 isoprostane and RAGE, as the percentage of HbA1c increases. From the multiple linear regression vs HbA1c, a model, $r = 0.667$ and $p < 0.0001$ was obtained. A direct and significant relationship was found with F2-isoprostanes and RAGE ($r = 0.392$, $p < 0.0001$; $r = 0.308$ $p = 0.005$ respectively), as well as an inverse relationship with SOD (r=0-0.322 p=0.003), TAS (r=-0.401, p<0.0001), GAP (r=-0.354, p= 0.001), uric acid (r=-0.261 p<0.0001) and albumin (r=-0.401, p<0.0001). These findings support the proposal linking excess circulating glucose with the activation of metabolic pathways that alter the balance between oxidants and antioxidants. The results suggest that there is a direct relationship between the percentage of HbA1c and oxidative stress in elderly diabetics.

ACTIVITY OF NOX-2 AS A FUNDAMENTAL PIECE OF THE MICROGLIAL RESPONSE TO EXCITOTOXIC DAMAGE

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Key words: NADPH oxidases, Excitotoxicity, Interleukins.

Introduction: The excitotoxic damage is a common phenomenon in various pathologies of the central nervous system (CNS). The mechanism of neuronal damage depends on several factors, including an increase in the intracellular concentration of calcium and the production of reactive oxygen species (ROS). Recently, several evidences point out to the NADPH oxidases (NOX), particularly NOX2, as the main source of ROS responsible for oxidative stress during this process. The NOX production of ROS in high quantities has been observed in necrosis, apoptosis and inflammation. The inflammation resulting from excitotoxic damage, can culminate in injury or the generation of a secondary damage, subsequent of a deregulated inflammatory process.

Objective: Characterize the role of ROS produced by NOX2 in the inflammatory response, secondary to excitotoxic damage in the striatum of mice.

Methodology: The excitotoxic damage was produced by intracerebral (IC) injection of glutamate (1M) in the striatum of C57-BL6 (wild type) and gp91^{phox-} mice (NOX2-KO); additionally, other wild type mice were treated with recombinant mouse IL-10 (200 pg/mL) at the time of injection of glutamate. Subsequently, striatum homogenates were obtained at 1, 3, 6, 12 and 24 hrs. NOX activity and the amount of active caspase 3 were performed by fluorescence spectrometry, as well as the production of interleukins 1β,4,6,10 and 12, using ELISA methods (Abcam kit). Before the mice were sacrificed, they were subjected to the cylinder and the adhesive removal test for the evaluation of motor behavior.

Results: We observed a biphasic increase of NOX activity (1hr to 12 hrs.), which was not observed in animals owing the deficient enzyme. Mice NOX2-KO showed a better performance in the cylinder and adhesive removal test, after glutamate administration compared to wild type animals. The NOX-2 deficient animals also showed smaller amounts of activated caspase 3. As for interleukins, an increase in pro-inflammatory interleukins was observed in both groups, however, NOX-2 deficient animals showed a significant increase in the production of IL-4 and IL-10, cytokines characterized as anti-inflammatory factors.

Conclusions: The absence of NOX-2 activity promotes the production of anti-inflammatory interleukins, which favor neuronal survival by decreasing caspase-3-mediated apoptotic death, which is reflected in a more effective recovery of motor performance

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PHYSIOLOGICAL ADAPTATIONS AGAINST STRESS IN THE FISHING BAT *MYOTIS VIVESI* IN A HIGHLY SEASONAL ENVIRONMENT.

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Key words: antioxidants, immunocompetence, ecophysiology

All animals confront stressful situations through their lives, whether due to changes in environmental conditions, predation and competition. These situations are potentially amplified by human activities, which cause habitat loss or alterations, leading to rapid and often stressful and deteriorating changes. Those, environmental stress may have a significant impact on the evolutionary and ecological processes that affect and shape the genetic structure and evolution of populations. The fishing bat *(Myotis vivesi*) is endemic to desert islands in the Gulf of California, Mexico, where summers are extremely hot (up to 45^{o}C), and reproductive season occurs; and winters are cold (~5^{o}C), and bats hibernate. The main objective of this work is to determine if bats adapted to live in drastic seasonal environments, can withstand such conditions without suffering cellular damage, assuming they have evolved the physiological mechanism to counter them. We evaluated how physiological parameters related to stress (e.g. antioxidant activity [a.a], protein carbonylation and bactericidal killing capacity of plasma [BK]) fluctuate through the seasons and how are they affected by an acute stress stimulus (inflicted by means of movement restriction in small cotton bags for 6 and 12 h) during these seasons. Our results showed that antioxidant enzymes activities were in general higher during autumn in contrast to summer and winter. Along with less protein damage during autumn, and higher damage during summer, thus suggesting low antioxidant protection during summer and antioxidant accumulation as a protective measure for the upcoming winter season. The acute stress resulted in an enhancement of enzymatic antioxidant activity, especially during harsh seasons (summer and winter), but also protein damage increase. Basal BK was higher during summer, highlighting the importance of increasing the defence against infections during this period. Also BK was enhanced by acute stress during summer. These results show that bats are adapted to modulate their immune and antioxidant defences seasonally, and they are capable of increasing some of them further when energetic requirements sums up with an unpredictable source of stress.

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ASSOCIATION OF POLYMORPHISMS OF REACTIVE OXYGEN SPECIES MODULATOR 1 (ROMO1) GENE WITH NONALCOHOLIC STEATOHEPATITIS DEVELOPMENT

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Key words: SNPs, ROMO1 and NASH

Introduction: The Reactive Oxygen Species Modulator 1 (ROMO1) is involved in the regulation of mitochondrial Reactive Oxygen Species (ROS) generation and it has been associated with the development of some liver diseases. The Nonalcoholic Steatohepatitisis (NASH) is characterized by a ROS overproduction, leading to an imbalance with the antioxidant enzymes that can induce hepatocellular carcinoma. Single-Nucleotide Polymorphisms (SNPs) present in different regions of ROMO1 gene as rs1279454 (promoter) (A→G) and rs6058303 (3'UTR) (A→G) could have an effect in NASH development and they could be used as biomolecular prognostic factors.

Objective: To evaluate the association between NASH development and the genetic variants rs1279454 and rs6058303 of ROMO1 in the Mexican population.

Methodology: The investigation includes patients diagnosed with NASH (n=50) and people without presence of NASH as a control (n=308). It was assayed the allelic discrimination by RT-PCR using the Applied Biosystems StepOne and TaqMan assay previously designed. To determine the difference between genotype frequencies in the study groups we used the Fisher Exact Test. The Odds Ratio (OR) was calculated as a measure of association with the disease by logistic regression models.

Results: Regarding the genotypes of rs1279454 polymorphism there was a higher frequency of A/G among patients than controls (p=0.036). Furthermore, the OR obtained for this polymorphism heterozygous genotype indicates that the ones that carry it have three times more possibilities of have NASH than the carriers of the A/A genotype (p=0.013). On the other hand, for the rs6058303 polymorphism A/G genotype we obtained an OR of 2.0 (p=0.037), even though the difference in the genotype frequencies of this polymorphism among patients and controls was statistically marginal (p=0.059).

Conclusions: The heterozygous genotype (A/G) of the rs12479454 SNP, increase the risk to develop NASH, due to presence of the minor allele G, which may have an effect in the ROMO1 gene expression rate, leading to a deregulation in the ROS modulation present in the disease. The heterozygous genotype (A/G) of the rs6058303 was associated with an increased risk of having the disease.

AUTOPHAGY INHIBITION WITH SPAUTIN-1 LEADS TO CELLULAR SENESCENCE THROUGH OXIDATIVE STRESS RELATED MECHANISMS IN PRIMARY CULTURED ASTROCYTES.

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Keywords: Senescence, Autophagy, Proteostasis

Senescence is a phenomenon defined by a permanent growth arrest as a response to damage or stress. It has mainly been related to aging and age-related diseases. The role of senescent cells in age-related neurodegenerative diseases has become a topic of increasing interest in the past few years due to the increasing number of people who suffer from these diseases.

Astrocytes are the most numerous cells in the brain. They are responsible for brain homeostasis, and the loss of their functions by acquiring the senescent phenotype, has been related to neurodegeneration.

Proteostasis loss has been associated to the origin and progression of many diseases. At the same time, it has also been revealed as a triggering mechanism for senescence, in particular proteasome and autophagy inhibition. However there are no studies related with autophagy inhibition and senescence induction in the central nervous system. Thus, the aim of this work was to evaluate if it is possible to induce senescence in primary astrocytes using Spautin-1 to impede autophagy.

Our results showed that Spautin-1 decreased astrocyte proliferation rates. At the same time, it increased the number of positive cells to the senescence marker SA ß-galactosidase and the cell cycle inhibitor protein p16 after 6 days of treatment. Spautin-1 treated cells also had higher levels of carbonylated proteins when compared with non-treated astrocytes, concurring with previous results from our lab and suggesting that the senescent state is triggered and/or maintained by redox mechanisms.

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EVALUATION OF OXIDATIVE STRESS INDICATORS IN GREEN TURTLE *CHELONIA MYDAS* AFLICTED WITH FIBROPAPILLOMA IN MEXICAN CARIBBEAN.

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Introduction. Fibropapillomatosis (FP) is a worldwide epizootic threat for the survival of sea turtles, documented worldwide in green turtle (*Chelonia mydas*) populations. The etiology and pathogenesis of FP remains unknown; it is a multifactorial neoplastic chronic disease frequently associated to herpesvirus. The increased prevalence of FP in the last years in the coast of Mexican Caribbean may be an indicator of the altered habitat conditions. Cumulative production of reactive oxygen species (ROS) through endogenous (*e.g.* activated immune system) or exogenous factors (*e.g.* xenobiotics) are involved in the pathophysiology of several diseases and carcinogenesis.
Objective. The goal of this study was to evaluate differences in the oxidative stress indicators between healthy sea turtles and those afflicted with FP, and to evaluate their relationship with hematological parameters and frequency of nuclear abnormalities.
Methods. Immature green turtles inhabiting foraging grounds were captured alive in the coast of Quintana Roo, Mexico. Differential, total white blood cell counts and frequency of nuclear abnormalities in erythrocytes were determined. Superoxide radical production ($O2^{\bullet-}$), activity of antioxidant enzymes (SOD, CAT, GST, GPx, GR) and indicators of oxidative damage (TBARS and carbonyl proteins) were determined in intraerythrocytic material.
Results. During summer 2015, 11 green turtles were captured in Punta Herrero (control group) inside of the Natural Reserve of Sian Ka'an; 22 individuals were captured in the touristic bay of Akumal. Prevalence of FP in Akumal was 22.7% (n=5) while FP was not observed in sea turtles from Punta Herrero. Nuclear abnormalities determined in Akumal were 94% times higher than in Punta Herrero. Statistical differences were not found for indicators of oxidative stress when healthy individuals were compared between zones, neither between green turtles from Akumal with and without FP. In Akumal, the median of the CAT activity and concentration of TBARS concentration was higher (percentage change 87% and 44%, respectively) in both healthy turtles and those with FP compared to the control group, while lower activity of SOD, GST and GPx (percentage change -16%, -9% and -43% respectively) was found in turtles afflicted with FP. Antioxidant defenses and oxidative damage indicators presented significant correlations with monocyte, basophil and heterophil counts (cells μL^{-1}) in both study sites.
Conclusions. Results suggest that sea turtles from Akumal show adaptive biochemical and immune responses not observed in Punta Herrero, probably to cope with their particular habitat conditions. Increase of sample size including new study sites is required in order to continue monitoring the health state of this population.
Keywords: fibropapillomatosis, oxidative stress, sea turtles

REACTIVE OXYGEN SPECIES DYNAMICS IN DEVELOPING ZEBRAFISH EMBRYOS.

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Keywords: ROS, zebrafish, Nox.

Introduction

Reactive oxygen species (ROS) are oxygen products generated during aerobic metabolism and by specific enzymatic activities. ROS play pivotal roles in the regulation of major cellular behaviors such as proliferation, cell death, cell migration and cell differentiation; all fundamental in animal development. Previously, we reported that ROS participate in the control of cell death during morphogenetic remodeling of different developing tissues in mouse embryos, suggesting an extensive role in development.

Objective

Characterize the ROS localization patterns and functions during early zebrafish development.

Methodology and Results

To gain further insight into the role of ROS during animal development, zebrafish embryos were stained with a widely used ROS-sensitive dye and visualized by confocal time-lapse microscopy. Interestingly, we found that ROS present highly dynamic patterns that correlate with key developmental process. Remarkably, we found that throughout gastrulation and particularly in epiboly, that is the first major morphogenetic event in which massive movement of cells occur; ROS exhibited dynamic fluctuations localizing at the interstitial space among blastoderm cells. In addition we observed a distinctive region of ROS accumulation at the blastoderm margin through whole epiboly progression. We found that ROS are generated by NADPH oxidase (Nox) activity and that specific pharmacological inhibition of Nox, decreased cell motility and delayed epiboly; an effect that can be fully rescued by hydrogen peroxide treatment.

Conclusions

In the present study we show evidence that ROS participates in the control of cell motility during a major developmental process fundamental in animal development.

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EVOLUTION OF THE HEMERYTHRIN-LIKE DOMAIN SUPERFAMILY

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Key words: oxygen-carrier, hemerythrin, non-heme iron

Introduction
Accumulation of free atmospheric oxygen during the Precambrian is, undoubtedly, one of the major changes in the history of the planet and may be considered the most significant biogeochemical process after the origin of life itself (Alvarez-Carreño et al., 2016). The evolution of oxygenic photosynthesis during Precambrian times entailed the diversification of strategies minimizing reactive oxygen species-associated damage. Four families of oxygen-carrier proteins (hemoglobin, hemerythrin and the two non-homologous families of arthropodan and molluscan hemocyanins) are known to have evolved independently the capacity to bind oxygen reversibly, providing cells with strategies to cope with the evolutionary pressure of oxygen accumulation.
Methods
we studied the phylogenetic distribution of hemerythrin-like sequences in 2521 completely sequenced bacterial, archaeal and eukaryotic genomes, and correlated the possible evolutionary scenarios with available structural and functional data.
Results
Oxygen-binding hemerythrins are a monophyletic sub-group of the hemerythrin/HHE cation-binding domain. Overall, oxygen-binding hemerythrin homologues were found in the same proportion as single-domain and as long protein sequences. The associated functions of protein domains in long hemerythrin sequences can be classified in three major groups: signal transduction, phosphorelay response regulation, and protein binding. This suggests that in many organisms the reversible oxygen-binding capacity was incorporated in signaling pathways. A maximum-likelihood tree of oxygen-binding hemerythrin homologues revealed a complex evolutionary history in which lateral gene transfer, duplications and gene losses appear to have played an important role.
Conclusions
Hemerythrin is an ancient protein domain with a complex evolutionary history. The distinctive iron-binding coordination site of oxygen-binding hemerythrins evolved first in prokaryotes, very likely prior to the divergence of Firmicutes and Proteobacteria, and spread into many bacterial, archaeal and eukaryotic species. The later evolution of the oxygen-binding hemery- thrin domain in both prokaryotes and eukaryotes led to a wide variety of functions, ranging from protection against oxidative damage in anaerobic and microaerophilic organisms, to oxy-gen supplying to particular enzymes and pathways in aerobic and facultative species.

POSTER SESSIONS

H_2O_2 IS THE SECOND MESSENGER FOR α_1-ADRENERGIC RECEPTORS IN HEPATOCYTES

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The NADPH oxidase (NOX) family catalyzes the electron transport of cytosolic NADPH to molecular oxygen and generates O^{2}- which dismutase to H_2O_2.We reported that α_1-adrenergic receptors (AR) activation increased Nox2 activity and enhanced extracellular H_2O_2 pool in isolated hepatocytes; whereas β-AR activation decreased Nox2 activity and lowered extracellular H_2O_2 pool in the same cells.Because the ARs also lead to activate the rates of hepatic glycogenolysis, ureagenesis, and glyconeogenesis (GUG), we explored an eventual role of this extracellular H_2O_2 once generated by α_1-AR activation, on the above mentioned metabolic routes.Results: a) H_2O_2 generated by α_1-AR activation stimulates the rate of GUG in isolated hepatocytes; b) this H_2O_2 effect was prevented if H_2O_2 uptake by hepatocytes was impaired by $AgNO_3$ or with Aquaporine-8 (Aq) antibodies; c) when H_2O_2 generated by α_1-AR activation is allowed to get inside the hepatocyte, β-AR activation did not stimulate the rate of GUG in the cell; d) when H_2O_2 uptake we impaired with Aq antibodies, β-AR activation stimulates the rate of GUG in isolated hepatocytes; e) H_2O_2 synthesis by α_1-AR activation demanded the presence of cytosolic Ca^{2+}; f) H_2O_2 increased, in a doses dependent form, cytosolic Ca^{2+} pool in hepatocyte at expenses of other intracellular pools; g) β-AR activation does not increase cytosolic Ca^{2+} pool.These data strongly suggest the role of H_2O_2 as the second messenger of activated α_1-AR, at least in hepatic cells. Partially supported by PAPIIT IN214616 and CONACYT 166733

EFFECT OF N-ACETYLCYSTEINE ON MITOCHONDRIAL BIOENERGETIC AND H_2O_2 PRODUCTION ON EXPERIMENTAL ACUTE KIDNEY DISEASE INDUCED BY FOLIC ACID.

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Key words: Mitochondrial bioenergetic, acute kidney disease (AKI), N-acetylcysteine (NAC).

Introduction. The term acute kidney disease (AKI) is used to include a wide range of pathologies characterized by an abrupt deterioration of kidney function and an increased in renal damage markers. Since mitochondrial function and regulation is essential for the maintenance of renal function, the idea that mitochondrial dysfunction plays a fundamental role in the genesis and progression of AKI has raised in recent years. Nevertheless, the bioenergetics and oxidative stress alterations involved in AKI model induced by folic acid administration (FA) (widely used to study clinical AKI given their likenesses) are not fully understood. On the other hand, N-acetylcysteine (NAC), a selective antioxidant, could be a powerful agent to prevent both mitochondrial and renal dysfunction in AKI, given its ability to control the mitochondrial ROS production. However, this hypothesis has not been explored

Objective. To characterize the mitochondrial bioenergetics and oxidative stress alterations in the AKI induced by FA, as well as the effect of the of NAC in these parameters.

Methods. Four groups of Wistar male rats (250-300gr) were used: 1-Vehicle group. 2-FA, single injection of folic acid (300mg/kg). 3-NAC+FA, two dose of NAC (300 mg/kg) plus FA (300mg/kg). (4) NAC, only NAC (300 mg/kg). The animals were sacrificed 24 h after the FA administration and the blood (for renal damage markers) and kidneys were extracted (for mitochondria isolation). Mitochondrial respiration was determined feeding CI, CII and both. As well as the total uncoupled respiration, the activity of each mitochondrial complex, mitochondrial membrane potential and ATP production. The redox state in mitochondria was evaluated by the production of hydrogen peroxide.

Results. FA administration increases the renal damage markers creatinine and blood urea nitrogen (BUN), as well the rise in the urinary volume and kidney weight. That increase in renal damage markers was reduced by NAC preadministration. Furthermore, FA also induced the decrease in: State 3, State 4o, respiratory control (RC) and OXPHOS capacity (P) in CI-feeding respiration; reduction in State 3, RC and P in CI+CII-feeding respiration; besides it decreases the mitochondrial membrane potential (in state 3), ATP synthase and complex I activities and increase the H_2O_2 mitochondrial production. Moreover, NAC prevent partially all these alterations.

Conclusions. Folic acid induces mitochondrial bioenergetic and ROS production alterations in the kidney which might contribute to the development of AKI in this model. Additionally, NAC protected the kidney function preventing the emergence of these alterations.

MAIZE SCUTELLUM EPIDERMIS TRANSFORMATION TO EPITHELIUM AND FACTORS THAT ENABLE THE MORPHOLOGICAL CHANGE.

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Key words: Class III Peroxidase, Scutellum, Maize.

From the nutritional point of view, maize is one of the three most important cerals. The kernel include an embryo composed by the embryo axis and the scutellum, and is separated of the endosperm by the fibrous layer. The scutellum works as a storage structure of lipids, phytate, and some ions that are used in germination to support the growth of the radicle. During postgermination, the growth of the radicle requiere more nutrients, wich are stored in the endosperm and need to be mobilized. In early postgermination, the scutellum secretes amylolitic enzymes that breakdown the starch close to it. For this, the scutellum epidermis wich initially is a monolayer and must be transformed to a functional epithelim cells enabled for absorption and secretion. Results from our investigation group shows that epidermic cell transformation is an asinchronic process that starts from the base to the apical scutellum surface, and this occur from the 18 to the 28 h after the initiation of imbibition. During this morphological changes, the cuboidal cells of scutellum epidermis increase in size and develop a finger-like extensions that projects them into the endosperm, and acquire characteristic of papillate cells that constitute an epithelium. 24 h after imbibition we detected the production of reactive oxigen species (ROS) in the frontier of the scutellum and the fibrous layer, and this correlated with *in situ* Class III peroxidase (POD) activity and immunolocalization. It has been demostrated that POD has two catalitic cycles: the peroxidative, in wich the enzyme acts as an antioxidant, and the hydroxilic in wich ROS are produced. The last mechanism has been linked to the cell wall relaxation needed to cell expansion driven by turgor pressure. Related with this, we have demostrated that maize scutellum can secrete POD at 24 h of imbibition, and this secretion is induced by an acid pH (5.5), several ions as Ca^{2+}, K^{+}, Na^{+} y $H_2PO_4^{-}$; and a synthetic auxin (1 to 10 μM). With this evidence we propose that the morphological changes in the scutellum epidermis are induced by auxin and the H^{+}-ATPase activity that generates an acidic pH and a transmembrane potential; that are analogs to other systems, in wich the flow of K^{+}, $H_2PO_4^{-}$ and Ca^{2+} lead to POD secretion, whose activity is related to cell wall modifications that allow the cell expansion and finally the transformation scutellum epidermis to epithelium.

EVALUATION OF THE ANTIGENOTOXIC CAPACITY OF A MICROENCAPSULATED OF GRANADA AGAINST THE OXIDATIVE DAMAGE PRODUCED BY ACRYLAMIDE

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Acrylamide (AA) is an α,β-unsaturated carbonyl compound with a high chemical activity that participate in different reactions to generate free radicals. The best known is the metabolic transformation to glycidamide, which binds to DNA and can cause genetic damage. AA is considered a pro-carcinogen agent class 2A and different studies have evidenced its presence in starchy foods and reducing sugars processed at high temperatures (above 120 °C). On the other hand, the granada (*Punica granatum* L.), besides being a fresh seasonal fruit, has been traditionally used for its anti-inflammatory, anti-angiogenic and anti-tumor capacity; properties attributed to antioxidants present in its chemical composition.
The aim of this study was to determine the antigenotoxic potential of a microencapsulated of granada (MEGr) against oxidative damage caused by acrylamide evaluated by micronucleus assay. We include a negative control, a control batch of MEGr (3 g/kg), a positive batch (AA in a dose of 50 mg/kg), and a combined lot with MEGr plus AA. The MEGr was administered intragastrically for 14 days before the intraperitoneal administration of AA. For a week, we perform blood smear, were stained and observed microscopically to quantify the number of micronucleated erythrocytes normochromic (ENCMN).
The results indicated that the genotoxic effect of AA increased with the time of administration; on the contrary, the MEGr is not a micronucleus inducer agent; and significantly reduces the frequency ENCMN to the 48hr of treatment. The greater protection was at the end of the experiment (40%).
These results suggest that the process of microencapsulation used (conversion of natural juice in small particles of water soluble powder) maintains the natural properties of the fruit and allow their antioxidant compounds reach the digestive tract. Whereby, the MEGr may be considered a chemopreventive agent, and its mechanism of action probably is related to their antioxidant capacity.

Keywords: *P. granatum*, acrylamide, micronucleus.

REDOX STATUS AND ANTIOXIDANT RESPONSE DURING FASTING

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Key words: Antioxidant defense, reactive oxygen species (ROS), fasting.

Introduction. Food deprivation imposes a tremendous metabolic challenge to organisms. In this regard, there is a general idea that food deprivation also causes redox imbalance promoting oxidative stress in the liver. However, it remains unknown whether fasting has different effects on intracellular redox status in distinct tissues.

Objective. To characterize the impact of food deprivation on redox status and antioxidant defense in the liver and kidney.

Methods. Antioxidant enzymes and oxidative stress markers were evaluated in the liver and in the heart of male Wistar rats fasted for one to three days.

Results. We found that both the liver and the heart exhibit specific antioxidant defense against nutritional challenge. While in the liver fasting causes redox imbalance and reduces antioxidant defense, in the heart fasting increases both total native thiol content and protection against free radicals. Intriguingly, carbonylated proteins were reduced in the liver of fasted rats, revealing an unexpected beneficial effect of fasting in reducing damage to hepatic proteins.

Conclusions. Fasting modifies intracellular redox status in a tissue-specific manner.

APOCYNIN ACTION ON THE GROWTH OF CELL POPULATION AND MIGRATION IN MDA-MB-231 BREAST CANCER CELLS.

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Keywords: Apocynin, migration, ROS

Background: Cancer is one of the main health problems worldwide, being breast cancer the most frequently diagnosed cancer in women; in Mexico, breast cancer is the second cause of death in women. Its development depends on several internal and external factors, and one of this are the reactive species of oxygen (ROS) and their effect in cell metabolism and function. ROS are generated from diatomic oxygen and they have high reactivity and a double role in the cells because they can take part in several signaling pathways like second messengers, but when there is an excess of these molecules, they can damage macromolecules like proteins, lipids and DNA. This damage can take the cell to the develop of a malignant phenotype, because of this, researchers have seen in ROS possible therapeutic targets, not only in cancer but in several chronic-diseases. Recently, researches have shown that antioxidant compounds have an inhibitory effect in the progression of several types of cancer, including, breast cancer, and can to prevent the expression of proteolytic enzymes involved in cell migration.
Objective: Here, we studied the effect of different concentrations of apocynin, an inhibitor of NADPH oxidase, one of the main sources of ROS, on the growth of cell population, migration, and the expression of the serine protease, urokinase plasminogen activator (uPA).
Methods: MDA-MB-231 cell population growth and migration were assessed using 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) and scratch-wound assays, respectively. The expression of uPA was assessed using Western blotting and gel zymography.
Results: We found that apocynin had an inhibitory effect on the growth of cell population and migration of MDA-MB-231 invasive cells, this effect was counteracted by the presence of fetal bovine serum (FBS) in the culture media. It was also found that the apocynin did not modify the expression of uPA.
Conclusions: From our data, we can conclude that apocynin reduces cellular proliferation and migration of MDA-MB-231 cell, indicating that proliferation and migration of these cells are dependent on the production of ROS by NADPH oxidase. The inhibitory action of apocynin on cell migration was not mediated by changes in the expression of uPA, probably other proteases and anti-migratory mechanism must be implied.
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MORPHOLOGICAL CHANGES AND ROS PRODUCTION BY IMMUNEPOTENT CRP IN CANCER CELLS: TOWARDS THE CHARACTERIZATION OF A CELL DEATH MECHANISM

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Keywords Cancer • IMMUNEPOTENT CRP • ROS

Introduction Cancer, one of the major causes of morbidity and mortality worldwide, represents the third-leading cause of death in Mexico. The main therapeutic options include surgery, chemotherapy or radiotherapy, unfortunately most of them are non-specific. As a therapeutic alternative, immunotherapy arose in order to restore or enhance the immune system's ability to fight cancer. A promising immunotherapy is the use of IMMUNEPOTENT CRP, a bovine dialyzable leukocyte extract (bDLE) obtained from bovine spleen. Previous studies have demonstrated the cytotoxic effects of the ICRP in different cancer cell lines and revealed that it induces a caspase-independent death. **Objective** The aim of the present study was to evaluate the morphological changes and the production of reactive oxygen species induced by the ICRP in human cervical (HeLa) and breast (MCF7) cancer cell lines. **Methods** Changes in cell morphology were assessed by means of light microscopy after treatment with ICRP. To determine the type of vacuoles in the cell, the chromatin condensation and the mitochondrial membrane potential, cells were analyzed by confocal fluorescence microscopy using different dyes (CYTO ID Green, LYSO ID Green, Hoechst 33258 and TMRE). Cell death analysis (phosphatidyl-serine exposure / membrane permeability) and oxidative stress-dependent changes were evaluated by flow cytometry using a labeling with Annexin V / Propidium Iodide (PI) and DCFDA, respectively. **Results** Data shows that treatment with ICRP induces: autophagosome formation, loss of mitochondrial membrane potential and chromatin condensation in both cell lines. Moreover, ICRP induces PS exposure, PMP and ROS production. However, cells pre-treatment with a ROS inhibitor (N-acetyl cysteine, NAC) prevents these characteristics of cell death. Furthermore, ROS inhibition also impedes autophagosome formation. **Conclusion** ICRP induces ROS-dependent RCD in HeLa and MCF7 cancer cells, provoking autophagosome formation, loss of mitochondrial membrane potential and chromatin condensation.

KINETICS OF LIGNOLYTIC ENZYMES BY *TRAMETES POLYZONA* USING SOLID-STATE FERMENTATION (SSF) IN COCONUT MESOCARP

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Coconut mesocarp, lignolytic enzymes, solid-state fermentation.

The lignolytic enzymes of basidiomicetes are necessary for an efficient bioconversion of plant residues. The fermentation of fungi produces three extracellular enzymes that are essential for the degradation of lignin: lignin peroxidase (LiP), manganese peroxidase (MnP), and laccase. The fibers coconut are mainly made up of lignin, cellulose and hemicellulose, this components represents 34, 30 and 10% of coconut chemical composition, respectively. The coconut mesocarp has high water retention capacity, which makes it a suitable material for use in solid state fermentation and the production of metabolites of interest. Therefore, the aim of this study was to quantify the enzymatic activities of the lignolytic enzymes produced during the solid-state fermentation for the delignification of the coconut mesocarp. In the fermentation, 1 g of sterile coconut mesocarp was used as carrier and source of carbon. *Trametes polyzona* was growed in Pontecorvo medium using the mycelium during 24 h of growth, the medium was additioned to adjust the humidity of the substrate to 80 %. The inoculum was kept at a constant temperature of 28 °C during 10 days, taking samples for the enzymatic kinetics. Protein enzymatic extracts were determined by Bradford method and the enzymatic activity were studied by spectrophotometric methods. The enzymatic activity of laccase was quantified by the oxidation reaction from ABTS to ABTS + cation and manganese peroxidase activity was determined by oxidation of phenol-red. Moreover, the enzymatic activity of lignin peroxidase was evaluated through the formation reaction of veratraldehyde. At the end of the fermentation process, a maximum protein value was obtained: 586 µg protein/g (dry simple) in the enzymatic extracts. The laccase presented its maximum activity on the eighth day (5.3 U/g), whereas the maximum values of lignin peroxidase and manganese peroxidase were observed at ninth day, 0.096 U/g and 0.5 U/g, respectively. The presence of laccase in the last days indicates that the microorganism has consumed the easily metabolizable compounds of the medium. The increase in the activity of MnP and LiP was due to the formation of reactive species such as peroxides, the main inducers of enzymatic expression, and as a result of the metabolism of laccase activity products.

ANTIOXIDANT ACTIVITY IN A FUNCTIONAL BREAD ADDED WITH FERMENTED COCONUT MESOCARP USING *TRAMETES POLIZONA*

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Coconut mesocarp, solid-state fermentation, antioxidant activity

The generation of reactive oxygen species and reactive nitrogen species in the body is a consequence of the exposition to different physicochemical conditions or pathological states in various endogenous systems. The free radicals are very unstable and they react quickly with other groups or substances in the body. These components are present in all the essential structures of cells, and it is possible that may origin a cellular or tissue damage. The antioxidants from a diet can protect against disease related with the oxidative stress, through oral administration. Besides these antioxidants can be addition for producing functional food. The aim of this work was to evaluate the phytochemical content and the antioxidant activity of dried and fermented coconut mesocarp using solid-state fermentation (SSF) with *Trametes polyzona* and to elaborate bakery product (baguette). For the solid-state fermentation, 1 g of sterile coconut mesocarp was used as carrier and source of carbon. The methanolic extracts of the samples were obtained in order to evaluate their antioxidant properties. The polyphenols content was determinated using the Folin–Ciocalteu method. The phenolic content in the dried sample was 138.15 µg gallic acid equivalents (GAE) and fermented coconut mesocarp was 26.33 µg GAE, the lowest concentration was 2.36 µg GAE for bakery product. The highest antioxidant activity evaluated with DPPH method in dried coconut mesocarp was 76.4 % inhibition, followed by fermented coconut mesocarp with 42% inhibition, and the lowest activity was 18.5% inhibition in the bakery product. The highest antioxidant activity evaluated with ABTS method was 177.56 µM trolox equivalents (TE), followed fermented by coconut mesocarp with 42 µM TE, and the lowest was 4.16 µM TE in baguette. The antioxidant activity in baguette with fermented coconut mesocarp was 51.8 % higher than the control bread. The results showed that coconut mesocarp could be used in the formulation of bakery product with a higher content of phenols, which improves the antioxidant capacity and nutritional quality of the baguette with respect to the conventional one.

EVALUATION OF PHYTOCHEMICALS AND ANTIOXIDANT CAPACITY OF THE SEMINAL INTEGUMENT IN PEANUT (ARACHIS HYPOGAEA)

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Key words: phytochemycals, peanut, antioxidant capacity

Mexican agroindustry generates approximately 96,000 tons of peanut (*Arachis hypogaea*) waste per year, most of which are not used and contribute to the problem of environmental contamination. Several studies have shown that these byproducts contain phytochemicals, such as polyphenols, whose concentrations depend on variety, maturity and culture conditions. The antioxidant properties of phytochemicals have the potential to prevent damage caused by lipid alterations in the body associated with obesity and its manifestations of hepatic steatosis and its involvement in liver function. The aim of this study was to characterize one of the residues of peanut (*Arachis hypogaea*): the seminal integument for use as a functional ingredient in a product with nutraceutical properties. The raw material was obtained by peeling the peanut by hand. The content of polyphenols and flavonoids in the integument as well as the antioxidant activity were quantified by the DPPH method, FRAP and iron chelating activity. The content of polyphenols and flavonoids was 72.11 ± 7.81mg / GAE/g and 44.43 ± 1.55 mg/CAE/g. The following data were found for antioxidant capacity: 208.95 ± 7.28 µmol TE/ g by the DPPH method; 491.4 ± 54.2 µmol TE/g by FRAP method and 10.60±1.71µg EDTA/g for iron chelating activity. It is important to emphasize that the polyphenol content in the peanut integument is related to a high antioxidant capacity, which means: it is directly proportional. Due to the high content of phytochemicals (polyphenols) present in the peanut integument, we proposed the addition of this component as an ingredient in the formulation of functional products.

EFFECTS OF ROS INHIBITION IN CELL DEATH AND CELL CYCLE ARREST INDUCED BY IMMUNEPOTENT-CRP IN CANCER CELL LINES

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Keywords: Cancer, Cell death, N-acetylcysteine.

Introduction. Cancer is a group of diseases mainly characterized by uncontrolled proliferation and cell death deficiencies, representing a serious health problem in Mexico and all around the world. Today, several therapies against this set of diseases are available, and the most implemented (chemotherapy and radiotherapy) are well known to affect normal cells and reducing, consequently, the patient's quality life. Therefore, alternative therapies have been developed, such as immunotherapy. Immunotherapies include IMMUNEPOTENT CRP (I-CRP), molecules smaller than 12kDa obtained from the dialyzable extract of bovine leukocytes, which is used in patients as an adjuvant to reduce the negative effects of the chemotherapy and radiotherapy. It has also been shown to present cytotoxic capacity *per se* over different cancer cell lines. As cell death and cell cycle arrest are implicated in viability diminution, in the present work we assessed cell death and cell cycle arrest induced by I-CRP on A549 (lung cancer), HeLa (cervical cancer) and MCF-7 (breast cancer) cell lines. Moreover, as reactive oxygen species (ROS) are involved in both processes, the antioxidant N-acetylcysteine (NAC) was used to study the role of ROS on these effects. **Objective**. Assessment of cell viability diminution, cell death, and cell cycle arrest induced by I-CRP in cancer cell lines, and the effect of ROS inhibition on these effects. **Methodology.** In all the assays, cells were treated with I-CRP, and NAC was used as an antioxidant. First, cell viability was evaluated by MTT assay. Afterward, we implemented a fluorescence-activated cell sorting (FACS) by flow cytometry in different experiments to observe multiple characteristics associated with the loss of cell viability, such as cell membrane permeabilization (propidium iodide), phosphatidylserine exposure (annexin V), ROS production (DCFDA), loss of mitochondrial membrane potential (TMRE), and the effects on cell cycle (propidium iodide). **Results.** Our data shows that I-CRP is cytotoxic in a dose-dependent manner in all cancer cell lines tested, as it reduced cell viability, and induced phosphatidylserine exposure, plasma membrane permeability, and cell cycle arrest. It also causes mitochondrial damage as observed by loss of mitochondrial membrane potential and ROS production. Cells pretreatment with NAC inhibited cell death caused by I-CRP, but did not affect cell cycle arrest. **Conclusion.** ROS play a main role in the reduction of cancer cells viability induced by I-CRP. Altogether, these results improve the knowledge about the mechanism in which I-CRP operates.

EXPRESSION OF FIBROSIS-RELATED GENES IN PULMONARY FIBROBLASTS EXPOSED TO HYPOXIA

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Keywords: Hypoxia, Pulmonary fibrosis, Smooth muscle alpha-actin

ABSTRACT

Background: Idiopathic pulmonary fibrosis is a chronic, progressive, lethal disease of unknown etiology associated with aging. It might possibly be the result of a complex interaction between environmental and genetic factors. In this work we analyze the effect of hypoxia on gene expression of fibrosis-related proteins in pulmonary fibroblasts. **Objetive**: To measure smooth muscle alpha-actin (ACTA), TGF-β1 and the transcription factors HIF1α, HIF2α and HIF3α gene expression in fibroblasts from healthy and fibrotic lungs exposed to 1% oxygen for 12, 24, 48, 72 and 96 hours, in order to determine if hypoxia could play a role in the development and maintenance of fibrosis. **Methods**: Healthy and fibrotic fibroblasts were cultured under conditions of hypoxia (1% O_2) during several periods and gene expression was measured by real-time PCR. Possible differences between experimental cells exposed to hypoxia and unexposed control cells were analyzed by a Student's t-test. **Results**: When comparing basal gene expression (0 hours) against the different exposure periods (12, 24, 48, 72 and 96 hours) of hypoxia in normal and fibrotic fibroblasts, we observed significant differences in the expression of ACTA, HIF1a and HIF2α ($p < 0.05$) whereas HIF3α expression was not observed in any condition. Additionally, we observed no significant increase in the expression of TGF-β1 in normal fibroblast in hypoxia, while significant differences were observed in fibrotic cells after 12 hours exposure. When corresponding exposure period groups were compared (normal vs fibrotic fibroblasts) significant differences in the expression of ACTA, HIF1a and HIF2α were observed, while no differences in TGF-β1 expression were found. Fibrotic fibroblasts express more ACTA, TGF-β1, HIF1α and HIF2α than normal fibroblasts when subjected to 1% hypoxia. Regarding protein levels it was observed by Western blot that the fibrotic fibroblasts express since the basal more α–SMA than the healthy and hypoxia at 48 hours increases slightly more. In healthy fibroblasts little protein is observed in the basal and a significant increase in hypoxia at 48 hours, but the increase is not major than in fibrotic fibroblast in normoxia and hypoxia. **Conclusion**: Hypoxia may play a role in the development of fibrosis probably involving overexpression of pro-fibrotic markers such as ACTA and TGF-β1.

SEPTIN CYTOSKELETON INTERACTS WITH NOX4 IN SPERMATOZOA OF GUINEA PIG

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Keywords: Sperm capacitation, NADPH oxidases,Septin cytoskeleton.

Septins are a family of GTP-binding proteins which form hetero-oligomeric high-ordercomplexes such as filaments and rings, they have been related withtheformation of scaffolds and diffusion barriers for sub-cellular compartmentalization. In the spermatozoa, a highly polarized and compartmentalized cell, septins were detected between the middle piece and the principal piece. For example,septin4 has been related whit bothdifferentiation andmechanical development of sperm in mice. Although somatic cells from mutant *ΔSept4* mice do not have a different phenotype from wild-type cells, the spermatozoa of these mice aremotionlesscells that lose their annulus and fail to reach the last step of the maturation process. In mature spermatozoa,septinshave also beenassociated with the formation of a membrane barrier in the annulus.

In a different process, the rice blast fungus infection, septins seems to interact and regulate the polymerization of both actin and tubulin cytoskeleton in somatic cells; it has been found that some NADPH oxidases (NOX's) are implicated in the septin-mediated cytoskeletal remodelingduringthis infection process. NOX's are proteins that produce reactive oxygen species (ROS) in a tightly regulated way. Members of NOX family have been found in a varietyof mammalian sperms in which they seem to play an important roleduring the correct capacitationprocess of these cells. Further investigation is necessary to identify the regulation mechanisms of septins and NOX's interactions in spermatozoa.

The aim of this project is toidentifyNOX's and septinsin guinea pig spermatozoa, and to find out if there is any direct interaction between them. Using WB, we detected Nox2and Nox4 as well asSeptin4 and Septin7. By immunodetection usinga gold-labeled antibody and transmition electronmicroscopy (TEM), we locatedSeptin4 and Septin7 inthe spermatozoa middle piece. The identified septins showed a different relative-mobility pattern after capacitation, which suggests that the modification of thecytoskeleton is necessary for a correct capacitation process. By immunoprecipitation, we also found that both identified septins interact with Nox4, by using Septin4, Septin7 and Nox4-labeled antibody columns. Interaction with Nox2 has not been tested yet.

CURCUMIN: A POWERFUL ANTIOXIDANT THAT REGULATES THE EXPRESSION OF PROTEINS IN THE LIVER OF DIABETIC *db/db* MICE

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Introduction. DM2 is a metabolic disease characterized by hyperglycemia resulting from defects in the secretion and/or action of insulin. In the liver, elevated levels of glucose and insulin modify gene expression. In Addition, DM2 is a model of oxidative stress. Curcumin is a powerful antioxidant and antidiabetic agent that regulates the gene expression of different signaling pathways through various transcription factors.

Objective. To evaluate the effect of curcumin on the protein expression profile in liver of diabetic *db/db* mice.

Methodology. Four groups of five mice, two healthy groups (WT) and two diabetic groups (*db/db*) were formed. Two groups were given curcumin (WT+C and *db/db*+C) at 0.75% w/w in diet. Liver proteins were separated by 2D electrophoresis. Differential protein expression analysis was performed on ImageMaster 2D Platinum software, proteins were identified by MALDI-TOF and subjected to enrichment analysis using STRING, DAVID and BRENDA databases.

Results. We found 41 proteins with differential expression change, of which 36 changed in the *db/db* group. Among the proteins modified in diabetic mice are the PRDX1 and GPDX1 -related redox regulation, as well as the TXNDC5 involved in cell apoptosis. Curcumin prevented the expression change of eight of the 36 proteins, and two appeared again. Curcumin decreased IDH3A and increased DHTKD1, involved in the Krebs cycle as well as carbohydrate, lipid and protein metabolism; Decreased PP1R14D which regulates glycogen metabolism; decreased MUP2 and MUP6 related to hormonal metabolic regulation, and increased SERPINB1 and PDX3X involved in the insulin signaling pathway.

Conclusion. Although curcumin had no effect on proteins related to redox regulation, it was able to prevent the change in the expression of proteins in the liver of diabetic mice, involved in the metabolism of carbohydrates, lipids, proteins, in the Krebs cycle, in regulation of glycogen metabolism and in the signaling pathway of insulin. The results shown in this paper highlight the beneficial effect of curcumin, and may serve to select molecular biomarkers useful for the diagnosis and treatment of DM2.

Keywords: curcumin, diabetes mellitus, proteomics.

MEASUREMENT OF ANTIOXIDANT CAPACITY IN AQUEOUS, ACETONIC AND ALCOHOLIC EXTRACTS OF LEAVES AND FLOWERS of *Cuphea wrightii*

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Key words: Antioxidant activity, medicinal plants, DPPH.

Introduction. The antioxidant capacity helps to reduce the pro-oxidative state inflicted by reactive oxygen species which are produced either from natural cell metabolisms or from external sources and increased in different chronic and degenerative diseases. Antioxidant compounds have received attention in recent years, particularly the ones from medicinal plants. Several studies reported about the bioactive compounds present in plants called phytochemicals, these are produced in the secondary metabolism. Among the different medicinal plants use in Michoacán is the *Cuphea wrightii* known in Spanish as "hierba de la calavera" and is used in for the treatment of several diseases. There are no information available concerning the compounds or its properties.

Objective. Evaluate the antioxidant capacity of *Cuphea wrightii* from the aqueous, acetone and alcoholic extracts.

Methods. Aqueous (AQE), acetone (ACE) and alcoholic extracts (OHE) of the leaves and flowers were obtained and evaluated the antioxidant activity using the 1-1-dyphenyl-2-picrylhydrazyl (DPPH) method (Bonet and Brand-Williams). Sample solutions were diluted in methanol. The absorbance was measured at 517 nm. The control used was ascorbic acid Results are presented as EC_{50} (µg/ ml): the concentration required to inhibit 50% of DPPH free radicals. T_{EC50} is the time needed to reach the steady state to EC_{50} concentration and AE (antiradical efficiency) obtained with the inverse of the EC_{50} multiplied by TEC_{50}.

Results. Flower AQE have the highest antioxidant capacity compared with leaves, when comparing the results with ACE and OHE, the strongest activity was obtained with leaves ACE and flower OHE respectively. Combination of leaves and leaves also exhibit high antioxidant activity.

Conclusions. The extracts analyzed from *Cuphea wrightii* exhibited a high antioxidant capacity. Individually, Flowers have the strongest antioxidant capacity. To our knowledge, this is the first report where this evaluation is reported.

DETERMINATION OF ANTIOXIDANT ACTIVITY OF EXTRACTS OF *Cordia elaeagnoides*.

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Keywords: Antioxidant activity, DPPH, Cúeramo.

Introduction: For decades, the direct relationship between overproduction of free radicals in the body and certain disease states as cancer, autoimmune disorders, rheumatoid arthritis, aging, cardiovascular and neurodegenerative diseases has been studied. The human body has several mechanisms to counteract oxidative stress through the production of antioxidants, these antioxidants act to prevent and repair damage caused by reactive oxygen species (ROS), and therefore can improve immune defense and reduce risk of cancer and degenerative diseases. Several compounds present in plant products have the property of acting as antioxidants. Among the most studied compounds are phenols, polyphenols and flavonoids. Their metal-chelating capabilities and radical-scavenging properties have enabled phenolic compounds to be thought of as effective free radical scavengers and inhibitors of lipid peroxidation.

Objective: Determine the antioxidant activity of aqueous, acetone and alcoholic extracts from different parts of the plant *Cordia elaeagnoides* (a medicinal plant used in the state of Michoacán).

Materials and methods: Aqueous, acetone and alcoholic extracts of leaves, flowers, seeds, bark and branches of *Cordia elaeagnoides*. The antioxidant activity was determined using the stable 1, 1-diphenyl-2-picril hydrazyl (DPPH) at 517 nm at different time intervals. The percentage of remaining DPPH obtained against the standard concentration was then plotted to obtain the amount of antioxidant necessary to decrease the initial DPPH concentration by 50% (IC_{50}). We obtained the time needed to reach the steady state to IC_{50} concentration (TEC_{50}). Also the antiradical efficiency (AE) was calculated by the following formula: $AE= 1/IC_{50}xTEC_{50}$.

Results: In all the different sections of the plant analyzed a greater antioxidant activity was observed in the aqueous extracts compared to the acetone and alcoholic extracts. The alcoholic extracts had higher antioxidant activity than the acetone extracts.

Conclusions: With the results obtained, the antioxidant activity of the extracts of the *Cordia elaeagnoides* is reported for the first time.

EFFECT OF A FUNTIONAL FOOD WITH RICE BRAN ON OXIDATIVE STRESS IN PERSONS WITH OVERWEIGHT AND OBESITY

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Keywords: Obesity, oxidative stress, antioxidants

Introduction: Several studies have showed that obesity is associated with oxidative stress and it is a factor to develop comorbidities like insulin resistance and Diabetes mellitus type 2 (DM2). For that reason it is important to develop strategies to reduce oxidative stress. Nowadays, it is known that the rice bran contains antioxidants, for example polyphenols and tocopherols. In this study, our **aim** was to determine if a functional food made from rice bran (marzipan) has the capacity to reduce the oxidative stress present in people with overweight or obesity. **Methods:** A functional food made with rice bran, in the form of a marzipan was crafted and given to 23 persons (14 male and 9 female) ranging from 22 to 53 years old, with a BMI >25, with no other comorbidities. Marzipan was given daily for 20 days. Before and after consumption of marzipan measuring anthropometric parameters (BMI, height, weight and waist circumference), biochemical parameters (total cholesterol, HDL cholesterol, triglycerides, fasting serum glucose, insulin) and biomarkers of oxidative stress (carbonyl groups, quinones groups, thiobarbituric reactive substances (TBARS), malondialdehyde (MDA), thiol groups (SH), arginase activity and Glutathione peroxidase (GSH-Px) activity) were evaluated in order to determine and analyze systemic oxidative stress state. Student´s *t-test* was used to determine differences statistically significant. P-values <0.05 were considered statistically significant. **Results:** Our results reveal a significant decrease on TBARS, MDA, carbonyl groups, GSH-Px and arginase activity. The concentrations of the SH groups showed no changes but quinones group increase. **Conclusion:** The functional food made with rice bran has the capacity to reduce the oxidative stress of people with overweight or obesity.

CHITOSAN-COATED GOLD NANOPARTICLES INDUCE REACTIVE OXYGEN SPECIES-DEPENDENT CELL DEATH IN LEUKEMIC CELL LINES

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Key words: Leukemia, regulated cell death, ROS

INTRODUCTION. Cancer represents a serious health problem around the world. It is known that first-line therapies are frequently characterized by affecting normal cells, moreover deregulations in tumor cells, such as their capacity to evade cell death, promote treatment resistance. Therefore, the development of new therapies that can be specific to cancer cells and overcome cell death resistance is necessary. Recent studies show that both gold nanoparticles (AuNPs) and chitosan have interesting biological activities including potential antitumor effects.
OBJECTIVE. Synthesis of chitosan-coated gold nanoparticles, and analysis of their effect in cell death of leukemic (K562 and CEM) and non-cancerous cells (PBMC).
METHODS. Chitosan-capped gold nanoparticles (chAuNPs) were synthethized by chemical method. Cell death was evaluated by cell morphology, phosphatidilserine exposure, and plasma membrane permeabilization in leukemic and Peripheral Blood Mononuclear Cells (PBMC). Expression of cleaved caspases 8, 9, and 3 were assessed by western blot. The dependence of caspases on cell death was assessed using a pancaspase inhibitor (Q-VD.OPH). The loss of mitochondrial membrane potential and ROS generation were measured using TMRE and DCFDA, respectively. Finally, the effect of ROS in cell death was assessed using the antioxidant N-Acetyl Cystein (NAC) as a ROS inhibitor.
RESULTS. Data shows that chAuNPs are cytotoxic in a dose-dependent manner in leukemia cells, while the show low citoxicity on PBMC. Additionally, they induce caspase activation, but the cell death is not dependent of caspases. They also induce mitochondrial damage and ROS production. Finally, our results also show that cell death and mitochondrial damage induced by chAuNPs are inhibited in the presence of NAC.
CONCLUSION. Altogether these results improve the knowledge of chitosan-capped AuNPs as selective cytotoxic agents, and open the way to the design of new pharmacological strategies that include these agents against cancer.

MODULATION OF REACTIVE OXIGEN SPECIES GENERATION AND THE PHAGOCYTIC PROCESS BY HEPATOCYTE GROWTH FACTOR IN ALVEOLAR MACROPHAGUES J774A.1.

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Keywords. Tuberculosis, Hepatocyte Growth Factor and Mycolic Acid. **Introduction.** The Tuberculosis (Tb) is a disease initiated by *Mycobacterium tuberculosis (Mtb)*. This disease is characterized by the formation of granulomes in lung tissue inhibiting gaseous exchange. Furthermore, *Mtb* have in its cell wall mycolic acids (MA) that provide it resistance and survival. MA are hidroxilipids with antigen capacity that are recognized for alveolar macrophagues for phagocytosis and bacterial elimitation. The phagocytosis is a specially endocytic process responsible in degradation of pathogens or strange bodies. NADPH oxidase is one of the main component in the process, especially the form Nox2. This complex is responsible for generation of reactive oxygen species (ROS) as superoxide anion radical ($O^{\cdot -}_2$). It is known that hepatocyte growth factor (HGF) is a modulator of the cell redox state, so in the present work our **Aim** was to determine if HGF improve and regulate the phagocytosis process and Nox2-derived ROS production in alveolar macrophagues in response to MA. **Material and Methods.** We performed neutral red assay for cell funtionality in the cell line J774A.1. We fixed optimal MA concentration at 250ng/mL. Moreover, we generated a plasmidic vector (pEGFP-Nox2) that contains Nox2 and the green fluorescence protein (eGFP). Cells were treated with HGF (50ng/mL). c-Met immunofluorescence, phagocytosis assay and ROS assay (DHE) in presence or not of HGF and MA, Western blot was performed following standard methods. Our **results** indicated that HGF increase the phagocytosis process and ROS production in macrophages at short times, in addition, the Nox2 transfected cells confirmed previous result at short times, since a loss in the location of Nox2 in plasmatic membrane was recorded at 15 minutes of treatment, and after 30 min it is relocated again in plasmatic membrane. Furthermore the Western blot indicate that the subunits of NADPH oxidase are increasing with the HGF treatment at long times indicating a better immune response. In **conclusion**, we are demonstrating that HGF is an immunomodulator agent in the phagocytic process and ROS production in the cells improving the phagocytic response against MA. Conacyt CB 252942

EVALUATION OF LIPID PROFILE AND OXIDATIVE DAMAGE IN HDL AND LDL OF WOMEN WITH PREECLAMPSIA

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KEYWORDS: Lipoproteins, oxidative stress, preeclampsia.

INTRODUCTION. Preeclampsia (PE) is a multisystemic syndrome that represents a public health problem. Its etiology is unknown; however, it has been proposed that abnormal implantation and maternal complications may be related to changes in lipid metabolism and an increase in oxidative stress (OS). OBJECTIVE. To evaluate the lipid profile and oxidative damage in HDL and LDL lipoproteins of women with preeclampsia. MATERIALS AND METHODS. We included 30 women without a diagnosis of PE (control) and 30 with PE. From plasma, the lipid profile was determined and HDL and LDL were isolated. In these lipoproteins were determined biomarkers of lipid damage: conjugated dienes (CD), malondialdehyde (MDA) and lipohydroperoxides (LHP) and biomarkers of protein damage: reduction of nitroblue tetrazolium (NBT), protein carbonylation (PC) and dityrosines (DT). The antioxidant activity of PON-I was evaluated in HDL. RESULTS. The PE group exhibited LDL levels that were 51.56% higher than the control group. The levels of total cholesterol, triglycerides, HDL, Apo-A and Apo-B did not present a significant difference between groups. The results shown in HDL and LDL of women with PE an increase in the concentrations of CD (23%) (18%), MDA (25%) (42%) and LHP (70%) (80%) respectively, compared with the control group. Even, an increase in NBT (34%) (27%), PC (41%) (34%) and DT (88%) (93%) was observed in HDL and LDL, respectively. The PON-I activity in women with PE decreased by 60%, compared to the control group. CONCLUSIONS. In women with PE, there is an evident lipid and protein damage in HDL and LDL, that can be caused by an increase in OS and by an inadequate antioxidant defense, translated as a decrease in PON-I activity. This process may be involved in the structural alteration of lipoproteins, which results in the formation of oxidized LDL that can generate damage in the vascular endothelium, characteristic of this syndrome.

EVALUATION OF THE PROCESS OF LIPOPEROXIDATION IN NEONATES OF WOMEN WITH PREECLAMPSIA

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KEYWORDS: Preeclampsia, Oxidative Stress, Neonates.

INTRODUCTION: Preeclampsia (PE) is a public health problem with unknown etiology; however, its close relationship with oxidative stress (OE) has been demonstrated. It has been reported that in plasma of women with PE, there is an increase of lipohydroperoxides (LHP) and malondialdehyde (MDA), both products of lipid oxidation, which promotes the vascular endothelium dysfunction. Currently, the term "Fetopathy by preeclampsia" has been coined, to the alterations observed in neonates of women with PE. At present, there are no studies that evaluate at the biochemical level, the impact that the PE generates in the neonates. OBJECTIVE: To evaluate the process of lipoperoxidation in neonates of women with preeclampsia compared with a control group. METHODS: We included 54 neonates (n = 27 represents the control group and n = 27 neonates born to women with PE). From the arterial cord blood, plasma was obtained, in which markers of oxidative damage to lipids (conjugated dienes (CD), LHP and MDA) and oxidative damage to proteins (protein carbonylation (PC)). Antioxidant defense was determined by the enzymatic activity of paraoxonase (PON-I) and total antioxidant capacity (CUPRAC). To determine the availability of nitric oxide, Nitrites and Nitrates were determined. RESULTS: Neonates of women with PE showed a significant increase in oxidative lipid damage, for CD 41%, LHP 38% and MDA 86%, for the CP increased by 42%, compared to the control group. The antioxidant defense presented an increase of 40% and 14% for CUPRAC and PON-I respectively. The Nitrites/Nitrates concentration presented a 57% decrease in the neonates of women with PE. CONCLUSION: OE present in women with PE affects the circulating lipids of the neonates, resulting in an increase in the markers of lipoperoxidation CD, LPH and MDA, the latter form adducts with proteins, which promotes the increase in the carbonylation of proteins. These oxidative modifications cause damage to the vascular endothelium, which affects the availability of nitric oxide that is reflected in the decrease of nitrites / nitrates, which could explain the activation in the antioxidant systems of the neonates, translated into an increase of CUPRAC and PON-I, so that in future research the use of an antioxidant therapy in women with PE is proposed to reduce OE in neonates.

EFFECT OF THE STATE OF CHRONIC OXIDATIVE STRESS ON THE P2X7 RECEPTORS AND THE METABOLISM OF GLUCOGEN IN HYPOCAMPUS OF RATS EXPOSED TO OZONE

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Keywords: Ozone, P2X7, glycogen

Environmental pollution by ozone is a health problem in highly populated cities. Exposure to this gas causes a state of oxidative stress, which is present in neurodegenerative diseases. In an oxidation-reduction balance, extracellular ATP activates nucleotide receptors found in the cell membrane, activating intracellular signaling pathways, which have a trophic effect. These pathways are involved in the development, growth, and proliferation of different cell types including neurons. In a chronic oxidative stress state, there is an increase in ATP extracellular levels, by changes in its metabolism within the cell. This promotes changes in cell signaling, which contribute to the progressive neurodegeneration process caused by chronic oxidative stress.
The objective of this work was to study the effect of the chronic oxidative stress state on the expression of P2X7 purinergic receptors, and changes in glycogen storage in rats hippocampal cells exposed chronically to low doses of ozone.
For this purpose, 72 male Wistar rats with free access to water and food were divided into 6 random groups (n = 12). Each group received one of the following treatments: Group 1) control (exposed to ozone free air), group 2, 3, 4, 5 and 6 were exposed to ozone (0.25 ppm for 4 hours daily from Monday to Friday), for 7 , 15, 30, 60 and 90 days respectively. Six rats from each group were processed for immunohistochemistry techniques, and the other 6 animals for Western blotting using the following antibodies: P2X7, GSK3β GS, and Pas staining.
The results indicate that the P2X7 receptor increases its expression at 60 days of ozone treatment, as well as, increases the phosphorylation of GSK3β protein at 90 days of ozone exposure ($p > 0.05$). Also, the exposure to ozone generates GSK3β kinase translocation to the nucleus at 90 days of exposure. Western blot results show a significant increase for P2X7 receptors at 60 ($p > 0.05$), as well as an increase in GSK3β phosphorylation from 15 days to 90 days of exposure.
In conclusion, chronic exposure to low ozone doses causes an increase in P2X7 receptors. Which induces GSK3β phosphorylation and its translocation to the nucleus, leading to a decrease in glycogen stores in rat hippocampal cells, like to what may be happening in Alzheimer's Disease.
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CONTROLLED AND UNCONTROLLED PHYSICAL ACTIVITY ON OXIDATIVE STRESS IN OLDER ADULTS WITH OBESITY

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Introduction

Human aging is a universal and inevitable phenomenon. Average life expectancy has improved in the last century. Currently 60-70% of older adults are independent, 30% are fragile or at risk and 3% are bedridden. However, the excessive increase in body weight of this population due to changes in eating habits and poor physical activity is one of the major problems. Overweight and obesity are a major risk factor for death, with a mortality rate of about 3 million adults per year. Physical exercise has a specific incidence preventing diseases and contributing to maintain motor independence and its biological, psychological, socioeconomic and affective benefits. Increased oxidative damage to biomolecules, insufficient repair capacity of lesions produced, and decrease in the level and activity of exogenous enzymes and antioxidants have been shown to contribute to the etiopathogenesis of chronic degenerative diseases. Therefore, the objective of this work was to determine the most effective activity, whether controlled or uncontrolled, to decrease the damage to biomolecules in obese elderly adults.

Material and methods

An experimental, randomized controlled clinical study was conducted, forming 2 groups of obese elderly adults with N = 7 each (women); Who underwent a physical activity program for 12 weeks. The first AFC group with elevated BMI, received controlled physical activity; And the second group with high BMI uncontrolled physical activity (AFNC). The two groups underwent anthropometric evaluation to determine body composition, strength, endurance and flexibility; In addition, a blood sample was taken. The determinations were before and after the physical activity program, to determine biomolecule damage by determination of 1.- Lipid Damage, Malondialdehyde (MDA). 2.- Protein damage. Quantification of carbonyl groups. 3.- Antioxidant activity. Total SH or thiol groups.

Results

A decrease in anthropometric and biochemical parameters was found; As well as an increase in muscle% in older adults who had controlled physical activity (AFC), likewise AFC showed a decrease in biomolecule damage in markers of MDA lipid damage and carbonyl groups as a marker of protein damage and An increase in the antioxidant defense determined by the SH groups, as opposed to NAFTA, which had no changes in their anthropometric parameters or markers of molecular damage.

Conclusion

Controlled physical activity is a strategy that improves the anthropometric, morphofunctional and biochemical parameters; And decreases the damage to biomolecules in obese elderly adults.

SARCOPENIC OBESITY IN ELDERLY ADULTS AND DECREASE OF OXIDATIVE STRESS AND RISK OF FRAGILITY WITH A PROGRAM OF EXERCISE

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Summary:

Obesity is observed in the aging process, sarcopenic obesity is a term that has been coined to describe the simultaneous occurrence of obesity in individuals with low muscle mass.

Objectives: To evaluate the effects of a sports medical program with isometric exercises on the production of ERO and muscle mass in adult {healthy and with sarcopenec obesity.

Method: Two groups of female adults aged 60 to 75 years were included. The first group consisted of theoretically healthy older adults (AMTS, n = 30) and the second group of older adults with sarcopenia obesity (AMOS, n = 30). Both groups underwent a sports medical program with isotonic exercises with progressive weight loads for 90 days practicing anthropometric assessments, body composition, strength, physical performance, reaction times, flexibility, as well as clinical biochemical profile and markers of oxidative damage (MDA, Carbonyls and SH groups) were determined before and after the intervention.

Results: The implementation of the sports medical program generated changes in anthropometric parameters, morphofunctional parameters and clinical biochemical variables. Lipid oxidative damage markers (MDA) decreased in both groups, the marker of protein damage increased in the AMOS group after the program. An increase in antioxidant defense systems was observed in both groups.

Discussion: the AMOS group was at an important degree of fragility at day 0 of the intervention; When implementing the sports medical program achieved an optimum redox state necessary to potentiate the diminished muscle mass and achieved to reverse the degree of fragility.

EFFECT OF MELATONIN ON OXIDATIVE STRESS MARKERS IN HEART, LIVER, KIDNEY AND BRAIN OF BALB/c MICE SUBJECTED TO CHRONIC SLEEP DEPRIVATION

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Keywords: oxidative stress, sleep deprivation, melatonin.

Introduction: Chronic sleep deprivation is an important factor in neurobehavioral, cardiovascular and metabolic morbidity in both developing and adult patients. Cellular protection is favored by melatonin, a hormone that besides regulating the sleep cycle has an antioxidant function. **Objective:** Evaluate the effect of melatonin on oxidative stress markers in brain, heart, liver and kidney of BALB/c mice under chronic sleep deprivation. **Methodology:** Sleep deprivation (SD)12:12h cycle with time inversion was performed using the multiple platform method and mice were divided into 3 groups (I, Control group; II, Sleep deprivation; and III, Sleep deprivation plus melatonin). Oxidative stress markers were quantified in tissue homogenates. **Results:** Lipoperoxides in the cerebral cortex of mice subjected to SD increased significantly compared to the control group and this increase is reversed by melatonin (MEL). While in other brain tissues no change was detected in the content of lipoperoxides, nitrate-nitrite, and membrane fluidity under the different treatments. Glutathione peroxidase activity in hippocampus and basal ganglia of the SD mice decreased significantly as compared to the control group, whereas in cerebral cortex no change was observed in any treatment. The content of lipoperoxides in comparison to the control group decreased in the group submitted to SD with MEL in heart and increased in the group submitted to SD in kidney; while in the liver did not exhibit changes in the different treatments. Membrane fluidity in the heart and kidney showed a significant increase in the MEL treatment group compared to the control group. While the liver showed no change in fluidity under the different treatments. Nitrate-nitrite content in heart and liver of mice submitted to SD with MEL treatment increased significantly compared to the control group. In the kidney, the significant increase was presented in the group submitted to SD and was reversed by the MEL. Glutathione peroxidase activity in heart and liver showed no change in any of the treatments, while, in kidney, this activity significantly decreased in the SD group with MEL treatment compared to the SD group. **Conclusion:** melatonin administration has beneficial effects on some OS markers in mice under chronic sleep deprivation.

EFFECT OF OXIDATIVE STRESS ON THE EXPRESSION OF INFLAMMATORY MARKERS IN HYPOCAMPUS OF RATS EXPOSED TO LOW OZONE

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Keywords: ozone, inflammation, neurodegeneration

Exposure to ozone caused by photochemical air pollution is a serious health problem in large cities. It has been shown that exposure to such gas causes a oxidative stress state. It is also known that neurodegenerative diseases present a oxidative stress state, which causes alterations in the response of the endogenous antioxidant systems, as well as a loss of regulation of the inflammatory process. In our laboratory, we have developed a noninvasive murine model of Alzheimer's disease. This model is produced by chronically exposing animals to low ozone doses, similar to what happens in a day of environmental contingency.

The objective of this study was to evaluate the effect of oxidative stress on peroxidized lipids, Mn-SOD activity, and expression of TNF alpha, COX 2, NFkB, IL2 and beta amyloid 1-42 in rats hippocampus chronically exposed to ozone.

For this purpose, 72 male Wistar rats with free access to water and food were randomly divided into 6 groups (n = 12), each group received one of the following treatments: 1) Control (ozone free air) 2) ozone 7 days, 3) ozone 15 days, 4) ozone 30 days, 5) ozone 60 days and 6) ozone 90 days of exposure (0.25 ppm for 4 hours daily from Monday to Friday). Two hours after the last treatment, the animals were deeply anesthetized, the hippocampus was obtained and 6 rats from each group were processed to evaluate oxidized lipids and Mn-SOD activity by spectrophotometry. By western blot, the levels of TNF alpha, COX 2, NFkB, IL2 and BA 1-42 were determined. The other 6 brains were processed for immunohistochemical techniques against the above-mentioned antibodies.

Results show an increase in lipid peroxidation from 7 days to 90 days of ozone exposure ($p < 0.05$). Mn-SOD activity shows an increase in its expression from 15 days to 60 days, and a decrease in its activity after 15 days of ozone exposure ($p < 0.05$). The TNF alpha, COX 2, NFkB, IL2 expression showed a significant increase ($p < 0.05$) from the 15 days of exposure and during the rest of the treatments to ozone. The number of cells expressing beta-amyloid 1-42 increases significantly at 30, 60 and 90 days of exposure to ozone in control comparison ($p < 0.05$).

With these results, we can conclude that the chronic oxidative stress state, caused by low doses of ozone exposure, leads to a loss of regulation of the inflammatory response and the expression of amyloid beta 1-42 like what happens in Alzheimer's disease.

This work was supported by DGAPA IN221417 to S.R-A.

EFFECT OF CONTROLLED PHYSICAL ACTIVITY ON COGNITIVE DETERIORATION AND OXIDATIVE DAMAGE IN ELDERLY ADULTS WITH MELLITIS TYPE 2 DIABETES

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Introduction

Diabetes Mellitus (DM) represents a leading cause of mortality in Mexico, and DM and hyperglycemia has been linked to oxidative stress as a triggering factor of cognitive impairment in older diabetics. On the other hand, regular exercise has been shown to be effective in improving glucose control, which could probably improve cognitive function. In addition to reducing the risk of dementia and Alzheimer's. The psychosocial benefits of exercise gain special prominence, combating isolation, depression, anxiety and favoring self-esteem and social cohesion.

Objective: The main objective of this study is to determine whether through programmed aerobic exercise accompanied by memory and association exercises can improve glycemic control in older adults with DM and improve cognitive impairment.

Material and methods: A prospective, longitudinal, controlled clinical study was conducted with n = 16 of older adult women diagnosed with type 2 diabetes mellitus, who underwent a program of controlled physical activity and memory and association exercise. His physical condition, anthropometry, was initially evaluated to determine: his body composition, strength, endurance and flexibility. In addition, a blood sample was taken before and 90 days after the program of controlled physical activity and memory and association exercises to determine the damage to biomolecules (malondialdehyde (MDA), protein damage, (quantification of carbonyl groups), Clinical biochemical variables (triglycerides, cholesterol, capillary glucose, glycosylated hemoglobin), antioxidant activity (SH or total thiol groups) and cognitive impairment (MMSE, MOCA).

Results: In older adults with DM, a reduction in weight and percentage of body fat was found, including a reduction in systolic blood pressure. In clinical biochemist's cholesterol and triglycerides were reduced as well as glucose and glycosylated hemoglobin levels. As for cognitive impairment, an or of 250mg / dl glucose was found. The physical activity program, memory and association exercises generated a better performance in the MMSE and MOCA tests.

Conclusions: Controlled physical activity, memory exercise and association improve glycemic levels (glycosylated hemoglobin) and clinical biochemical variables such as triglycerides and cholesterol, as well as anthropometric variables (weight and fat percentage) in diabetic elderly adults. Damage to biomolecules. There was also a significant improvement in the performance of the MMSE and MOCA tests at the end of the program.

Effect of linolenic acid (omega-3) on function and lipoperoxidation of liver mitochondria of rats during lactation.

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Introduction. The lactationis a fundamental period for the development of individuals, not only because of the higher cell plasticity during this period, but also because it is a period for the adaptation of the individuals and their metabolism to its environment. Currently, the omega-3 fatty acids (ω3)isrecommended not only as an antioxidant source, but also as promoters for brain development and plasticity in infants, however, this is a hypothesis not yet demonstrated. In recent researches the supplement of ω3 was given in the diet to individuals, it was reported adverse effects of these kind of fatty acids that point out that far from stimulating beneficial effects, they are promoting Reactive Oxygen Species formation and damage to the tissues. So, a fundamental question arises: What is the effect of ω3 in the generation of Reactive Oxygen Species and in the metabolism of mitochondria?. **Methodology**. Newborn Wistar ratas (10 males and 10 females) were used; 5 males and 5 females were supplemented with a daily doses of ω3 (125 mg/kg of body weight) during lactation. At 1 month-old, the animals were sacrificed, the liver was removed and the mitochondria obtained by centrifugations. It was measured the mitochondrial activity with a Clark-type electrode and MDA formation. **Results**. The results show that the animals supplemented with ω3, males and females,have a 65% higher concentration of MDAin liver mitochondria compared to controls without ω3. In male rats, the mitochondrial Respiratory Control is 60% lower than the control males, but in female rats there was an increment of 21% in the Respiratory Control compared with the respective female controls. A statistical difference $p<0.05$ was considered in both parameters.**Conclusions**. The results obtained show that the supplementation of ω3 during lactation induces the increase of Reactive Oxygen Species and consequently the lipoperoxidation in both, males and females. The results of oximetry show an alteration in the Respiratory Control in liver mitochondria; however, the Pearson Correlation analysis did not show an important association between these two variables. Therefore, it is possible that the changes in oxygen consumption are associated to changes in the composition and membrane fluidity of the mitochondrial membrane, possibly derived from the lipoperoxidation.

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NADPH OXIDASE ACTIVITY IS REQUIRED FOR EPIBOLY CELL MIGRATION IN ZEBRAFISH EMBRYOS.

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Keywords: ROS, zebrafish, Nox.

Introduction

NADPH oxidase (Nox) enzymes catalyze the formation of reactive oxygen species (ROS), superoxide and/or hydrogen peroxide. ROS are important signaling molecules involved in the regulation of major cellular behaviors such as cell migration. In zebrafish five *nox* genes have been reported *nox1*, *nox2*, *nox4*, *nox5* and *duox*, from which *duox* was found to participate in hydrogen peroxide formation during wound response using 3 days post fertilization fishes. However, detailed studies on the participation of *nox* genes in early zebrafish development are still lacking. During zebrafish gastrulation a major cell migration developmental process occurs, which is known as epiboly. During epiboly cells migrate covering the yolk cell from the animal pole to the vegetal pole. Recently, we detected a distinctive ring of deep blue formazan deposition at the epiboly leading edge in zebrafish embryos stained with nitroblue tetrazolium salt. This pattern indicates the presence of superoxide in the leading epiboly region. Due to the particular capacity of Nox enzymes to form superoxide; we propose that Nox are responsible for ROS formation at the epiboly leading edge and these molecules could participate in the control of cell migration.

Objective

Analyze the pattern of expression of *nox* genes during zebrafish development and characterize the effect of Nox activity knock down.

Methodology and Results

To characterize the expression of *nox* genes, RT-PCR reactions were carried out with RNA samples of zebrafish embryos obtained at different developmental stages. We found that *nox* genes present interesting temporal expression patterns suggesting their involvement in early development and in particular during gastrulation. To identify the Nox enzyme(s) that is (are) involved in superoxide formation during early gastrulation, we knocked down two genes, *duox* and the common regulatory subunit p22phox of Nox1, Nox2 and Nox4 with morpholinos. We found that neither morpholino alone affected epiboly. However when we coinjected both morpholinos we found that cell motility of epiboly is severely affected.

Conclusions

These results indicate that the activities of several *nox* genes are required for the cell motility and epiboly progression.

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EFFECTS OF AGED GARLIC EXTRACT ON NPY mRNA AND MARKERS OF OXIDATIVE STRESS IN HYPOTHALAMUS AND BLOOD OF DIABETIC RATS

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Keywords: Diabetes, oxidative stress, aged garlic extract.

Introduction: Diabetes mellitus is a chronic degenerative disease, with impact worldwide. It is characterized by hyperglycemia, this leads overproduction of Reactive Oxygen Species (ROS) and depletion of antioxidant defense system to generate oxidative stress, which is responsible of neuroinflammation and subsequent neurodegeneration, and progressive cognitive deterioration in diabetics. On the other hand, hypothalamus plays a central role on regulation of feeding behavior and homeostasis of glucose, it can be affected during diabetes. Neuropeptide Y (NPY) is a key molecule abundantly expressed in this region, this molecule has orexigenic activity, and therefore its increased expression in diabetes induces hyperphagia, which could be counterproductive for a diabetic patient. It has been shown that endogenous antioxidants are involved in NPY-mediated appetite control. Therefore, it is necessary to study the effects of exogenous antioxidants as Aged garlic extract (AGE) in relation to appetite control in diabetic rats. ***Objective***: Evaluate the effects of AGE on NPY mRNA level and markers of oxidative stress in hypothalamus and blood of diabetic rats. ***Methodology***: Male Wistar rats (10 weeks old) were used, and they were divided randomly into 4 groups: 1) Control, 2) Control with AGE; 3) Diabetic; 4) Diabetic with AGE. Diabetes was induced by treatment with streptozotocin (60mg/kg b.w.) freshly prepared in sodium citrate buffer administered intraperitonally (i.p.). The treatment with AGE (200mg/kg b.w.) was given via oral every day for four weeks. The superoxide dismutase 1 and 2 (SOD1 and SOD2), catalase (CAT), glutathione peroxidase (GPx) and NPY mRNA levels were measured by real-time RT-PCR, and ROS levels by flow citometry in hypothalamus. Markers of oxidative stress: Malondialdehyde (MDA) and carbonyl groups levels were measured in blood samples at the end of treatment. ***Results***: Diabetic rats showed increase of SOD2, CAT, GPx and NPY mRNA levels in hypothalamus compared with control group ($p<0.05$), and there was a tendency of MDA levels to increase. Administration of AGE decreases CAT mRNA levels ($p<0.05$), while, SOD2 and GPx mRNA decrease not significatively ($p>0.05$). NPY mRNA level shows a tendency to increase in hypothalamus. Markers of oxidative stress in blood not present important changes. The treatment of AGE in control rats, induces a decrease ROS level ($p<0.05$), and shown a tendency to increases SOD1, CAT and GPx mRNA levels. Moreover, it increases NPY mRNA level in hypothalamus ($p<0.05$), and tends to decreases MDA in blood. ***Conclusion***: These findings suggest that in this model of diabetes, the AGE administered via oral, could regulate the mRNA expression of antioxidant enzyme genes and it has an important effect on mRNA expression of NPY in hypothalamus.

CARDIAC HORMETIC RESPONSE IN AGED RATS, EVALUATED IN A HUNTINGTON´S DISEASE MODEL

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Administration of the mitochondrial neurotoxin 3-NP recapitulates many of the pathological characteristics in Huntington's disease (HD). 3-NP induces spontaneous bilateral striatal lesion and reactive gliosis, along with increased oxidative stress (OS). Its effect is linked to the irreversible inhibition of succinate dehydrogenase (SDH), complex II in the mitochondrial electron transport chain. Although HD is unequivocally a neurological disease, cardiac dysfunction is the second most common cause of death in these patients. Therefore, our goal was to evaluate whether cardiac dysfunction coexists with 3-NP-induced neurodegeneration and if this condition is aggravated during aging. We administrated tert-buthylhydroquinone (tBHQ) to induce the activation of the transcription factor Nrf2, in order to diminish 3-NP-induced oxidative damage. To accomplish these objectives, four groups of adult (9 months) and aged (24-months) Wistar rats were treated either with tBHQ (100 mg/Kg, 7 days, ip), 3-NP (10 mg/Kg, 2 doses per day/4 days, ip) or tBHQ+3-NP (100 mg/Kg+10 mg/Kg). At the end of the protocols, heart function, tissue morphology, oxidative stress markers and Nrf2 activation were evaluated. Both age groups developed HD neurological characteristics after 3-NP treatment. Changes in cardiac structure were associated with aging and fibers disarray were observed after 3-NP treatment in both groups; however, these conditions were not accompanied by cardiac dysfunction. In contrast with adult animals, in which the oxidation of proteins and lipids significantly increased, we observed an hormetic response related to chronic exposure to OS in the hearts of old animals, which was not surpassed by 3-NP intoxication. OS diminution was related with Nrf2 nuclear translocation, increased Nrf2-ARE binding and protein expression (γGCS y GST) in the tBHQ+3-NP adult group. In contrast, we found an unregulated antioxidant response in the aged group. These results suggest that aging is accompanied by a different redox-stress response, than that observed in younger individuals, and that the transcriptional regulation of the antioxidant genes in aging might depend on specific conditions and on particular stimulus.**Keywords:** Heart, aging, hormetic response

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TERT-BUTYLHYDROQUINONE (TBHQ) HORMETIC INDUCTION, AS A PROTECTIVE MECHANISM AGAINST FATTY ACIDS IN THE L6 RAT MYOBLAST CELL LINE

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The presence of saturated fatty acids has recently been related with the development and progression of sarcopenia, due to oxidative stress increase. In recent years, treatments have been developed to counteract oxidative damage. Among the most prominent, are those tratments that promote or induce an adaptive response to changes in the physiological or cellular homeostasis. A process that is based on these mechanisms is “hormesis”. Hormesis can be defined as “a process in which exposure to a low dose of a chemical agent or environmental factor that is damaging at higher doses induces an adaptive beneficial effect on the cell or organism”.

An interesting molecule that might activate an hormetic response due to its chemical-biological properties is the phenolic compound Tert-butylhydroquinone (tBHQ), which has been used in the food industry as an excellent food preservative. It has been shown that tBHQ treatments are capable of inducing the expression of different enzymes involved in detoxification and in antioxidant defense through the activation of transcription factor Nrf2.

In the present work, an hormetic model was developed in the rat myoblast cell line L6 in order to decrease the oxidative damage induced with palmitic acid, by pre-treating L6 cells with tBHQ at different concentrations (20, 50, 100 µM) and time points (6, 12, 18, 24 h). Our results showed that the best hormetic dose was 50 µM for 12 h. As a model of oxidative damage, sodium palmitate was used at concentrations of 0.25, 0.5, 0.75 and 1 mM on the L6 cell line. Fatty acids incorporation was corroborated with the red Oil technique. The results obtained in the present work will allow us to evaluate the antioxidant hormetic model that might be able to counteract the damage observed during aging in muscle tissue.

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PROTECTIVE EFFECT OF KAFIRIN PEPTIDES AGAINST OXIDATIVE STRESS OF HUMAN SKIN

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There is evidence that oxidative stress is involved in human skin lesions caused by UVB irradiation, which is mediated predominantly by reactive oxygen species (ROS) immediately after irradiation. Likewise, there is an antioxidant defense system limiting the noxious actions of these radicals; it can remove reactive species through enzymatic and non-enzymatic antioxidants. In this sense, the main compounds used in skin care are vitamins (A, B_3, C and E), polyphenols and carotenoids due to their antioxidant activity (AOX). However, peptides with AOX are not commonly used for skin protection. Thus, the aim of the present study was to assess the protective effect of peptide fractions obtained from white sorghum (*Sorghum bicolor* L. Moench) grain on the activity of endogenous antioxidant defense enzymes during oxidative stress in human skin cultures.

Sorghum kafirins (prolamin fraction) were extracted with *t*-butanol and were hydrolyzed with alcalase by using an enzyme/substrate ratio of 1.6 U/g protein for 9 h, at pH 7.5 and 50°C (Degree of hydrolysis = 18 %). For peptide separation, hydrolysates were fractionated through an ultrafiltration (UF) system with a M_W cut-off (M_WCO) of 3000 Da. Peptide concentration was determined by TNBS method. Antioxidant activity was measured by enzymatic activity of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) by the use of antioxidant assay kits. Skin samples (n=6) were obtained from healthy adults (25 – 35 years of age) and tissue cultures were prepared according to previously reported protocols. Four groups were assessed for each sample: non-UVB-exposed skin (negative control); UVB-exposed skin (positive control); non-UVB-exposed skin plus peptides (group 1) and UVB-exposed skin plus peptides (group 2). Prolamin extraction showed the presence of the three main Kafirins (α-, β- and γ-Kaf). UF achieved the separation of peptide fractions (PF; M_W < 3000Da), with 37.71 ± 3.69% of permeate with a peptide concentration of 818.56 ± 112.63 mg/mL eq L-Leucine. UVB exposed controls resulted in a reduction of (10 – 52 %) SOD, CAT and GPx activity in comparison to negative controls, which is due to the presence of oxidative stress.

However, when applying the PF, it was observed that the activity of the enzymes did not decrease (SOD, CAT and GPx), whereby the PF have a protective effect during the oxidative stress on the human skin caused by exposure to UVB irradiation. Our results show that fractions of sorghum peptides had a protective effect on the activity of antioxidant enzymes in the presence of oxidative stress. Therefore, PF derived from kafirins could are incorporated as ingredients in health skin products.

Keywords: oxidative stress, skin, peptides.

A NITRIC OXIDE SPIN TRAP SHORTENS SURVIVAL OF RATS WITH SEPTIC SHOCK, INDEPENDENT OF THE STEP OF SEPTIC SHOCK OF ADMINISTRATION

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The goal of the study was originally to make a time course of nitric oxide (NO) production in septic shock. Septic shock was induced in male Wistar rats (10-week old, 280-300g) through the intravenous administration of lipopolysaccharide (LPS, 6 mg/Kg). NO production was determined by electron paramagnetic resonance (EPR) and Griess reaction in blood samples taken before or 1, 2, 3 and 6 hours after LPS. The animals were divided into 4 groups for each phase (phase 1: survival, phase 2: sampling) two of the four groups were treated with the NO spin trap sodium diethyldithiocarbamate (DETC, 500 mg/kg, intraperitoneal), iron sulfate and sodium citrate (35 and 187.5 mg/kg, respectively, both subcutaneous) to form the adduct Fe-DETC-NO and increase NO EPR detection. This treatment was given at the same time (DETC-0) or three hours (DETC-3) after LPS. The other two groups were treated with LPS. DETC treatment significantly reduced the survival mean time: 11.49 h for ISS, 6.4 for DETC-0 and 5.0 for DETC-3 ($p<0.01$, Mantel Cox Test). NO converted in nitrate/nitrite was also reduced by DETC ($p<0.01$, two way ANOVA, Bonferroni post hoc). Fe-DETC-NO EPR spectrum was detected even two hours after DETC administration. Later, Fe-DETC-NO spectrum was slowly replaced by HbNO (adduct of hemoglobin and NO) spectrum. It is concluded that DETC effects on NO last longer that it was previously reported (up to 30 min). Even though it is thought that NO is needed in the first phase of shock (compensation) whereas its excess contributes to vasoplegia and multiple organ failure during decompensation, our results show that NO is needed in both phases.

CURCUMIN MODULATES MITOCHONDRIAL BIOENERGETIC ALTERATIONS AND REACTIVE OXYGEN SPECIES PRODUCTION IN CISPLATIN-INDUCED RENAL DAMAGE.

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Key words: Curcumin, cisplatin, kidney.

Introduction: Curcumin is a polyphenol extracted from the plant *Curcuma longa* rhizome that exhibits antioxidant and anti-inflammatory activity. On the other hand, cisplatin is widely utilized as quimioterapeutic agent for diverse type of cancer, but a side effect is acute kidney injury (AKI). Diverse mechanisms have been reported for this injury, such as oxidative stress, increase in the production of reactive oxygen species (ROS), inflammation, DNA damage and apoptosis, as well as mitochondrial damage. Previous studies have shown that curcumin can have protective effect; however, it is unknown whether curcumin protection it is related to attenuate hydrogen peroxide (H_2O_2) production and bioenergetics mitochondrial alterations.

Objective: To determine the effect of curcumin on mitochondrial bioenergetics in acute kidney injury induces by cisplatin.

Methods: We used 4 groups of Wistar rats (200-250 g) for the experiments. 1) Vehicle, 2) Cisplatin (CP), 3) CP and Curcumin (Cur) and 4) Cur. Rats were treated with curcumin (200 mg/kg/day) for 3 days and/or a single dose of cisplatin (5 mg/kg). Blood urea nitrogen (BUN) and creatinine were measured in plasma. In isolated mitochondria we measured oxygen consumption in state 3 and state 4, respiratory control index (RCI), ADP/O, hydrogen peroxide production, and mitochondrial membrane potential using as substrate malate-glutamate and succinate, furthermore to evaluating the activity of complex I.

Results: We showed that curcumin protects against cisplatin-induced AKI, which is related to decrease bioenergetics as oxygen consumption alterations in state 3, ADP/O and RCI. In addition, recover mitochondrial membrane potential and activity of complex I. Furthermore, curcumin reduced H_2O_2 production caused by cisplatin.

Conclusions: Curcumin can reduce alterations in mitochondrial bioenergetics and H_2O_2 production in the AKI induced by cisplatin.

DETECTION OF NITRIC OXIDE THROUGH EPR IN BLOOD AND BRAINS OF TYPE II DIABETIC RATS

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Key words: Diabetes, Nitric Oxide, EPR.

The prevalence of diabetes has dramatically increased worldwide due to the vast enhancement in the obesity rate. Nephropathy and cerebrovascular disease are two of the major complications of diabetes and the leading cause of end-stage renal disease and stroke respectively. Hyperglycemia seems to be 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) and reactive nitrogen species (RNS) seem to play a role in diabetic cardiovascular complications. Nitric Oxide (NO) has beneficial or detrimental effects depending on its concentration and the environment where it is produced.

The goal of the present study was to detect the systemic and cerebral NO by means of Electron Paramagnetic Resonance (EPR) in type 2 diabetic rats. Diabetes was induced by streptozotocin in Male Wistar rats. Eight weeks later, NO was significantly greater in blood of diabetic compared to normal rats, whereas this mediator was significantly reduced in diabetic compared to normal brains.

NO in blood is the sum of NO production in the entire organism. Even though the significance of our results is unknown we could postulate that a large quantity of systemic NO comes from organs where smooth muscle relaxation is massive in diabetes such as gastrointestinal tract. The reduction of NO in diabetic brains could explain cerebral deficit and brain vascular fragility in diabetes.

EFFECTS ON CANCER CELL PROLIFERATION AND ON MITOCHONDRIAL DYNAMICS OF THE PHARMACOLOGICAL INHIBITION OF THE ESTROGEN RELATED RECEPTOR ALPHA (ERRα)

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Estrogen related receptors are 3 transcription factors, ERRα, ERRβ and ERRγ, that have been studied in breast and ovarian cancer because of their homology with the estrogen receptor. The overexpression and/or increased activity of ERRα correlates with cancer progression. In turn, ERRα's inhibition or silencing decreases the proliferation of several cancer cell lines as well as tumor growth in animal models. In some studies decreased cell proliferation due to ERRα's inhibition has been linked to increased production of reactive oxygen species.
ERRα controls the expression of hundreds of genes involved in energy metabolism, including many genes involved in glicolysis, OXPHOS and mitochondrial biogenesis. Many cancer cells maintain high levels of glycolisis even in the prescence of oxygen. This type of metabolism is referred to as the Warburg effect and it is proposed to be an effective way by which tumors generate both ATP and biomass while being tolerant to hypoxia. However, a prominent function of ERRα is the induction of mitochondrial biogenesis, which would seem contrary to the Warburg effect. It is unclear if ERRα's ability to induce mitochondrial biogenesis is necessary for the cancer promoting functions of this transcription factor.
We evaluated whether the pharmacological inhibition of ERRα with its inverse agonist XCT-790 affects the ammount and morphology of mitochondria in ovarian and breast cancer cell lines. We also explored whether any mitochondrial effects correlate with the inhibition of cell proliferation that has been reported with XCT-790. We used two genomically related and aggresive cancer cell lines: the ovarian cancer cell line, SKOV3 and the triple negative breast cancer cell line, MDA-MB-231. Mitochondria were visualized with the fluorescent dye Mitotracker Red and evaluated in flow cytometry and fluorescence microscopy (Zeiss PALM, equiped with APOTOME). Cell proliferation and migration was evaluated in an Incucyte Zoom system (Bioessence). We found that inhibition of ERRα with XCT-790 reduces cell proliferation and induces extensive fragmentation of mitochondrial networks. Mitochondrial fragmentation could be upstream of the increased ROS production that has been reported with XCT-790. Our results furhter suggest that ERRα activity modulates mitochondrial networks in breast and ovarian cancer cells.

Keywords: ERRα, breast cancer, ovarian cancer, XCT-790, mitochondria, mitochondrial dynamics.

Denitrase activity of yeast *D. hansenii* tested in liver proteins of *M. musculus* with colitis

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Keywords: 3-nitrotyrosine, denitrase activity, *Debaryomceshansenii*

Introduction. In degenerative diseases such as Alzheimer's or diabetes mellitus type II or colitis have been reported conditions of oxidative stress and oxidation of biomolecules. Although mammalian cells have antioxidant systems to prevent, reduce or repair oxidation damage, there are irreversible oxidations such as 3-nitrotyrosine, which is formed by the reaction of peroxynitrite and tyrosine of proteins.It has been determined that the yeast *Debaryomyceshansenii* can degrade residual free 3-nitrotyrosine.**Objective**. Evaluation of the denitrase activity of *D. hansenii*in liver proteins of *Musmusculus* with colitis.**Methods and Results**. In liver protein extracts of *M. musculus* with and without colitis were measured ROSlevels.In mice with colitis significantly increased ROS levels up to two-fold in relation to the control (t-Student, $p<0.06$).The concentration of 3-nitrotyrosine decreased significantly in the liver protein extracts of *M. musculus* with colitis incubated with the yeast extracts(Mann-Whitney, $p<0.001$). The concentration of nitrites increases in the samples incubated with the yeast extract (Mann-Whitney, $p<0.001$),this result corroborates the degradation of 3-nitrotyrosine.**Conclusions**. The formation of 3-nitrotyrosine can be reversed by denitraseenzymes of *D. hansenii.***Acknowledgments.**This work was partially financed by the grant DGAPA-PAPIIT-IN226716 "Evaluation of genetic expression and detoxification of oxidation in yeast *Debaryomyceshansenii*: towards biotechnology in chronic degenerative diseases."

LOW INTENSITY TRAINING AS A PREVENTION OF SARCOPENIC OBESITY ASSOCIATED WITH AGING: A MODEL IN RAT

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Sarcopenic obesity (SO) is a medical condition which is defined as the presence of both sarcopenia and obesity. Sarcopenia is the gradual loss of strength, quality, and quantity of muscle during aging and obesity is associated with having an excess of body fat.
The increase in the adipose tissue with the decrease of the muscular mass initiates at 60 years of age and leads to a greater functional impairment that in each of the separated process (Rolland et al., 2009). Harmans theory of aging says that the accumulation of oxidative damage caused by free radicals generated throughout life produces greater susceptibility to present chronic diseases like SO in aged organisms. It is known that the generation of Reactive Oxygen Species (ROS) is three times higher in old mice (28 to 32 months old) than in young mice (10 months old), and this has been associated with 30% of the mass loss of the gastrocnemius muscle (Muller et al., 2007). According to the European Working Group on Sarcopenia in Older People (EWGSOP), the Dual-energy X-ray absorptiometry (DXA) is the gold standard for diagnosis for SO. Our interest was to establish a SO model using sedentary Wistar female rats of 4, 8, 12, 16, 20, 24 and 28 months old, and rats of the same ages under a low-intensity exercise regimen along with the life. We compared both groups by determinations of DXA, biochemical parameters, histological analysis, and oxidative protein damage. Our preliminary results, comparing both groups, showed significant differences ($p < 0.005$) in the composition of fat and muscle, protein oxidative damage and triglycerides levels. Hematoxylin and eosin stain revealed morphological changes in the proportion of fat and muscle in myocytes. These data indicate that physical training of low intensity throughout life is a good therapeutic strategy for the prevention of SO in comparison of a sedentary lifestyle. Our results provide a good model for the molecular study of SO and oxidative stress in aging that could be a used for the evaluation and comparison of therapeutic strategies for effective SO prevention.

Keywords: Sarcopenic obesity, protein oxidative damage, low-intensity exercise

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SENESCENCE ASSOCIATED SECRETORY PHENOTYE ANALYSIS IN RAT PRIMARY SENESCENT ASTROCYTES

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Keywords: Senescence, SASP, Cytokines.

Cellular senescence (CS) has been accepted asone of the hallmarks of aging, because iscontributes with the development of chronic illnesses. Senescent cells secrete a pool of cytokines, chemokines and growth factors with a proinflammatory profile known as Senescence Associated Secretory Phenotype (SASP) that is able to promote the deleterious effects observed in damaged or aged tissues.Recently, is has been suggested that the SASP of senescent astrocytes might be related withneurodegenerative diseases establishment. It has been hypothesized that lessen the pro-inflammatory character of the SASP could be helpful to prevent or alleviate the inflammatory state of some neurodegenerative diseases such as Alzheimer's and Parkinson's Diseases.

The aim of this work was to evaluate the ability of some anti-inflammatory molecules to modify the SASP secretion from senescent astrocytes in culture. Three potentially SASP modulators were chosen: Acetylsalicylic acid (AA), Dehydroepiandrosterone (DHEA) and Sulforaphane. The experiments were performed in rat astrocytes primary cultures and senescence was induced by oxidative stress (SIPS, Stress Induced Premature Senescence) and by proteasome inhibition (PIIPS, Proteasome Inhibition Induced Premature Senescence) using H_2O_2 and epoxomicin, respectively. Senescence was validated using some classical hallmarks: proliferation arrest, SA-βGalactosidase activity staining and cell cycle inhibitors overexpression. SASP analysis was carried out by Enzyme-Linked Immunosorbent Assay (ELISA) for IL-6 (the most common SASP factor) and by multiplex cytokine analysis for other 22 SASP components.

Our results showed that both stressors (oxidative and proteasome inhibitor) were effective to induce senescence in the primary astrocytes with similar characteristics concerning with the classical hallmarks. SASP obtained from SIPS and PIIPS astrocytes displayed subtle differences and the modulators reduced specific SASP components in each senescence type.

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EFFECT OF THE ETHANOLIC EXTRACT OF AVOCADO POLYPHENOLS ON THE RETICULUM ENDOPLASMIC STRESS OF HEK-293T CELLS STIMULATED WITH HYDROGEN PEROXIDE

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Key words: Polyphenols, BiP, ROS.

Introduction: Endoplasmic reticulum (ER) stress represents an accumulative of misfolding protein that causes activation of the unfolding protein response (UPR). Under stress conditions, the chaperone BiP/GRP78 is dissociated from PERK a transmembranal ER protein and triggering UPR pathway activation. Under hyperglucemic conditions like in diabetic disease, high levels reactive oxygen species (ROS) are produced. Stimulation of renal human cells HEK 293T in culture with hydrogen peroxide provoques a high BiP expression due to an increasing ROS leading to ER stress. The polyphenols are antioxidants compounds that reduce oxidative stress caused by ROS. We suggest that ethanolic extract of avocado polyphenols could reduce UPR activaction through decline BiP expression in culture cells HEK 293T stimulated with hydrogen peroxide. **Objetive:** To evaluate effect of the ethanolic extract of avocado polyphenols on BiP expression in cells HEK 293T. **Methods:** Renal human cells (HEK 293T) were incubated in D-MEM medium during 62 hours, 37 °C and 5% CO_2. After that, cells were treated with 50 and 250 µM of H_2O_2 by 4 hours. Afterward ethanolic extract of avocado polyphenols were added during 6 hours. After treatment, cellular homogenates were obtained and BiP expression was evaluated by Western Blot. The statistical analyses used were student's T-test for independent groups and ANOVA $p< 0.05$. **Results:** The ethanolic extract of avocado polyphenols reduced BiP expression in renal human cell stimulated with H_2O_2 ($p= 0.0019$ and $p= 0.0024$ respectively). **Conclusion**: Ethanolic extract of avocado polyphenols decreased activation of PERK pathway and relieve ER stress by a reduction of the BiP expression, suggesting its evaluation for diminishing oxidative stress under hyperglucemic conditions.

RESPONSE TO OXIDATIVE STRESS IN TWO SPECIES OF VAMPIRE BATS: *DESMODUS ROTUNDUS* AND *DIPHYLLA ECAUDATA,* AS A MODEL TO UNDERSTAND AGING.

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Aging is a natural process of all living things, which involves deleterious aspects and is a risk factor for the development of various diseases including diabetes and hypertension. Despite of the great efforts to understand the mechanism of aging and counteract its negative effects, this process has not yet been fully understood.
Oxidative damage and cellular responses to stressors have been related to alterations in physiological processes within cells and organs, resulting in the deterioration associated to aging. This kind of damage can be caused by an imbalance between reactive oxygen species (ROS) and antioxidant enzymes, which is called oxidative stress. Vampire bats are a very interesting model; they are long-lived species (8 - 20 years) compared to other mammals its size. Even though they feed exclusively on blood, which contains high levels of iron. Iron is a ROS inductor through the Fenton reaction, causing DNA damage. There are only three known species of vampire bats in the world and two of them live in Mexico, *Desmodus rotundus* that feeds on mammal's blood and *Diphylla ecaudata* that feeds on wild bird's blood. So it is interesting to study the mechanisms by which these species are able to counteract ROS damage in order to and live long.
In order to do it, DNA oxidative damage was quantified measuring 8-OH dG by HPLC-EC and protein oxidation was quantified measuring carbonyl formation by spectrophotometry. The GSH/GSSG ration was evaluated by HPLC-UV-VIS, along with antioxidant enzymes superoxide dismutase, catalase and glutathione peroxidase activity. All determinations were performed in liver, brain and intestine of those two vampire bat species. In addition, primary cultures of lung fibroblasts were established to determine cell resistance to stress.
Our results indicated that oxidative damage increased with age, which was not the case for the antioxidant enzymes.

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SPATIAL AND TEMPORAL EXPRESSION OF *p22phox* A NADPH OXIDASE ACCESORY SUBUNIT IN EARLY ZEBRAFISH DEVELOPMENT

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Keywords: ROS, zebrafish, Nox.

Introduction

Aerobic organisms generate reactive oxygen species (ROS) through different enzymatic activities. ROS are known to play a signaling role relevant for the control of different cell behaviors important for animal development. NADPH oxidases (Nox) are a set of enzymes specialized in the production of ROS that are conserved in eukaryotes. Nox complexes are formed by multiple subunits of which P22phox is required for activation of Nox1, Nox2 and Nox4 isoforms.

Objective

Analyze the expression pattern of *p22phox* gene in early zebrafish development and characterize the effect of activity loss of function.

Methodology and Results

Since the expression pattern of NAPDH oxidases accessory subunits might confer an important fine level of regulation for ROS production during animal development, we are characterizing the spatial and temporal expression pattern of *p22phox* gene during the first 24 hours of zebrafish embryo development. cDNA was generated from total mRNA obtained from whole embryos at different developmental stages and gene expression was characterized by RT-PCR reactions. *p22phox* was found to be maternally inherited and expressed through different developmental stages. Preliminary immunofluorescence microscopy characterization of P22phox protein localization, indicates that it shows dynamic patterns. Currently, we are performing different control experiments to validate the used P22phox antibody in zebrafish embryos.

Conclusions

p22phox is expressed and localized in early zebrafish development in a dynamic pattern that suggests its participation in determining the patterns of ROS accumulation in different embryonic regions and apparently in subcellular domains.

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ACTIVITY ANTIOXIDANT OF HYDROALCOHOLIC EXTRACT OF CALENDULA OFFICINALIS DETERMINING THE PROPORTION OF ITS MAIN COMPOUNDS

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In recent years the food industry has increased interest in plant foods also provide nutrients, bioactive compounds containing a beneficial effect in the prevention of certain diseases. These foods are called "functional foods". An example of functional food is the flower of *Calendula officinalis* is a plant of the family asteraceae, for the compounds phenolic, carotenoids, coumarins, saponins and other constituents have effects for health for example have property antiinflamatory, inmunoestimulation, antispasmodic, antiviral and quimioprotect. The extract was obtained from lyophilized petals and adding 20ml of 70% ethanol for 48 hours by maceration. The total content of phenols compounds was 335.5 mg of galic acid/mL for the method Folín Ciocalteu. For the activity antioxidant in aqueous-alcoholic extract of C. officinalis for ABTS and DPPH was 33.70µM/mL y 34.89 mM equivalent of trolox. Tests in vitro antioxidant activity with five positive controls which are EDTA, BHT, gallic acid, caffeic acid and quercetin and 5 dilutions for the hydroalcoholic extract were used. It is known that by evaluations extract has a capacity reduction as it has an ability to donate electrons and act as an antioxidant, since a greater percentage of activity compared to the positive control (BHT). It was observed that has a high ability to chelate copper, since the results are compared with similar positive control (EDTA), this neutralizes free radicals present. Iron chelation to the last dilution of the extract has a similar positive controls (EDTA and BHT) activity. Chromatographies were performed by TLC, HPLC-UV and HPLC-MS, for chromatography to TLC the following phenolic compounds catechin, quercetin, gallic acid, caffeic acid, vanillin and routine it was identified. For TLC his Rf were 0.87, 0.85, 0.78, 0.76, 0.87 and 0.84 respectively. For chromatography by HPLC-UV concentrations for phenolic compounds and carotenoid identified, for caffeic acid 2.41, coumaric acid 5.80, ferulic acid 19.40, gallic acid 3.22 p-coumaric acid 5.80, catequin 2.88, quercetin 13.97, routine 15.84, vainillin 5.67 and lutein reported as 7.2 ug / ml of extract they. With these results a cytotoxic effect on the cell line A549 (lung adenocarcinoma) is expected.

Keywords: Activity antioxidant, phenolic compounds, *Calendula officinalis*.

HYDROGEN PEROXIDE OF HEPATIC AND HEART MITOCHONDRIA IN DIABETES MELLITUS TYPE TWO IN ABSENCE OF MACROPHAGE MIGRATION INHIBITOY FACTOR (MIF).

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Key words: non-insulin dependent diabetes (NIDDM), macrophage migration inhibitory factor (MIF), hydrogen peroxide (H_2O_2).

Introduction: MIF is a proinflamatory cytokine expressed on different tissues; it participates in the immunologic and inflammatory response. Diabetic patients have high plasmatic levels of MIF in comparison to non-diabetic patients. Deletion of MIF reduces cytokines production and the diabetic manifestations are less severe, which indicates that inhibition of MIF production represents an alternative approach for diabetes treatment. The hepatic or heart failure is another alteration on diabetic patients and mitochondria have a central function in cellular energy production, participating in multiple metabolic pathways. Therefore any negative effect on mitochondrial function may affect cell viability.

Objective: The aim of this work is to evaluate the mitochondrial respiratory chain activity on a murine model of NIDDM in the absence of MIF. We investigated the production of H_2O_2 and the activity of complexes I, II, III and IV in isolated mitochondria from heart and liver and the effect of adding superoxide dismutase (SOD) and catalase (CAT) to the assay.

Methodology: The diabetes mellitus experimental model was established on male BALB/c mice (wild type; WT healty) using a 130 mg/kg dose of streptozotocin (STZ) and in knockout mice of MIF gene (*Mif*$^{-/-}$). Mitochondria were isolated from livers and hearts extracted from each group. Activity of mitochondrial complexes was measured spectrophotometrically with or without antioxidant enzymes. Hydrogen peroxide (H_2O_2) production was measured with Amplex red methodology in metabolically active (succinate) mitochondria.

Results: The physiological parameters showed an experimental induction of NIDDM. Blood glucose levels were maintained over 200 mg/dL in the diabetic group (WT/STZ) while in the other groups the blood glucose levels oscillated between 100-120 mg/dL. Activity of respiratory complexes was similar between groups in liver but in heart complex I was significantly different between WT/STZ and *Mif*$^{-/-}$, complex II had significant differences between *Mif*$^{-/-}$ and *Mif* -/- STZ and the highest activity for complex III was obtained in WT. We observed that presence of SOD y CAT in the assay caused a significant decrease of activity of the respiratory complexes of both mitochondria. *Mif*$^{-/-}$ liver mitochondria showed higher H_2O_2 production than WT mitochondria. This value was comparable to mitochondria of WT/STZ liver but it was lower for *Mif*$^{-/-}$/STZ. Inhibition of electron transport with rotenone, stigmatellin or antimycin had no effect on the H_2O_2 production, but it diminished when the three inhibitors were added simultaneously.

Conclusion: Absence of MIF does not increase blood glucose levels therefore DM2 or is milder in the *Mif*$^{-/-}$ STZ mice. These mitochondria produce a higher amount of H_2O_2 that seems to be modulating the cell against disease.

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THE ANTIOXIDANT SYSTEM AS THE KEY TOLERANCE MECHANISM DURING THE FIRST HOURS OF ALUMINUM TREATMENT IN *FAGOPYRUM ESCULENTUM* SEEDLINGS.

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Keywords: Aluminum, *Fagopyrum esculentum*, Antioxidant-enzymes

Aluminum toxicity is one of the major constraints for plant growth in acid soils. This stress factor causes mainly root inhibition especially during the first hours of exposure. *Fagopyrum esculentum*, var Mancan (Polygonaceae) regardless is an aluminum tolerant plant, during the first hours of exposure presents Al toxicity, primarily root growth inhibition and cell damage. In this study we hypothesized that during these early hours, *F. esculentum* cope with Al toxicity by regulating and enhancing its antioxidant system. First, we are evaluating the relative root growth (RRG) and the entrance of Al to the root in seedlings of 3 days old exposed to 50 μAl during 3, 6, 12, 24 and 48 h of exposure. We are measuring the following enzyme activity of cytosolic: catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GR) and ascorbate peroxidase (APX), as well as the levels of H_2O_2. Results showed that Al exposure inhibits radicle growth during the first 12 h (30 % RRG), but since 24 h hours the RRG started to be recovered (> 50%). Since 3h, Al penetrates to the root, however it reaches a maximum and at 24 h and 48 h it does not increase more. At the same time, CAT activity shows a significant increase at 12 h treatment, correlating with the recovery of growth and not entrance of Al. Currently we are determining the other antioxidant enzyme activities and the levels of H_2O_2. So far our results confirm that during the first hours of Al exposure there is damage in roots of *F. esculentum* seedlings, and that Al penetrates to the roots, but at 24 h the antioxidant system might help to cope the damage and seedlings can recover root growth rate.

OESTRADIOL REGULATES ANTIOXIDANT ENZYME ACTIVITY IN AN EXPERIMENTAL MODEL OF CEREBRAL MALARIA.

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Key Words: Malaria, Oxidative Stress, Sex Hormones

Introduction

Malaria is an infectious disease caused by the protozoa *Plasmodium.* In 2015, 214 million new cases and 458 thousand deaths. Malaria is a dimorphic disease, where males present more severe pathology than females. Sexual hormones may influence this phenomenon, because they are responsible of the main physiological differences between sexes. We hypothesise that sexual steroids can also modify the oxidative stress which is the main route of parasite elimination, the activity of antioxidant enzymes such as: catalase (CAT), glutathione peroxidase (GPx) and superoxide dismutase (SOD), which are responsible for the elimination of ROS and constitute a powerful antioxidant barrier.

Objective

To determine the effect of oestradiol on the activity of SOD, CAT and GPx, and on the concentration of malondialdehyde (MDA) in CBA/Ca mice infected with *P. berghei* ANKA.

Methodology

Groups of 5 mice, both male and female, were treated for 3 weeks with oestradiol at a dose of 12µg/50µL subcutaneously. Mice were then infected with $1x10^3$ erythrocytes parasitized with *P. berghei* ANKA. Parasitemia was evaluated in blood smears Giemsa stained. On the 8^{th} day post-infection mice were sacrificed and blood and brain tissues were extracted where the antioxidant activities of SOD, CAT and GPx were evaluated. The MDA concentration was evaluated as a measure of oxidative stress.

Results

Oestradiol decreased SOD activity in the blood of no infected intact female mice, which increased MDA levels. Infected female mice developed higher SOD activity than males in the same condition, administration of oestradiol increased CAT activity in intact infected female mice, thereby reduced the MDA concentration in blood.

In the brain, intact infected female mice displayed higher SOD activity than male in the same condition. Oestradiol did increased CAT and GPX activity in the brain of Gx infected mice.

Conclusions

Oestradiol enhances the antioxidant activity in females so it can prevent damage by ROS.

ROLE OF ROS IN THE REGULATION OF NEUROINFLAMMATION INDUCED BY EXCITOTOXIC DAMAGE IN CEREBELLAR GRANULAR NEURONS

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Key words: Excitotoxicity, NADPH oxidases, neuroinflammation.

Introduction: Excitotoxicity is the pathological process by which neurons are damaged and killed by an overactivation of the neurotransmitter glutamate, and it has been associated with several neurological diseases. This event triggers the production of reactive oxygen species (ROS) from different sources, among them, the members of the NADPH oxidases (NOX) family are important. There are evidences that ROS regulate some inflammatory mediators such as the inflammasomes, which are the responsible for the processing of proinflammatory cytokines. For instance, the NLRP3 inflammasome is responsible for the maturation of the cytokines IL-1β (proinflammatory) and IL-18 (anti-inflammatory). However, the process of activation and regulation of the inflammatory response by inflammasomes by excitotoxic damage, has not yet been clearly understood.

Objective: Demonstrate that production of ROS from NOX by an excitotoxic damage in neurons regulates the inflammatory process.

Methodology: Primary cultures of cerebellar granule neurons from Wistar rats of 7-8 days, were treated with glutamate (100µM for 20 minutes) after 8 days *in vitro*. Some cells were treated with a NOX inhibitor (DPI, 200 nM) or a mimetic of superoxide dismutase (EUK, 100 µM). After 1, 6, 12, 24 and 48 h of incubation, the production of ROS was determined by the administration of dihydroethidium (DHE) and the subsequent observation of the cultures by a confocal microscope. The activity of NOX and caspase-1 was determined by fluorescence spectrometry and the levels of IL-1β and IL-18 were assessed in cell supernatants by an ELISA test (Affymetrix eBioscience kit).

Results: The NOX activity showed a biphasic increase in response to glutamate administration, increasing after one and 48 h of treatment, which coincided partially with the levels of ROS. Moreover, the activity of caspase-1 showed an increase over time with a maximal at 48h that was not observed in the presence of DPI and EUK, suggesting that the activity of caspase-1 is regulated by ROS produced by a NOX. Finally, the production of cytokines showed a temporary production, with an increase of IL-1β at early times, and IL-18 at longer times. This profile of cytokines production was modified by the antioxidants.

Conclusions: The production of reactive oxygen species produced by NOX appears to regulate the activation and maintenance of the cytokine processing activity of caspase-1, which could suggest the involvement of a redox regulation of NLRP3 inflammasome by NOX during the inflammatory process induced by excitotoxicity.

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Accumulation of H_2O_2 in a non-lethal line of Arabidopsis with low polyamines levels.

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Key words: Arabidopsis thaliana, arginine decarboxylase, polyamines.
Introduction. Salinity represents one of the most serious problems for growth and development of plants. Plants have generated several strategies to ensure their survival. In a dynamic cellular scenario, the metabolites production is critical to reestablish the plant homeostasis under stress conditions**.** In particular, polyamines (PAs) are metabolites closely associated in the response and tolerance to adverse conditions. However, the stress response in plants when polyamines are decreased have been particularly difficult to study, due to failure for produce a PAs auxotrophic line. In a previous work, we reported the generation of a non-lethal line of *A. thaliana* (amiR:*ADC*-L2) who have a drastic decrease in PAs levels (Sánchez-Rangel et al., 2016).
Aim. Characterization of Aradidopsis amiR:*ADC*-L2 line under abiotic stress.
Materials and methods. Wassilewskija (Ws) parental and the amiR:*ADC*-L2 transgenic lines of A. thaliana were used. For the analysis of sensitivity to salt stress, 5-day-old seedlings of both lines were transferred to MS (control), MS + 200U/ml CAT, MS +150 mM NaCl, MS +175 mM NaCl, MS +150 mM NaCl + 200 U/ml CAT and MS + 175 mM NaCl + 200 U/ml CAT for a period of 7 days. Visualization of H_2O_2 production was performed by the test with 2',7'-dichlorofluorescein diacetate (DCFH2-DA) as described by Fu et al 2011. Cell viability analysis was performed in 10-days old seedlings of Ws and amir:*ADC*-L2 lines by staining with trypan blue.
Results. We observed an increment of sensibility of the amiR:*ADC*-L2 line under NaCl treatment, as compared to the WS parental.
In this sense, the seedlings of amiR:*ADC*-L2 line treated with 175mM NaCl showed a severe damage in cotyledons. In contrast, the amiR:*ADC*-L2 line exhibited a significant recuperation in fresh weight under the co-treatment with 175mM NaCl and 200U/ml Catalase. In addition, our results indicate that amiR:*ADC*-L2 line shows an increase of H_2O_2 under normal growth conditions. Likewise, the silencing line of ADC genes showing a decrease in cellular viability, as well as the induction of transcripts related to the response to oxidative stress.
Conclusion. These results show that the low PA levels in this Arabidopsis mutant line (amiR:*ADC*-L2) triggers the accumulation of H_2O_2 levels, which would explain the phenotypes of amir:*ADC*-L2, such as slow growth and sensitivity to stress.

ROLE OF ROS PRODUCED BY MITOCHODRIA AND NOX (NADPH-OXIDASE) IN APOPTOTIC DEATH OF CEREBELLAR GRANULE NEURONS.

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Keywords: Mitochondria, NADPH-oxidase, Neuronal death.

Introduction: It has been described that reactive oxygen species (ROS) play a role in multiple processes during physiological and pathological conditions. There are several sources that produce ROS in the cell, including xanthine oxidase, CYP450, the mitochondria and NADPH-oxidase (NOX). Recent lines of evidence show an interplay between different ROS sources, suggesting that ROS produced by the mitochondria induce the ROS production by NOX. One of the sources involved in apoptotic neuronal death is NOX; however, it is unknown if there is a crosstalk between ROS produced by the mitochondria and those produced by NOX. In cerebellar granule neurons (CGN) treated with staurosporine (ST) or potassium deprivation (K5), ROS production occurs at different times along of the death process, which is critical for apoptotic cell death of CGN.

Objective: Evaluate the mitochondrial ROS generation induced by two apoptotic conditions (ST and K5) and their possible role in the NOX-mediated ROS production.

Methods: Briefly**,** we used rat cerebelar granule neurons (CGN) cultured in a medium with 25mM of potassium (K25). Apoptotic death was induced after 7 days *in vitro* (DIV) by transferring the cultures to a medium with 5 mM potassium or treating cells with staurosporine (0.5μM). Under these conditions, we measured cytoplasmic ROS production by using dihydroethidium or mitochondrial ROS levels with Mitotracker red CM-H_2XRos. To evaluate cell viability we used calcein-propidium iodine stain. Mitochondrial activity was estimated by MTT reduction and the changes of mitochondrial membrane potential were determined with JC-1.

Results: ST or K5 treatment induced a significant increase of cytoplasmic ROS levels after five hours. Interestingly, we also found an early cytoplasmic ROS production (0-30 min), which was less extensive than that observed at five hours. Moreover, an early increase in mitochondrial ROS levels was observed under the apoptotic conditions. Also, ST and K5 induced a decrease in the mitochondrial membrane potential. On the other hand, antioxidants treatment partially reduced cell death induced by ST and K5.

Conclusions: These results suggest that early mitochondrial ROS produced during this process could participate in the neuronal death process mediated by ST or K5; we hypothesize that mitochondrial ROS induce the ROS production by NOX, which are the final mediator in this process.

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Hst1-Sum1 COMPLEX IS INVOLVED IN OXIDATIVE STRESS AND FLUCONAZOLE RESISTANCE IN *C. glabrata*.

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Key words: *C. glabrata*, oxidative stress response, Hst1-Sum1 complex

Abstract

Candida glabrata is a commensal yeast that can act as an opportunistic pathogen in immunocompromised patients. In the last years, there has been an increased incidence of *C. glabrata* as etiologic agent of candidemia. In *C. glabrata*, oxidative stress response and multidrug resistance are negatively regulated by the Hst1-Sum1 complex. Hst1 is a histone deacetylase that uses NAD^+ as cofactor and Sum1 is a DNA-binding protein. Deletion of *HST1* or *SUM1* (*hst1*Δ or *sum1*Δ) decreases susceptibility of *C. glabrata* to fluconazole and hydrogen peroxide. Expression of *PDR1* (transcription factor) and *CDR1* (drug efflux pump) mediates the fluconazole resistance in *C. glabrata*, whereas *CTA1* (catalase) and *MSN4* (transcription factor) are necessary to provide oxidative stress resistance. Previously, we have observed that decreased susceptibility of *C. glabrata hst1*Δ to fluconazole is affected by the growth media. We are interested in determining if the oxidative stress response mediate by *CTA1* and *MSN4* are also dependent on the growth media. We are evaluating if *CTA1* and *MSN4* expression are affected in rich media (YPD) or casamino-acids media. In addition, we are evaluating the fluconazole and hydrogen peroxide susceptibility in clinical isolates of *C. glabrata*.

"DESIGN AND CONSTRUCTION OF PLASMIDS FOR OVEREXPRESSION AND SILENCING *romo1* AND ITS EFFECT ON Huh-7 CELL LINE"

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Introduction. Mitochondria are the main reactive oxygen species (ROS) generator in cells.. Recently, it has been identified a new protein located in these organelles identified as Reactive Oxygen Species Modulator 1 (Romo1). Romo1 is a 79 aminoacids proteins encoded by *romo1* gene, the principal function assigned is the ROS regulation and its expressed in many tissues predominantly lungs, liver and kidneys. This protein is overexpressed in different cancer cell lines inducing an elevation in ROS levels, however the role and mechanism of action is not well understood. It is know that hepatocyte grown factor (HGF) is a modulator of cellular redox state by interfering with prooxidant systems such as NADPH oxidase. **AIM** In the present work we were focused to figure out if Romo1 is regulated by HGF and its receptor c-Met in the human hepatocellular carcinoma cell line Huh-7, based in plasmid constructions for overexpression or silencing by RNAi of *romo1* mRNA. **MATERIAL AND METHODS.** For overexpression of *romo*1 we use the vector pcDNA3.1+ (invitrogen), first the *romo1* cds was obtained by RT-PCR of RNA extracted from HepG2 cell line, due to pcDNA3.1+ vector does not any "tag" or signal that can be easily detected, has been designed the binding EGFP (enhanced green fluorescent protein) gene, downstream of *romo1* cds. For the *romo1* silencing by RNAi mechanism, we used the plasmid pSingle-tTs-shRNA (clontech), a pair of oligos were designed form a region of 22 pb *romo1* gene (s-romo1) and from this the antisense was obtained (as-romo1) in order to generate a shRNA. Both oligos were hybridized using the following conditions: 95ºC for 5 min, 2 cycles of 95ºC for 15 sec, 65ºC for 30 sec and 72ºC for 20 sec, and an final elongation at 72ºC for 5 min. The hybrid product generated *Xho*I sites at the 5´ end and *Hind*III site at the 3' end. This product was cloned into the pSingle-tTs-shRNA vector. All ligation products were transformed into *E. coli* Stabl3 cells and selection on LB plates with ampicillin (100ug/ ml). Transformants were analyzed using the miniprep kit (Promega) and by restriction analysis depend of the plasmid was the selection. The plasmids for overexpression and silencing were transfect each one of them in Huh-7 cells line with Lipofectamine 2000. **RESULTS.** Our data demonstrated by confocal microscopy, that both constructions were functional, Romo1 was overexpressed and this expression was localized in mitochondrial membrane. The *romo1* silencing was checked out in Huh-7 cell line, and their expression was depleted 33% with Dox addition. By other hand, we treated the Huh-7 with 50 ng/ml for 5, 15, 30 and 60 min of HGF, the results indicated that *romo*1 expression is stimulated by HGF addition from 5 min and this effect was maintained up to 60 min. Plasmid-based strategy designed in this work will be used for a complete characterization of HGF-induced redox regulation in a Romo1-dependent process. CONACYT No. 222578.

EVALUATION OF THE ACTIVITY OF ACHIOTE EXTRACTS FROM YUCATAN ACCESSIONS ON THE *C. elegans* MODEL

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Keywords: Achiote, Antioxidant Capacity, *C. elegans*

Introduction. Achiote (*Bixa orellana*) is a shrub native to South America. In Mexico, it is manly grown in Quintana Roo, Tabasco and Yucatan. It is possible to extract important industrial and biological compounds from its seeds, main among which are the carotenoids bixin and norbixin, which have shown to have antioxidant activity. Furthermore, achiote is the main natural source of tocotrienols, which are compounds of the vitamin E family, that possess higher antioxidant activity than tocopherols.

Objective. Determine the effect of achiote lipophilic extracts on the lifespan and heat and oxidative stress resistance in the *C. elegans* nematode model.

Methods. Ground achiote seeds were extracted with ethyl lactate; the lipophilic compounds were precipitated with water, dried and resuspended in ethanol 96% (v/v). 65, 100 and 170µg/ml of extract in NGM/OP50 plates were tested. For the stress assays, the nematodes were pre-treated for 48h at 20°C with extracts. Heat stress was performed at 35 °C and oxidative stress with 1mM H_2O_2. The survival rate of the worms was measured in the treatments and the controls.On the other hand, the effect of 170µg/ml of extract on the lifespan was determined.

Results. It was observed that achiote extracts at all tested concentrations had a protective effect against oxidative stress, increasing the survival between 20% to 35% in comparison with the control. In the case of heat stress, the concentrations of 100µg /ml and 170µg/ml increased the survival 40 and 50% respectively, while 65µg /ml concentration had no significant effect. The average half-life increase conferred by the 170µg/ml extract was 28%.

Conclusions. Achiote seed extracts protected the *C. elegans* nematodes against heat and oxidative stress. Extracts also increase the lifespan of the nematodes in the survival curve.

CHARACTERIZATION OF STRESS RESPONSE IN THE OPPORTUNISTIC PATHOGENIC FUNGUS*CANDIDA GLABRATA* WITH THE ABSENCE OF PEROXIREDOXINS TSA1 AND TSA2

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Key words: peroxiredoxins, ROS, resistance.

*Candida glabrata*is an opportunistic pathogenic yeast becauseit can cause candidiasis in immunocompromised patients. It has special features that make it resistant to the immune response of the human body as its ability to form biofilms, innate resistance to azoles and its high resistance to oxidative stress. Particularly, we are interested in systems that help it to withstand reactive species. We know that *Candida glabrata* has enzymatic and non-enzymatic systems for such action, in addition these systems can be found in nucleus, cytosol and mitochondria. Our work has focused on an enzymatic system: peroxiredoxins. In *Candida glabrata* there are three peroxiredoxins: Tsa1, Tsa2 and Aph1. We focused on Tsa1 and Tsa2; weevaluated the induction of the gens individually as well as their sensitivity to some oxidative agents.

The objectives of our work are knowing if these enzymes are induced under the presence of oxidative agents and if so, whether they confer resistance to reactive oxygen species.

To determine the induction, we performed cytometric assay under different mutant backgrounds:*yap1Δskn7Δ, msn2Δmsn4Δ, yap1Δskn7Δmsn2Δmsn4Δ*, and the simple mutants. To evaluate the sensitivity to oxidative agents'spot assayswere performed in different phases (logarithmic and stationary) and with different exposure times (acute and chronic).

The cytometry of tsa1 and tsa2 under the background of the wild-type strain tells us that the gene is induced with oxidizing agents after 2 hours, but when performed under single, double and quadruples mutants of some transcriptional factors the result obtained is variable. On the other hand, the spot assays in the single and double mutants are observed equal to the wild-type strain. For the mutants under the background of *cta1*Δ are observed slightly more resistant than the simple mutant *cta1*Δ and the triple mutant is more resistant than its parental,*cta1*Δ mutant.

We can conclude that the peroxiredoxin genes are induced in the presence of oxidizing agents and the transcriptional factors that regulate other systems against oxidative stress may not be directly related to our genes. Also, we observe that these genes are not essential to the response against reactive oxygen species, but may be interacting directly with the other systems which increase or decrease resistance to these agents.

DISRUPTION OF RRM75 GENE IN *Ustilago maydis* SHOWS TEMPERATURE STRESS-SENSITIVE AND ACCUMULATES H_2O_2.

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Keywords: RNA binding proteins (RBP), Ustilago maydis, ROS

Introduction: Eukaryotic organisms have the ability to respond to the stress through RNA binding proteins (RBP). In fungi, the RBP regulates very important biological functions, such as metabolite signaling, mating, transport, and development. In *Ustilago maydis,* we identify the *UmRrm75* gene that encodes a protein with an RNA binding domain (RRM). The null mutant strains of the *UmRrm75* gene, exhibited phenotypic alterations, such as slow growth, donut type morphology, as well as a reduction in mating rate and virulence ability.
Aim: We analyze whether the UmRrm75 protein displays RNA chaperone activity. In addition, we analyze the thermosensitivity of the null mutant strains and their peroxide accumulation.
Method: We used FB2, 1/2 and SG200 parental strains and null mutant strains 1/46, 1/40 and 1/53 of the *UmRrm75* gene. For RNA chaperone activity of the UmRrm75 protein, we used strain RL211 as a system to evaluate the anti-termination transcript activity (Landick et al., 1990) and strain BX04 which lacks RNA chaperones and is unable to grow at 15 ° C (Xia et al., 2001). For thermosensitivity test, four serial dilutions (10^{-2} to 10^{-5}) of the parental and mutant strains were subjected to 28°C, 37°C and 15°C temperatures for 6 days (CM Holliday, 1975). We analyzed the *UmRrm75* and *Yap1* transcripts in FB2 parental strain using qRT-PCR under optimum conditions and abiotic stress treatments. The generation of H_2O_2 in parental and null mutant strains was performed by test with 2-7 dichlorofluorecein diacetate (DCFH2-DA) as described by Fu et al. 2011)
Results: The transcriptional analysis in parental strain FB2 revealed that *UmRrm75* gene exhibited a high induction by stress conditions. Through the heterologous system in *E. coli*, we observed that the *U. maydis* UmRrm75 protein exhibited RNA chaperone activity and anti-transcription terminator activity. When we analyzed the null mutant strains in non-optimal conditions, we detected a severe growth decrease. Interestingly, we noticed that Δ*UmRRm75* null mutant strains accumulate a brown pigment under optimal and non-optimal conditions. However, the production of pigment in null mutant strains could refer to the presence of ROS. The 2,7-DFCHA assays showed that the null mutant strains accumulate more H_2O_2 in optimal conditions compared to parental strains; then, in stress conditions, it was observed a high accumulation of H_2O_2. In this sense, we observed that the null mutant strains induced expression of the *Yap1* gene, a sensor redox, which active genes from detoxification enzymes.
Conclusions: These results show that UmRRm75 protein has a chaperone activity and the null mutant of UmRrm75 gen is temperature sensitive and accumulates H_2O_2. Our next step will be identify the relationship between the *UmRrm75* gene and ROS accumulation.