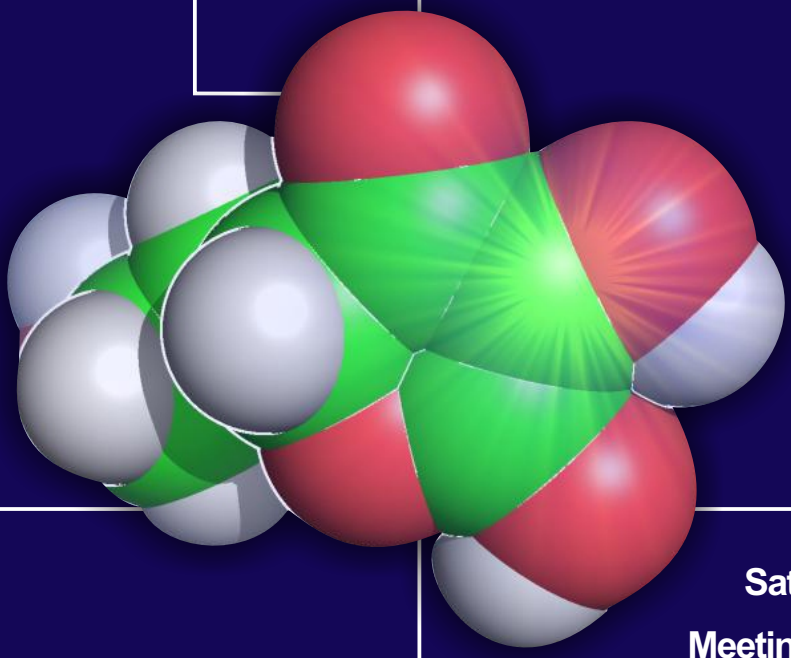
Antioxidants and Redox Process in Health

Bilateral Meeting Brazil - Japan

Universidade de São Paulo
Instituto de Química
Departamento de Bioquímica



Brasiliana **USP**

São Paulo, Brazil
October 21 to 22, 2013

Satellite Meeting of 6th ICPH and VIII Meeting of the SFRBM - South America

Antioxidants and Redox Process in Health 2013

Program and Abstracts

Antioxidants and Redox Process in Health

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Satellite Meeting of 6th International Conference of Polyphenols and Health (ICPH) and VIII Meeting of the Society for Free Radical Biology and Medicine (SFRBM) - South America

Program and Abstracts

Universidade de São Paulo
Instituto de Química – Departamento de Bioquímica
Brasiliana USP
São Paulo, Brazil
October 21 to 22, 2013.

Cover Page: Alexandre Takashi Bando
Diagramming: Cynthia Sayuri Bando
Logo and homepage: Leandro de Rezende

Dear Participants

On behalf of the organizing committee, we sincerely welcome you to the Antioxidants and Redox Process in Health – Bilateral Meeting Brazil Japan and Satellite Meeting of 6th International Conference of Polyphenols and Health and VIII Meeting of the Society for Free Radical Biology and Medicine-South America, to be held in São Paulo, Brazil, October 21 to 22, 2013.

Brazil has an enormous biodiversity, where the potential pharmacological/therapeutic properties of antioxidant compounds isolated from their biome is still little explored. In contrast, the area of redox process has a consolidated excellence in Brazil and Japan has a long tradition on research of antioxidants and other phytochemicals with therapeutic potential.

The objective of this event is to bring together Japanese and Brazilian researcher to discuss the latest advances in the area of antioxidants and redox processes. We hope this meeting stimulate and accelerate collaborations and joint research efforts between Brazilian and Japanese research groups.

We look forward to welcoming you in São Paulo.

The Organizing Committee

Chair: Sayuri Miyamoto, Universidade de São Paulo, Brasil
Co-Chair: Junji Terao, The University of Tokushima, Japan
Paolo Di Mascio, Universidade de São Paulo, Brasil
Marisa H. G. Medeiros, Universidade de São Paulo, Brasil
Yoji Kato, Hyogo Prefectural University, Japan

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Scientific Program

October 21st, 2013	
8:30	**Opening address**
	Chair: Paolo Di Mascio, University of São Paulo, Brazil
8:45-9:30	**Plenary Lecture: Helmut Sies, University of Düsseldorf, Germany**
	"Some Reflections on Oxidative Stress and Redox Biology"
9:30-9:55	**Session one: Brain/neurodegenerative diseases/inflammation**
	Chair: Junji Terao, The University of Tokushima, Japan
	"Role of dietary flavonoids on oxidative stress in the central nervous system"
9:55-10:15	De-Xing Hou, Kagoshima University, Japan
	"Garlic organosulfur compounds as a potential anti-inflammatory agent: molecular and animal evidence"
10:15-10:30	Ana Helena S. Oliveira, Federal University of Rio Grande do Norte, Brazil
	"Proteomic analysis in response to vitamin b6 therapy during oxidative stress induced by Pneumococcal meningitis in rats"
10:30-11:00	**Coffee break**
	Session two: Advanced analytical strategies for oxidant and antioxidant analysis
	Chair: Sayuri Miyamoto, University of São Paulo, Brazil
11:00-11:25	Norberto Peporine Lopes, University of São Paulo, Brazil
	"Advances in MALDI-MS/MS for polyphenols analysis"
11:25-11:45	Ana Paula Loureiro, University of São Paulo, Brazil
	"Saliva as a promising matrix for biomonitoring of redox-modified DNA bases"
11:45-12:00	Priscila R. Santos, University of São Paulo, Brazil
	"Behavior of the thermal diffusivity of native and oxidized human low-density lipoprotein solutions"
12:00-14:00	**Lunch**
14:00-14:25	**Session three: Vascular function and metabolic syndrome**
	Chair: Francisco Laurindo, University of São Paulo, Brazil
	"Nox Family NADPH oxidases in vascular cells: mechanisms and therapeutic implications"
14:25-14:45	Naomi Osakabe, Shibaura Institute of Technology, Japan
	"Flavan 3-ols bioactivities on metabolic syndrome-a new angle of observation"
14:45-15:05	Yoshichika Kawai, Nagoya University, Japan
	"Specific localization of quercetin glucuronides in macrophages: new insights into the biological actions of dietary flavonoids at sites of inflammation"
15:05-15:20	Alexandros Papadimitriou, University of Campinas, Brazil
	"Theobromine cocoa prevents extracellular matrix accumulation via activation of NAD+-dependent deacetylase sirtuin-1 in experimental Diabetes mellitus"
15:20-15:35	Eliziane Patricio, University of São Paulo, Brazil
	"Underlying the pro-oxidant effects of urate hydroperoxide on redox modulated proteins: a pivotal role in inflammation"
15:35-16:05	**Coffee break**
	Chair: Paolo Di Mascio, University of São Paulo, Brazil
16:05-16:35	**Special Lecture: Prof. Jean Cadet, CEA-Grenoble - France**
	"Oxidatively generated damage to celular DNA"
16:35-18:50	**Poster presentation**
19:00-21:30	**Coquetel/Banquet with Brazilian Music at Institute of Chemistry**

October 22nd, 2013	
8:45-9:10	**Session four: Redox signalling/Cancer/System biology**
	Chair: Hitoshi Ashida, Kobe University, Japan
	"Effects of flavonoids on the expression system of drug-metabolizing enzymes"
9:10-9:30	Glaucia R. Martinez, Federal University of Paraná, Brazil
	"Effects of flavonoids and melanin on DNA oxidation by singlet molecular oxygen"
9:30-9:50	Kayoko Shimoi, Shizuoka University, Japan
	"Chemopreventive effects of flavonoids on the breast cancer development"
9:50-10:10	Diego Bonatto, Federal University of Rio Grande do Sul, Brazil
	"Toxicological and oxidative stress effects of the different substances in tobacco smoke on human embryonic development by a systems chemo-biology approach"
10:10-10:25	Juliana D. Zeidler, Butantan Institute, Brazil
	"PMA triggers oxidative stress and apoptosis in H-RasV12-dependent human malignant keratinocytes"
10:25-11:00	**Coffee break**
	Session five: Carotenoids and other phytochemicals
	Chair: Paolo Di Mascio, University of São Paulo, Brazil
11:00-11:25	Delia Rodrigues Amaya, University of Campinas, Brazil
	"Processing effects on food carotenoids: Impact on human health"
11:25-11:45	Koichi Aizawa, Kagome Company, Japan
	"Enhancement of in vivo radical reducing ability of lycopene"
11:45-12:05	Lydia Fumiko Yamaguti, University of São Paulo, Brazil
	"Occurrence and biosynthesis of biflavonoids"
12:05-12:25	Dulcineia Abdalla, University of São Paulo, Brazil
	"Identification of microRNAs involved in the antiangiogenic activity of polyphenols"
12:25-14:00	**Lunch**
14:00-14:25	**Session six: Stress response/Adaptation/Proteasome**
	Chair: Akira Murakami, Kyoto University, Japan
	"Low and medium but not high doses of green tea polyphenols ameliorate dextran sodium sulfate-induced hepatic and kidney dysfunctions "
14:25-14:45	Hirofumi Arai, Kitami Institute of Technology, Japan
	"Anti-allergic functions of polyphenols"
14:45-15:05	Marilene Demasi, Butantan Institute, Brazil
	"Redox Control of 20S Proteasome Gating"
15:05-15:20	Kaio Vitzel, University of São Paulo, Brazil
	"The involvement of oxidative stress in skeletal muscle dysfunction of streptozotocin-induced diabetic rats"
15:20-16:00	**Coffee break**
16:00-16:25	**Session seven: Biomolecule damage and biomarker analysis**
	Chair: Yoji Kato, Hyogo Prefectural University, Japan
	"Detection of protein tyrosine modifications by mass spectrometry and antibodies"
16:25-16:45	Sayuri Miyamoto, University of São Paulo, Brazil
	"Lipid hydroperoxides: singlet oxygen generation and protein modification"
16:45-17:00	Thiago C. Genaro-Mattos, University of São Paulo, Brazil
	"Cytochrome C adduction promoted by cholesterol derived aldehydes – implications to protein-membrane binding"
17:00-17:45	**Chair:** Marisa H.G. Medeiros, University of São Paulo, Brazil
	Plenary Lecture: Ohara Augusto, University of São Paulo, Brazil
	"Oxidative mechanisms in protein aggregation"
17:45	Closing remarks

Plenary Lecture
(PL-01)

SOME REFLECTIONS ON OXIDATIVE STRESS AND REDOX BIOLOGY

Helmut Sies

Institute for Physiological Chemistry I

Heinrich Heine University Düsseldorf (Postfach 10 10 07, D-40001 Düsseldorf)

Recent advances in noninvasive monitoring of cellular and subcellular redox processes allow addressing exciting new questions in redox biology. The lecture will attempt to bridge these new developments to the longstanding research endeavors in this field, characterized by the terms of oxidative stress, redox signaling, bioactive phytochemicals and redox switches. Differences and common properties of two major redox systems, NAD and thiol systems, will be discussed, pertaining to thermodynamic and kinetic control of biological redox processes.

Keywords: oxidative stress, redox biology, redox signaling, redox switches, phytochemicals

Special Lecture
(SL-01)

OXIDATIVELY GENERATED DAMAGE TO CELLULAR DNA

Jean Cadet

CEA - Institut Nanosciences & Cryogénie (Grenoble), Dép de Médecine Nucléaire et Radiobiolog - Université de Sherbrooke (Sherbrooke, Quebec)

More than the 100 oxidized purine and pyrimidine nucleosides including hydroperoxide precursors and diastereomers have been characterized so far in model studies. However, much less information is currently available on the mechanisms of hydroxyl radical (•OH) and one-electron oxidation-induced degradation of bases in cellular DNA mostly due to analytical difficulties. At the present, using the accurate and sensitive HPLC/MS/MS assay, 11 single modified nucleosides and bases were found to be generated in cellular DNA upon exposure to gamma rays and heavy ions. The concomitant decrease in the yields of oxidized bases with the increase in the linear energy transfer of heavy ions may be accounted for by the preponderance of indirect effects in the damaging action of ionizing radiation on DNA. Further evidence for the major role played by •OH was provided by the results of exposure of cells to high intensity 266 nm laser pulses. Thus 8-oxo-7,8-dihydroguanine is mostly produced as the result of bi-photonic ionization of DNA nucleobases and subsequent hole migration to guanine bases. It is likely that some of the oxidized bases that have been isolated as single lesions are in fact part of clustered damage. In that respect it was found that a single oxidation hit is capable of generating complex lesions in cellular DNA. Thus •OH-mediated abstraction at C4 of the 2-deoxyribose moiety gives rise to DNA strand cleavage together with the formation of a highly reactive aldehyde that undergoes an addition reaction to the amino group of a proximate cytosine, giving rise to 4 diastereomeric cycloadducts as likely interstrand cross-links. It was also shown that the (5'R)-5',8-cyclo-2'-deoxyadenosine, a tandem lesion, that arises from intramolecular addition of the •OH-mediated C5' radical to the C8 position of the adenine moiety, is generated in cellular DNA, however, in a very low yield, upon gamma irradiation. Information is also available on the formation of DNA-protein cross-links, inter- and intra-strand DNA cross-links in cellular DNA. This may be rationalized in terms of nucleophilic addition of amino groups of either lysine or cytosine as well as the N3 position of thymine to the guanine radical cation generated by one-electron oxidation. Recent articles: J. Cadet, J.-L. Ravanat, M. Tavernaporro, H. Menoni, D. Angelov. Cancer Lett, 327 (2012) 5-15 J. Cadet, J.R. Wagner, Cold Spring Harb Perspect Biol, 5 (2013) a12559 G.S. Madugundu, J.R. Wagner, J. Cadet, K. Kropachev, N.E. Geacintov, V. Shafirovich, Chem Res Toxicol, 26 (2013) 1031-1033 J. Cadet, J.R. Wagner, Mutat Res, (2013) in press. **Keywords:** DNA-protein, 5',8-cyclo-2'-deoxyadenosine, hydroxyl radical

Closing Lecture
(CL-01)

OXIDATIVE MECHANISMS IN PROTEIN AGGREGATION

Ohara Augusto

USP-IQ - Universidade de São Paulo (São Paulo)

Protein aggregates are oligomeric complexes of unfolded or misfolded proteins that are unrelated to the original function of the proteins. Protein aggregates import a new component to cellular metabolism, which might be toxic. The levels of unfolded proteins in a cell may increase due to the presence of mutated proteins and of proteins that possess marginally stable native states. The latter property is characteristic of most proteins involved in neurodegenerative diseases. The levels of unfolded proteins and protein aggregates in cells may also increase as a consequence of protein oxidation. Here, I will summarize recent studies that argue for a role of an oxidative lesion, the ditryptophan cross-link1, in the non-amyloid protein aggregation characteristic of amyotrophic lateral sclerosis (ALS). This disease is characterized by the degeneration of motor neurons, resulting in progressive muscle weakness, atrophy, paralysis and death. Most cases of ALS are sporadic but a large subset of familial ALS presents mutations in human superoxide dismutase 1 (hSOD1). Currently, the predominant view is that hSOD1 mutants cause motor neuron death because of their spontaneous tendency to misfold, unfold and aggregate. However, hSOD1 has pro-oxidant activities that may contribute to the pathogenic mechanism of ALS, such as its bicarbonate-dependent peroxidase activity. In this activity, hSOD1 acts as the source and the target of the carbonate radical being carbonylated and covalently dimerized through a ditryptophan cross-link in the process1. Previously, we showed that the non-classical antioxidant tempol prevented hSOD1 carbonylation and dimerization caused by its bicarbonate-dependent peroxidase activity in vitro2. In vivo, tempol extended moderately the survival of a rat model of ALS (hSOD1G93A rat) while inhibiting neuronal cell loss and levels of oxidized and unfolded hSOD1G93A forms in spinal cord tissues3. More recently, we showed that the oxidation products of hSOD1 formed by the bicarbonate-dependent peroxidase activity of hSOD1WT and of the ALS-associated mutant hSOD1G93A trigger hSOD1 unfolding, oligomerization and non-amyloid aggregation4. Taken together, the results indicate that formation of the hSOD1-W32-W32-hSOD1 cross-link in a “loose” mixed tetramer is crucial for triggering the aggregation process. 1Medinas DB, Gozzo FC, Santos LF, Iglesias AH, Augusto O. (2010) Free Radic Biol Med. 49,1046-1053. 2Queiroz RF, Paviani, V, Coelho FR, Marques EF, Di Mascio, P, Augusto, O (2013) Biochem J 455, 37-46. 3Linares, E, Seixas, LV, Dos Prazeres JN, Ladd, FVL, Laad, ABLA, Copi, AA and Augusto, O (2013) PLoS One 8, e55868. 4Coelho FR, Iqbal A,Linares E, Silva DF, Lima F, Cuccovia IM, Augusto, O (2013) In preparation.

Keywords: Oxidative Process, Protein aggregates, hSOD1

Conferences

CF.1-01 - ROLE OF DIETARY FLAVONOIDS IN OXIDATIVE STRESS OF THE CENTRAL NERVOUS SYSTEM

Junji Terao

[1]Department of Food Science - Institute of Health Biosciences, the University of Tokushima Graduate School (Tokushima 770-8503)

Flavonoids are ubiquitously present in fruits and vegetables and recognized to act as dietary antioxidants by scavenging ROS and/or inhibiting oxidative enzymes. Their prooxidant activity may participate in the attenuation of oxidative stress by inducing the expression of antioxidative enzymes, although this activity also leads to cytotoxicity. We confirmed by LC-MS analysis that detoxified conjugated metabolites accumulated in the brain tissue of the rats fed a typical flavonol-type flavonoid quercetin (1). In addition, blood-brain barrier (BBB) transport of conjugated quercetin could be reproduced in vitro by using rat brain capillary endothelial cells. Mouse microglial cells were found to release quercetin aglycone from its glucuronide conjugate (2). It is therefore likely that dietary flavonoids target central nervous system by crossing the BBB and act as their aglycones through deconjugation reaction. Deconjugation and conjugatin seem to be critical steps in the attenuation of oxidative stress. We focused on the effect of quercetin on mitochondrial monoamine oxidase-A (MAO-A), because MAO-A reaction may result in oxidative stress by generating ROS through electron transfer to oxygen molecule. Quercetin aglycone exerted the inhibitory effect on mitochondrial MAO-A activity in the mouse brain (3,4). Interestingly, quercetin was less toxic than luteolin (a flavone-type flavonoid) toward a nerve cell line. These data suggest that quercetin is a weak (but safe) MAO-A inhibitor in the brain without induction of cytotoxicity.

(1) Ishisaka et al. Free Radic Biol Med 51:1329-1336 (2011);(2) Mukai et al. Free Radic Res 46:1019-1028(2012);(3) Yoshino et al. Nutrition 27:847-852(2011); (4) Bandaruk et al. J Agric Food Chem: 60:10270-10277(2012)

Keywords: flavonoid, quercetin, deconjugation, monoamine oxidase-A

CF.1-02 - GARLIC ORGANOSULFUR COMPOUNDS AS A POTENTIAL ANTI-INFLAMMATORY AGENT: MOLECULAR AND ANIMAL EVIDENCE

De-Xing Hou [1], Sixiang You [1,2], Xi He [2], Jianhua He [2]

[1] Dep. Biochem.l Sci. & Tech - Kagoshima University (Korimoto 1-21-24, Kagoshima 890-0065), [2] Botanical Functional Ingredients - Hunan Agriculture University, (Changsha)

Garlic is used for both culinary and medicinal purposes by many cultures. The garlic organosulfur compounds (GOSCs) are thought to be bioactive components. This study aims to clarify the anti-inflammatory effects and molecular mechanisms of GOSCs in both cell and animal models. RAW264.7 cells were treated with six kinds of GOSCs to screen their influence on COX-2 and iNOS expression by Western blotting. PGE2 and nitrite were measured by ELISA and Griess reaction, respectively. Cytokines in culture medium were assayed by the multi-plex technology. Proteins were detected by Western blotting. Mouse paw edema was induced by LPS. The results revealed that DATS was a strongest inhibitor for COX-2 and iNOS among GOSCs, and reduced the levels of LPS-induced IL-6, IL-10, IL-12(p70), KC, MCP-1 and TNF-α. Cellular signaling analysis revealed that DATS downregulated AKT1/TAK1-mediated MAPK and NF-&KappaB pathways. Furthermore, DATS activated Nrf2-mediated expression of HO-1 and NQO1 and reduced LPS-induced intracellular ROS, which might contribute to suppress inflammatory mediator production. Finally, in vivo data demonstrated that DATS attenuated LPS-induced mouse paw edema. Taken together, we can conclude that DATS revealed anti-inflammatory effect in both cell and animal models and will be a potential inhibitor for anti-inflammation.

Keywords: Garlic organosulfur compounds, Inflammatory mediators, Antioxidant enzymes, Cellular signaling, Mouse paw edema

CF.2-01 - ADVANCES IN MALDI-MS/MS FOR POLYPHENOLS ANALYSIS

Norberto Peporine Lopes [1]

Departamento de Física e Química - USP Ribeirão Preto (São Paulo)

There is ongoing interest in understanding the main physiological and ecological functions of phenolic compounds. The biosynthesis occurs in the cytosol and the products can be transported for specific storage. MALDI-MS imaging procedures are normally applied for protein analyses from animal tissues. However, it is just starting to be used for plant analyses. In MALDI-MS it is possible to obtain the molecular formula of a specific compound, but systematic MS/MS studies furnish structural information and can highly improve the data confidence. Recently, our group provided the first High Throughput MALDI-MS/MS screening for forest dereplicatio as one of the aims of a BIOTA/FAPESP Program presented at the Rio+20 meeting. Based on this model, the aim of this work is the development of a HT-MALDI-MS/MS screening method using herbarium voucher samples from herbaceous species of Campus Rupestris (Brazilian Savana) and construct ion maps based on sequential MALDI-MS/MS. Using HT-MALDI-MS/MS we screened up to 60 extracts from voucher samples within an hour time-frame. The results show conserved accumulation of a specific di-C-glycosyl flavonoid (vicenin-2) in several species from Campus Rupestris. Quantitative UPLC-MS/MS analysis reinforce the observation. Transverse sections of Lychnophora leaves were analyzed by MALDI-MS/MS Imaging showing vicenin-2 accumulation in the epidermal cells on the adaxial side of Lychnophora. Flavonoid aglycones do not show any specific compartmentalization. We developed a new HT-MALDI-MS/MS herbarium voucher samples methodology and an uncommon MALDI-Imaging system based on MS2 data. To the best of our knowledge, this is the first evidence of a chemical barrier performed of a specific flavonoid for sunlight absorption in plants reported by the simultaneous quantification and structural elucidation of a specific flavonoid. It was clearly observed that vicenin-2 produced a layer on top of the leaf in epidermal tissues, and like this can act as a chemical UV light barrier.

Keywords: epidermal, protein, MALDI-MS/MS, polyphenols

CF.2-02 - SALIVA AS A PROMISING MATRIX FOR BIOMONITORING OF REDOX-MODIFIED DNA BASES

Tiago Franco de Oliveira [1], Alliny Souza Bastos [2], Fabiana Almeida dos Santos [1], Antonio Anax Falcão de Oliveira [1], Marisa Helena Gennari de Medeiros [3], Paolo Di Mascio [3], Silvana Regina Perez Orrico [2],
Ana Paula Melo Loureiro [1]

[1]Clinical and Toxicological Analyses - Universidade de São Paulo, [2]Department of Diagnosis and Surgery - Universidade Estadual Paulista (UNESP) (Araraquara-SP)[3]Department of Biochemistry - Universidade de São Paulo

Oxidative stress has been implicated in inflammation, aging, and disease development. Cell death, senescence, and transformation may be accomplished either by direct modification of targeted biomolecules by the exceeding reactive species, or by inducing posttranslational modifications of proteins involved in gene transcriptional regulation, as well as interfering in signaling cascades that regulate gene expression. Quantifying damaged biomolecules in accessible body fluids, such as urine, blood, and saliva is emerging as an attractive tool to assess the whole body redox state and disease risk. Among the biomolecule targets, DNA is interesting for damage biomarker validation due to its stable nature and equal distribution throughout the body. So, the occurrence of DNA adducts reflects the availability of damaging reactive species in a tissue. This also gives information about risks of disease, as DNA damage is closely related to DNA mutations. Saliva is yet poorly explored for DNA adduct assessment. There is evidence that salivary 8-oxodGuo correlates with plasma and urine 8-oxodGuo, which poses saliva as a possible matrix for whole body oxidative stress assessment. Following this tendency, we validated a SPE, isotope dilution HPLC-ESI-MS/MS method for analysis of 8-oxodGuo, N2-carboxyethyl-2'-deoxyguanosine (CEdG, a DNA advanced glycation marker), and 1,N6-etheno-2'-deoxyadenosine (1,N6-ethenodAdo) in human saliva. The validated method was applied to saliva samples collected from 87 individuals, from which data of fasting glycemia, HbA1c, total cholesterol, HDL, LDL, TGL, and insulin were also obtained. Good Spearman correlations were found for fasting glycemia and 8-oxodGuo ($r=0.3767$, $p=0.0005$) or CEdG ($r=0.4305$, $p<0.0001$), HbA1c and 8-oxodGuo ($r=0.3119$, $p=0.0043$) or CEdG ($r=0.3044$, $p=0.0041$), circulating insulin and 8-oxodGuo ($r=0.2984$, $p=0.0065$) or CEdG ($r=0.2331$, $p=0.0298$). On the other hand, 1,N6-ethenodAdo levels were not correlated with any variable. This is the first work pointing to the possible use of 8-oxodGuo and CEdG in saliva to monitor diabetes related oxidative and glycoxidative stress.
Financing: FAPESP,CAPES,CNPq,PRP/USP>

Keywords: Diabetes, DNA adducts, Saliva

CF.3-01 - NOX FAMILY NADPH OXIDASES IN VASCULAR CELLS: MECHANISMS AND THERAPEUTIC IMPLICATIONS

Francisco Rafael Martins Laurindo

FM-USP-Incor - University of São Paulo School of Medicine

Redox homeostasis depends on the regulated production of reactive oxygen species (ROS) via enzymatic sources such as the Nox family NADPH oxidase complex, the only known enzymatic system dedicated to generate reactive oxygen species (ROS), mainly with signaling purpose. Understanding regulatory mechanisms underlying activation and expression of Nox(es) has become a major goal, particularly because Nox(es) are one of the most important emergent therapeutic targets in the area. This family is composed by 7 transmembrane catalytic subunit members (Nox1-5 and Duox 1-2), with each of these isoforms being regulated by a variable combination of one to several transmembrane or cytosolic canonical regulatory subunits (ex: p22phox, p47phox, p67phox, Rac1 and others). Each isoform has particular structural features confering distinct subcelular locations, as well as type and rates of ROS generation. Thus, the first level of Nox regulation is the type of isoform expressed by the particular tissue or cell, which is specific to the cell type, proliferative or differentiation status, confluence, exposure to stress and many other factors related to disease pathophysiology in distinct systems. For example, in the vascular system, Nox1 is the most prominent growth factor and inflammatory cytokine-driven isoform and supports smooth muscle cell migration, while Nox4 is driven by (patho)physiological stresses or cytokines such as TGF-beta to induce cellular quiescence and differentiation. Nox(es) integrate signaling associate with specific kinases and phosphatases to enhance or target specific signals to a particular subcelular location. Thus, the second level of regulation is the integration of Noxes with specific subcellular compartments. A third level of regulation is the association and/or interaction with non-canonical regulatory proteins that connect Nox signaling with particular physiological programs. Our laboratory has identified Nox interaction with the redox chaperone protein disulfide isomerase (PDI), the founding member of a large thioredoxin superfamily group of enzymes mainly dedicated to redox protein folding and disulfide isomerization in the endoplasmic reticulum (ER) lumen. PDI associates with and regulates Nox NADPH oxidases, as follows. PDI supports growth factor-dependent Nox1 activation and mRNA expression, as well as migration in smooth muscle cells, and PDI overexpression induces acute spontaneous Nox activation. Mechanisms of PDI effects include possible support of complex formation and RhoGTPase activation. In phagocytes, PDI supports phagocytosis, Nox activation and redox-dependently interacts with p47phox. Together, results implicate PDI as possible Nox organizer. PDI exerts many other functions outside of the ER, including redox modulation of cell surface events associated with thrombosis and, as described by our group, vascular remodeling after injury. Specific flavonoids have been shown to inhibit PDI and prevent thrombosis. The Nox NADPH oxidase family, and its associated branches of interaction, such as with the PDI family, may offer a host of opportunities for therapeutic modulation of disease-related processes. (Research supported by FAPESP – Individual projects and CEPID Redoxoma and CNPq – INCT Redoxoma).

Keywords: NADPH, Nox, Redox

CF.3-02 - FLAVAN 3-OLS BIOACTIVITIES ON METABOLIC SYNDROME – A NEW ANGLE OF OBSERVATION

Naomi Osakabe

Department of Bioscience and Engineering - Shibaura Institute of Technology (College of Systems Engineering and Science)

Flavan 3-ols, a type of polyphenolic substance, are known to be rich in chocolate. There is evidence that cacao products have the potential to contribute to the risk reduction of cardiometabolic disorders according to recent epidemiological or intervention studies. However, the detail of alteration on the metabolic syndrome risk factors by flavan 3-ols remains unclear. We attempt to elucidation of flavan 3-ols acute or repetitive bioactivity against circulating and energy metabolism system using several experiments. In our recent study, repeated ingestion of flavan 3-ols reduced mean blood pressure, in contrast, a single administration of flavan 3-ols in rats was shown to cause an immediate elevation in blood pressure and heart rate. In microcirculation, a single oral administration of flavan 3-ols elevated blood flow and recruitment of capillaries in skeletal muscle by the in vivo intravaital microscopy study. In addition, ingestion of flavan 3-ols altered energy expenditure estimated by an indirect calorimetric. We found that the average of respiratory exchange ratio was significantly reduced by flavan 3-ols. The mitochondria copy number in gastrocnemius, soleus and blown adipose tissue are significantly increased, and the expression of uncoupling protein (UCPs) and βoxidation relative enzyme was also significantly elevated by the administration of flavan 3-ols. According these results, a part of alteration on metabolic syndrome risk factors by the ingestion of chocolate may be induced by this mitochondrial biogenesis promotion.

Keywords: mitochondrial, metabolic, elucidation

CF.3-03 - SPECIFIC LOCALIZATION OF QUERCETIN GLUCURONIDES IN MACROPHAGES: NEW INSIGHTS INTO THE BIOLOGICAL ACTIONS OF DIETARY FLAVONOIDS AT SITES OF INFLAMMATION

Yoshichika Kawai

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Dietary flavonoids have long been recognized to protect blood vessels from atherogenic inflammation by yet unknown mechanisms. We have previously discovered the specific localization of quercetin-3-*O*-glucuronide (Q3GA), a major metabolite of quercetin, in macrophage cells in the human atherosclerotic lesions [1]. In addition, we have also found the specific localization of Q3GA in macrophages in human brain during cerebral infarction [2]. However, the biological significance of the specific localization of Q3GA is poorly understood. We here demonstrate the molecular basis of the interaction between quercetin glucuronides and macrophages, leading to deconjugation of the glucuronides into the active aglycone. It was found that Q3GA binds to cell surface proteins on the macrophages through anionic binding, followed by the deconjugation by β-glucuronidase secreted from the macrophages. The macrophage-derived β-glucuronidase activity was significantly enhanced upon mitochondrial dysfunction, characterized using antimycin-A (a mitochondrial inhibitor) and siRNA-knockdown of Atg7 (an essential gene for autophagy). The deconjugated aglycone, quercetin, acts as an anti-inflammatory agent in the lipopolysaccharide-stimulated macrophages, whereas Q3GA acts only in the presence of extracellular β-glucuronidase activity. Furthermore, we found that quercetin and related analogs significantly induced the autophagic degradation, presumably inhibiting the inflammatory responses in macrophages. These results showed that mitochondrial dysfunction and autophagy in macrophages could be the key players in the anti-inflammatory actions of dietary flavonoids. This study may provide one mechanism for the anti-inflammatory/anti-atherosclerotic actions of dietary flavonoids within the inflammation sites.

[1] Kawai *et al*., *J. Biol. Chem.* **283**, 9424-34 (2008).
[2] Ishisaka *et al*., unpublished results.

Keywords: flavonoid, macrophage, inflammation, mitochondria, autophagy

CF.4-01 - EFFECTS OF FLAVONOIDS ON THE EXPRESSION SYSTEM OF DRUG-METABOLIZING ENZYMES

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Modulation effects of flavonoids on the expression system of drug-metabolizing enzymes will be discussed. Certain flavonoids can modulate phase 1 drug-metabolizing enzymes through the aryl hydrocarbon receptor (AhR) while phase 2 drug-metabolizing enzymes through the nuclear factor-erythroid-2-related factor 2 (Nrf2). Recently, we found procyanidins and cyanidin 3-glucoside down-regulated benzo[a]pyrene-induced cytochrome P4501A1 (CYP1A1) expression by inhibiting the transformation of AhR, while they increased expression of glutathione S-transferases (GSTs) via increasing the binding of Nrf2 to antioxidant response elements. We also found that luteolin affected the expression of these phase1 and phase 2 enzymes including CYP1A1 and GSTs through modulation of the function of AhR and Nrf2. Especially, luteolin induced phase 2 enzymes at 1 nM. Therefore, dietary flavonoids may be act as a functional modulator for the expression system of drug metabolizing enzymes in the body.

Keywords: flavonoids, drug-metabolizing enzymes, Nrf2, glutathione S-transferases, cytochrome P4501A1

CF.4-02 - EFFECTS OF FLAVONOIDS AND MELANIN ON DNA OXIDATION BY SINGLET MOLECULAR OXYGEN

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Singlet molecular oxygen ($^1O_2$) is generated by energy transfer to molecular oxygen. The resulting $^1O_2$ is able to oxidize the nucleoside 2'-deoxyguanosine (dGuo), which leads to the formation of 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodGuo) and spiroiminodihydantoin 2'-deoxyribonucleoside diastereomers (dSp) in an aqueous solution. Melanins have been associated with the development of melanoma and its resistance to photodynamic therapy (PDT) and flavonoids are phenolic compounds found in plants and present in the human diet. Here, we investigated the effect that these factors have on DNA damage and repair. Of the tested flavonoids (flavone, apigenin, quercetin, morin and catechin), flavone possessed antioxidant activity, as determined by a decrease in the formation of both products (dSp and 8-oxodGuo). Apigenin, morin, quercetin and catechin all increased the formation of 8-oxodGuo at a concentration of 100 &muM. The quantification of plasmid strand breaks after treatment with formamidopyrimidine-DNA glycosylase showed that flavone protected and quercetin and catechin enhanced DNA oxidation. In relation to melanins, our results show that both types of melanin (eumelanin and pheomelanin) lead to DNA breakage in the absence of light irradiation and that eumelanin is more harmful than pheomelanin. Interestingly, melanins were found to bind to the minor groove of DNA, guaranteeing close proximity to DNA and potentially causing the observed high levels of strand breaks. We also show that the interaction of melanins with DNA can impair the access of repair enzymes to lesions, contributing to the perpetuation of DNA damage. Moreover, we found that after melanins interact with $^1O_2$, melanins exhibit a lower ability to induce DNA breakage; we propose that these effects are due to modifications of their structure.

Keywords: flavonoids, melanin, singlet oxygen, melanoma, DNA

CF.4-03 - CHEMOPREVENTIVE EFFECTS OF FLAVONOIDS ON THE BREAST CANCER DEVELOPMENT

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Epidemiological and animal studies indicated that daily stress promote the development of breast cancer. And recent preclinical and epidemiological studies have shown an association between β-blockers and breast cancer progression. The results obtained from these studies have suggested that the β2-adrenergic receptor signaling pathway can modulate breast cancer progression and metastasis. On the other hand, epidemiological studies also suggested an inverse association between a higher intake of flavonoids and breast cancer risk. In order to clear the chemopreventive action mechanism of flavonoids, we have focused on estrogen metabolism and adrenergic receptor-mediated responses in human mammary epithelial MCF-10A cells and breast cancer MCF-7 cells. It was found that methoxyflavonoids such as crysoeriol selectively inhibited CYP1B1 activity without affecting CYP1A1 and consequently reduced the formation of carcinogenic 4-OHE2, and that quercetin and its glucronide reduced phosphorylation of histone H2AX(γ-H2AX), one of the earliest indicators of DNA damage, through the α2-adrenergic receptor(AR) induced by co-treatment with 4-OHE2 and noradrenaline. These flavonoids inhibited the binding of noradrenaline to α2-AR. These inhibitory activities of these flavonoids were observed at physiological concentrations. Effects of these flavonoids on AR mediated signaling and the breast cancer development will be discussed.

Keywords: flavonoid, breast cancer, estradiol, CYP1B1, adrenergic receptors

CF.4-04 - TOXICOLOGICAL AND OXIDATIVE STRESS EFFECTS OF THE DIFFERENT SUBSTANCES IN TOBACCO SMOKE ON HUMAN EMBRYONIC DEVELOPMENT BY A SYSTEMS CHEMO-BIOLOGY APPROACH

Diego Bonatto

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The physiological and molecular effects of tobacco smoke in adult humans and the development of cancer have been well described. In contrast, how tobacco smoke affects embryonic development remains poorly understood. In this study, we applied interactome data mining tools and small compound interaction networks to elucidate possible molecular pathways associated with the effects of tobacco smoke components during embryonic development. Our analysis showed a relationship between nicotine and 50 additional harmful substances involved in a variety of biological process that can cause abnormal proliferation, impaired cell differentiation, and increased oxidative stress. Our work provides the first approach describing how different tobacco constituents affect a broad range of biological process in human embryonic development.

Keywords: oxidative stress, chemo-biology, Toxicological

CF.5-01 - PROCESSING EFFECTS ON FOOD CAROTENOIDS: IMPACT ON HUMAN HEALTH

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Recent years have seen continued interest and research on the effects of processing on food carotenoids. This presentation will integrate the wealth of information of information that has been generated so as to present the current knowledge on this important topic. The bulk of studies still involves monitoring of carotenoid losses during processing, including emerging processing technologies, generally confirming variable and often considerable losses during thermal processing and no or minimum losses during non-thermal processing.There has been a resurgence of investigations on the chemical reactions (i.e., E-Z isomerization and oxidation) responsible for such losses, along with underlying influencing factors (e.g., exposure to oxygen and light, disruption of food matrix, water activity, pH, presence of pro-oxidants and antioxidants). There is more evidence that E-Z isomerization accompanies oxidation, both all-E- and Z-carotenoids being oxidized. However, direct oxidation of all-E-carotenoids appears to be the main route. The initial oxidation products are epoxy carotenoids and apocarotenoids; subsequent successive fragmentations lead to the formation of low mass compounds (e.g., hydrocarbons, aldehydes, ketones), The impact of the alterations brought about by processing, not only on food quality but also on human health, is increasingly being investigated. Current findings on the effects of oxidation products are, however, conflicting, with some studies reporting positive effects while other studies are showing negative effects.

CF.5-02 - ENHANCEMENT OF *IN VIVO* RADICAL REDUCING ABILITY BY LYCOPENE

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Lycopene is the most abounding carotenoid in tomato fruits and tomato-based products, and reported to be one of the most potent singlet oxygen quencher among various dairy antioxidants. But, there are few reports of its ability on radical reducing effect *in vivo*. So, we investigated the *in vivo* radical reducing ability of dietary lycopene using electron paramagnetic resonance (EPR) method. Rats were fed either a control diet or a diet containing lycopene for 2 weeks, and *in vivo* EPR measurements were conducted. As the result, dietary administration of lycopene significantly increased the radical reducing ability on rat kidney. This is the first verification of the *in vivo* enhancement of the antioxidant ability of lycopene. Further analysis for understanding potential molecular mechanisms by lycopene treatments in the study is under the way.

CF.5-03 - OCCURRENCE AND BIOSYNTHESIS OF BIFLAVONOIDS

Lydia F. Yamaguchi[1], Andre W. Santos[2], Eny I.S. Floh[2] and Massuo J. Kato[1]

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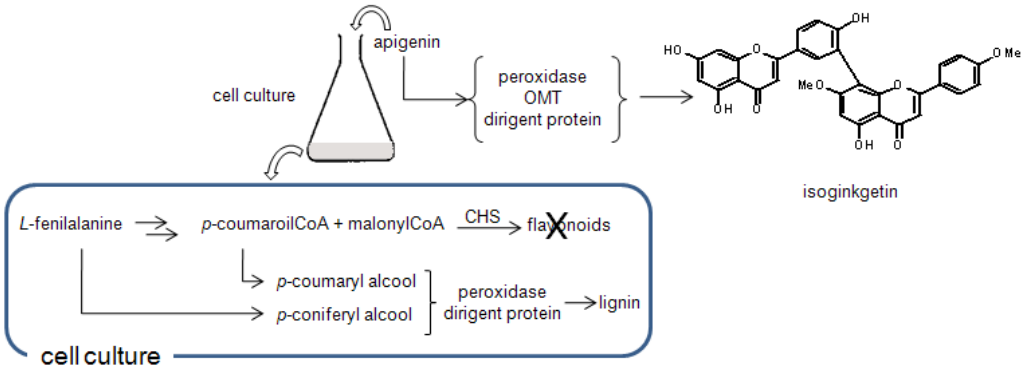
The occurrence of biflavonoids is restricted to some families, such as, Ginkgoacea and Araucariaeae in Gimnospermae and to only few species of Angiospermae. Phytochemistry investigations of *Araucaria angustifolia*, South American endemic pine, identified amentoflavone-type biflavonoids and lignans. These biflavonoids presented several biological properties as antioxidant and photoprotective activities. Their precursors and the biosynthetic pathways have not described yet but the oxidative coupling, mediated by a peroxidase, of two apigenins or chalcones has been proposed. Biosynthetic studies were carried out using callus culture that produced only p-coumaroyl/feruloyl esters but no flavonoids. Nevertheless, the administration of apigenin in the cell culture promoted the formation of ginkgetin, a methoxylated amentoflavone-type biflavonoid. Then, the biosynthesis of biflavonoids in *A. angustifolia* proceeds through a long sequence of steps involving the apigenin production followed by the action of peroxidase and dirigent protein to mediate oxidative coupling forming the 3'-8" linkage, and methylation reaction by O-methyl transferase. These enzymes and the dirigent protein were detected and characterized in cell cultures. Acknowledgments: FAPESP, CNPq and CAPES



CF.5-04 - IDENTIFICATION OF MICRORNAS INVOLVED IN THE ANTIANGIOGENIC ACTIVITY OF POLYPHENOLS

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Aim: The neovascularization of atherosclerotic plaque is an important factor that contributes to its instability. Polyphenol extracts have been shown to decrease the progression of atherosclerosis, as well as an inhibitory effect on angiogenic factors. However, the molecular mechanisms involved are not completely known. Thus, this study aims to identify microRNAs involved in the modulation of angiogenic factors in the atherosclerotic plaque in mice treated with a polyphenols from propolis (PCP). Methods: 50 Ldlr-/ - mice were divided into two groups: control and PCP-treated and further fed a hypercholesterolemic diet (0.5% cholesterol) for 3 months. Then mice were treated with PCP (250 mg total polyphenols/kg/day) for more one month. The screening of microRNAs in the aortic sinus lesions was performed using the platform Exiqon's LNA ™ microRNA Array miRCURY (SA Exiqon, Denmark). Possible microRNA-target interactions were identified with the database mirWALK. The identification of signaling pathways and biological processes modulated by differentially expressed microRNAs and their relationship with angiogenic factors were studied using the platform IPA (Ingenuity Systems). Gene and microRNAs (miRs) expression was quantified by qPCR. Results: We identified 29 miRs differentially expressed, involved in biological processes including cell cycle, cell migration, cell growth and proliferation. Gene expression of angiogenic factors in atherosclerotic plaque showed that Vegf-A, Fgf-2 and Pdgf were downregulated. The bioinformatic analysis identified 8 miRs (miR-181a, miR-106a, miR-19b, miR-15a, miR-16, miR-17, miR-20a, miR-20b) associated with Vegf-A, the major inducer of angiogenesis in the atherosclerotic plaque. Microarray validation by qPCR showed that only miR-181a, miR-106a, miR-20b and miR-19b were upregulated, highlighting that miR-106a and miR-20b have sites of high affinity (7mer-m8) for the 3' UTR region of the Vegf-A mRNA with a higher probability of preferential conservation (Pct 0.86). In addition, the evaluation of signaling pathways modulated by miRs suggested that the IL-8 pathway (target of miR-106a) and NOTCH (target of miR-181a) could be suppressed. Conclusion: Data analysis provides evidence that the antiangiogenic effect of polyphenols can be modulated by microRNAs. Financial support: FAPESP; CNPq; CONICYT-CHILE.

Keywords: microRNAs, polyphenols, atherosclerotic

CF.6-01 - LOW AND MEDIUM BUT NOT HIGH DOSES OF GREEN TEA POLYPHENOLS AMELIORATE DEXTRAN SODIUM SULFATE-INDUCED HEPATIC AND KIDNEY DYSFUNCTIONS

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Green tea is a popular and widely consumed beverage, and contains characteristic polyphenolic constituents, generally known as green tea polyphenols (GTPs). They have been reported to have anti-oxidative properties and versatile preventive effects toward several chronic diseases, including cancer and inflammatory bowel disease. Our previous study, however, showed that the diets containing high doses (0.5-1%) of GTPs deteriorated dextran sodium sulfate (DSS)-induced intestinal inflammation and carcinogenesis (1). In addition, we showed that high-doses GTPs disrupted liver and kidney functions via reduction of antioxidant enzyme and heat shock protein (HSP) levels in both colitis and non-treated ICR mice (2). On the other hand, we assessed the effects of 0.01%, 0.1% and 1% dietary GTPs on liver and kidney physiological functions in DSS-exposed and normal mice (3). GTPs at 0.01% and 0.1% significantly suppressed DSS-increased serum AST and ALT levels. In contrast, GTPs at 1% increased kidney weight, serum creatinine level, and thiobarbituric acid-reactive substances (TBARS) in both the kidneys and livers in normal mice, as compared with DSS-exposed mice. Interestingly, GTPs at 0.01% and 0.1% remarkably up-regulated the expressions of heme oxygenase-1 (HO-1) and HSP70 mRNA in livers and kidneys of DSS-exposed mice, whereas GTPs at 1% abolished them. Collectively, low and medium doses of GTPs have beneficial effects on DSS-induced hepatotoxicity and nephrotoxicity via up-regulation of self-protective enzymes, while those disappeared at a high dose. Our results indicate that the effects of GTPs on hepatic and kidney functions have some associations with hormesis, which was reported by Calabrese and Baldwin who described the U-shaped toxicity of environmental chemicals (4). References: (1) Kim M, et al., Biofactors, 2010;36:43-51, (2) Inoue H, et al., Cell Stress Chaperon, 2011;16:653-62, (3) Inoue H, et al., Biosci Biotechnol Biochem., 2013;77:1223-28, (4) Calabrese EJ, Baldwin LA., Trends Pharmacol Sci. 2002;23:331-7.

Keywords: heat shock protein, inflammation, pro-oxidant, side-effect, supplement

CF.6-02 - ANTI-ALLERGIC FUNCTIONS OF POLYPHENOLS

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It has been reported that polyphenols have various physiological functions. In the present study, we investigated effects of tellimagrandins, major hydrolyzable tannins in Rugosa rose, on the release of chemical mediators such as histamine and leukotriene B4 (LTB4) from mast cells in vitro, which are responsible for type I allergy symptoms. Rat basophilic leukemia cell line (RBL-2H3) and mouse mast cell line (PB-3c) were used for histamine and LTB4 release assay, respectively. RBL-2H3 and PB-3c were stimulated by calcium ionophore (A23187) or the cross-linking of high-affinity IgE receptors via IgE-antigen complexes in Tyrode buffer. Then the secreted histamine and LTB4 were determined by HPLC. Calcium influx into the cytoplasm was monitored by spectrofluorometry. The phosphorylation of intracellular signaling molecules was analyzed by western blotting. Histamine and LTB4 releases from mast cells were suppressed by tellimagrandin I in a dose-dependent manner. Tellimagrandin I inhibited calcium influx in RBL-2H3 stimulated by IgE-antigen, whereas there was no effect on the cells stimulated by calcium ionophore. The phosphorylation of protein kinases was prevented in the presence of tellimagrandin I. These results suggest that tellimagrandin I may have the anti-allergic function.

Keywords: tannins, histamine, leukotrienes, mast cells, allergy

CF.6-03 - REDOX CONTROL OF PROTEASOMAL GATING

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The proteasome is a multimeric and multicatalytic complex responsible for the degradation of poly ubiquitinylated proteins, most of them involved in cellular regulation and signalling, antigen presentation and, control of protein synthesis. It consists of a central core named 20S proteasome (20SPT) coupled to regulatory units (e.g., 19S) in one or both sides. The 20SPT is able to degrade oxidized proteins through a process independent on ATP and protein poly ubiquitinylation. Latter mechanism is considered an important anti-oxidant defense. The 20SPT is formed by a central unit formed by two heptameric rings called β, where the active sites are located, flanked in both sides by heptameric rings called α that regulate the gating of the catalytic chamber. Very few cysteine residues into the 20SPT α-rings of the yeast S. cerevisiae are prone to the oxidative post-translational modification namely, S-glutathionylation. The 20SPT S-glutathionylation promotes the gate opening of the catalytic chamber that increases the degradation of oxidized proteins. This mechanism was shown to be coupled to decreased intracellular reductive ability and so, increased pool of oxidized proteins in yeast cells grown to stationary phase into glucose-containing medium. Phenotypic and biochemical consequences of site specific mutation of those proteasomal Cys residues prone to S-glutathinynalion will also be addressed in the present talking.

Keywords: proteasome, S-glutathionylation, proteolysis, redox modulation of the proteasome, metabolism of oxidized proteins

CF.7-01 - DETECTION OF PROTEIN TYROSINE MODIFICATIONS BY MASS SPECTROMETRY AND ANTIBODIES

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Biomolecules are often modified by reactive oxygen species (ROS). The modified biomolecules will be good biomarkers for evaluation of human health in respect to oxidative balance. Oxidized lipids form covalent adducts with an amine of a protein. Protein tyrosine is one of the targets for ROS and then generates dityrosine, halotyrosines, and nitrotyrosine residues. To measure these molecules, specific antibodies has been developed and applied. Mass spectrometry has also been applied for accurate quantification of the modified molecules. These markers were present in higher levels in urine from a diabetic patient than in that from a healthy person. Some investigations of functional food ingestion with measurements of oxidative stress markers suggest that intake of some kinds of food actually protect our health at least in part. Hence, oxidative stress markers might serve as useful biomarkers for not only the evaluation of health status but also for gauging the effectiveness of functional foods.

Keywords: oxidative stress, biomarker, tyrosine modification, antibody, mass spectrometry

CF.7-02 - LIPID HYDROPEROXIDES: SINGLET MOLECULAR OXYGEN GENERATION AND PROTEIN MODIFICATION

Sayuri Miyamoto

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Lipid hydroperoxides (LOOH) are formed by enzymatic and non-enzymatic mechanisms. If not efficiently reduced, these hydroperoxides can participate in reactions leading to the generation reactive oxyl radicals, such as, alkoxyl and/or peroxyl radicals. This process is known to enhance radical chain reactions responsible for the accumulation of biomolecule damages. In this context, we have characterized in details the generation singlet molecular oxygen ($^1O_2$) from reactions involving LOOH with several biologically relevant oxidants, such as, metal ions, peoxynitrite, hypochlorite and cytochrome c. For mechanistic studies we used ^{18}O-labeled hydroperoxides and confirmed the formation of ^{18}O-labeled $^1O_2$ by chemical trapping and detection of the corresponding products by LC-MS/MS. Using this approach we have identified that cytochrome c interactions with polyunsaturated cardiolipin species induces the generation of a continuous flux of excited species, including both triplet carbonyl species and $^1O_2$. More recently, we have also characterized protein modifications induced by cholesterol derived aldehyde formed by singlet oxygen mediated oxidation of cholesterol. This aldehyde has been detected in biological tissues, implicating the involvement of singlet oxygen in the oxidation mechanism. Altogether, our studies indicate that $^1O_2$ is an important intermediate formed in biochemical reactions involving lipid peroxidation and LOOH formation. Acknowledgements: FAPESP, CNPq, CEPID-Redoxoma, INCT de Processos Redox em Biomedicina-Redoxoma, NAP-Redoxoma, Pró-Reitoria-USP.

Keywords: lipid hydroperoxides, singlet molecular oxygen, protein modification, cholesterol aldehydes, cytochrome C

Selected Oral Presentations

SP-01 - PROTEOMIC ANALISYS IN RESPONSE TO VITAMIN B6 THERAPY DURING OXIDATIVE STRESS INDUCED BY PNEUMOCOCCAL MENINGITIS IN RATS

Ana Helena Sales Oliveira[1,2,3], Leonam Gomes Coutinho[1], Diogo Ribeiro Demartini[3], Célia Regina Ribeiro da Silva Carlini[3,4], João Antônio Pegas Henriques[2,3], Stephen Leib[5], Lucymara Fassarella Agnez-Lima [1]
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Bacterial meningitis caused by Streptococcus pneumoniae leads to death in up to 30% of patients. More than half of the survivors have neurological impairments due the current treatment is focused on the bacterial elimination by antibiotic. Despite effective prophylaxis, the inflammatory host response against pneumococcal meningitis is the main cause of hippocampal apoptosis, which is associated with learning and memory deficits. Vitamin B6 is related to limit the accumulation of neurotoxic metabolites and preserve the cellular energy status. Thus, the aim of this study was to investigate the effect of vitamin B6 in vivo on protein expression profile in hippocampal brain region by proteomic approach using a rat pneumococcal meningitis model. Eleven day old Wistar rats were infected with 3x106 cfu/ml of S. pneumoniae and randomized for treatment with vitamin B6 or saline buffer (placebo) as controls. Hippocampal brain region was dissected and the total content of protein was extracted. Proteins were resolved by 1D-SDS-PAGE. Further, in-gel protein digestion and UPLC-ESI-MS/MS analysis were performed. Data collected was processed using Mascot Destiller and Daemon followed by Scaffold. According to UniProt functional annotation, proteins involved in processes of inflammatory and apoptosis were down-regulated while antioxidants were mostly up-regulated, except superoxide dismutase (SOD) which was up-regulated in placebo animals. Indeed, the SOD activity is associated with low DNA repair efficiency. These results evidence that vitamin B6 had neuroprotective effects by modulation of protein expression and is a potential target for strategies development to attenuate brain injury in bacterial meningitis
Keywords: brain inflammation, bacterial meningitis, vitamin B6, oxidative stress , proteomic analysis

SP-02 - Behavior of the thermal diffusivity of native and oxidized human low-density lipoprotein solutions

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Low-density lipoprotein (LDL) in vivo oxidative modifications play an important role in the development of atherosclerotic plaques. It is possible to distinguish native from in vitro oxidized LDL (oxLDL) samples using Z-scan (ZS) technique in millisecond time scale [1]. Part of the beam is absorbed and converted into heat, generating a refractive index gradient. The normalized transmittance, measured as a function of the sample position around the beam focus, provides a typical peak-to-valley curve, which is related to the thermal diffusivity of the sample by using the Thermal Lens model [2]. We prepeared copper-mediated oxLDL samples, which were oxidized sequentially from 10 to 90 minutes, in steps of 10 minutes. Our results show that the thermal diffusivity increases as a function of the LDL oxidation degree. This behavior can be explained by the increase of the hydroperoxides production due to the oxidation process. The oxidation products translocate from one LDL to another, disseminating the oxidation process and caring the heat across the sample. This phenomenon leads to a quick thermal homogenization of the sample, avoiding the formation of the thermal lens in highly oxidized LDL solutions. Acknowledgements: CNPq, FAPESP, CAPES, INCT-FCx and INCT Redoxoma. References [1] S.L. Gómez et al., Chem. and Phys. Lipids 163, 545-551 (2010). [2] Carter et al., Appl. Opt. 23, 476-481 (1984).
Keywords: lipoprotein, oxidation, Z-scan, thermal diffusivity

SP-03 - THEOBROMINE COCOA PREVENTS EXTRACELLULAR MATRIX ACCUMULATION VIA ACTIVATION OF NAD+-DEPENDENT DEACETYLASE SIRTUIN-1 IN EXPERIMENTAL Diabetes mellitus

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Background and aims: In *Diabetes mellitus* (DM), sirtuin 1 (Sirt1) reduction is associated with kidney extracellular matrix (ECM) accumulation. How Sirt1 reduction in diabetes occurs and its link with ECM accumulation are not known. Theobromine (TB), a methylxanthine of cocoa, contributes to its beneficial actions. The aims of this study were to assess the mechanism of Sirt1 reduction, its relationship with ECM accumulation, and whether TB increases Sirt1 activity protecting the kidney in DM. **Experimental Models:** 12-week-old spontaneously hypertensive (SHR) rats rendered diabetic via streptozotocin (60 mg/Kg), whereas control rats received citrate buffer. Diabetic SHR rats received or not TB (5 mg/Kg per day) for 12 weeks. *In vitro,* human mesangial cells (HMCs) cultured for 24 h in normal glucose (NG, 5mM) or high glucose (HG, 30mM) or hydrogen peroxide (H2O2, 10μM) alone or TB (41 nM) or poly(ADP-ribose) polymerase-1 (PARP-1) blocker (PJ-34, 5 μM) or NAD+ (300 μM) or Sirt1 blocker (EX-527, 10 μM). **Results:** TB reduced albuminuria, collagen IV expression, activated PARP-1 and increased NAD+ levels and Sirt1 activity (P<0.0001) in SHR diabetic rats. In HMCs under HG or H2O2, TB ameliorated collagen IV and ROS formation induced by NADPH oxidase (P><0.0001). PJ-34 or NAD+ supplementation increased Sirt1 and reduced fibronectin. TB prevented Smad3 acetylation under HG which was reversed by EX-527, a Sirt1 blocker (P><0.0001). >Conclusion: In DM, Sirt1 activity is reduced by PARP-1 activation and NAD+ consumption, leading to ECM accumulation via transforming growth factor beta-1 (TGFb-1). Activation of Sirt1 by theobromine may have therapeutic potential for diabetic nephropathy. Supported by: FAPESP (Fundação de Amparo a Pesquisa do Estado de São Paulo) and CNPq.
Keywords: diabetic nephropathy, extracellular matrix accumulation (ECM), poly(ADP-ribose) polymerase-1 (PARP-1), sirtuin 1 (Sirt1), theobromine cocoa

SP-04 - UNDERLYING THE PRO-OXIDANT EFFECTS OF URATE HYDROPEROXIDE ON REDOX MODULATED PROTEINS: A PIVOTAL ROLE IN INFLAMMATION

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Aim: Myeloperoxidase can oxidize uric acid to form the urate hydroperoxide (HOOU) (J Biol Chem. 286:12901-11, 2011). Protein disulfide isomerase (PDI) regulates NADPH oxidase in a redox-dependent manner (J Leukoc Biol. 90:799-810, 2011). Thus, this work investigates the effect of HOOU on redox modulation of PDI and if it would contribute to inflammation and atherosclerosis. Methods: HL-60 cells were incubated with urate and PMA (phorbol-12-myristate-13-acetate, 100 ng/mL), the oxygen consumption was evaluated by a Clark electrode and superoxide production was quantified by 2-hydroxiethidium formation. Chemical synthesis of HOOU was performed by phootoxidation. Results and discussion: urate (200 and 500 μM) increased oxygen consumption by 7 and 5% and superoxide production by 44 and 51%, respectively. The chemically synthesized HOOU presented absorption coefficient ε308 6537 M-1.cm-1 and half-life (t½) 32 min at 22ºC. Seventy and 100% of total HOOU (3 μM) was consumed after 30 and 120 seconds of incubation with PDI (10 μM). When exposed to HOOU (140 μM) for 30 min, PDI (23 μM) presented only 2.19 μmol SH/μmol protein; control PDI presented 5.70 μmol SH/μmol protein. HOOU reacted with catalytic sites of PDI as demonstrated by mass spectrometry analysis using modification of cysteine residues by alkylation with iodoacetamide. The oxidant action of HOOU upon PDI may explain the pro-inflammatory effect of the HOOU in HL-60 cells. Supported by: FAPESP
Keywords: uric acid, urate hydroperoxide, protein disulfide isomerase, inflammation

SP-05 - PMA TRIGGERS OXIDATIVE STRESS AND APOPTOSIS IN H-RASV12-DEPENDENT HUMAN MALIGNANT KERATINOCYTES

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PMA (Phorbol 12-myristate 13-acetate), diterpene largely used as a tumor promoter in skin mouse carcinogenesis assays, has also shown anticancer activity. This study aims to investigate PMA effects in H-RasV12-transformed human keratinocytes. HaCaT-H-RasV12 clonal sublines, but not HaCaT parental cells, grew colonies in suspension of soft agar, which were substantially inhibited by PMA. Furthermore, PMA induced caspase activation and DNA fragmentation in HaCaT-H-RasV12 cells, but not in HaCaT parental cells. Considering that PMA is a known NADPH oxidase activator, we investigated whether ROS could mediate PMA cytotoxicity. In four hours of PMA treatment ROS intracellular levels increased in HaCaT parental line and also in HaCaT-H-RasV12 sublines. However, within 24h PMA treatment, HaCaT parental cells reestablished its normal ROS levels, while HaCaT-H-RasV12 cells displayed sustained high ROS levels at 24h, 48h and 72h of PMA treatment. In addition, PKC inhibitors have shown that PMA induction of both cytotoxicity and ROS generation are dependent on PKC activity. The antioxidant NAC (N-acetyl cysteine) and apocynin, an inhibitor of the superoxide-producing enzymes NADPH oxidases, both protected HaCaT-H-RasV12 sublines from PMA cytotoxic effects, suggesting that ROS underlies PMA cytotoxicity. In comparison to HaCaT control, H-RasV12 subclones were shown to display a higher oxidative status and were shown to express higher amounts of Nox3 and Nox5 transcripts. PMA treatment triggered upregulation of Nox3 transcript in H-RasV12 subclones, but not in HaCaT empty vector control. All these findings suggest that PMA triggers apoptosis in H-RasV12-transformed keratinocytes as a consequence of oxidative stress through Nox3 upregulation. Supported by FAPESP, CAPES and CNPq.
Keywords: PMA, H-RasV12, oxidative stress, keratinocytes, NADPH oxidase

SP-06 - THE INVOLVEMENT OF OXIDATIVE STRESS IN SKELETAL MUSCLE DYSFUNCTION OF STREPTOZOTOCIN-INDUCED DIABETIC RATS

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The aim of the study was to evaluate the association between the contractile function alterations and oxidative stress (OxS) that occur in the skeletal muscle of streptozotocin-induced diabetic rats. Male Wistar rats were kept diabetic for 21 days and then treated with the antioxidant N-acetyl-L-cysteine (NAC), 300 mg/kg b.w. for 5 days. In diabetic rats, soleus and *extensor digitorum longus* (EDL) muscles had signs of OxS, as shown by the increased H_2O_2 content when compared to the control group. This effect was abolished by the NAC treatment, inducing specific alterations on soleus and EDL muscles. In the soleus muscle, OxS may take part in the early onset of fatigue and decreased expression of hypoxia-inducible factor-1 alpha (HIF-1α), vascular endothelial growth factor (VEGF), peroxisome proliferator-activated receptor gamma co-activator 1alpha (PGC-1α), cytochrome c oxidase subunit IV (COX IV), peroxisome proliferator-activated receptor alpha (PPARα), citrate synthase and glucose transporter 4 (GLUT4), suggestive of impaired energy metabolism. In the EDL muscle, OxS may be involved in the slower relaxation and conversion of glycolytic/fast fibers to oxidative/slow fibers, as shown by the increased expression of PGC-1α, COX IV and peroxisome proliferator-activated receptor beta (PPARβ).
Keywords: Antioxidants, Diabetes mellitus, Muscle contractile activity, N-acetyl-L-cysteine, Oxidative stress

SP-07 - CYTOCHROME C ADDUCTION PROMOTED BY CHOLESTEROL DERIVED ALDEHYDES – IMPLICATIONS TO PROTEIN-MEMBRANE BINDING

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Mitochondrial cholesterol has been reported to be increased under specific pathological conditions associated with enhanced oxidative stress parameters. In this scenario, cholesterol oxidation would be increased, leading to the production of reactive aldehydes, including cholesterol carboxyaldehyde (ChAld). By using SDS micelles as a mitochondrial mimetic model we have demonstrated that ChAld covalently modifies cytochrome c (cytc), a protein known to participate in electron transport and apoptosis signaling. This mimetic model induces changes in cytc structure in the same way as mitochondrial membranes do. Tryptic digestion of cytc-ChAld adduct followed by MALDI-TOF/TOF analyses revealed that modifications occur at Lys residues (K22 and K8) localized at cytc site L, a site involved in protein-protein and protein-membrane interactions. Interestingly, ChAld ligation prevented cytc detachment from liposomes even under high ionic strength conditions. Overall, it can be concluded that ChAld ligation to Lys residues at site L creates a hydrophobic tail at cytc, which promotes cytc anchoring to the membrane. These modifications, for instance, could prevent cytc movement along the membrane and its release to the cytosol, influencing, therefore, the electrons transport and the apoptosis signaling. Supported by: FAPESP, INCT de Processos Redox em Biomedicina-Redoxoma and CNPq.
Keywords: lipids, oxysterols, mass spectrometry, lipid peroxidation, singlet oxygen

Poster Presentations

A – Brain / Neurodegenerative Disease / Inflammation
B – Advanced Analytical Strategies for Oxidant Analysis
C – Vascular Function and Metabolic Syndrome
D – Redox Signaling / Cancer / System Biology
E – Carotenoids and Other Phytochemicals
F – Stress Response / Adaptation / Proteasome
G – Biomolecule Damage and Biomaker Analysis

A – Brain / Neurodegenerative Disease / Inflammation

A-01 - DISRUPTION OF NEUTROPHILS AND MACROPHAGES BACTERICIDAL ACTIVITY BY URATE

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Patients with sepsis present elevated serum total antioxidant capacity and uric acid (UA) is the principal contributor for this index. However, the actual pathophysiologic role of UA in critically ill patients is still a matter of debate. UA is oxidized to urate hydroperoxide by myeloperoxidase (MPO) in a situation that mimics the oxidative burst by inflammatory cells. Urate hydroperoxide can oxidize proteins susceptible to redox modulation and alter their functions. Therefore, we hypothesized that urate or its oxidation product urate hydroperoxide would modulate the killing activity of neutrophils and macrophages against *Pseudomonas aeruginosa*, a Gram-negative bacterium that causes opportunistic infections in hospital bed. Neutrophils or macrophages (2x10^6 cells/well) were incubated with *P. aeruginosa 14* (2x10^7 cells/well) with increasing concentrations of UA (0; 0.2; 0.5 and 2.0 mM). The suspension was used to evaluate bacterial viability by colony forming unit (CFU) counting. UA (2 mM) prevented bactericidal activity of neutrophils and macrophages. The supernatant was subjected to measurement of cytokines IL-β; and TNF-α; by Enzyme-Linked Immunosorbent Assay. The incubation of neutrophils or macrophages with *P. aeruginosa* in the presence of UA, reduced the cytokines release in both cases compared to the control. These data indicate that urate can disrupt neutrophils and macrophages bactericidal activity and down regulate cytokines release by these cells. Thus, this propitiates a new perspective about UA effect in opportunistic bacterial infection.
Keywords: Inflammation, Pseudomonas aeruginosa, Urate hydroperoxide

A-02 - PROTECTIVE EFFECTS OF TEMPOL ON A RAT MODEL OF AMYOTROPHIC LATERAL SCLEROSIS (ALS)

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by the progressive dysfunction and death of motor neurons by mechanisms that remain unclear. Evidence indicates that oxidative mechanisms contribute to ALS pathology, but classical antioxidants have not performed well in clinical trials. Cyclic nitroxides are an alternative worth exploring because they are multifunctional antioxidants that display low toxicity in vivo. Here, we examine the effects of the cyclic nitroxide tempol (4 hydroxy-2,2,6,6-tetramethyl piperidine-1-oxyl) on ALS onset and progression in transgenic female rats over-expressing the mutant hSOD1G93A. Starting at 7 weeks of age, a high dose of tempol (155 mg/day/rat) in the rats drinking water had marginal effects on the disease onset but decelerated disease progression and extended survival by 9 days. In addition, tempol protected spinal cord tissues as monitored by the number of neuronal cells, and the reducing capability and levels of carbonylated proteins and non-native hSOD1 forms in spinal cord homogenates. Intraperitoneal tempol (26 mg/rat, 3 times/week) extended survival by 17 days. This group of rats, however, diverted to a decelerated disease progression. Therefore, it was inconclusive whether the higher protective effect of the lower i.p. dose was due to higher tempol bioavailability, decelerated disease development or both. Collectively, the results show that tempol moderately extends the survival of ALS rats while protecting their cellular and molecular structures against damage. Thus, the results provide proof that cyclic nitroxides are alternatives worth to be further tested in animal models of ALS. (Supported by INCT-REDOXOMA, FAPESP and CNPq)
Keywords: Amyotrophic Lateral Sclerosis, cyclic nitroxides, neurodegenerative disease, tempol

A-03 - EFFECTS OF POLYPHENOLS ON ANTI-INFLAMMATORY ACTIVITY OF BIFIDOBACTERIUM ADOLESCENTIS

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Excessive oxidative stress can cause chronic diseases such as inflammatory bowel disease, cardiovascular disease, and cancer. Polyphenols and probiotics are a general tool for the maintenance and improvement of human health. Polyphenols have some beneficial effects including anti-oxidative and anti-inflammatory activities. Probiotics are known to improve the condition of not only the gastrointestinal tract but also the whole body. We have recently found that quercetin, galangin, and fisetin enhance anti-inflammatory activity of probiotic bacteria *Bifidobacterium adolescentis* (BA) that is usually detected in the human intestine (Kawabata K., BioFactors, 2013). Here, we investigated whether other polyphenols increase the anti-inflammatory activity of BA. BA were incubated with polyphenols (quercetin, 46 polyphenols, and 7 quercetin glucosides and conjugates) in DMEM under anaerobic conditions for 3 h, and the supernatants were estimated their effects on nitric oxide (NO) production in lipopolysaccharide-stimulated RAW264 macrophages. Although the supernatants of neither BA nor quercetin mono-culture affected the NO production, the co-culture supernatant markedly suppressed it. On the other hand, 46 tested polyphenols showed a slight or no effect on the NO suppression activity of BA, indicating that quercetin may be the most potent stimulant of BA. Next, we examined actions of quercetin glucosides and conjugates to the anti-inflammatory activity of BA. Among them, quercetin-3-glucoside, quercetin-4'-glucoside, quercetin-3-glucuronide, and isorhamnetin significantly increased the NO suppression activity in the co-culture supernatant. Thus, these results suggest that various forms of quercetin may be capable of enhancing the anti-inflammatory activity of BA in the intestine by a daily intake of quercetin-rich foods.
Keywords: Anti-inflammaotry activity, Bifidobacteria, Functional interaction, Quercetin

A-04 - POLYPHENOL-ENRICHED COCOA PROTECTS THE DIABETIC RETINA FROM GLIAL REACTION AND OXIDATIVE STRESS THROUGH THE SIRTUIN (SIRT-1) PATHWAY

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BACKGROUND: Cocoa widely studied for its beneficial properties in protecting against disease and is a rich source of polyphenols, particularly (-)-epigcatechin. In this present study we presented protective effects of polyphenol-enriched cocoa in diabetic retinas and in retinal cells under diabetic conditions. METHODS: Rat Muller cells (rMCs) were exposed for 24h to normal or high glucose or H2O2, submitted to polyphenol-enriched cocoa treatment combined or not to inhibitors. The animal study was conducted in 12-week-old hypertensive rats experimentally diabetic by streptozotocin. The animals received cocoa by gavage daily for 16 weeks. A vehicle was administered orally to the control animals. RESULTS: rMCs exposed to HG or H2O2 increased GFAP, acetyl-Lys310-p65 subunit-NFkB-complex and decreased SIRT1 activity/expression. These effects were abolished by cocoa treatment, which decreased intracellular ROS production and in turn diminished PARP-1 activity augmenting intracellular pool of NAD+ hence improving SIRT1 activity. Diabetic rats displayed higher expressions of GFAP and nitrotyrosine and decreased occluding accompained by electroretinogram markedly impairment. Similar to in vitro findings, the presence of diabetes activated PARP-1, lowered NAD+ levels following SIRT1 activity impaired. This in turn augmented acetylation of Lys310-p65 subunit-NFkB-complex which modulated the GFAP-upregulation. The oral administration of cocoa restored all these alterations. CONCLUSION: This study reveals, for the first time, that the beneficial mechanism of cocoa is depend of intracellular NAD+ content, enhancing SIRT1 activity which in turn deacetylates the Lys310-p65 subunit-NFkB-complex, lowering GFAP expression on rMCs. These observations were also present in retina from diabetic rats treated with oral administration of polyphenol-enriched cocoa. Fomentation:FAPESP. **Keywords:** SIRT1, Polyphenol, Antioxidants, Inflamation, Diabetes

A-05 - FORMATION OF REACTIVE QUINONE FROM 5-HYDOROXYINDOLEACETIC ACID CATALYZED BY MYELOPEROXIDASE - COVALENT ADDUCTION OF QUINONE WITH PROTEIN THIOLS -

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Serotonin (5-hydroxytryptamine; 5HT) is a monoamine neurotransmitter and also used to regulate intestinal movement in gut. It might be participated in encephalopathy such as depression. 5HT is metabolized into 5-hydroxyindoleacetic acid (5HIAA) by monoamine oxidase and then aldehyde dehydrogenase. We have found that 5HT is oxidized by myeloperoxidase (MPO) and a highly reactive quinone, tryptamine dione (TD), is then formed (Kato et al., CRT 2012). However, the oxidation of 5HIAA by MPO has not been examined. In this study, we have investigated the in vitro oxidation of 5HIAA by MPO in detail. At first, we confirmed the formation of quinone compound from 5HIAA by chemical trapping with o-phenelenediamine as a phenazine derivative. We then succeeded the chemical preparation of quinone from 5HIAA by Fremy's reagent. In the presence of a thiol compound, the quinone-thiol adduct was formed. We have prepared a novel monoclonal antibody specific to quinone-modified protein and characterized in detail. Since TD is known as a putative neurotoxin, we investigated the effect of quinone from 5HIAA on neuronal cells, SH-SY5Y. The cells were treated with the quinine and the lysate of cells were then separated by 1D or 2D-PAGE. Some proteins were selectively modified. The modified proteins were immunoprecipitated and then separated by 1D-PAGE. Cytoskeletal proteins, tubulins, vimentin, and peroxiredoxin, were identified as targets proteins. Modification of proteins by the quinone could alter their biological function and might be related to development of some diseases.
Keywords: Serotonin, 5-hydroxyindoleacetic acid, Myeloperoxidase, Quinone, neuronal cell

A-06 - ANTI-INFLAMMATORY ACTIVITY OF A MIXTURE OF BREIN AND MANILADIOL, TRITERPENES FROM PROTIUM SP., IN LPS-ACTIVATED J774 MACROPHAGE CELLS.

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Introdution: Inflammation acts as a central executor in the pathogenesis of many diseases such as heumatoid arthritis, arteriosclerosis, infections, cancer, metabolic disorders. Natural plant compounds which are able to suppress the production of inflammatory mediators from activated macrophages can act as potential anti-inflammatory agents. Objective: This study is aimed to explore and evaluate the anti-inflammatory potential of mixture of brein and maniladiol, triterpenes from Protium sp. Methods: J774 cells line (1x106 cell/well) were challenged with 2.5, 5 and 10 µg/mL of Brein/maniladiol and LPS (1 µg/mL) for 24h. Cells untreated and treated with LPS were used as negative and positive control, respectively. Cell viability was evaluated by a colorimetric method for the determination of cell densities using alamar blue assay. Culture supernatants were collected to analyze the production of nitric oxide using the Griess reaction assay. Results: The results showed that treatment with Brein/maniladiol decreased the formation of nitrite (nitric oxide-derived products) with an IC50 value of 4.05±0.45µg/mL. The cytotoxic effects of brein exhibited only moderate cytotoxicity at 10 µg/mL. Conclusion: Our results suggest that Brein/maniladiol mixture potentially decrease the inflammation in vitro, and might be a therapeutic agent against inflammatory diseases.
Keywords: Anti-Inflammatory , Protium , triterpenes

A-07 - CHOLESTEROL AND ITS OXIDIZED DERIVATIVES INDUCES ALPHA-SINUCLEIN SECONDARY STRUCTURE ALTERATIONS

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Cholesterol, an essential component of cell membranes, is the major component of the plasma membrane and it plays an important role in the maintenance of cellular homeostasis. The highest level of cholesterol is located in the brain. The human brain contains as much as 25% of total body cholesterol and cholesterol derivatives (~20 mg/g). Recently, two oxidative products from cholesterol, 3-hydroxy-5-oxo-5,6-secocholestan-6-al (CSec) and 3-hydroxy-5-hydroxy-B-norcholestane-6-carboxaldehyde (ChAld), called as secosterols, have been detected in human atherosclerotic tissues and in human brain from patients with Alzheimer disease. Moreover, it has been reported that secosterols can react with the beta-amyloid protein in Alzheimer disease resulting in the misfolding and aggregation of this protein. Secosterols can also accelerate alpha-synuclein fibrilization, which has been associated with Parkinson disease and Lewy body dementia. Considering that ChAld is the major aldolization product in biological system, we compared the effect of each secosterol on alpha-synuclein. CD analysis results show that both secosterols induces similar alteration in the protein secondary structure. These alterations were pH dependent and were not related to covalent modifications of the protein, as suggested by mass spectrometry analysis. Overall, these data suggests that CSec and ChAld induced alterations in alpha-synuclein secondary structure is mediated by hydrophobic interactions.
Keywords: Cholesterol, Secosterol, Alpha synuclein

B – Advanced Analytical Strategies for Oxidant Analysis

B-01 - BLACK SOYBEAN SEED COAT POLYPHENOLS PREVENT OXIDATIVE DNA DAMAGE IN HEPG2 CELLS

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Black soybean seed coat contains abundant polyphenols and has been reported to have various beneficial functions. In the present study, we investigated the protective effects of black soybean seed coat extract (BE) on 2,2′-azobis (2-amidinopropane) dihydrochloride (AAPH)-induced oxidative DNA damage in HepG2 cells. BE and procyanidin (PC)-rich BE were reduced AAPH-induced oxidative stress in HepG2 cells. PC-rich BE has slightly higher antioxidant ability than BE at the same concentration without significance. Moreover, both BE and PC-rich BE revealed almost the same oxygen radical absorbance capacity (ORAC) values, indicating that these extrtacts can suppressed radical formation and/or radical trap. To elucidate the protective mechanism of BE against oxidative DNA damage, measurement of the formation of 8-hydroxy-2′-deoxyguanosine (8-OHdG) was developed by LC-MS/MS. Treatment with BE and its polyphenols to the cells for 1 or 24 h completely inhibited AAPH-induced 8-OHdG formation in the dose- and time-dependent manners. Notably, PCs and cyanidin 3-glucoside (C3G) showed stronger inhibitory effect than catechin and epicatechin. Together all the results, PCs and C3G in BE will protect oxidative DNA damage in hepatocytes effectively.
Keywords: oxidative DNA damage, black soybean seed coat extract, 8-hydroxy-2′-deoxyguanosine, procyanidins, cyanidin 3-glucoside

B-02 - ANTIOXIDANT ACTIVITIES OF SPRAY DRIED EXTRACTS OF PSIDIUM GUAJAVA L. LEAVES AND THE CORRELATION WITH TOTAL PHENOLIC CONTENT

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Psidium guajava (guava) is important as food crop and as medicinal plant. In traditional medicine it is widely used for treatment of several diseases, including diarrhoea and gastroenteritis. The leaves of P. guajava are rich in polyphenols, which exhibit powerful antioxidant activity. The aim of this research was to evaluate the antioxidant capacity of spray dried preparations of guava extracts (SDP). The drying were carried out in a spray dryer (SD) using maltodextrin, arabic gum, colloidal silicon dioxide and β-cyclodextrin at concentration of 8% (wet base) as carriers. The ABTS, DPPH, FRAP and ORAC assays were used for estimating antioxidant activity (AA) of SDP. The chemical markers were evaluated through determination of the total phenolic (TP) and flavonoid (TF) content. Averaged AA [μM Trolox equivalent (TE)/g] were 3.296, and 1.913 as determined by the ABTS and ORAC respectively. The IC50 of the SDP ranged from 7.96 to 8.11 μg/mL using the DPPH• method. The FRAP values ranged from 4.210 to 4.540 (μM FeSO4 equivalent/g). Pearson's correlation coefficients show that the AA determined by DPPH assay had strong negative correlation with TF (r = -0.976) and TP (r = -0.980). The FRAP assay showed a strong positive relationship comparing to ORAC (r = 0.965). FT was strongly positive correlated with PT (r = 0.947). The guava SDP presented significant antioxidant activity presenting high potential as an active phytopharmaceutical ingredient for herbal medicine.
Keywords: Psidium guajava, antioxidant activity, total phenolic

B-03 - ANTIOXIDANT AND PROTECTIVE PROPERTIES OF BRAZILIAN PAMPA BIOME HONEYS IN VITRO AND IN VIVO

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Honey is a natural substance and complex mixture produced by honey bees with many medicinal effects such as antibacterial, hypoglycemic, reproductive and antioxidant effects. The aim of this study was to evaluate the antioxidant properties of Brazilian Pampa Biome honeys in vitro and its potential protective effect against oxidative stress induced by Fe and paraquat (PQ) in Drosophila melanogaster (in vivo). A total of 10 honey samples were tested for in vitro antioxidant activity (phenols, flavonoids, DPPH-ABTS scavenging activity and FRAP). Female flies (20 per group) were exposed for 48h as follows: control (1% sucrose), honey (10% solution), 15mM Fe (in 1% sucrose), 20mM PQ (in 1% sucrose), Fe + honey and PQ + honey. Honey samples were obtained from local suppliers and all samples were within the parameters required by Brazilian law. The survivorship and locomotor activity (negative geotaxis) were analyzed. Statistical analysis was done by One Way ANOVA followed by Duncan's post hoc test. All honey samples presented antioxidant activity in vitro. It was observed a substantial increase in dead flies exposed to iron (p
Keywords: Oxidative Stress, Honey, Drosophila, Iron, Paraquat

B-04 - FORMATION OF AMIDE-TYPE LIPID-LYSINE ADDUCT IN MODEL SYSTEMS

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Polyunsaturated fatty acids are known as highly oxidizable lipids because of their double bonds in the structure. Lipid hydroperoxides are formed as an initial stage of lipid peroxidation. In 1999, as a novel amide-type lipid-lysine adduct, hexanoyl lysine (HEL) was identified in the reaction mixture of lipid hydroperoxide derived from n-6 fatty acid and lysine (Kato et al., JBC). Subsequently propanoyl lysine (PRL) derived from n-3 fatty acid has also reported (Hisaka et al., FRBM). The contribution of these amide-type adducts to pathogenesis of diseases are still unknown. Moreover, the formation mechanism and physiological significance of these adducts are not clear. Then, we have currently performed the following researches. Research 1: We have investigated the possibility of participation of singlet oxygen and some other species in the formation of amide-linkage. The supplementation of sodium azide into a reaction system showed the suppressive effect on the amide-adduct formation, suggesting that singlet oxygen might be one of the players of amide-adduct generation. We also found the minor contribution of aldehyde pathyway to amide-adduct formation. Research 2: We have constructed model systems to make clear biological significance of the amide-adduct. First, the uptake of hexanoylated LDL by RAW264.7 cells has been evaluated by the antibody to HEL and also a fluorescent lipid probe. Next, hexanoylated/propanoylated protein in a liposome was added to the cultured cell. By using these model systems, we would like to investigate the participation of amide-adduct formation in apoptosis or neurological disease.

Keywords: Lipid peroxidation, Amide-type lipid-lysine adduct, Hexanoyl lysine, Propanoyl lysine, Liposome

B-05 - INCREASED VIRULENCE FACTOR OF FUNGAL BANANA PATHOGEN MYCOSPHAERELLA FIJIENSIS: GENERATION OF SINGLET MOLECULAR OXYGEN BY MELANIN AND MELANIN BIOSYNTHESIS INTERMEDIATES

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For plant fungal pathogens, melanin contributes to virulence by allowing tissue invasion, inactivation of the defence system and possibly cell death. In this work, we suggest that melanin synthetized by the fungal banana pathogen *Mycosphaerella fijiensis* is an increased virulence factor through the generation of singlet molecular oxygen ($^1O_2$). We demonstrated the generation of $^1O_2$ through monomol light emission at 1270 nm from melanin in the mycelia and melanin secreted to the culture media. The analyses of mycelia and culture media by elemental analysis, ultraviolet/infrared absorption spectrophotometry and MALDI-TOF mass spectrometry demonstrated that pigment content is 1,8-dihydroxynaphthalene-melanin (DHN-melanin), a polymeric melanin. Using melanin biosynthesis inhibitors (tricyclazol and pyroquilon), we identified many melanin biosynthesis intermediates by HPLC-MS/MS. Our results demonstrated that fungal source of $^1O_2$ could be DHN-melanin and melanin biosynthesis intermediates. The $^1O_2$ generated by M. fijiensis probably acts as a virulence factor of two ways: first it protects fungus from oxidative attack of plant and second, indirectly it causes necrotic death of plant cells.

Keywords: melanin, singlet molecular oxygen, HPLC-MS/MS, MALDI-TOF

B-06 - CHEMICAL INCORPORATION OF ANTIOXIDANTS INTO A BIODEGRADABLE POLYMER FOR CONTROLLED RELEASE

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Reactive oxygen species (ROS) in life organisms are produced as well as antioxidants, which are bioactive compounds that can counteract the effect of such harmful species. Under UV light and other stress situations, inhibitors aren't enough to react with ROS, causing redox misbalance and aging. In order to replace these antioxidants, we will show the development of a biodegradable and biocompatible catechol-based poly(anhydride-esters). This polymer is designed to break down and release the antioxidant after hydrolysis of anhydride and ester bonds. In order to synthesize this macromolecule, catechol was reacted with glutaric anhydride by ring opening forming the diacid, which was activated via acetylation generating the monomer, precursor of the polymer. Thus, catechol glutaric poly(anhydride-ester) was synthesized via melt-condensation. Unlike some systems in which the bioactive is simply mixed into a polymer, this system demonstrates the chemical incorporation of the bioactive into polymer backbone by Infrared Spectroscopy (FT-IR), proton Nuclear Magnetic Resonance (1H-RMN), Gel Permeation Chromatography (GPC) and Mass Spectroscopy (MS). For further characterization thermal properties were elucidated by Thermogravimetric Analysis (TGA) and Differential Scanning Calorimetry (DSC). Release studies were performed by HPLC. A slow and controlled releasing profile was observed, i.e. 0.5% of the loaded catechol were released over 20 days.
Keywords: oxidative stress, antioxidant, polymer, biodegradable, controlled release

B-07 - ELECTROACTIVE IN SITU GENERATED OLIGOMERS BASED ON NATURAL PHENOLS APPLIED AS REDOX MEDIATORS FOR THE ANALYSIS OF ANTIOXIDANTS

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Phenols are widely distributed in all the natural kingdoms. Some of them exhibit a wide range of biological activities, behaving also as antioxidants. In electrochemistry, they have several rules, mainly as analytes as well as molecular catalysts. The electrooxidation of phenolic compounds could lead to a noticeable decrease in the anodic current, due to the formation of electroinactive polymeric films on the electrode surface [1]. However, the electrooxidation of these compounds on MWCNT modified electrodes may lead to redox active couples [2], which may be successfully applied as mediators, in the analysis of important antioxidants, for instance, NADH, Ascorbic Acid (AA), Uric acid (UA), thiols and others. This was the case of three natural phenolic compounds presently studied: Xanthurenic (XA), Ferulic (FA) and Vanillic acids (VA). A glassy carbon (GC) electrode was modified with a dispersion of MWCNT in DMF (1 mg mL-1). Then, electroactive polymeric layers were electrodeposited by cyclic voltammetry. All of them were used as redox mediators in the analysis of AA, XA, gluthatione, cystein, NADH with good analytical figures of merit. The characterization, advantages and disadvantages of the use of each of them will be presented. [1] FERREIRA, M. et al. Electrochimica Acta 52 (2006) 434–442
[2] SILVA, F.A.S. et. al. Electrochemistry communications 12 (2010) 450-454
Keywords: Antioxidants, Bioelectrochemistry, Oxidative Stress, Phenols, Redox catalysis

B-08 - EFFECTIVE ANTIOXIDANT POTENTIAL OF RESINOUS EXTRACTS COLLECTED BY BRAZILIAN BEES

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Many diseases have their origins in erroneous molecular combination involving free radicals, especially when these molecules are in excess in the organism. The antioxidant properties of some compounds are fundamental in preventing the recombination of free radicals, blocking their damage to the metabolism and cellular structure. Due to the exponential increase connected to this kind of problem, various studies have been made with various methods in the field of biophysical together with other studies of specific areas of natural compounds in the search for non-toxic compounds with antioxidative capacity. Propolis, a product resulting from the collection of resinous compounds processed by bees, has a broad spectrum of preventive actions and diseases treatment, especially antimicrobial, anticancer and antioxidant activities. It is known that the resinous compounds that bees collect in vegetation, such as terpenoids, flavonoids and cafeic acids, are closely linked to the therapeutic action scavenging free radicals and affecting the properties of biological membranes of target cells. In this study, we measured the antioxidant activity of propolis collected by four bee species (one type was collected by Africanized bees specie and the others by Indigenous species) showing their high potential to be used against free radicals. We analyzed the efficiency of the antioxidant activity by reduction of the optical spectra signal from formazan, a dye formed when anion superoxide ($O_2\bullet^-$) interacts with Nitroblue Tetrazolium (NBT) salt. Through analysis of IC_{50}, we verified which propolis that has the greatest potential to scavenging the superoxide radical and inhibit that salt formation. Acknowledgement: CAPES, Cnpq, FAPESP;
Keywords: Brazilian propolis, antioxidant activity, Nitroblue Tetrazolium, IC50

B-09 - ANTIOXIDANT ACTIVITY AND CYTOTOXICITY OF SEED, PEEL AND PULP EXTRACTS OF *Myrciaria dubia*

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Myrciaria dubia, commonly known as camu-camu, is a fruit tree species native from the Amazon rain forest. It is one of the richest sources of vitamin C. The present study focuses on the investigation of the antioxidant activity and cytotoxicity of the seed, peel and pulp of camu-camu extracts on the cellular levels. The antioxidant properties were evaluated by assays *in vitro* for the potential to inhibit total ROS generation using 2',7'dichlorodihydrofluorescein diacetate (DCHF-DA) in cultured MRC-5 fibroblast cells. Free radical scavenger methods as 1,1-diphenyl-2-picrylhydrazyl (DPPH) and ABTS was also performed. For the cell viability assay, cytotoxicity in MRC-5 was assessed by Alamar Blue method. The results demonstrate strong inhibitory activity on total ROS generation increases in fibroblast cells and the DPPH and ABTS assays also indicated a high antioxidant activity, especially of the fractions obtained from the seed extract with IC_{50} = 16.77 ± 0.14 and 29.77 ± 0.72 μg/ml, respectively. For cell viability, the extracts of camu-camu in maximum concentrations of 50 μg/ml were not toxic in vitro, showing no significant cell death over time (24h/48h/72h).Our results suggest that camu-camu may be considered as a good source of bioactive compounds of biotechnological applications.
Keywords: antioxidant, cytotoxicity, camu-camu

B-10 - UPLC-MS IDENTIFICATION OF PHENOLIC COMPOUNDS AND ANTIOXIDANT CAPACITY OF MURICI (*Byrsonima crassifolia* (L.) Kunth)

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Byrsonima crassifolia (L.) Kunth (Murici) is a plant native to tropical America and in Brazil it has been used with medicinal purposes as anti-inflammatory. Among the compounds present in food that have functional properties, polyphenols receive significant attention because they protect the human body against inflammation and oxidative stress. However, there are few researches about murici bioactive compounds. Therefore, the aim of the study was to provide a comprehensive characterization of phenolic constituents in the murici by UPLC-MS. In addition, the antioxidant capacity was assessed to evaluate their biological activity. Polyphenol extraction was extracted with acetone/water (43:57, v/v) for 51 min at 29 °C. The antioxidant capacity was determined by using oxygen radical absorbance capacity (ORAC) and trolox equivalent antioxidant capacity (TEAC) assays. A total of 13 phenolic compounds could be at least tentatively identified as proanthocyanidins, catechin and quercetin derivatives. Identification of gallic acid and quercetina was assured by comparison of the fragments with those of an authentic standard. The mean concentration of quercetin was 3.0 mg/100 g in murici while that gallic acid was 1.8 mg/100 g. Lastly, murici extract acted effectively against ABTS•+(TEAC of 158.48 μM Trolox/g fresh murici) and peroxyl (ORAC of 106.49 μM Trolox/g fresh murici) radicals.
Keywords: Quercetin, Gallic acid, ABTS, ORAC

B-11 - ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITIES OF AMAZON FRUIT, PIQUIÁ (Caryocarvillosum Aubl)

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In the Amazon, is a very common treatment of diseases through the use of medicinal plants. The rich biodiversity that Amazon offers coupled with the traditional use of plants already known has aroused increasing interest in the study of plant species in the region. Caryocarvillosum (piquiá) is a fruit native to the Amazon region, considered a source of bioactive substances. In this study, we obtained the hydroethanolic extracts of peel, pulp and seeds piquiá and evaluated the antioxidant potential in fibroblast murine 3T3L1 line, measurement of nitric oxide of J774 murine macrophages line stimulated with LPS as indicative of anti-inflammatory activity. The extracts of peel, pulp and seed concentration of 20 μg/mL showed antioxidant activity of 70.69 ± 2.77%, 79.89 ± 6.50 and 79.48 ± 8.6, respectively. To evaluate the effect on the production of oxide, extracts were tested at concentrations of 25, 10 and 5 μg/mL. Only the seed hydroethanolic extract at a concentration of 25 μg/mL showed the ability to inhibit around 72.51 ± 1.65% the production of nitric oxide. According to the results, few data are available in literature on the chemical composition of the waste Amazonian fruits, making it necessary for characterizing a better utilization of the fruit. * PhD student scholarship from FAPEAM Financial support: FAPEAM
Keywords: ANTIOXIDANTE, CITOCINAS, HIDROETANÓLICO

B-12 - BIOACTIVE COMPOUNDS OF THE COLD PRESSURE GRAPE SEED OIL AND ITS CORRELATION WITH OXIDATIVE STABILITY

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The residues from winery are high and its use has been improved. The cold pressed grape seed oil (CPGO) can be contained high amount of minor components. The present study aimed to characterize minor constituents and evaluate the oxidative stability of the CPGO obtained from Brazilian agro-industrial waste of wines and juices. The quantification of bioactive components such as phenolics, flavonoids and carotenoids (spectrophotometric methods) and vitamin E (High performance liquid chromatography) were evaluated. In order to understand the contribution of these components on the oil oxidative stability (Rancimat assay - 110 •C/20 mL/h) and antioxidant capacity - oxygen radical absorbance capacity (ORAC) were measured. Spearman's correlations tests between the variables were analyzed. Total phenolic, flavonoids and carotenoids content were 59.63 ± 2.44 mg gallic acid equivalent.Kg^{-1}, 12.36 ± 0.54 mg quercitin equivalent.Kg^{-1} and 5.09 ± 0.05 mg.Kg^{-1}, respectively. The γ-tocotrienol isomer represents 57 % of the total vitamin E content quantified (492.9 ± 0.32 mg.Kg^{-1}). The total carotenoid content were statistically correlated with ORAC (28.34 mmol trolox equivalent.g^{-1}) and vitamin E (γ-tocotrienol, specifically) with the Rancimat assay (induction time 4.53 ± 0.05 hours). Phenolics and flavonoids no showed significant correlation between antioxidant capacities. In conclusion, it can be stated that Brazilian CPGO has a suitable profile of minor compounds and good correlation between the oxidative stability of the oil.
Keywords: agro-industrial waste, antioxidant capacity, vegetable oil

B-13 - BIOACCESSIBILITY AND ANTIOXIDANT CAPACITY OF PHENOLIC COMPOUNDS FROM JUA (Ziziphus joazeiro MART)

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Introduction The bioaccessibility of phenolic compounds is crucial to knowledge the beneficial effects of their on human health. the Jua (Ziziphus joazeiro Mart) is an endemic species of the Brazilian savanna, its fruit is sweet and has a high economic potential. Objective The aim of this work was to estimate the bioaccessibility and antioxidant capacity of phenolic compounds from Jua, by an in vitro gastrointestinal digestion. Methods Samples of Juá water extract and the yield of Jua in vitro enzymatic digestion (stomach and small intestine) were analyzed for total phenolics by Folin-Ciocalteau and oxygen radical absorbance capacity (ORAC) using fluorescein as the fluorescent probe. Analysis of variance (ANOVA) and significance test (p
Keywords: bioavailability, antioxidant, juá

C – Vascular Function and Metabolic Syndrome

C-01 - CHARACTERIZATION OF A METABOLIC SYNDROME ANIMAL MODEL IN C57BL/6J AND C57BL/6J LDLR -/- MICE.

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Metabolic Syndrome (MS) can be defined like a group of conditions that increases the cardiovascular and diabetes risk with obesity, dyslipidemia, arterial hypertension and insulin resitance as clinic features. In 20-70 years olds population have a prevalence of 24% in Brazil and world. An inflammatory reactions serie and insulin resistance, also triggered by obesity, are demonstrated in metabolic syndrome, showing that metabolic syndrome animal models is important to research development with the objective of understand not only the desease ethiology, but also to base and complement therapeutic intervention estudies. This study was developed metabolic syndrome animal models in C57BL/6J and C57BL/6J LDLr -/- mice, that demonstrated hyperglycemia, hyperinsulinemia and hyperleptinemia features, associated with insulin resistance and leptin resistance, both obesity and hypoadiponectinemia, also relevant features in metabolic syndrome.
Keywords: Metabolic Syndrome, C57BL/6J, C57BL/6J LDLr -/-

C-02 - DIMERIC AND MONOMERIC BETA 2-GLYCOPROTEIN I DIFFERENTIALLY CONTRIBUTE TO In vitro ANGIOGENESIS

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The β_2-glycoprotein I (β_2GPI) is an endothelial cell ligand, accessible for systemic, autocrine and paracrine signaling. *In vivo*, β_2GPI binds to the endothelial cell membrane heparan sulfate, to anionic phospholipids, and to functional receptors. The β_2GPI was attributed anti-angiogenic properties *in vitro* and *in vivo*. This work was designed to evaluate the effects of native, monomeric, and dimeric β_2GPI. Monomeric as well as dimeric forms were purified from human plasma, and the native protein was obtained as a balanced mixture of both components. The proliferation and differentiation of Human Umbilical Vascular Endothelial Cells (HUVEC) were considered in an in vitro angiogenesis model based on tridimensional cultures and quantitative digital image processing techniques. The *in vitro* HUVEC growth and differentiation in the tridimensional cultures microenvironment were addressed by the morphological analysis. The morphological aspects were correlated to the cell growth, oxidative balance outcome and mitochondrial toxicity assays, leading to the evidence that non-confluent HUVEC cultures temporarily stop growing in the presence of the native protein, but remain competent to proliferate. The β_2GPI monomer allowed the in vitro differentiation of the HUVECs into typical trabeculæ and incomplete capillary-like tubes, along with lowering the available proliferation fraction. The dimer rich purification fraction exposure halted cell elongation and migration, and prevented the organization of the tubular structures, maintaining cell growth. The morphological approach was useful to attribute to β_2GPI dimerization the cell migration inhibition modulation, which potentially leads to overcome the diminished sprouting antiangiogenic effect of the monomer fraction of the native protein.
Keywords: Beta 2 - glycoprotein I, Angiogenesis, Digital image processing, HUVEC

D – Redox Signaling / Cancer / System Biology

D-01 - REDOX STATUS EVALUATION OF RAT HEPATOCYTES TREATED WITH 5-AMINOLEVULINIC ACID

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5-Aminolevulinic acid (ALA) is the first precursor of heme, accumulated mainly in the liver in some types of hepatic porphyrias, as acute intermitente porphyria (AIP) and in lead poisoning, due to the diminished activity of porphobilinogen deaminase. Symptomatic patients of AIP present increased incidence of hepatocellular carcinoma (HCC). In vitro, ALA produces reactive oxygen species (ROS), through oxidation catalized by metals, deflagrating oxidative damages to DNA and proteins. This process can be related to initiation and development of cancer. It has been demonstrated that ALA is able to produce damage to DNA like plasmidial DNA strand breaks, increase of 8-oxodGuo and 5-OhdCyd in DNA of some rat cells treated with ALA and increase in generation of several modified bases in vitro. The objective of this work is to investigate the oxidative stress in primary culture of rat hepatocytes promoted by ALA, through analysis of qualitative and quantitative changes in composition of lipids in biomembranes that can led to modifications of their fluidity and selective permeability to biomolecules and quimioterapics. This study aims a better comprehension of molecular mechanisms involved in the relationship between ALA and major incidence of HCC in AIP.
Keywords: aminolevulinic acid, porphyria, cancer

D-02 - BLACK SOYBEAN SEED COAT EXTRACT INHIBITS GENOTOXICITY INDUCED BY BENZO[A]PYRENE THROUGH MODULATING OF DRUG-METABOLIZING ENZYMES

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Benzo[a]pyrene [B(a)P] is a polycyclic aromatic hydrocarbon and widespread in the environment. B(a)P is activated to epoxide form by cytchrome P4501A1 (CYP1A1), and this activated form induces oxidative DNA damage. Black soybean seed coat extract (BE) contains various polyphenols, such as catechins, cyanidin 3-glucoside (C3G), procyanidins and other flavonoids. We previously demonstrated that BE prevents B(a)P-induced oxidative DNA damage, but its mechanism is not clarify. To investigate the underlying mechanism, we examined the effects of BE and its polyphenols on B(a)P-induced CYP1A1 expression and the specific binding between aryl hydrocarbon receptor (AhR) and dioxin responsive element in HepG2 cells and ICR mice. We further examined the effects of BE and its components on glutathione S-transferase (GST) α, μ and π expression and the specific binding between nuclear factor erythroid 2-related factor 2 (Nrf2) and anti-oxidant responsive element (ARE). As the result, BE suppressed the expression of CYP1A1 by inhibiting AhR transformation, whereas BE enhanced DNA-binding activity of Nrf2 to ARE and subsequent induction of GSTs expression both in vitro and in vivo. Our findings demonstrate that procyanidins and C3G, which are the major polyphenols in BE, contributed to suppress formation of epoxide of B(a)P and promote its detoxification. From these results, polyphenols in BE have the potential to protect against B(a)P-induced oxidative DNA damage through modulating expression of drug-metabolizing enzymes.
Keywords: black soybean seed coat extract, benzo[a]pyrene, drug-metabolizing enzymes

D-03 - PARALLEL DAMAGE IN MEMBRANES OF LISOSOMES AND MITOCHONDRIA CAUSE PHOTO-INDUCED CELL DEATH BY AUTOPHAGY

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In order to understand the relationship between the photosensitizer (PS) structure and the efficiency in triggering specific cell death after PDT, we have used two PS - methylene blue (MB) and 1,9-dimethyl methylene blue (DMMB) in HaCaT cells. IC50 of DMMB and MB are 10nM and 2microM, respectively, showing 2 orders of magnitude higher efficiency of DMMB. Only MB induced a significant increase of ROS in a dose-dependent manner as measured by DCF fluorescence and the GSH/GSSG ratio. MB did not show autophagy induction. In the case of photosensitization with DMMB, we have observed autophagy induction by interruption of the normal autophagic flux (immune assays with LC3II and acid vacuoles by acridine orange). The mechanism of autophagy induction was shown to be the parallel damage in mitochondria and lysosomes, initiation of mitophagy, accumulation of autophagolysosomes and consequent autophagic cell death. Cell death induced by DMMB is not related exclusively to the general level of oxidative stress, but instead, with the generation ROS in the proper cell location and induction of specific cell death mechanisms. Developing concepts to improve specificity of the photosensitization reactions can be an interesting alternative to develop more efficient PDT photosensitizers.
Keywords: PDT, cell death, autophagy, membrane

D-04 - NOVEL KOJIC ACID DERIVATIVES ACT ON MELANOGENESIS AND SKIN AGE-RELATED ENZYMES

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The aging process is characterized by skin hyperpigmentation and degradation of extracellular matrix. Tyrosinase inhibitors such as kojic acid, are widely known and used as depigmenting agents, however, limitations such as stability and insufficient activity has stimulate researchers to synthetize derivatives to improve activity. This work was aimed to evaluate the cytotoxicity and effects of two novel kojic acid derivatives on the pigmentation and skin age-related enzymes. This study evaluated the cytotoxicity, antioxidant activity, inhibitory activity of collagenase, elastase and hyaluronidase (spectrophotometric assays) and depigmentant activity (. New synthetic compounds showed no cytotoxicity at 100 µM and low scavenging antioxidant activity. The iron chelating activity was better for Oleikojate cooper (Ok-Cu) (76.0 ± 5.7 %) than kojic acid (AK) (58.9 ± 5.63 %) and kojato cooper (Cu-Ak) (57.4 ± 5.24). The tyrosinase activity was strongly inhibited by derivatives, Cu-Ak (86.7 %) and Ok-Cu (92.5 %), while AK inhibited only 15 % of the tyrosinase activity, and the melanin content was reduced in 40.8 % and 38.4 %, respectively, observing low influence of AK in decreased melanogenesis and without alteration in tyrosinase gene expression by all samples. The derivatives of kojic acid used in this study have shown to be more stable and effective for inhibiting melanogenesis of the kojic acid. This makes them novel targets for possible cosmetic depigmenting products.
Keywords: melanogenesis, tyrosinase, antioxidant, matrix metalloproteinases

D-05 - S-NITROSOGLUTATHIONE OPHTHALMIC DROP INHIBITS INDUCIBLE NITRIC OXIDE SYNTHASE UP REGULATION BY ITS REDOX POST-TRANSLATIONAL MODIFICATION IN EXPERIMENTAL DIABETIC RETINOPATHY

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Diabetic retinopathy (DR) is associated with up regulation of inducible nitric oxide synthase (iNOS) and nitrosative stress. Retinal pigment epithelial (RPE) cells forms the blood retinal barrier (BRB) and high glucose been shown to increases iNOS expression. The purpose of this study was to evaluate the beneficial effects of S-nitrosoglutathione (GSNO) eye drop treatment through balance of nitrosative stress in experimental model of DR. Experimentally induced diabetic (DM) or controls rats were randomized to receive low (900nM) or high (10μM) dose of GSNO eye drop twice daily during 20 days. In vitro study was conducted with RPE cell line (ARPE-19) exposed to normal glucose (NG) or high glucose (HG) with or without GSNO (100nM-100uM) for 24 hours. In DM animals, GSNO eye drop diminished iNOS expression and peroxynitrite formation, resulting in improvement of retinal function. In cells exposed to HG, an increase in superoxide and nitric oxide productions were observed, accompanied by iNOS upregulation, peroxynitrite formation, and a decrease in GSNO, as well as reduced glutathione levels and GSNO reductase (GSNO-R) expression. Treatment with GSNO under HG conditions counteracted the nitrosative stress due to iNOS down regulation by its S-glutathionylation. This post-translational modification was probably promoted by the release of oxidized glutathione through GSNO denitrosylation via GSNO-R. A new therapeutic modality (GSNO eye drop) targeting nitrosative stress by redox post-translational modification of iNOS was efficient against the early damage in experimental DR showing the potential clinical implications of S-nitrosoglutathione/glutathione system balance in the treatment of DR.
Keywords: diabetic retinopathy, nitrosative stress , retinal pigment epithelial cells, S-glutathionylation, S-nitrosoglutathione

D-06 - THE TREATMENT EFFECTS OF ASCORBIC ACID AND FLAVONOIDS IN THE GUT OF A RAINBOW TROUT ONCORHYNCHUS MYKISS (WALBAUM 1792)

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The occurrence of diseases in fish farming is the result of several factors involved in the zootechnical methods and environmental conditions changes. Inadequate conditions may affect homeostasis and make animals more susceptible to potential pathogens. Antibiotics are commonly used in the bacterial diseases control, but their indiscriminate use can lead to selection of resistant pathogens as addition to being a source of environment pollution. An alternative is the use of immunostimulants and additives in disease prevention. Considering that the gastrointestinal tract serves as the first line of defense against pathogens, the present study aimed to analyze the influence of an commercial additive, with organic acids and flavonoids in the rainbow trout growth performance and gut histology. The 90 days of treatment do not influence the mass gain, carcass yeld or hepatosomatic index, whereas in the evaluation of gut can be observed an decrease density of goblet cells of pyloric cecae and increased in the anterior intestine as well as increased intestinal epithelial height of that portion, thus it is possible that due to changes in the dynamics of cell populations in the villi a population increased or a larger renovation rate have collaborated with this changes.
Keywords: Goblet cells, Epithelial height, Quercetin, Rutin, Citric flavonoids

D-08 - DYNAMIC MODULATION OF NRF2/KEAP1 SYSTEM BY BAICALEIN, A CHEMOPREVENTIVE COMPOUND

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Baicalein, a major component of Scutellaria Baicalensis Georgi (Huang Qin), is widely used in the traditional Chinese medicine. However, the mechanisms underlying cancer chemoprevention are still not clear. The present study aimed to clarify how baicalein modulate Nrf2/Keap1 system to exert its cytoprotection and cancer chemoprevention. In the upstream cellular signaling, baicalein stimulated phosphorylation of MEK1/2, AKT and JNK1/2, which were demonstrated to be essential for baicalein-induced Nrf2 expression by their inhibitors. Immunoprecipitation with Nrf2 found that baicalein increased the amounts of Nrf2-bound phosphorylated MEK1/2, AKT and JNK1/2. Baicalein not only up-regulated the expression of Nrf2 mRNA and protein, but also stabilized Nrf2 protein by inhibiting the ubiquitination and proteasomal turnover of Nrf2. Simultaneously, baicalein down-regulated Keap1 by stimulating modification and degradation of Keap1 without affecting the dissociation of Keap1-Nrf2. Silencing Nrf2 using Nrf2 siRNA markedly reduced the ARE activity under both baseline and baicalein-induced conditions. Thus, the modulation of Nrf2/Keap1 system by baicalein involved the upregulation of Nrf2 at both transcription and posttranscription sites, and the downregulation of Keap1 by affecting the posttranscription site. These finding provide an insight into the mechanisms underlying baicalein in cytoprotection and cancer chemoprevention.
Keywords: Baicalein, Nrf2, Keap1, Transcriptional Regulation, Posttranscriptional Regulation

E – Carotenoids and Other Phytochemicals

E-01 - AN ANTIOXIDANT FIBER FROM CASHEW APPLE (Anacardium occidentale, L.) AND ITS ASSOCIATED COMPOUNDS

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The cashew bagasse is an abundant agroindustrial residue and can generate a serious environmental problem, but it can also be a rich source of products with technological and functional properties. In this study the antioxidant activity, directly measured on the fiber, flavonoids and anacardic acids, from a cashew apple residue were analyzed, before and after the extraction of non-polar and polar compounds. The cashew bagasse was subjected to five extractions with water at 1:1 (w/w), on an expeller press. After extraction two samples (control and treated) were dried at 60 °C, milled and sorted into four different sizes. Subsequently, anacardic acids and flavonoids were extracted, leading to a fiber without extractable compounds. Cashew fiber antioxidant activity ranged from 6.1±0.7 to 79.9±7.7 mmol Trolox/Kg, while that in the fiber without extractables the values ranged from 150.0 ± 10.5 to 387.9 ± 5.3 mmol Trolox/Kg. The methanolic extract presented the highest antioxidant activity (210.0 to 526.3 mmol Trolox/Kg). This extract was analyzed by LC-MS and 22 peaks were detected, being quercetin and myricetin glucosides the main compounds. The major anacardic acids were also quantified and the highest concentrations, especially for anacardic acid C15:3 were observed on the control samples. Due to its antioxidant activity and the profile of compounds associated, namely anacardic acids and flavonols, cashew fiber is a good candidate to be a functional ingredient on food products.
Keywords: anacardic acids, dietary fiber, flavonol, LC-MS

E-02 - PREDICTION OF THE FUNCTIONALITY OF YOUNG SOUTH AMERICAN RED WINES BASED ON CHEMICAL PARAMETERS

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Wine functionality is an emerging parameter that may affect consumers' decision to purchase a wine. Thus, our objective was to classify young South American red wines according to their functionality. Four factors were considered for sample selection: vintage (2009/2010), variety (Cabernet Sauvignon, Malbec, Carménère, Merlot, Syrah and Tannat), country (Argentina, Brazil, Chile and Uruguay) and price (1.0-50.0 USD/bottle). Functionality of the wines was defined by their antioxidant activity (DPPH and ORAC), total polyphenols (TP), total anthocyanins (TA) and color. After applying multivariate analysis, wines were separated into three clusters. Wines grouped into the highest functionality cluster presented higher values of TP, TA, antioxidant activity, darkness and more purple color than wines classified as Intermediate or Low Functionality. Using discriminant analysis, more than 96% of the wines were correctly classified according to their functionality, using only seven markers. Applying multivariate analysis, 666 South American wines were classified according to their functionality based on chemical markers, color and price. Malbec and Tannat wines produced in Argentina, priced above 15.00 USD/bottle, showed the best functionality. The present study brings relevant information that may help wine consumers on their choices regarding wine functionality.
Keywords: functionality, phenolic, antioxidant, wine, cluster

E-03 - CORRELATION BETWEEN PHENOLIC COMPOUNDS AND ANTIOXIDANT ACTIVITY IN BROWN RICE BEFORE AND AFTER PARBOILING

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The purpose of the study was to evaluate the effect of parboiling brown rice on the phenolic contents and their correlation with antioxidant capacity. Twenty seven non-pigmented brown rice samples were grown in 2008 by different producers and provided by Itajaí Experiment Station/EPAGRI (Santa Catarina State Agricultural Research and Rural Extension Agency) in Brazil. Soluble phenolic compounds were extracted before and after parboiling with EtOH 80%. Bound phenolics were released by NaOH, then partitioned with ethyl acetate and all extracts analyzed for their phenolic contents. Antioxidant activity was measured by the oxygen radical absorbance capacity (ORAC) and the 1,1-diphenyl-2-picrylhydrazyl (DPPH•) free radical scavenging methods. Total phenolic content in non-parboiled brown rice was about 1150 mg ferulic acid eq./kg and the ratio between soluble and insoluble phenolics was 55:45. Parboiling decreased in almost 60% the contents of soluble phenolics which were the most affected by the process. However, the insoluble phenolic fraction showed in average a slight increase after rice parboiling, probably due to polymerization and complexation reactions. Correlation coefficients between levels of soluble phenolics and antioxidant activity were high for ORAC (r=0.825, p
Keywords: antioxidant, parboiling, phenolic compounds, rice

E-04 - CONTENTS OF PHENOLIC COMPOUNDS AND ANTIOXIDANT ACTIVITY IN WHOLE BLACK, RED AND WILD RICE, BEFORE AND AFTER COOKING

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This study describes a comparative approach of phytochemical contents and antiradical efficiency of whole 16 black, 9 red and 6 wild rice accessions, before and after cooking. The black and red (*Oryza sativa* L.) genotypes were grown at Itajai Experiment Station/Epagri (Santa Catarina Agricultural Research and Extension Institute) from 2009 to 2011, whereas the wild rice (*Zizania spp.* L.) was from Canada. Total phenolic compounds, flavonoids, proanthocyanidins and anthocyanins contents were determined by spectrophotometry. Antioxidant activity was determined by ORAC and DPPH• methods and anthocyanins were identified by HPLC-DAD-MS/MS. Black rice showed higher contents of total phenolics (446±79 mg ferulic acid eq./100g) and flavonoids (390±42 mg catechin eq./100g) than red and wild rice. Anthocyanins were the predominant flavonoids in black grains (365±88 mg cyanidin glucoside eq./100g) and the main compounds were cyanidin-3-*O*-glucoside and peonidin-3-*O*-glucoside. Wild rice had the lowest contents of total phenolics (215±17 mg ferulic acid eq./100g) and flavonoids (106±4 mg catechin eq./100g). Red rice had intermediary phytochemical contents and proanthocyanidins were the major flavonoids. ORAC method was more suitable than DPPH• to differentiate black, red and wild rice regarding their antioxidant activity. Black rice had the highest antiradical efficiency (18±2 mmol trolox eq./100g), strongly correlated with the anthocyanin and flavonoid contents (r=0.951, r=0.937; p
Keywords: anthocyanins, pigmented rice, thermal processing

E-05 - ANTIOXIDANT AND ANTIPROLIFERATIVE ACTIVITIES OF A HYDROLYZED DERIVATIVE OF RUTIN

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Rutin (quercetin-3-O-rutinoside) is a flavonoid diglycoside which shows several pharmacological functions such as antioxidant and antitumor activities. Bioavailability and biological properties of flavonoid glycosides can be improved after the hydrolysis of specific glycosyl groups. In this study, we evaluate the antioxidant and antiproliferative activities of a derivative obtained after rutin enzymatic hydrolysis by hesperidinase. The enzyme was heated at 70°C for 30 min to inactivate the undesirable β-D-glucosidase activity. Hydrolysis was carried out using 100 µL of enzyme preparation (50 mg L-1) and 4 mL of a 1% (m/v) rutin solution, at 40°C, for 2, 4, 8 and 12 h. The antioxidant potential was investigated using DPPH radical scavenging method, β-carotene bleaching and xanthine oxidase inhibition assay. Antiproliferative in vitro activity was studied in ten human cancer cell lines, including melanoma, breast, renal, ovarian, prostate, colon, leukemia and lung cancer. Rutin was predominantly converted into quercetin-3-glucoside (70%), as evaluated by UPLC-MS, which demonstrated high DPPH radical scavenging activity. There were no significant differences in the inhibition of lipid peroxidation, estimated by the β-carotene bleaching method, and in the inhibition of xanthine oxidase before and after hydrolysis of rutin. In vitro antiproliferative assays showed that quercetin-3-glucoside exerted a more powerful activity than quercetin and rutin on various cancer cell lines, especially glioma and ovarian and breast adenocarcinomas. The removal of the rhamnoside terminal of rutin improves its radical scavenging and antiproliferative activities. Quercetin-3-glucoside can be a promissory functional derivative obtained by selective hydrolysis of rutin.
Keywords: Rutin, Antioxidant, Antiproliferative, Enzymatic hydrolysis

E-06 - TOXICITY-INDUCED BY Psidium guajava ESSENTIAL OIL ON FRUIT FLY Drosophila melanogaster: EVIDENCES FOR A BIOINSECTICIDE ACTION

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Chemical pesticides have been used for decades in agriculture and are extremely harmful to humans and the environment. This has motivated the search for natural insecticides, which are less noxious than chemical ones. Because of its redox properties, plant derived essential oils have been widely investigated for their bioinsecticide activity. Thus, our study aimed to evaluate the bioinsecticide effect of the essential oil from two varieties of *Psidium guajava* (red guava and white guava) in the fruit fly model (*Drosophila melanogaster*; Harwich strain). Flies were exposed to essential oils by a fumigation procedure, in which 30 flies per group were exposed for 6 to 12 hours in the presence of oils in a closed vial. Flies concomitantly fed on 1% sucrose solution during oil exposure. Controls were administered sucrose only. Essential oil toxicity was evaluated by mortality and locomotor alterations (climbing test). A survival curve for determining the LD50 was performed. It was observed a significant decrease in survival of the flies exposed to the red guava essential oil, when compared to control. White guava oil also produced some degree of mortality, however in a lesser extent when compared to the red guava oil. We also observed a significant decrease in locomotor activity in flies treated with red guava oil. This study shows the potential bioinsecticide effect Psidium guajava, however more studies are being conducted to elucidate the toxicological mechanisms of essential oils, as well as the phytochemical constituents responsible for this effect.
Keywords: Psidium guajava, biopesticide, Drosophila, essential oil

E-07 - EFFECT OF HESPERITIN AND ITS GLYCOSIDES SUPPLEMENTATION ON THE LIPID PROFILE AND OXIDATIVE STRESS MARKERS IN RATS

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Many flavonoids exist in both aglycone and glycone forms. Different biochemical and pharmacological activities between these two forms have been reported to a number of flavonoids. Hesperitin is the aglycone form of the flavonoid glycoside hesperidin, an abundant flavonoid in citrus fruit which has biological beneficial effects. In this study, we compared the effects of hesperitin (HT) on the lipid profile and oxidative stress markers with those of hesperidin (HD) and G-hesperidin (GHD), a water-soluble derivative of hesperidin, in rats. Male Wistar rats that were fed with standard rat chow diet received saline solution (control group) and 1mmol/Kg of HT, HD or GHD in 2 mL of saline solution by gastric intubation for 30 days (n=6/group). After the study period, the levels of total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides were measured in serum. Thiobarbituric acid reactive substances (TBARS), glutathione peroxidase, (GPx), superoxide dismutase (SOD) and catalase (CAT) levels were measured in hepatic tissue samples. The levels of total cholesterol, LDL-cholesterol, HDL-cholesterol and triglyceride were not significantly different between the groups. Hepatic measurements of SOD and GPx levels were found to be significantly higher in group HT and HD than in GHD and control groups. There were no significant differences between the groups in respect to TBARS and CAT levels. These results indicates that both HT and HD, but not GHD, showed to exhibit positive changes in oxidative-antioxidative balance and the administration of both HT and HD significantly increased the levels of GPx and SOD in the liver.
Keywords: Antioxidant, Hesperidin, Hesperitin, Lipid profile, Oxidative status

E-08 - STUDIES ON LEPTOSIN IDENTIFIED IN MANUKA HONEY. - ITS METABOLISM AND APPLICATION OF PLAUSIBLE CERTIFICATION FOR MANUKA HONEY -

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Manuka (Leptospermum scoparium) is often used as therapeutics in New Zealand. Honey from manuka has high antibacterial and anti-inflammatory activities. Although methylglyoxal (MGO) has been reported as the major antibacterial compound, the MGO can't explain the whole biological activities. Recently we have found a novel glycoside, leptosin (methyl syringate 4-O-beta-D-gentiobiose), from manuka honey (Kato et al., JAFC2012). We would like to present the following two studies on leptosin. 1) Metabolism of leptosin and methyl syringate in cultured cells It's important to examine the metabolism of phytochemicals because most biological actions by phytochemicals arise in a body. Leptosin is composed of methyl syringate (MSYR) and gentiobiose. At first, leptosin was treated with commercially available alpha or beta-glucosidases. Only beta-glucosidase digested the leptosin, accompanied by the formation of MSYR and monoglycoside of MSYR. By incubation of leptosin or MSYR with Caco2 or HepG2 cells, both the glucuronic acid conjugate and sulfate conjugate of MSYR were detected. On the other hand, leptosin in the culture mediums was hardly changed. We are going to perform animal experiments to know in vivo metabolism of leptosin. 2) Plausible certificate of manuka honey bland by measuring leptosin and MSYR Manuka honey contains MGO, which is transformed from dihydroxyacetone during storage of the honey. This means that the amount of MGO is not constant in a jar. We have established a method for simultaneous sensitive detection of leptosin and MSYR. Currently we have analyzed the amounts of leptosin and MSYR in some commercial products.
Keywords: manuka honey, Leptosin, Caco2, metabolism

E-09 - CAROTENOID INTAKE IN THE BENEFICIARIES OF "BOLSA FAMÍLIA" PROGRAM DIET, ACCORDING TO GENDER

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Background and objective: Carotenoids are natural colors that act as antioxidants and are mainly found in fruits and vegetables. The objective of this study was to estimate the intake of these phytochemicals in the diet of beneficiaries of a national conditional cash transfer program – the "Bolsa Família" (BFP) – according to gender. Methods: Individual food intakes of National Dietary Survey (n = 34,003; 10 years old or more), conducted by the Brazilian Institute of Geography and Statistics in 2008-2009 (cross-sectional study) were assessed. From all of the sample individuals 7,600 were identified as BFP beneficiaries. A database was built with food descriptions from the survey (n = 1,121; 15 different culinary preparations) and the composition of beta-carotene, lycopene, lutein, zeaxanthin, beta-cryptoxanthin and alfa-carotene were obtained at a national source, preferably, and at a North American one. The daily prudent ingestion values recommended by the Institute of Medicine of United States of America were used as reference: for beta-carotene (3,000 to 6,000 μg) and total carotenoids (9,000 to 18,000 μg). Results: Total carotenoid consumption by beneficiary men (7,298.77 μg) and the others (7,334.06 μg) were similar. Non-beneficiary women, however, ingested an intake considerably higher (8,300.52 μg) when compared with beneficiary women (6,751.35 μg). Lycopene was the most consumed carotenoid (among 3,840.3 μg for beneficiary women and 4,848.41 μg for non-beneficiary women), followed by beta-carotene, lutein and zeaxanthin. Conclusions: Consumption by men and women is insufficient, especially by low-income women. Public policies that stimulate healthy diets are recommended, especially the ones focusing on this population group. de Fomento: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior.
Keywords: carotenoids, antioxidant, ingestion, Brazil, Bolsa Família

E-10 - ANTIOXIDANT ACTIVITY OF HYDROETANOL EXTRACT OF ACAÍ (Euterpe oleracea, Mart.) SEEDS

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The metabolic syndromes such as diabetes and atherosclerosis can be their origin in the harmful effects caused by free radicals. Several studies have demonstrated high content of phenolic compounds in açai *Euterpe oleracea*, Mart.) and are known the beneficial effects of these compounds in metabolic diseases. This work aimed study antioxidant activity of hydroetanol extract of açaí seeds. Samples were collected in the city of Coari/AM - Brazil. The extraction of phenolic compounds was by cold maceration for 48 hours with a hydroalcoholic extraction solution 5:5 (v/v) with the drug/solvent of 10%. The extracts (ASE) obtained were dried by spray dryer. Antioxidant assays DPPH, ABTS, superoxide, TBARS was performed and also quantified phenolic and flavonoids content. In antioxidant cellular assay were used MRC-5 fibroblast human cells. In these assays we used eight different concentrations of the extract for obtaining the minimum concentration in 50% (IC50). The content phenolic of ASE was 37.42 ± 1.87%, and the totals flavonoids was 1.69 ± 31%. In DPPH and ABTS assays present an excellent antioxidant with the IC50 of 9.56±0.46 µg/mL and 17.88±0.49 µg/mL, respectively. However, ASE presented a minimum inhibitory action against the superoxide anion with IC50 of 50.14±1,25 µg/mL and inhibited only 17.22±2.88% of lipid peroxidation products at 100 µg/mL. In cell antioxidant assay, the ASE inhibited 82.33±3.62% at 100 µg/mL. Then, the ASE proved be a promising antioxidant and can be used as materials for cosmetic and food industry.
Keywords: antioxidant, Euterpe oleracea, Amazonian, Phenolics

E-11 - OPTIMIZATION OF EXTRACTION OF PHENOLIC COMPOUNDS FROM PASSION FRUIT SEED RECOVERED FROM JUICE PRODUCTION RESIDUE USING RESPONSE SURFACE METHODOLOGY

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The extraction of phytochemicals (EP) from agricultural residues is particularly interesting, since it is a valuable source of natural antioxidants substances that can be used in food, drugs and cosmetic industries. More recently, statistical techniques have been used for evaluating the effects of the factors and determine optimal conditions of extraction factors for desirable responses. In order to optimize EP, a complete factorial design 33 and response surface methodology (RMS) were applied in designing the experiments. Passion fruit seed were submitted to a conventional solid–liquid extraction method, using ethanol as solvent at different concentrations (30–80 %), extraction time (40–120 min) and temperature (30–70 °C) and the influence of these independent operational variables on the content of total phenols (TPC, g gallic acid equivalents (GAE)/100 g extract) and antioxidant activity by oxygen radical absorbance capacity assay (ORAC, µmol Trolox equivalent (TE)/ g extract), of the produced extracts were evaluated. The statistical analysis was performed using the analysis of variance including the F-ratio. Regression analysis showed that more than 93% of the variation was explained by the models. Using the RSM the optimal conditions were selected: 80 °C, 70 % at 30 min. Under that condition the predicted values for TPC and ORAC were 3.14 g GAE/100 g extract and 11.7 µmol TE /g extract. Those variables were statistically significant correlated (r=0.802, p
Keywords: Passion Fruit Seed, Antioxidant Capacity, phenolic compounds

E-12 - ELLAGIC ACID PROTECTS WILD-TYPE AND SOD-DEFICIENT YEAST FROM MENADIONE-INDUCED STRESS.

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Yeasts deficient in the CuZn-SOD (Δsod1) present a reduced growth rate under aerobic conditions and greater sensitivity to redox-cycling drugs, such as menadione. The Δsod1 mutant phenotype is, in part, explained by superoxide toxicity, which oxidizes [4Fe-4S] clusters from key metabolic enzymes, causing iron release and enzyme malfunction. Increased "free iron" also promotes oxidative stress in yeast. In this study, we analyze the effect of the polyphenol ellagic acid (EA) against a menadione-induced oxidative stress in wild-type and Δsod1 yeasts. Menadione was toxic to Δsod1 strains when present in concentrations higher than 20 μM, while the wild-type strain was resistant up to 50 μM menadione. In the absence of menadione, EA improved Δsod1 viability in a dose-dependent manner. Moreover, 50 μM EA increased, by over 30%, the viability of the wild-type strain treated with 15 μM menadione, an effect not observed in the Δsod1 strain treated with EA. Menadione also increased by 10 fold GSSG levels (due to GSH oxidation) in SOD-deficient yeast while no effect was observed in the wild-type. The administration of EA, on the other hand, increased total-GSH levels in both strains (by about 60%) and induced a decrease in GSSG formation in the Δsod1 strain trated with menadione. Furthermore, menadione did not cause a significant alteration on yeast membrane integrity nor in the concentration of ergosterol, a highly oxidizable sterol present in fungi membranes. Altogether, these results suggest that EA presents an antioxidant effect demonstrated by improvement of cell viability and minimization of GSH oxidation. Further studies are under way to evaluate EA capacity to modulate yeast antioxidant enzymes.
Keywords: elagic acid, menadione, SOD-deficient, yeast

E-13 - CAROTENOIDS PROTECT HUMAN ERYTHROCYTES AGAINST OXIDATIVE DAMAGE INDUCED BY PEROXYL RADICALS

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Despite the presence of endogenous antioxidants in erythrocytes, such as glutathione, glutathione peroxidase, and catalase, these cells are highly susceptible to oxidative damages due to their high concentration of oxygen and iron. Here, we evaluated the potential of β-carotene, zeaxanthin, lutein, β-cryptoxanthin and lycopene, which are carotenoids usually detected in human blood plasma, to prevent oxidative damage in erythrocytes induced by peroxyl radicals (ROO●) generated by 2,2´-azobis (2-methylpropionamidine) dihydrochloride (AAPH). Human erythrocytes (n=4) were subjected to induced oxidative damage and the following biomarkers of oxidative stress were monitored: hemolysis, lipid peroxidation, oxidation of hemoglobin, depletion of glutathione (GSH) and the formation of oxidized glutathione (GSSG). Among the tested carotenoids (0.09-3 μM), lycopene (IC50 =0.24±0.05 μM) was the most efficient one to inhibit erythrocyte hemolysis, followed by β-carotene (0.32±0.02 μM), lutein (0.38± 0.02 μM) and zeaxanthin (0.43±0.02 μM). However, β-cryptoxanthin did not present any anti-hemolytic effect up to the highest tested concentration. β-carotene and zeaxanthin were the most efficient in preventing the oxidation of hemoglobin (IC50=2.9±0.3 μM and 2.9±0.1 μM, respectively), whilst lutein presented 45% of inhibition at the highest tested concentration. Regarding lipid peroxidation, lutein was more efficient (IC50=2.5±0.7 μM) than zeaxanthin (2.8±1.1 μM) and β-carotene (3.0±0.5 μM). It is interesting to notice that β-cryptoxanthin and lycopene did not inhibit hemoglobin oxidation or lipid peroxidation even at the highest tested concentration. Furthermore, ROO●- mediated GSH depletion and GSSG formation were not prevented. Our study demonstrates that carotenoids may act as natural antioxidants to prevent ROO●-induced toxicity in human erythrocytes.
Keywords: carotenoids, erythrocytes, oxidative damage, peroxyl radicals

F – Stress Response / Adaptation / Proteasome

F-01 - STUDY OF THE ROLE OF RTG-DEPENDENT RETROGRADE SIGNALING PATHWAY IN MITOCHONDRIAL ACTIVITY MAINTENANCE OF S. cerevisiae

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RTG-dependent retrograde signaling is a communication pathway between the mitochondrion and the nucleus. Through a mechanism still being elucidated, the protein complex Rtg1/3 is translocated from the cytoplasm to the nucleus, where it acts as a transcription factor. The Msk1/Bmh1/2 complex is a negative regulator of retrograde signaling, preventing Rtg1/3 nuclear translocation. However, if Msk1p is bound to Rtg2, the signaling pathway is unblocked. Our main goal is to study the contribution of the Rtg1 and Rtg2 proteins to mitochondrial function and mass, using S. cerevisiae grown in fermentative or respiratory media. After 7 days of growth in fermentative medium, WT cells present lower respiratory rates than RTG1Δ and RTG2Δ cells, indicating that cells lacking the retrograde signaling pathway are unable to react to a substrate shift. We confirmed higher mitochondrial activity in RTGΔ cells by measuring citrate synthase activity. By using isolated mitochondria, we identified that not only RTGΔ cells have more mitochondria than WT, but also each mitochondrion has a higher respiratory rate, though similar membrane potentials. Measurements of hydrogen peroxide production show that WT cells produce more oxidants, indicating that the signaling pathway is not acting to remove defective mitochondria, but may possibly provide a nutrient source. The assessment of mitophagy, the quantification of mitochondrial cytochromes as well as lifespan assays in minimum medium will help to elucidate these questions.
Keywords: MITOCHONDRIA, RETROGRADE SIGNALING, RTG

F-02 - ANALYSIS OF THE GLUTATHIONYLATED CYSTEINE RESIDUES IN THE A-SUBUNIT OF THE 20S PROTEASOME FROM THE YEAST Saccharomyces cerevisiae

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The proteasome is a multicatalytic complex responsible for the degradation of poly ubiquitinylated proteins involved in cellular regulation and signalling, antigen presentation and control of protein synthesis. The proteasome consists of a central unit named 20S proteasome (20SPT) coupled to regulatory units (*e.g.*, 19S) in one or both sides. The 20SPT is able to degrade oxidized proteins independent on ATP and poly ubiquitinylation. This mechanism is considered an anti-oxidative defense to prevent protein aggregation. The 20SPT is formed by two heptameric rings called β, where the active sites are located, flanked by two heptameric rings called α that regulate the gating of the catalytic chamber. Our group demonstrated that the 20SPT is prone to the oxidative post-translational modification namely glutathionylation. The glutathionylation of the 20SPT promotes gate opening, therefore increasing the degradation of oxidized proteins. The Cys residues prone to glutathionylation in vivo are C76 and C221 both into the α5 subunit. Strains with site-specific mutations of C221- and C76-α5 subunit were obtained. Assays performed with these strains revealed that the C221S strain exhibited very similar growth when compared with the wild-type strain, higher resistance to oxidative challenge induced by H2O2 and increased proteolytic activity accompanied by increased frequency of the open gate conformation. The C76S strain showed opposite phenotype. The glutathionylation of the C221-α5 residue is most probably a negative regulation of the 20SPT gate opening. The true positive regulation through glutathionylation seems to be centered on the C76-α5.
Keywords: Proteasome, Proteolysis, Redox modulation, S-glutathionylation

F-03 - A MODEL FOR PROTEOTOXICITY IN THE YEAST S. cerevisiae BASED ON REDOX POST-TRANSLATIONAL MODIFICATION OF THE 20S PROTEASOME

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The proteasome is a multimeric and multicatalytic complex responsible for the degradation of poly ubiquitinylated proteins involved in cell regulation and signaling, antigen presentation, and quality control of protein synthesis. It consists of a catalytic central unit called 20S proteasome (20SPT) and regulatory units (19S) coupled in one or both extremity to form the 26S proteasome. The 20SPT is able to degrade oxidized proteins via an ubiquitin- and ATP-independent process. This mechanism is considered an antioxidant defense to prevent protein aggregation. The 20SPT consists of a central unit formed by two heptamers called β, where the active sites are located, flanked by two heptamers named α that regulate the gating of the catalytic chamber. It was described by our group that the 20SPT of the yeast *S. cerevisiae* undergoes oxidative post-translational modification called S-glutathionylation. The in vivo S-glutathionylated cysteine residues are the C76 and C221 in the α5 subunit. The 20SPT S-glutathionylation promotes the opening of the catalytic chamber which results on increased degradation of oxidized proteins. Strains with site-specific mutations of C221- and C76-α5 subunit presented opposite phenotypes related to growth and resistance to oxidative stress. Based on that, these strains have been utilized as a model of proteotoxicity. They were transformed with the human wild type SOD1 gene and the same gene carrying the mutation G93A, found in familial cases of Amyotrophic Lateral Sclerosis.
Keywords: Proteasome, S-glutathionylation, Proteotoxicity, SOD1, ALS

F-04 - SOCIAL ISOLATION STRESS INDUCES THE EXPRESSION OF HEME OXYGENASE-1 IN MOUSE LIVER

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There are few reports about the effects of social stress in our daily life on HO-1 expression. Moreover, the intrahepatic localization of HO-1 expression in response to social stress is unclear. Then, in this study, we investigated whether isolation stress induces HO-1 in the mouse liver. The male BALB/c mice (4-week old) were exposed to isolation stress. The control mice were housed at five per cage and the five isolated mice were housed individually. After 2 days, the mice were anaesthetized and sacrificed. A significant increase in urinary excretion of biopyrrins was observed in mice exposed to isolation stress for 2 days compared with control mice. HO-1 gene expression was significantly increased in the proximal part of the hepatic portal and central part of lobes in the liver of the isolated mice. HO-1 protein was preferentially stained in the hepatocytes around the central vein and the interlobular portal vein in mice exposed to stress. It was notice to note that the expression of HO-1 was observed before the appearance of morphological changes in response to stress, such as plasma concentrations of corticosterone, the weight of the spleen and adrenal glands. In conclusion, we presented that isolation stress induced HO-1 preferentially around the central vein and interlobular portal vein in hepatocytes. These suggests that social stress generates ROS, resulting in expression of HO-1. To clarify the action mechanisms in detail, and to regulate this by food phytochemicals, the examination using human hepatocyte, HepG2, exposed to stress hormone are now in progress.
Keywords: social isolation stress, HO-1, ROS, liver

F-05 - ENDOPHYTES MICROORGANISMS OF Agave tequilana: ORGANIC NITROGEN TRANSFER EVALUATION USING MASS SPECTROMETRY MALDI-TOF AND LC-MS.

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Agave tequilana, is a plant that is an important economic product of Mexico. Endophytic microorganisms that colonize tissues of plants healthy without symptoms apparent of diseases. The majority of endophytes are horizontally transmitted to its plants host through of spores or inactive forms dispersed by the wind or by transmission vertical to the generations later of plants by seeds. This work aims investigate the role of endophytes bacteria at the plant A. tequilana in local with little or total absence of soil organic to support the growth. A hypothesis proposal it is that the achievement nitrogen organic by plants it is beneath a mechanism of oxidation digestion of bacteria by the H2O2 important investigate as the growth of plants it is held in environment poor of nitrogen. Until now it was possible to identify the bacteria isolated from A. tequilana, Bacillus sp. The bacteria were cultured in a medium mineral using as source of nitrogen 15NH4Cl and 14NH4Cl. Although the results are still preliminary, it was observed that DNA bases incorporated 15N present in the medium. Further analysis of feoftina were performed and it was also possible to observe an increase in the mass of plants inoculated with bacteria grown in medium containing 15N. This was a test illustrative to have the certainty of the incorporation of 15N in the biosynthesis the plant and microorganism.
Keywords: Agave tequilana, microorganism, mass spectrometry

F-06 - DIET-SENSITIVE SOURCES OF REACTIVE OXYGEN SPECIES IN LIVER MITOCHONDRIA: ROLE OF VERY LONG CHAIN ACYL-COA DEHYDROGENASES

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High fat diets and accompanying hepatic steatosis are highly prevalent conditions. Previous work (Cardoso, 2010) has shown that steatosis is accompanied by enhanced generation of reactive oxygen species (ROS), which may mediate further liver damage. Here we investigated mechanisms leading to enhanced ROS generation following high fat diets (HFD). We found that mitochondria from HFD livers present no differences in maximal respiratory rates and coupling, but generate more ROS specifically when fatty acids are used as substrates. Indeed, many acyl-CoA dehydrogenase isoforms were found to be more highly expressed in HFD livers, although only the very long chain acyl-CoA dehydrogenase (VLCAD) was more functionally active. Studies conducted with permeabilized mitochondria and different chain length acyl-CoA derivatives suggest that VLCAD may be the source of enhanced ROS production in the mitochondria of HFD animals. This production is stimulated by the lack of NAD+ and modulated by pH, but it is not changed by the addition of others classic metabolic regulators: NADH, FAD, AMP, ADP, ATP, GDP or GTP. Overall, our studies uncover VLCAD as a novel, diet-sensitive, source of mitochondrial ROS.
Keywords: beta oxidation, very long chain acyl CoA dehydrogenase, reactive oxygen species, high fat diet

F-07 - MODULATION OF THE LIPID PEROXIDATION AND ANTIOXIDANT ENZYNES BY OILS SUPPLEMENTATION FROM PEQUI ALMOND AND PALM IN RATS KIDNEYS

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Depletion of antioxidants in the body, including antioxidant enzymes causing oxidative stress and may increase the risk of some diseases such as cardiovascular events, diabetes and cancer. In order to investigate such effects, a study was performed to evaluate the effect of oil supplementation, from pequi almond and from palm, on lipid peroxidation and antioxidant enzyme activity in rats. Healthy Wistar male rats were divided into four groups and one control group (water). Each group received a daily dose of 3 or 6 mL/kg (weight/animal) of pequi almond oil (manufacture of handmade) or refined palm oil, both by gavage for a period of four weeks. The animals kidneys were collected and from their homogenates were evaluated peroxidation assay by measuring the thiobarbituric acid reactive substances (TBARS) and activity of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GR). TBARS levels showed significant changes only in animals treated with palm oil at the highest dose, indicating variation in redox state. Significant statistical changes in SOD, CAT and GPx activities were observed in both groups that received palm oil and higher dose of pequi oil. This increased activity may be associated with an induction of oxygen reactive species. In conclusion, the results suggest that increased activity of antioxidant enzymes in animals could signal an attempt to combat oxidative stress. CAPES
Keywords: Antioxidants, Oxidative stress, Enzymes, Oils, Rats

F-08 - GUARANÁ POWDER (Paullinia cupana) PRE FEEDING PROTECTED FRUIT FLIES (Drosophila melanogaster) FROM TOXIC EFFECT OF PARAQUAT

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Guaraná (*Paullinia cupana*) is an Amazon native fruit considered a functional food due their phitochemicals compounds, like caffeines, catechin, tanines and theobromine. Several studies has been described the biological proprieties as antioxidant effect, antiobesogenic, antitumorogenic. However, there are few experimental models studies that test the safety and efficiency of Guaraná. Paraquat (PQ), a quaternary nitrogen herbicide, is a highly toxic substance for humans and animals. The toxicity of PQ is due to the generations of the superoxide anion which can lead to the synthesis of more reactive oxygen species (ROS). The aim of this study was to verify if Guaraná powder could safely protect fruit fly (*Drosophila melanogaster*) against PQ. For this, five days old flies were pre feed with 1, 5, 10, 20, 50 and 100mg/mL of Guaraná powder for 72h. Then the flies were transferred to 15mM PQ treatment until the last flies die. For each concentration 120 flies were treated. The results showed that concentrations of 1, 5, 10, 20 mg/mL of Guaraná powder the fruit flies survived ($p=0.03$), and the 20mg/mL has the best results when compared with control group treated only with PQ. The 50 and 100mg/mL had presented toxic effects on fruit flies during the pre feeding phase, causing the flies dead. These results suggest that the antioxidant effect of Guaraná against the toxicity of PQ. A minimum Guaraná doses causes protective effect, otherwise it can become toxic.
Keywords: Guaraná powder, Paraquat, Drosophila melanogaster, Oxidative stress

G – Biomolecule Damage and Biomaker Analysis

G-01 - STUDY AND CHARACTERIZATION OF ERGOSTEROL OXIDATION PRODUCTS GENERATED THROUGH THE REACTION WITH SINGLET OXYGEN

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Ergosterol is a steroid that is highly susceptible to oxidation mediated by reactive oxygen species. Compared with cholesterol, its oxidation is much faster, due to the presence of an additional double bond at C7. The oxidation products of ergosterol modify plasma membrane properties, affecting cell viability and cell growth. Stable products of ergosterol oxidation may be used as biomarkers for fungal oxidation. This work aims through a detailed characterization study by NRM, HPLC and mass spectrometry identify the major oxidation products of ergosterol and their properties, especially those related to stability and reactivity of the formed products. Through this study we aim to contribute to the identification of oxidation products that could serve as oxidation markers in studies involving yeasts. Preliminary analysis of ergosterol's oxidized products showed the existence of several products, including endoperoxides, hydroperoxides and aldehydes. The probe 2,4-dinitrophenylhydrazine (DNPH) reacted with some of the products purified through silica column, indicating the generation of an aldehyde. This product's is being purified for NMR structural characterization. Also, luminescence experiments showed the generation of excited species by the thermal decomposition of the product's mixture. Thus, this study also aims to identify the oxidation products responsible for these potentially toxic properties.
Keywords: ergosterol, reactive oxygen species, singlet oxygen, lipids oxidation

G-02 - NON-SELECTIVE DISTRIBUTION OF ISOMERIC CHOLESTEROL HYDROPEROXIDES TO MICRODOMAINS IN CELL MEMBRANES AND ACTIVATION OF MATRIX METALLOPROTEINASE ACTIVITY IN A MODEL OF DERMAL CELLS

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Cholesterol hydroperoxides (ChOOHs) are included as lipid peroxidation products in the skin exposed to ultraviolet (UV) light irradiation. They may exert physicochemical actions affecting biomembrane rigidity because cholesterol is one of the major components of cell membranes. We investigated the distribution of isomeric ChOOHs in heterogeneous cell membranes with different lipid profiles using mouse fibroblast NIH-3T3 cells as a model of the dermis. Before and after UVA irradiation in the presence of hematoporphyrin, cell membranes were partitioned to microdomains (lipid rafts and caveolae) containing a higher amount of cholesterol and non-microdomains (containing a lower amount of cholesterol) by ultracentrifugation. By a combination of diphenylpyrenylphosphine-thin-layer chromatography blotting analyses and gas chromatography-electron ionization-mass spectrometry/selected ion monitoring analyses, ChOOH isomers were determined as their trimethylsilyloxyl derivatives. Cholesterol 5α-, 7α- and 7β-hydroperoxide were found before irradiation. The amounts of the three ChOOH isomers increased significantly after photoirradiation for 2 h. No difference was observed between microdomains and non-microdomains with regard to the ratio of the amounts of isomeric ChOOHs to that of cholesterol, suggesting that these ChOOH isomers were distributed equally in both parts depending on cholesterol content. When cells were treated with a purified mixture of ChOOH isomers, cell membranes incorporated ChOOHs into microdomains as well as non-microdomains evenly. Cellular matrix metalloproteinase-9 (MMP-9) activity was elevated by treatment with the purified mixture of ChOOH isomers. These results strongly suggest that ChOOHs accumulate in cell membranes irrespective of the heterogeneous microstructure and promote MMP activity if dermal cells are exposed to photodynamic actions. **Keywords:** Cholesterol hydroperoxide, Microdomain, Lipid rafts, Matrix metalloproteinase, UVA irradiation

G-03 - BINDING AND PEROXIDASE ACTIVITY OF CYTOCHROME C IN THE PRESENCE OF LIPOSOMES CONTAINING CARDIOLIPIN HYDROPEROXIDE

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Cytochrome c (cytc) is a highly conserved heme protein in eukaryotic cells that acts as an electron carrier between complexes III and IV in the respiratory chain. As a peripheral protein, cytc is anchored to cardiolipin (TLCL), an anionic phospholipid found in the mitochondrial inner membrane by a combination of electrostatic and hydrophobic interactions, forming a cytochrome c-cardiolipin complex which exhibit peroxidase activity. In this study we have investigated the effect of tetralinoleoil cardiolipin mono-hydroperoxide (TLCL(OOH)1) on the membrane binding and peroxidase activity of cytc.The results of ultracentrifugation assays showed that around 95±1 % cytc binds to liposomes, which are mitochondrial membrane mimetic, containing DPPC:TOCL:TLCL(OOH)1 in which TLCL(OOH)1 varied from 0% to 50% in the total cardiolipin content of the membrane. In the presence of liposomes containing 100% TLCL(OOH)1 of total cardiolipin, cytc exhibited a lower binding than in the absence of the mono-hydroperoxide, but most of the protein (85%) was still bound in the membrane.The peroxidase activity of cytc determined for TLCL(OOH)1 was kpr = 29 ± 2 M-1.s-1. The activities for H2O2 and 13-HpODE were kpr = 10 ± 1 M-.s-1 and kpr = 92 ± 8 M-1.s-1, respectively. These results show that cytc binds to membranes containing cardiolipin mono-hydroperoxide, and this binding would only slightly decrease in a not physiological condition, in which all cardiolipin is converted to cardiolipin mono-hydroperoxide and the peroxidase activity of cytc decreases in this order 13 HpODE > TLCL(OOH)1> H2O2.
Keywords: cardiolipin, cytochrome c, bindind, peroxidase activity

G-04 - THE DEVELOPMENT OF A SPECIFIC AND SENSITIVE LC-MS-BASED METHOD FOR THE DETECTION AND QUANTIFICATION OF HYDROPEROXY- AND HYDROXYDOCOSAHEXAENOIC ACIDS AS A TOOL FOR LIPIDOMIC ANALYSIS

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Docosahexaenoic acid (DHA) is an n-3 polyunsaturated fatty acid that is highly enriched in the brain, and the oxidation products of DHA are present or increased during neurodegenerative disease progression. The characterization of the oxidation products of DHA is critical to understanding the roles that these products play in the development of such diseases. In this study, we developed a sensitive and specific analytical tool for the detection and quantification of twelve major DHA hydroperoxide (HpDoHE) and hydroxide (HDoHE) isomers (isomers at positions 4, 5, 7, 8, 10, 11, 13, 14, 16, 17, 19 and 20) in biological systems. In this study, HpDoHE were synthesized by photooxidation, and the corresponding hydroxides were obtained by reduction with NaBH4. The isolated isomers were characterized by LC-MS/MS, and unique and specific fragment ions were chosen to construct a selected reaction monitoring (SRM) method for the targeted quantitative analysis of each HpDoHE and HDoHE isomer. The detection limits for the LC-MS/MS-SRM assay were 1–- 670 pg for HpDoHE and 0.5–- 8.5 pg for HDoHE injected onto a column. Using this method, it was possible to detect the basal levels of HDoHE isomers in both rat plasma and brain samples. Therefore, the developed LC-MS/MS-SRM can be used as an important tool to identify and quantify the hydro(pero)xy derivatives of DHA in biological system and may be helpful for the oxidative lipidomic studies.
Keywords: docosahexaenoic acid, oxidation products, mass spectrometry

G-05 - SYNTHESIS, CHARACTERIZATION AND SIMULTANEOUS DETERMINATION OF CARNOSINE-ALDEHYDE ADDUCTS BY ELECTROSPRAY TANDEM MASS SPECTROMETRY ASSAY

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The lipid peroxidation generates as secondary products electrophilic derivatives including aldehydes and epoxides that are capable of reacting with protein or nucleic acid. Current evidences suggest the involvement of these species in several disorders, such as inflammatory, neurodegenerative diseases, and cancer. One of the detoxification mechanisms of these aldehydes is through conjugation with glutathione or, alternatively, reaction with other endogenous peptides as L- carnosine (CAR), widely found in skeletal muscle and central nervous system. In this work, a highly sensitive method involving HPLC-ESI/MS/MS detection was developed for the simultaneous quantification of CAR-adducts with 4-hydroxy-2-nonenal (HNE), 4-hydroxy-2-hexenal (HHE) and acrolein (ACR) using L-histidine ⌈15N3 as internal standard. Stable adducts were prepared isolated by HPLC and characterized by NMR and ESI/MS/MS analyses. The results showed that CAR reacts with HHE and HNE via Michael addition, forming cyclic products with [M + H]+ = 341 and [M + H]+ = 383 respectively. We elucidated, also, the structure of a compound [M + H]+ = 303, product of the reaction CAR-ACR and proposed a new route for this reaction. The new-developed HPLC/MS/MS methodology permits accurate quantification of concentrations of 2 pmol CAR and histidine, 1 pmol CAR-HHE, 500 fmol CAR-ACR and 200 fmol CAR-HNE with CV of 10 %. The method described here can be used to study the biological significance histidine-containing dipeptides and for the possible use of these molecules as biomarkers involving redox processes.
Keywords: Carnosine, acrolein, HHE, HNE, HPLC/MS/MS

G-06 - PROTEIN MODIFICATIONS INDUCED BY ELECTROPHILIC COMPOUNDS: POSSIBLE ROLE IN ALS

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Electrophilic compounds, like aldehydes, are endogenously produced and can react with nucleophilic biomolecules such as proteins, DNA, etc. In recent years, protein modifications induced by aldehydes have been studied because of their relation to several disorders involving pathological protein aggregation, which is the case of many neurodegenerative diseases. Moreover, such proteins can influence changes in various signaling pathways, further amplifying its deleterious effects in biological systems. Typical examples of such reactive aldehydes are 4-hydroxy-2-nonenal (HNE) and 4-hydroxy-2-hexanal (HHE). Therefore, this work aims to detect cellular proteins modified by aldehydes in tissue of transgenic mice, models for amyotrophic lateral sclerosis (ALS), which super express the mutated form of SOD1 (SODG93A), through proteomic techniques such as two-dimensional electrophoresis (2D PAGE), labeling of HNE adducts with specific antibody, and identification of possible modified proteins by mass spectrometry. Our preliminary results indicate that the reaction of 2.5 mmol L-1 of cytochrome C with 2 mmol L-1 of aldehyde, generated up to 4 Michael additions by HNE and three by HHE, as analyzed by MALDI-TOF MS. The adduct was digested and analyzed by ESI-MS/MS, where one Michael addition was found at the His 33 (peptide mass 1324.737 Da) when modified with 2 mmol L-1 HNE and at the Lys 86 (peptide mass 779.448 Da), Lys 54 (peptide mass 1813.941 Da), Lys 74 (peptide mass 920.545 Da) and Lys 100 (peptide mass 520.535 Da) when modified with 2 mmol L-1 HHE. The 2D PAGE conditions are being optimized in order to identify protein-aldehyde adducts from tissue of ALS models.
Keywords: ALS, HNE , HHE, MALDI-TOF MS, ESI-MS/MS

G-07 - A DI-CATIONIC WATER SOLUBLE NAPHTHALENE ENDOPEROXIDE DERIVATIVE AS A CHEMICAL SOURCE OF SINGLET MOLECULAR OXYGEN

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Singlet molecular oxygen plays an important role in chemical and biological systems. It is a powerfull electrophile, reacting with electron rich molecules through [2+2] cycloadditions, [4+2] cycloadditions and ene reactions. Naphthalene derivatives can trap singlet oxygen by [4+2] cycloaddition and release it in mild temperatures, which make these compounds suitable for biological studies. Herein, we describe a synthetic route for the synthesis of a di-cationic water soluble naphthalene derivative, which was prepared from commercial compound 1,4-dimethylnaphthalene. In the first step of the synthesis, 1,4-dimethylnapthalene was brominated, given an intermediate that was submitted to a nucleophilic substitution of bromide ion to cynamide ion in good yields (84%). The resulting compound was reduced with lithium aluminum hydride and aluminum chloride in dry ether and the amine compound obtained was alkylated with iodomethane, producing a quaternary ammonium salt. Our preliminary results has indicated that this di-cationic compounds can be a potential chemical source of singlet oxygen and may be explored in studies where singlet oxygen biological role is investigated. Finacial Support: CAPES, FAPESP, CNPq, CEPID-Redoxoma, INCT-Redoxoma and NAP-Redoxoma.
Keywords: di-cationic, naphthalene, singlet oxygen

G-08 - ANTIOXIDANT AND ANTI-OBESITY ACTIONS *IN VITRO* BY NEWLY SYNTHESIZED RESVERATROL DERIVATIVE

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Introduction: Resveratrol is one of polyphenol which has a potentially important activities on human health and known as a causative compound of ″French paradox″. Moreover, viniferin, which is identified as a resveratrol derivative, has similar effects with resveratrol. Therefore, it is considered that resveratrol and its derivatives have potential as a therapeutic of metabolic syndrome for human. The aim of this study was to investigate whether the newly synthesized resveratrol derivative (RS-2) has antioxidant and anti-obesity activities. **Methods**: RS-2 was synthesized from the mixture of resveratrol and sinapic acid with heat-treatment. The derivative was purified by LH-20 gel-column and reverse-phase HPLC, and the structure of the derivative was identified by the physical data of NMR and MS spectrum. Antioxidant activity was measured by 2,2-diphenyl-1-picrylhydrazyl (DPPH). On the other hand, Mouse 3T3-L1 preadipocytes were differentiated into mature adipocytes and cultured with resveratrol or RS-2. After the culture, adiponectin level in the medium and AMPKα phosphorylation activity in the cells were evaluated by Western blotting. **Results**: RS-2 showed higher antioxidant activity than precursor compounds. Meanwhile, RS-2 promoted higher expression of adiponectin than that of resveratrol. In addition, AMPKα phosphorylation was significantly increased in the RS-2 treated adipocytes. **Conclusion**: It was known that the functions of resveratrol were related to the beneficial effects such as anti-aging, anti-atherosclerosis and anti-obesity. In the present study, RS-2 exhibited stronger effects than resveratrol on the antioxidant and anti-obesity actions *in vitro*. Therefore, our results suggests that RS-2 might have higher health beneficial functions than resveratrol *in vivo*.
Keywords: Resveratrol derivative, DPPH, AMPK, adiponectin

G-09 - CELLULAR EFFECTS OF LOW CONCENTRATIONS OF B[A]P: BIOENERGETICS CHANGE AND MALIGNANT TRANSFORMATION

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Benzo[*a*]pyrene (B[*a*]P) is a polycyclic aromatic hydrocarbon found in tobacco smoke and widespread in the environment due to incomplete combustion of organic material. We evaluated here the cytotoxic effects and metabolic alterations promoted by exposure of the normal and metabolic proficient (CYP450) human bronchial epithelial cell line BEAS-2B to low concentrations of B[*a*]P. Cells were incubated with B[*a*]P (0.1, 0.5, 1 μM) for 1-168h, with renewal of the culture medium every 24 h. B[a]P absorption rate was determined by HPLC-UV. Cytotoxicity was assessed by crystal violet dye, XTT and glucose consumption assays. Cell cycle, DNA fragmentation and membrane integrity were analyzed by flow cytometry. Celular transformation was tested by soft-agar assay. Statistical analyzes were performed using one-way ANOVA. B[*a*]P significantly decreased cell growth by 72-168h in a dose-dependent manner. Cell membrane damage, DNA fragmentation, G0-G1 and G2-M cell cycle arrest were observed after 168h incubation with 0.5 and 1.0 μM. However, XTT assay revealed a boost in mitochondria enzyme activity with all tested concentrations. Glucose consumption rate and the number of soft-agar colonies also increased in cells incubated with 0.5 and 1.0 μM of B[*a*]P after 168h. The results indicate toxicity and changes in cell metabolism and bioenergetics. Metabolic disruption may promote cell transformation via epigenetic changes of gene expression, contributing to the malignant phenotype. So far, no study has shown a strong relationship between these changes and the action of B[*a*]P or other carcinogens. This assumption is under investigation. Acknowledgment: Prof. Sandro R. Almeida (FCFUSP).
Keywords: Benzo[a]pyrene, bioenergetics, malignant transformation, Cytotoxicity

G-10 - EPIGENETIC ALTERATIONS AND TOXICITY INDUCED BY BISPHENOL A ON HL-60 AND MCF-7 CELL LINES

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Bisphenol A (BPA) is largely used for plastic production and widely distributed in the environment, which favors chronic human exposure. As endocrine estrogenic disruption and damage to the bone marrow have been reported following BPA exposure, we evaluated here cytotoxic and epigenetic effects of BPA and nitro-BPA to MCF-7 and HL-60 cells. HL-60 and MCF-7 cells were exposed to 25, 100 and 250μM BPA or nitro-BPA for 1 or 24h. Cell viability, cell cycle, DNA fragmentation, and ROS generation were assessed by flow cytometry. Global DNA methylation was accessed by HPLC-PDA. Both BPA and nitro-BPA induced ROS generation in HL-60 cells after 1h of incubation. BPA led subsequently to a decrease in respiratory chain activity (XTT assay), increase in cell permeability, DNA fragmentation, G0/G1 or G2/M cell cycle arrest, and DNA hypermethylation. Cytotoxicity was less evident for nitro-BPA incubations, but DNA hypermethylation was also observed. Oxidative stress in MCF-7 cells occurred only after 24h of incubation with BPA, with less significant effects on the other parameters analyzed. Results show that ROS generation may be an important pathway for HL-60 cell toxicity induced by BPA. Myeloperoxidase activity and generation of BPA reactive metabolites may be involved.
Keywords: bisphenol A, oxidative stress, DNA methylation

G-11 - GLYCEMIC CONTROL IS NOT SUFFICIENT TO REVERT KIDNEY OXIDATIVE STRESS IN DIABETIC RATS

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In order to understand the pathophysiological mechanisms involved in the development of diabetic nephropathy, oxidative stress has emerged as an important pathway of kidney damage. Accordingly, this work aimed to evaluate the effects of insulin and metformin treatments regarding oxidative stress and kidney damage in a model of experimental diabetes induced by streptozotocin (40 mg/kg) in male Wistar rats. The animals were treated with metformin (100 or 200 mg/Kg) associated with insulin (4U/day) or insulin alone (8U/day) after four or six weeks of diabetes induction, until the total period of eight weeks. Glycemic levels and body weight were monitored weekly along the study. Total amount of proteins in urine was assessed by spectrophotometry and kidney MDA by HPLC/DAD. Diabetic rats presented hyperglycemia and reduced weight gain compared to non-diabetic. Significant increased urinary volume and proteinuria were detected after eight weeks of diabetes. All therapeutic schemes reversed these parameters. Regarding kidney damage, diabetic animals presented an enlarged kidney/body ratio, which was recovered by the employed therapies. Increased kidney MDA levels, indicating the occurrence of oxidative stress, were found in the diabetic group. Particularly, the four week metformin (200 mg/kg) treatment presented a protective role against kidney oxidative stress. However, kidney oxidative stress persisted despite glycemic and renal function recovery for the insulin and metformin (100 mg/kg) treatment schemes, which is an evidence of a delay in the oxidative stress control. This may lead to diabetic complications even under glycemic control, which needs further investigation.
Keywords: Diabetes Experimental, Estresse Oxidativo, Insulina, Metformina, Nefropatia diabética

G-12 - THE ROLE OF CYSTEINE RESIDUES ON SOD1 OLIGOMERIZATION INDUCED BY DOCOSAHEXAENOIC ACID

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Familial Amyotrophic Lateral Sclerosis (fALS) is associated to point mutations in the sod1 gene. These mutations are related to gain of toxic functions of the enzyme, including the formation of cytotoxic SOD1 aggregates. Our previous studies showed that docosahexaenoic acid (DHA) induces SOD1 aggregation. The aim of this study was to investigate the role of thiols in the aggregation mechanism. Protein aggregation was inhibited in the presence thiol reducing and alkylating agents, indicating that aggregate formation involves free thiol groups. Experiments with C6S and C111S mutants of SOD1 WT and G93A proteins revealed that aggregation is dependent on both Cys residues in WT, while for the G93A, only Cys 6 was critical for the aggregation. Although not completely elucidated, the mechanism seems to involve Cys residues in their thiolate forms since aggregates are not observed at pH
Keywords: docosahexaenoic acid, aggregates, thiol, amyotrophic lateral sclerosis

G-13 - EVALUATION OF OXIDATIVE AND GLYCATION DAMAGE IN A MODEL OF DIABETIC RATS

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Diabetes mellitus is growing worldwide and much research has focused on its complications. It is known that diabetes promotes oxidative stress, inflammation and the formation of advanced glycation products (AGEs), resulting in damage to biomolecules. To better understand its harmful effects, we evaluated malonaldehyde (MDA) levels in plasma and DNA lesions (8-oxodG, CEdG, 1,N^6-etenodA) in kidneys and liver of untreated diabetic rats, diabetic rats treated with insulin for 2 weeks (2w) or 6 weeks (6w) and healthy control rats. MDA (N=46) was determined by HPLC-DAD and DNA lesions were evaluated by HPLC-ESI-MS/MS (N=50). Data were analyzed using statistical *t*-test and one-way ANOVA. Untreated diabetic animals with blood glucose levels above 350 mg/dL and diabetic rats treated with insulin for 2w had higher plasma concentrations of MDA ($p<0,01$), whereas there was no statistical difference between control group and diabetic animals treated with insulin for 6w. This means that hyperglycemia induces plasma oxidative stress, leading to lipid peroxidation without reversal after short insulin treatment and glycemia normalization. However, an opposite trend was observed for kidney 8-oxodG, which was significantly increased in diabetic rats treated with insulin for 6w ($p<0,01$), compared to the other groups. Kidney CEdG levels were not augmented in the diabetic groups. Otherwise, liver samples from untreated diabetic animals showed significant higher levels of 1,N^6-etenodA compared to diabetic groups treated for 2w ($p<0,01$) and for 6w ($p<0,001$). Data show diverse effects of insulin administration in different body tissues, including induction of kidney oxidative damage, which needs further attention.
Keywords: Diabetes mellitus, DNA lesions, Oxidative stress

G-14 - MELATONIN AS A POTENTIAL THERAPEUTIC TARGET IN B-HEMOGLOBINOPATHIES: A PILOT STUDY

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The β-hemoglobinopathies are chronic hemolytic anemias, closely related to a hiperoxidative status. It is already established that melatonin is a direct free radical scavenger and can stimulate several antioxidative enzymes. Based on these roles of melatonin, the present work is an *ex vivo* study of melatonin modulatory effect on red blood cells (RBCs) under the influence of oxidant agents. The study was carried out on five healthy donors of both sexes (aged 20–30) by incubating the RBCs with oxidant agents (1 mM H_2O_2 and 1 mM *t*-BOOH) and the hormone at different doses for 1 hr, composing five experimental groups (Input, Output, 10 pM, 1 nM and 10 nM MEL). The biochemical parameters were measured using spectrophotometric methods (hemolysis degree and catalase and GPx activities) and chromatographic (MDA levels). Incubation period caused a ~2,5 fold increase in the hemolysis degree as well as in the MDA levels ($p < 0.01$; Friedman ANOVA followed by Dunn's test). However, melatonin treatment showed a dose-dependent diminish of both, hemolysis degree ($R^2 = -0.84$; $p < 0.01$) and MDA levels ($R^2 = -0.40$; $p < 0.01$). We did not find any significant difference in the antioxidative enzyme activities among the evaluated groups. Our findings suggest that melatonin usage could be a possible therapeutic target to reduce oxidative damage, alleviating the symptoms associated with, e.g, sickle cell anemia and β-thalassemia.
Keywords: melatonin, antioxidant therapy, sickle cell anemia, β-thalassemia

G-15 - PUNICIC ACID EFFECT ON OXIDATIVE STRESS INDUCED BY CCl4 IN RATS

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Punicic acid (PA; C18:3-9*c*,11*t*,13*c*) is a conjugated fatty acid (CFA) present in high percentages in the pomegranate seed oil (PSO). Several studies report that CFAs have anti-carcinogenic, anti-inflammatory and antioxidant both in vitro and in vivo. The aim of this study was to evaluate the effect of PSO supplementation on biomarkers in rats subjected to liver injury induced by carbon tetrachloride (CCl4). Twenty four male Wistar rats were divided into four groups: control- (H2O); control+ (H2O/CCl4); CLNA 2% (PSO/CCl4) and CLNA 4% (PSO/CCl4). The PSO were administrated daily by gavage for 21 days, and then subjected to liver injury induced by CCl4. The livers were evaluated for fatty acid profile by gas chromatography, the peroxidation level, and histological analysis. It was performed by one-way ANOVA test followed by Tukey. The PSO supplementation reduced serum concentrations of aminotransferases enzymes caused by CCl4. In the liver the PSO groups was not found PA, however were detected the both conjugated linoleic acids 9*c*,11*t* and 10*t*,12*c*. Although histological analysis has shown less injured areas in the liver parenchyma in the test groups, PSO provides substrate for the reaction of free radicals, increasing the level of lipid hydroperoxides in the liver. In general the PSO supplementation was not able to prevent the oxidative stress using this animal model. The results obtained in this study are a good addition to the literature once it provided more information about the effects of PSO and CFAs in oxidative stress-induced.
Keywords: punicic acid, pomegranate seed oil, conjugated fatty acid, oxidative stress

G-16 - PASSION FRUIT (Passiflora edulis) PEEL: ACTION IN THE ANTIOXIDANT STATUS OF WISTAR RATS

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Passion fruit (*Passiflora edulis*) peel is a source of bioactive substances with antioxidant potential, which acts against cell oxidative stress. This study evaluated the *in vivo* antioxidant effect of *Passiflora edulis* peel flour (PPF). Adult male *Wistar* rats (± 250g) were divided in PPF and Standard group (n= 6) and fed with standard diet (AIN-93M) up to 84 days old. Fifty percent of the fiber from the diet of the PPF group was replaced by PPF fiber. After 15 days, the animals were anaesthetized and sacrificed. The experimental group showed higher serum antioxidant potential compared to control according to FRAP assay (P> 0.001), but they presented lower antioxidant action according to ORAC assay (P< 0.005). In addition, there were an increase in GR activity, but also decreasing GPx and SOD activities (P< 0.005) in the liver of animals fed with PPF. However, the livers of the animals from PPF group showed higher lipid peroxidation (TBARS assay) (P< 0.005). The passion fruit peel may have improved the antioxidant status in the serum rats by bioactive compounds action, and this could reduce the activity of endogenous antioxidant enzymes. Furthermore, more investigations are necessary in order to elucidate the proprieties of PPF on oxidative status of the liver. This study is relevant considering that the peel is already marketed in the population.
Keywords: Antioxidant potential, Bioactive compounds, Oxidative stress, Passiflora edulis peel

G-17 - VIRTUAL SCREENING SEARCH FOR POTENTIAL INHIBITORS AND SELECTIVITY STUDIES FOR Trypanosoma cruzi LIPOAMIDE DEHYDROGENASE

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Chagas disease, caused by *Trypanosoma cruzi* parasite, affects ~8 million people worldwide, mostly in Latin America, leading to significant mortality, with devastating social and economic consequences. So far there is no effective treatment for the chronic phase of this disease. Lipoamide dehydrogenase (LipDH) is a FAD-dissulfide-oxidoreductase, considered as a putative target for novel antichagasic drug design. In most eukaryotes, this enzyme is a component of the mitochondrial 2-oxoacid dehydrogenase complexes. It is responsible for the NAD+ dependent reoxidation of dihydrolipoamide. LipDH was isolated from *T. cruzi* epimastigotes and its oxidized form bound to FAD, determined by X-ray crystallography, is available in PDB (2QAE; 1.90 Å resolution). In this work, in order to search for potential *T. cruzi* LipDH inhibitors, structure based drug design (SBDD) strategies were applied to generate virtual screening (VS) models, based on its 3D-structure and considering two different binding sites: (1) FAD binding site (Model 1) and (2) cavity adjacent to the NAD+ binding site (Model 2). The two VS models were proposed employing a sequence of pharmacophore, docking and visual inspection filters, which were applied to the ZINC database. Additionally, *T. cruzi* and human LipDH molecular interaction fields were calculated using GRID program and analysed by their PCA (principal component analysis) in order to identify the most highly selective ligand-protein interactions for these two isoforms. The generated VS Models 1 and 2 have selected, respectively, 3 and 7 compounds, from which 5 were acquired and will be tested as LipDH inhibitors for the VS experimental validation.
Keywords: T. cruzi LipDH inhibitors, Chagas disease, Virtual screening, GRID/PCA

G-18 - THE RELATIONSHIP BETWEEN THE INCREASE OF THE OXIDATIVE PROCESS OF ATHEROSCLEROSIS WITH PROTOPORPHYRIN IX

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Introduction: The protoporphyrin IX (PPIX) is an endogenous fluorophore . The increase of the intensity of PPIX reflect in changes in the optical parameters, when compared to normal endogenous levels. The formation of atheromatous plaques oxidation process results in the accumulation of LDL (low density lipoprotein) 1. The Z- scan technique scans enables the measurement of nonlinear refraction of the sample correlating the normalized transmittance function of the Z position of the sample and provides a direct and sensitive detection. Objectives: From the induction of a high calorie diet observe the change in transmittance with respect to the oxidation process of the formation process of atherosclerosis. Materials and Methods We used 12 male rabbits (Oryctolagus cunicullus) from New Zealand lineage divided into 3 groups : group - calorie diet 6 animals that were fed high-cholesterol (1 % cholesterol dissolved in chloroform) ; - control group 4 animals that received commercial chow soaked in chloroform and normal chow group - 2 animals were fed commercial . Blood was collected every 20 days through the ear headset animals. The PPIX blood was extracted with acetone. I have used a laser from Coherent Verdi V10 and power 401mW. Results and Conclusions: The increase in transmittance with the progression of the process of plaque formation in the experimental group compared to the control group.
Keywords: protoporphyrin IX, Z -scan technique, atherosclerosis

G-19 - SYNTHESIS AND CHARACTERIZATION OF GOLD NANOPARTICLES COATED WITH THIOLNAPHTHALENE ENDOPEROXIDE AS A SOURCE OF SINGLET MOLECULAR OXYGEN IN BIOLOGICAL SYSTEMS

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Many studies have demonstrated the potential of mesoporous materials nanoparticles in biological applications, once they have assisted in the insertion of certain molecules in biological medium. The aim of this work is synthesize spheric gold nanoparticles (AuNPs) containing 1,4-bisthiolmetilnaphthalene in its surface, which can trap singlet molecular oxygen ($^1O_2$) at low temperature via photosensitization reaction. AuNPs were used for immobilization of $^1O_2$ generator without the risks of them undergo modifications of their photochemical and photophysical properties within biological medium. Singlet molecular oxygen is a reactive oxygen species that can damage biomolecules, such as proteins, lipids and DNA. This species can be detected by the decay of its excited singlet state, emitting light in the near infrared region at 1270 nm. Polycyclic aromatic compounds react with $^1O_2$ by a Diels-Alder reaction [4 +2], forming the corresponding endoperoxide. The 1,4-naphthalene endoperoxide derivatives are widely used as $^1O_2$ generators in biological systems, delivering it when heated at 37ºC in good yields. Hence the development of these nanoparticles will contribute to study the role of $^1O_2$ in biological systems.

Keywords: singlet-oxygen generation, gold nanoparticles, thiolnaphthalenes
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G-20 - RADICAL ACYLATION OF LYSINE DERIVATIVES AND LYSINE-CONTAINING PEPTIDES BY PEROXYNITRITE/DIACETYL AND METHYLGLYOXAL SYSTEMS

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Diacetyl (2,3-butanedione) is a food and cigarette contaminant reportedly related to alcohol hepatotoxicity and lung disease. In turn, methylglyoxal (MG) is an α-oxoaldehyde associated with diabetes and aging putatively formed from trioses phosphate, acetone and aminoacetone. Peroxynitrite - a potent biological oxidant, nitrating agent and nucleofile – is formed *in vivo* by the diffusion-controlled reaction of superoxide radical with nitric oxide ($k \sim 10^{10}\ M^{-1}s^{-1}$). It is able to add to CO_2 and carbonyl compounds yielding NO_2, carbon-centered radicals and carboxylic acids. Treatment of diacetyl and MG with $ONOO^-$ in the presence of amino acids, peptides and nucleobases lead to radical acylation of their amino groups. The L-Lys derivatives Ac-Lys-OMe and Z-Lys-OMe, and the Lys-containing tetrapeptides (H-KALA-OH, Ac-KALA-OH and H-K(Boc)ALA-OH) were used here to distinguish the preferential Lys α- or ε-amino groups targeted by acyl radical generated by the α-dicarbonyl/peroxynitrite systems. RP-HPLC and ESI-MS analyses of reaction products confirmed $^{\alpha}$N- and $^{\varepsilon}$N-acetylation of Lys derivatives by diacetyl as well as acetylation and formylation by MG. The pH profile traced for acetylation of H-KALA-OH was found to be bell-shaped, peaking at approximately 7.6-8.0, which is in accordance with the previously reported reaction mechanism for the phosphate-catalyzed peroxynitrite addition to carbonyls. These data supports our previously proposed hypothesis that radical acylation of proteins may contribute to post-translational acetylation of proteins catalyzed by transacetylases at cellular sites where both peroxynitrite and α-dicarbonyls accumulate.

Keywords: α-Dicarbonyls, Peroxynitrite, Lysine peptides, Acetyl Radical, Formyl Radical

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