Oxidative stress

W369

SOD-1 AS A MARKER OF ANTIOXIDANT CAPACITY IN PATIENTS WITH DYSLIPIDAEMIA

J. Cepova[1], K. Kotaska[1], P. Bukovska[1], R. Prusa[1]
[1]*Department of Medical Chemistry and Clinical Biochemistry 2nd Faculty of Medicine, Charles University, Faculty Hospital Motol, Prague, Czech Republic*

BACKGROUND-AIM

The aim of this study was to confirm the relevance of superoxid dismutase (SOD-1) as a marker of antioxidant capacity in patients with dyslipidaemia.

METHODS

Serum levels of SOD-1 were investigated in group of 139 patients (72 males, mean age 54 years and 67 females, mean age 60) with dyslipidemia and in control group of 33 healthy individuals (14 males, mean age 55 years, 19 females, mean age 67 years). Patients with dyslipidemia were divided into groups according to the type of the therapy (1 st group – hypercholesterolemia with statin therapy – 68 patients, 2nd group – combined dyslipoproteinemia treated by statins and fibrates – 40 patients, 3rd group consisted of 31 patients with obesity and no hypolipidemic therapy). Serum SOD-1 levels were investigted by using commercially available enzymatic ELISA Kit (Cloud and Clone Corp, USA).

RESULTS

Serum SOD-1 levels in group of patients and in healthy individuals were as follows (results are expressed as mean+ SEM): Group 1: SOD-1 = 63.8 + 1.4 ng/ml, Group 2: SOD-1 = 65.8 + 2.0 ng/ml, Group 3: SOD-1 = 63.8 + 1.8 ng/ml, control group of healthy individuals: SOD-1 = 70.3 + 1.85 ng/ml. Significant differences in concentrations were found between control group of healthy individuals and group 1 consists of patients with hypercholesterolaemia treated by statin therapy ($p = 0.0077$) and between control group and group 3 - obese patients ($p = 0.01$).

CONCLUSION

We conclude, that patients with statin treated hypercholesterolemia have significantly decreased antioxidant capacity.

Oxidative stress

W370

OXIDATIVE PATTERNS OF RED BLOOD CELLS FROM PATIENTS WITH COMMUNITY-ACQUIRED PNEUMONIA AND CHRONIC OBSTRUCTIVE PULMONARY DISEASE

L. Muravlyova[1], V. Molotov- Luchanskiy[1], R. Bakirova[1], D. Klyuyev[1], Y. Kolesnikova[1], L. Demidchik[1]
[1]*State Medical University, Karaganda*

BACKGROUND-AIM

The impact of pre-existing chronic lung diseases on outcome in community-acquired pneumonia (CAP) is not well established (Loke et al., 2013; Dusemund et al., 2014). Insights into intracellular molecular transformations in red blood cells (RBCs) will need to be considered for strategies aimed to prevent inflammatory injury respiratory distress. The aim was to assess the oxidative patterns of RBCs from CAP patients with and without of chronic obstructive pulmonary disease (COPD).

METHODS

Patients were divided into 2 groups. 29 patients with community-acquired pneumonia moderate severity and respiratory insufficiency of grade 2 were included in the 1-st group. 36 COPD patients with community-acquired pneumonia moderate severity and with respiratory insufficiency of grade 2 were included in the 2-nd group. The control group consisted of 32 healthy persons. The protein reactive carbonyl derivatives, membrane-bounded hemoglobin, glycosylated hemoglobin, and malon dialdehyde (MDA) were detected in RBCs. Comparisons of the results obtained between patients and healthy persons were performed using non-parametric Mann-Whitney U-test (for independent variables).

RESULTS

Our results showed the significant increase in protein reactive carbonyl derivatives (by 26%, $p < 0.05$), and MDA (by 65%, $p < 0.01$) in RBCs from CAP patients with COPD in comparison with healthy persons. In RBCs from CAP patients with COPD the increase in glycosylated hemoglobin was observed (by 10%).
In RBCs from CAP patients the increase in MDA was observed (by 2,6 times in comparison with healthy ones, $p<0.001$).We also noted insignificant increase in protein reactive carbonyl derivatives and membrane-bounded hemoglobinin RBCs from CAP patients.

CONCLUSION

The data showed synchronous increase of modified proteins and MDA into RBCs of CAP patients with COPD. Accumulation of modified proteins and MDA leads to the alteration of RBCs metabolism and contributes hypoxemia progression in CAP patients with COPD. Biomarkers of oxidative metabolism may provide useful parameters for estimating the CAP severity.

Oxidative stress

W371

EVALUATION OF OXIDATIVE STRESS IN SICKLE CELL HOMOZYGOUS PATIENTS IN THE YAOUNDE CENTRAL HOSPITAL - CAMEROON

H. Manoji[1], V.J. Ama Moor[2], C.A. Pieme[1], B. Chetcha[3], B. Moukette Moukette[4], T. Asongany[1]
[1]*Faculty of Medicine and Biomedical Sciences – University of Yaounde 1, Cameroon.*
[2]*Faculty of Medicine and Biomedical Sciences – University of Yaounde 1, Cameroon. Yaounde University Teaching Hospital, Cameroo*
[3]*Faculty of Medicine and Biomedical Sciences – University of Yaounde 1, Cameroon. Yaounde Central Hospital, Cameroon*
[4]*Faculty of Sciences – University of Yaounde 1, Cameroon*

BACKGROUND-AIM

Sickle Cell Disease (SCD) is a class of hemoglobinopathy which results from a single mutation in the beta globin chain of the haemoglobin inducong the substitution of valin for glutamic acid at the sixth amino acid position. This disease can result in oxidative stress caused by Reactive Oxygen Species (ROS) which limits nitrogen oxyde (NO) bioavaibility and decreases anti oxydant status. The study aims to determine the level of oxydative stress marker in sickle cell homozygous patients (SS) in the Yaounde Central Hospital above fifteen years old.

METHODS

The study was an analytical and comparative study carried out from December 2013 to April 2014. It involved sickle cell homozygous patients at the Yaounde Central Hospital which were above 15 years of age, as well as healthy individuals (AA) which served as a control group. Blood samples were collected from the patients; the red blood cells were washed and used to determine the level of oxidative stress markers in these patients. The markers analyzed included: Malondialdehyde (MDA), NO, Catalase, Superoxide dismutase (SOD), Peroxidase, Total anti-oxydant capacity and total protein concentrations using spectrophotemeter methods. Ethical clearance was gotten from the Faculty of Medicine and Biomedical Sciences. The patients gave their consent to participate in this study.

RESULTS

Eighty four individuals, 42 males and 42 females participated with an age range between 15 to 55 years. There was a significant decrease in the catalase, superoxide dismutase, NO and activities in the sickle cell group compared to the healthy AA group. There is an increase of Total anti-oxydant capacity, MDA and peroxidase in the SS group. There were no significant variations in the levels of these stress markers with respect to sex except Total anti-oxydant capacity.

CONCLUSION

There is an increase in the oxydative stress level in sickle cell homozygous cell patients compare to healthy AA individuals.
Key words : Sickle cell disease, oxydative stress, antioxydants, Cameroon

Oxidative stress

W372

RELATIONSHIP OF TRACE ELEMENTS AND OXIDATIVE STRESS IN PATIENTS WITH BRUCELLA MELITENSIS

E. Belge Kurutas[2], M. Gungor[5], P. Ciragil[1], M. Aral[4], O. Kokoglu[3]
[1]*Cukurova University, Faculty of Pharmaceutic, Department of Pharmaceutical Microbiology, Adana, Turkey*
[2]*Sutcu Imam University, Faculty of Medicine, Department of Biochemistry, Kahramanmaras, Turkey*
[3]*Sutcu Imam University, Faculty of Medicine, Department of Infectious Diseases, Kahramanmaras, Turkey*
[4]*Sutcu Imam University, Faculty of Medicine, Department of Microbiology, Kahramanmaras, Turkey*
[5]*Toros University, Vocational School, Department of Medical Laboratory Techniques, Mersin, Turkey*

BACKGROUND-AIM

This is the first report regarding to investigate the activities of erythrocyte catalase (CAT) and superoxide dismutase (SOD), levels of plasma malondialdehyde (MDA), and serum zinc (Zn), copper (Cu) and selenium (Se) concentrations were measured in patients with brucella melintensis, and results were compared with those of healthy individuals.

METHODS

The investigation included 25 patient with Brucella melitensis (age: 34.4±4.5) and 20 healthy subjects (age: 33.6±5.0) as control group who were admitted to Department of Infection Diseases, Faculty of Medicine, Kahramanmaras Sutcu Imam University. The activities of erythrocyte catalase (CAT) and superoxide dismutase (SOD), levels of plasma MDA were measured as spectrophotometric. Serum Zn, Cu and Se concentrations were measured with flame atomic absorption spectrometry.

RESULTS

The mean of erythrocyte CAT and SOD activities and serum Zn and Se concentrations were significantly lower among patients compared with controls ($p<0.001$). However levels of plasma MDA in patients were comparable to controls and the mean NO levels in patients were significantly higher than controls ($p>0.001$). A significant positive correlation was found between levels of plasma MDA and serum Cu concentrations in patients with Brucella melitensis.

CONCLUSION

Decreased Zn and Se levels, antioxidant system insufficiency and increased levels of MDA and Cu were shown in patients with Brucella melitensis. Supplementary trace element antioxidative process may increase scavenger enzyme activities and also clinical symptoms may be amelioration in these patients.

Oxidative stress

W373

STATUS OF ANTIOXIDANT ENZYMES IN TUNISIAN PATIENTS WITH NON SMALL CELL LUNG CANCER

M.N. Belkhiria[1], J. Knani[2]
[1]*Laboratoire de Pharmacologie, Faculté de Médecine, Université de Monastir, Monastir, Tunisie*
[2]*Service de Pneumologie, Hôpital Universitaire Tahar Sfar, Université de Monastir, Mahdia, Tunisie*

BACKGROUND-AIM

Reactive Oxygen Species (ROS) can induce carcinogenesis via DNA injury. Both enzymatic and non-enzymatic antioxidants parameters participate in cell protection against harmful influence of oxidative stress. The present study aimed to determine the alterations of antioxidant activities from patients with non-small cell lung carcinoma.

METHODS

In total, 58 patients with non small cell lung cancer and 81 controls were assessed. Lung cancer patients were divided into those with early stage or advanced stage disease. The tumour type was squamous cell carcinoma in 34 patients and adenocarcinoma in 24. We analysed the activity of main antioxidative enzymes, superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx) in patients with non-small cell lung carcinoma and healthy subjects.

RESULTS

Statistically significant differences between the patient group and the control group were detected for all biochemical parameters. The levels of GPx were significantly lower in patients with early stage disease (27.86 ± 4.18 U/g Hb) and in patients with advanced stage disease (24.04 ± 3.83 U/g Hb) than in controls (38.04 ± 7.58 U/g Hb, P # 0.001). The levels of SOD in patients with early stage disease (1.09 ± 0.08 U/mg Hb) and in patients with advanced stage disease (1.03 ± 0.08 U/mg Hg) were significantly lower than controls (1.20 ± 0.13 U/mg Hg, $p < 0.01$ and $p < 0.001$ respectively). Also, the catalase concentrations were significantly lower in patients with early stage disease (120.53 ± 9.42 U/mg Hb) and in the group with advanced stage disease (110.93 ± 10.98 U/mg Hg) than in controls (141.96 ± 23.75 U/mg Hg, $p < 0.01$ and $p < 0.001$ respectively). Statistically significant differences between the group of the patient with advanced stage disease and the group of patients with early stage disease were detected for all biochemical parameters. No differences in GPx, SOD, or catalase activities were found between squamous cell carcinoma and adenocarcinoma.

CONCLUSION

The present study show that Lung cancer is associated with serious oxidative stress and that advancement of oxidative-antioxidative disorders is followed by progression of lung cancer.

Oxidative stress

W374

INHIBITIVE ACTION ON OXIDATIVE DAMAGE IN HUMAN ERYTHROCYTES AND ANTIOXIDANT ACTIVITIES OF DAPHNE GNIDIUM L.

S. Boumerfeg[1], A. Baghiani[2], D. Ameni[2], S. Aouachria[2], A. Benslama[2], L. Arrar[2]

[1]*Department of Biology. Faculty of Nature and Life Sciences, University Bordj Bou-Arreridj 34000 Algeria*

[2]*Laboratory of Applied Biochemistry, Department of Biology. Faculty of Nature and Life Sciences, University Ferhat Abbas, Setif 19000 Algeria.*

BACKGROUND-AIM

Oxidative stress is a general term used to identify the level of oxidative damage (OD) in a cell caused by free radicals (FR) such as superoxide ions (O2-.). One of the very important enzyme can produce O2-. is xanthine oxidase (XO). FR have been implicated in more than 100 diseases, including Alzheimer, diabetes, and cancer. Nevertheless, all aerobic organisms, have antioxidant defenses that protect against OD. However, this mechanism can be inefficient, so, there is an increasing interest in natural antioxidants present in medicinal and dietary plants, which might help prevent OD. Daphne gnidium L is common plant in North Africa used in the traditional medicine as diuretic agent and have an anti-inflammatory and anti antibacterial activities. In the present study, the antioxidant properties of D. gnidium extracts (DGE) were investigated.

METHODS

DGE were prepared using solvents of varying polarity. Total polyphenols were measured using Prussian blue. First the protective effects of DGE were determined by measuring the erythrocyte membrane resistance to FR.These to evaluate compound-iron interaction (chelating activity), the ferrozine test was performed. Finally the inhibition of XO by DGE was determined.

RESULTS

all the extracts are significant sources of polyphenols. In the cellular system data clearly indicate that hemolysis was inhibited by cud (CE), ethyl acetat (EAE), and chloofome (CHE) extracts with 81.21%, 70.62 % and 65.74%, respectively ($p \leq 0.01$) and have a protect effect against t-BH induced OD in human erythrocytes, these effect mainly due to phenolic compound present in extacts. To clarify this possibility, the chelating activity was examined, the CE showed an excellent chelating with IC50 of 8.171 ± 0.953 µM, lower than that of EDTA ($p \leq 0.01$). In the other hand the DGE exhibited a potent inhibitory effect on XO activity especially CE with IC50 (µM) of 1.828 ± 0.015 followed by CHE and EAE, which were too close to each other ($P \leq 0.01$), however Allopurinol (clinically used as a drug) gave a IC50 of 57.114 ± 1.093 µM

CONCLUSION

Our study may be considered as a new report based on antioxidant potential of Daphne gnidium L and could be used to treat conditions where inhibition of XO and FR scavenging action are warranted.

Oxidative stress

W375

THE EFFECTS OF ETANERCEPT ON SERUM SUPEROXIDE DISMUTASE, MALONDIALDEHYDE LEVELS IN RATS WITH EXPERIMENTAL ENDOMETRIOSIS.

C. EraldemİR[1], M. Çekmen[1], D. KeleŞ[3], D. Özsoy[1], H. Kir[1], B. Vural[3], C. BaydemİR[2]
[1]*Kocaeli University Faculty of Medicine Department of Biochemistry*
[2]*Kocaeli University Faculty of Medicine Department of Biostatistics*
[3]*Kocaeli University Faculty of Medicine Department of Obstetrics and Gynecology*

BACKGROUND-AIM

The purpose of this study was to evaluate the serum oxidative balance in rats with experimental endometriosis who received etanercept (anti-tumor necrosis factor alpha) treatment.

METHODS

Endometriosis was surgically induced in 30 female rats. The first blood samples were taken 4- weeks after this procedure, the viability of the endometriosis foci were recorded. Rats were then randomly divided into three groups: Control group (2 ml /day subcutaneous dose of saline, n = 10); etanercept group (2 mg/kg subcutaneous dose from three times per week, n = 10); Sham group (who received any treatment of endometriosis model, n=10). At the end of the 2-week treatment, second blood samples were taken. The MDA levels were measured spectrophotometrically. The activity of SOD was measured with ELISA.

RESULTS

There were no significant differences of serum MDA levels between etanercept and control groups ($p>0.05$). Additionally, the etanercept group had significant lower levels of MDA compared to sham group ($p<0.05$). Moreover, the levels of MDA were significantly lower after than before treatment in the etanercept group ($p<0.05$). There were no significant differences of serum SOD levels between the groups ($p>0.05$).

CONCLUSION

In summary, these results suggest that etanercept has protective effects against endometriosis in rats, which may be attributed to attenuating oxidative stress.

Oxidative stress

W376

COMBINATION OF RAPAMYCIN WITH THE ANTIOXIDANT SUPPRESSES TRASTUZUMAB-RESISTANT BREAST CANCER CELLS STEMNESS BY DOWN-REGULATING NRF2

S.H. Cheng[1], L.Y. Tsai[1]
[1]*Department of Medical Laboratory Science and Biotechnology, College of Health Sciences, Kaohsiung Medical University, Kaohsiung, Taiwan*

BACKGROUND-AIM

The her2 gene is overexpressed in ~20% to 30% of invasive breast carcinomas. Trastuzumab is a monoclonal antibody directed against the extracellular domain of the HER2 receptor. Although trastuzumab has been successfully used in patients with HER2-overexpressing metastatic breast cancer, resistance is a common problem in treatment failure. The nuclear factor erythroid 2–related factor 2 (Nrf2) is a regulator of cellular resistance to oxidants. Evidence indicates that an increase in Nrf2 activity is implicated in cancer chemoresistance. Besides, reactive oxygen species (ROS) is involved in the drug-induced tumor stem cell enrichment. Rapamycin, an inhibitor of the mTOR, is an incontrovertible treatment for breast cancer. In this study, we investigate the effects of combination of rapamycin with the antioxidant on trastuzumab-resistant breast cancer cells.

METHODS

The parental SK-BR-3 and trastuzumab-resistant SK-BR-3 (TR-SK-BR-3) cells were used. An antioxidant here we use was whey protein concentration (WPC), a precursor of glutathione (GSH). The cytotoxicity was assessed by MTT dye conversion at 570 nm. The levels of cellular GSH and ROS were analyzed by a capillary electrophoretic analyzer and flow cytometric analysis, respectively. By using sphere formation assay and side population (SP) analysis to evaluate the self-renewal capability and the drug efflux functions.

RESULTS

The IC50s of rapamycin in parental SK-BR-3 and TR-SK-BR-3 cells were 47.5 nM and 51.5 nM, respectively. After combined with the WPC, the IC50s of rapamycin were decreased to 33.5 nM and 41.5 nM, respectively. In addition, in TR-SK-BR-3 cells, the combination of rapamycin with the WPC could deplete the GSH levels and elevate ROS levels compare to the group treat with rapamycin only. The combination of rapamycin with the WPC attenuated the ability of sphere formation and SP in TR-SK-BR-3 cells. Furthermore, we found that the combination of rapamycin with WPC reduced the expression of protein levels including CD44 and Nrf2 in TR-SK-BR-3 cells.

CONCLUSION

This study suggested that the combination of rapamycin with the WPC suppresses cancer stemness and progression of trastuzumab-resistant breast cancer cells via down-regulation of Nrf2.

Oxidative stress

W377

COMPARISON OF THREE METHODS IN DETECTION OF URINE MALONDIALDEHYDE : THIOBARBITURIC ACID REACTIVE SUBSTANCES ASSAY, ELISA, AND HPLC METHOD

H. Kim[2], S. Lee[1], C.L. Chang[1]

[1]*Department of Laboratory Medicine, School of Medicine Pusan National University, Busan / Department of Laboratory Medicine and Molecular Genetics, Pusan Naitonal University Yangsan Hospital, Yangsan Gyeongnam, Republic of Korea*
[2]*Department of Laboratory Medicine, School of Medicine Pusan National University, Busan, Republic of Korea*

BACKGROUND-AIM

Malondialdehyde (MDA) is one of the major intermediates formed from the lipid peroxidation cascade, often be measured as oxidative stress marker in the clinical laboratory. Blood contains only the amount of MDA circulating in the body at a particular point in time. The amount of MDA in the urine is more of an end point product and the test is non-invasive. For this reason, we have established urinary MDA levels using three different methods.

METHODS

Spot urine samples were collected in the morning from 40 apparently healthy adults, the levels of MDA were measured by three different methods; thiobarbituric acid reactive substances (TBARS) assay (Cell Biolabs, Inc., USA), MDA adduct ELISA (Cell Biolabs, Inc., USA) and HPLC analysis. Absolute level of free MDA in urine be determined with HPLC without using any reactive component while total MDA level in urine by the TBARS assay with spectrophotometric detection. The MDA protein adducts content is determined by coparing with a standard curve that is prepared from predetermined MDA-BSA standards. In TBARS method, we replicate assays of the urine samples and yield mean concentration.

RESULTS

ELISA method has failed to detect MDA adducts in the most urine samples. Quantification of MDA by TBARS in urine shows from 4.84 to 16.9 μM (mean =8.5). Urine MDA level determined by HPLC shows significantly lower values than that of TBARS, 1.0~8.2 μM (mean = 2.7), in all of the test samples. Although, in urine samples, similary with plasma, a significant increase (up to 3-fold) in the concentration of total MDA by TBARS was noted than determined by HPLC ($P < 0.05$), correlation between two methodswas good ($r = 0.703$).

CONCLUSION

Consequently, it be suggested that HPLC be recommended for determination of free MDA levels in urine. Without appropriate reference range, TBARS methods may leads to an overestimation of the levels of urine MDA. Further studies focusing on the development of referece range and detection methods verification of urinary MDA quantitation as the oxidative stress markers.

Oxidative stress

W378

FEMALE REPRODUCTIVE HORMONES AND BIOMARKERS OF OXIDATIVE STRESS IN GENITAL CHLAMYDIA INFECTION IN WOMEN WITH TUBAL FACTOR INFERTILITY

A.C. Nsonwu-anyanwu[1], M.A. Charles-davies[2], V.O. Taiwo[5], L. Bin[6], A.A. Oni[3], F.A. Bello[4]
[1]*Chemical Pathology Unit, Department of Medical Laboratory Science, University of Calabar, Nigeria*
[2]*Department of Chemical Pathology, College of Medicine, University of Ibadan, Nigeria.*
[3]*Department of Medical Microbiology & Parasitology, University of Ibadan, Nigeria.*
[4]*Department of Obstetrics and Gynaecology, University of Ibadan, Nigeria.*
[5]*Department of Veterinary Pathology, University of Ibadan, Nigeria.*
[6]*Unit of Molecular Immunology, Institut Pasteur of Shanghai, Chinese Academy of Sciences.*

BACKGROUND-AIM

Genital Chlamydia Infection (GCI) and the associated pathologies have been implicated in tubal infertility. Though the actual pathologic mechanisms are still uncertain, oxidative stress and other factors have been implicated. This study was therefore aimed to determine the possible contribution of female reproductive hormones and biomarkers of oxidative stress to tubal occlusion in genital Chlamydial infection.

METHODS

Chlamydia trachomatis antibody (IgG), female reproductive hormones [Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH), Oestradiol (E2), Progesterone (P4), Prolactin (PRL)] and biomarkers of oxidative stress [Total Antioxidant Capacity (TAC) and 8-hydroxyl-2-deoxyguanosine (8-OHdG)] were determined by enzyme immunoassay (EIA) in the sera of Chlamydia positive women with tubal infertility (n = 50), fertile Chlamydia positive women (n = 50) and their corresponding fertile Chlamydia negative women as controls (n =50).

RESULTS

Higher levels of LH and 8-OHdG and lower TAC levels were observed in infertile Chlamydia positive women compared to fertile Chlamydia negative controls ($p<0.05$). Among women with GCI, higher levels of LH and 8-OHdG were observed in infertile Chlamydia positive women compared to fertile Chlamydia positive women ($p<0.05$).

CONCLUSION

Mechanisms involving oxidative DNA damage and lower TAC levels may be involved in the pathology of Chlamydia induced tubal damage. Enhanced total antioxidant capacity may protect against this pathologic state.

Oxidative stress

W379

EFFECT OF MORINGA OLEFERA LAM. LEAVES EXTRACT ON OXIDATIVE STRESS HUMAN T LYMPHOCYTE INDUCED BY ULTRAVIOLET AND URIC ACID

K. Usuwanthim[1], M. Yuchat[1], P. Adulyaritthikul [1], N. Kooltheat[1]
[1]*Allied health sciences, Naresuan University, Phitsanulok, Thailand*

BACKGROUND-AIM

Oxidative stress is induced by several environment factors including ultraviolet, smoke, bacterial pathogen and herbicide. Generation of reactive oxygen species and others free radical can damage DNA, proteins, lipids of cellular organelles leading to loss of function of immune cells which develop to some serious diseases. The antioxidant defense mechanism can reduce this phenomenon. In the study, we investigate the effect of Moringa olefera Lam. extract, a high antioxidant, on human T lymphocyte DNA damage induced by ultraviolet and uric acid.

METHODS

Moringa olefera Lam. was prepared from fresh leaves extract and shown to consist of high level of antioxidant activities. Oxidative stress of human T lymphocytes was induced by ultraviolet and uric acid and then treated with varying concentration of Moringa extract.

RESULTS

Human T lymphocytes induced by ultraviolet and uric acid showed decrease production of IL-2 and increase oxidative stress. Moringa treated T lymphocytes showed the extract activity can restore IL-2 production to close normal level and decrease oxidative stress. The evident were measured by DNA/RNA oxidative damage and both cytokine protein and mRNA levels.

CONCLUSION

The findings highlight the ability of Moringa extract to reduce oxidative stress induced by ultraviolet and uric acid which promote the restoring function of T lymphocyte of IL-2 production.

Oxidative stress

W380

ANALYSIS OF NON-CODING REGION OF CATALASE GENE IN HUNGARIAN PATIENTS WITH DECREASED BLOOD CATALASE

T. Nagy [1], L. Góth[1], E. Zabolai[1]
[1]*Department of Medical Laboratory and Diagnostic Imaging, Faculty of Medicine, University of Debrecen, Hungary*

BACKGROUND-AIM

Hydrogen peroxide is an oxidizing agent. The H2O2 can oxidize: DNA, RNA, fatty acids and proteins. Its small concentration is physiologic but in high concentration is toxic. The human body has developed catalase enzyme which could protect the cells from high concentration of H2O2. Decreased activity of catalase may lead to increased hydrogen peroxide concentration which may contribute to the manifestation of age-related disease. The human catalase gene(CAT,NCBI GENE ID:847) is localized on the short arm of chromosome13 (11p13) and the catalase gene includes 13 exons and 12 introns. Screening of 617 patients and 295 controls for blood catalase yielded 51 patients with less than half of blood catalase activity.
These 51 patients were examined for three polymorphisms in the non-coding region 20T/C(rs1049982), -21A/T(rs7943316) and -262C/T(rs1001179) which may be responsible for the decreased blood catalase activity.

METHODS

51 patients with less than half of normal blood catalase activity (<52MU/L) were examined. These patients had diabetes mellitus(type 1, type 2, and gestational), microcytic anemia, beta-thalassemia and presbycusis. They were examined with PCR-SSCP, RFLP(polyacrylamide gel electrophoresis and silver straining) and nucleotide sequencing.

RESULTS

For 20T/C substitution the frequencies of alleles T(0.59), C(0.41), genotypes CT:63%(32/51), CC: 27%(14/51), TT: 10%(5/51). The -21A/T substitution the frequencies of alleles T(0.68), A(0.32), genotypes AT:59%(30/51), TT:39%(20/51) and AA:2%(1/51). For -262C/T substitution the frequencies of alleles T(0.60), C(0.40), genotypes CT:26%(12/51), TT:47%(25/51) and CC:27%(14/51). Mutated alleles were detected for all three polymorphisms in 19 patients (37%), for two polymorphisms in 31 (39%) patients, and for one polymorphism (-21A/T) in one patient. There was no patients with the three wild type alleles of these polymorphisms.

CONCLUSION

Non-coding region of the catalase gene was examined in patients with decreased activity of blood catalase. We have identified three of the known polymorphisms in this area. We found that two or three SNPs are present in the majority of the samples. They may have a negative effect on transcription resulting in decreased enzyme activity.

Oxidative stress

W381

ANTIOXIDATIVE STATUS AND PARAOXONASE 1(PON1) ACTIVITY DURING UNCOPLICATED PREGNANCY AND AFTER DELIVERY

D. Ardalic[1], A. Stefanovic[2], S. Spasic[2], J. Kotur-stevuljevic[2], Z. Jelic-ivanovic[2], V. Spasojevic-kalimanovska[2], V. Mandic-markovic[1], Z. Mikovic[1], N. Cerovic[1]

[1]*Clinic of Gynecology and Obstetrics Narodni Front, Belgrade, Serbia*

[2]*Department of Medical Biochemistry, Faculty of Pharmacy, University of Belgrade, Belgrade, Serbia.*

BACKGROUND-AIM

Physiologically uncomplicated pregnancy is a condition with increased oxidative stress and suitable antioxidative response. Paraoxonase1(PON1) is significant part of the antioxidative system, but results in previous studies of PON1 activity during uncomplicated pregnancy were controversial.

METHODS

We monitored 43 healthy pregnant women throughout uncomplicated pregnancy and two months after delivery. Normal pregnancy was diagnosed on the basis of clinical and ultrasound examination. Blood was sampled towards the end of each trimester and two months after delivery. Additionally, 42 healthy women of reproductive age, but not pregnant, were recruited as controls. We measured serum total sulphydryl (SH) groups, superoxide dismutase (SOD) and paraoxonase1 (PON1) activity by appropriate assays.

RESULTS

SH groups concetrations (0,47±0,08 g/L; 0,40±0,06 g/L and 0,47±0,05 g/L) were significantly lower during pregnancy compared with controls (0,52±0,10g/L) also as SOD activities (101,1±27,38 kU/L; 73,7±34,52 kU/L) until 3rd trimester when SOD activity significantly increased (143,2±38,00 kU/L) compared with controls (119,2±41,19 kU/L). We also noticed significantly increase of SOD activities in 3rd trimester compared with 1st and 2 nd trimester. PON1 activities were significantly higher in first [17218 (15423,1 - 20667,2) U/L] and second trimester [18964(14373,9 - 22408,4) U/L] compared with controls, but in 3rd trimester were significantly lower [3828 (2814,9 - 5360,4) U/L] compared with controls [9109,3 (8148,2 - 10183,7) U/L] and compared with 1st and 2nd trimester. After the delivery the results of SH and SOD were significantly higher than controls, but PON1 activity were significantly lower.

CONCLUSION

Appropriate antioxidant response during uncomplicated pregnancy is accompanied by an increase in PON1 activity in the first and second trimester and increase in SOD activity in the third trimester of pregnancy. Enhanced antioxidant response is present also two months after vaginal delivery.

Oxidative stress

W382

SERUM PSA LEVELS IN RELATION TO TOTAL OXIDANT-ANTIOXIDANT STATUS IN HEALTHY MALES

A. Colak[1], B. Toprak[1], H. Yalcin[1], U. Bozkurt[1], E. Arı[1], I. Karademirci [1]
[1]*Department of Clinical Biochemistry, Tepecik Teaching and Research Hospital, Izmir*

BACKGROUND-AIM

Prostate cancer is the most frequently diagnosed malignancy in males. Aging, significant impairment of the oxidation/reduction balance, infection, and inflammation are recognized risk factors of benign hyperplasia and prostate cancer. Prostate cancer is mainly a disease of aging, with most cases occurring in men over the age of 55. Therefore, progressive inherent or acquired changes in cellular metabolism occurring over the years may play a very important role in the development of this disease. Hydroxyl radicals, peroxides and superoxides are ROS that are generated during every day metabolic processes in a normal cell and ROS generation has traditionally been associated with tissue injury or DNA damage. Association between prostate cancer risk and oxidative stress has been recognized, and epidemiological, experimental and clinical studies have unequivocally proven a role for oxidative stress in the development and progression of this disease. We aimed to investigate the relation between PSA levels and Serum oxidant/antioxidant status in healthy males.

METHODS

The study included 90 healthy males with no history of benign prostate hyperplasia or prostate cancer. PSA was measured by electrochemiluminescence immunoassay using a Total PSA Elecsys kit (Roche, Indianapolis, IN). Serum total oxidant status and total antioxidant status were measured using commercially available kits (Relassay, Turkey). Pearson correlation analysis and partial correlation analysis were used to determine the relationship between variables.

RESULTS

Mean age of the participants was 41 (range 18-77) mean BMI was 27,6 ± 4,6. In crude correlation analysis serum PSA was significantly negatively correlated with total oxidant status (R=-0.225 p=0.032) but not with total antioxidant status (R=-0.155 p=0.233). When controlled for age and BMI PSA was not significantly correlated with total oxidant and total antioxidant levels (p=0.24 p=0.84 respectively).

CONCLUSION

Our results showed that serum total oxidant status and total antioxidant status are not significant factors affecting serum PSA concentrations in healthy subjects.

Oxidative stress

W383

THE ASSOCIATION OF ANTIOXIDANT AND INFLAMMATORY MARKERS IN PATIENTS WITH THE EXUDATIVE FORM OF AGE-RELATED MACULAR DEGENERATION

E. Colak[1], S. Ignjatovic[2], A. Radosavljevic[3], N. Kosanovic-jakovic[3]
[1]*Institute of Medical Biochemistry, Clinical Center of Serbia, Belgrade*
[2]*Institute of Medical Biochemistry, Clinical Center of Serbia, Belgrade and School of Pharmacy, University of Belgrade*
[3]*Institute of Ophthalmology, Medical Retina Department, Clinical Center of Serbia and School of Medicine, University of Belgrade*

BACKGROUND-AIM

There are evidence that oxidative stress and inflammation are involved in the pathogenesis of the age-related macular degeneration (AMD) although the mechanism is still unknown.The aim of this study was to analyze the antioxidant defense parameters (SOD, GPx, GR and TAS) and inflammatory markers (CRP, IL-6 and fibrinogen) in patients with advanced-exudative form of AMD compared to patients with the early form, and to healthy subjects, in order to find their mutual correlations and association with the specific forms of AMD.

METHODS

The cross-sectional study, in the University clinical setting, included 75 patients with the exudative form, 31 patients with the early form of age-related macular degeneration, aged 71.25±7.14 yr,. and 87 aged-matched control subjects.

RESULTS

Significantly lower SOD and TAS values and higher GR activity and inflammatory markers (CRP, IL-6) were found in the exudative form of AMD compared to the early form ($p<0.05$). Significant positive correlations were found between SOD and IL-6 ($p=0.05$), GR and fibrinogen ($p=0.035$), and negative correlation between TAS and IL-6 ($p=0.045$), SOD and TAS ($p=0.016$) in the early form of AMD. GPx correlated negatively with CRP ($p=0.05$) and with TAS ($p=0.016$) in the late-exudative form of AMD. A significant association of CRP (OR:1.16; 95%CI 1.03#−1.32; P=0.018), fibrinogen (OR: 1.77, 95%CI 0.99−3.15; P=0.05), and TAS (OR: 7.45, 95% CI 3.97−14.35, P<0.000) was found with the advanced form of AMD ($\chi 2=27.3$, P=0.0003).

CONCLUSION

Based on the obtained results, it may be suggested that there is a significant impairment of antioxidant and inflammatory parameter levels in AMD patients and a significant association between this parameters with AMD exists, especially in the late-exudative form of the disease.

Oxidative stress

W384

RELATIONSHIP BETWEEN TESTOSTERONE TO CORTISOL RATIO AND OXIDATIVE STRESS PARAMETERS DURING EXERCISE IN ATHLETES

V. Ćosić[1], T. Ristić[1], L. Zvezdanović-Čelebić[1], S. Kundalić[1]
[1]*Center of medical biochemistry, Clinical center Niš, Serbia*

BACKGROUND-AIM

The testosterone/cortisol ratio (TCR) is used as an indication of the anabolic/catabolic balance. Also, in athletes this ratio may be important markers of the actual physiological strain in training and overtraining. Namely, TCR is in correlation with fatigue and underperformance of athletes. Physical activity increases oxygen consumption by 10- to 15-fold over common consumption and it resulting on produces an "oxidative stress" with excessive generation of free radicals and lipid peroxidation. On the other side, a defense system minimizes these dangerous radicals. One of the main antioxidative enzymes is superoxide dismutase (SOD) and he plays a significant role especially in the state of hypoxia, as a consequence of intense exercise.

METHODS

The effects of acute exercise on SOD activity and malondialdehyde concentrated (MDA - marker of lipid peroxidation), were determinate in plasma of 32 athletes and compared with TCR. All results were compared with non-athletes (healthy volunteers). Concentrations of hormones were determined by electrochemiluminescent assay, activity of SOD by UV spectrophotometry test, while MDA was measured by Andreeva spectrophotometry method.

RESULTS

Acute exercise showed effect on increased concentration of MDA after exercise in both investigated groups ($p < 0.001$), but with higher increase in non-athletes. We noted negative correlation between TCR and level of MDA ($r = -0.68$; $p < 0.001$). Simultaneously, we noted statistical negligible differences in SOD activity before and after exercise, but we noted the greater base level of SOD activity in athletes vs. non-athletes, as well as noted negative correlation between TCR and SOD activity ($r = -0.37$; $p < 0.01$).

CONCLUSION

The presence of high MDA level in athletes with low level of testosterone and high level of cortisol suggests an increased formation of free radicals in exercise, especially in state of training stress and overtraining, on the basis of predictor ratio. Increase of basic SOD activity is a consequence of subsequently compensated by an increase activity of antioxidants enzymes as a compensatory mechanism to prevent skeletal muscle damage.

Oxidative stress

W385

VALUE OF ASCITIC FLUID MALONDIALDEHYDE IN DIFFERENTIATING CIRRHOTIC AND MALIGNANCY RELATED ASCITES

P. Kiryttopoulos[2], P. Demetriades[1], E. Kotsifou[1], A. Agorastos[1], N. Platoyannes [1], A. Tsaparopoulos[1], M. Pasali[1], P. Doumtsis[1], Z. Migkos[1]

[1]*General Hospital of Imathia, Unit of Veria*

[2]*General Hospital of Imathia, Unit of Veria, Department of Internal Medicine*

BACKGROUND-AIM

In this perspective study, we examined malondialdehyde (MDA) of the ascitic fluid (AF) in a group of 50 patients. The purpose of the study was to determine whether the MDA of AF can be used as a marker in differentiating malignant related from cirrhotic ascites, in everyday practice.

METHODS

The method used to quantify the ascitic MDA was spectrophotometry and there were 2 groups of patients (25 with cirrhosis due to hepatitis or alcohol consumption and 25 with already known malignancies of the abdomen- 12 liver cancers, 6 pancreatic cancers, 2 biliary tract cancer and 5 stomach cancers).

RESULTS

The mean ascites level of MDA in the malignant group was 1,17+/-0,45 μmol/L whereas in the cirrhotic group it was 0,39+/-0,09 μmol/L. The SPSS statistical analysis confirmed strong correlation ($p<0,001$) between the existence of abdominal malignancy and the ascitic MDA levels, since MDA of the malignant group was statistically much higher than MDA of the non-malignant group.

CONCLUSION

In today's common practice in medical laboratories, serum-ascites albumin gradient is used to differentiate the malignant or not ascites.
This study suggests a discussion for use of further markers, such as MDA, a known biomarker of lipid peroxidation.

Oxidative stress

W386

CONTRIBUTION OF XANTHINE OXIDASE TO OXIDATIVE STRESS IN THE HEART OF RATS WITH METABOLIC SYNDROME INDUCED BY HIGH FRUCTOSE FEEDING

A. Kitagawa[2], Y. Ohta[1], K. Yashiro[1]
[1]*Department of Chemistry, Fujita Health University School of Medicine, Toyoake, 470-1192, Japan*
[2]*Department of Nutrition, Faculty of Wellness, Shigakkan University, Ohbu, 474-8651, Japan*

BACKGROUND-AIM

It is known that the symptom of metabolic syndrome occurs in rats with high fructose feeding. It is also known that oxidative stress is induced in the heart of rats with high fructose feeding. However, it is still unclear how cardiac oxidative stress occurs in rats with high fructose feeding. Therefore, we examined whether xanthine oxidase (XOD), an enzyme generating reactive oxygen species, contributes to cardiac oxidative stress in rats with high fructose feeding.

METHODS

Male Wistar rats were pair-fed either a diet containing 60% fructose (HFD) or a diet containing 60% dextrose (CD) used as the control diet for 4 or 6 weeks. Allopurinol, a XOD inhibitor, dissolved in drinking water, at a dose of 20 mg/kg body weight/day was administered to rats with and without HFD feeding everyday for 2 weeks, starting at 4-week HFD feeding. Rats fasted for 15 h were killed under pentobarbital anesthesia at 4 or 6 weeks of HFD feeding. Serum separated from the collected blood was used for assays of insulin, glucose, triglyceride, uric acid, free fatty acids, NOx (NO_2^-/NO_3^-), ascorbic acid (AA), and lipid peroxide (LPO). Hearts isolated from rats were used for assays of LPO, AA, reduced glutathione (GSH), and XOD.

RESULTS

Rats fed HFD for 4 and 6 weeks showed increased serum insulin, triglyceride, uric acid, free fatty acids, NOx, and LPO levels and decreased serum AA levels but had no change in serum glucose level. These changes were larger at 6-week HFD feeding than at 4-week HFD feeding. Allopurinol administration attenuated the changes in the levels of these serum components in rats fed HFD for 6 weeks. Rats fed HFD for 4 and 6 weeks showed increased cardiac LPO level and XOD activity and decreased cardiac AA level, although these changes were larger at 4-week HFD feeding than at 6-week HFD feeding. Rats fed HFD for 4 weeks had higher cardiac GSH level than CD-fed rats but rats fed HFD for 6 weeks had lower cardiac GSH level than CD-fed rats. Allopurinol administration to HFD-fed rats attenuated the increased cardiac LPO level and XOD activity and the decreased cardiac AA and GSH levels.

CONCLUSION

These results indicate that XOD contributes to oxidative stress in the heart of rats with metabolic syndrome induced by high fructose feeding.

Oxidative stress

W387

COMPARISON OF HEPARIN AND METHYLPREDNISOLONE POTENTIAL PROTECTIVE EFFECTS AGAINST ISCHEMIA-REPERFUSION INJURY OF THE TESTIS IN RATS.

C. Mertoglu[4], U. Senel[5], S. Cayli [2], U. Tas[1], Z. Kusku Kiraz[3], H. Ozyurt[4]
[1]*Anatomy, Medical of Faculty, Gaziosmanpasa Universty, TOKAT*
[2]*Department of Histology and Embryology, Faculty of Medicine, ANKARA*
[3]*Medical Biochemstry, Bingol State Hospital, BINGOL*
[4]*Medical Biochemstry,Faculty of Medicine , Gaziosmanpasa Universty, TOKAT*
[5]*Pediatric Surgery, Faculty of Medicine, Gaziosmanpasa Universty, TOKAT*

BACKGROUND-AIM

This study is aimed to invastigate and compare the potential protective effects of heparin and methlyprednisolone on ischemia-reperfusion injury in testis.

METHODS

24 male Sprague-Dawley rats were randomly divided into 3 groups, each containing 8 rats. Rats in the torsion-detorsion group, the left testis was rotated 720° for 2 hours. Rats in the treatment groups received the same surgical procedure as the torsion-detorsion group I, but methylprednisolone was administered in group II and heparin was administered in group III intraperitonealy before 30 minutes of detorsion. Left orchiectomy was performed all rats in each experimental group at 2 hours after detorsion for measurement of malondialdehyde (MDA, a lipid peroxidation product) , protein carbonyl (PC, a protein oxidation product), nitric oxide (NO), for evaluation of superoxide dismutase (SOD), Glutathione peroxidase (GSH-Px) and catalase activities, which are endogenous antioxidant enzymes and for histopathological and ımmunohistochemical (caspase 3,bax, Bcl2) changes.

RESULTS

MDA and PC levels found significantly low in methylprednisolone and heparin groups compered to control group. There was no statistically significant difference in NO level and activities of antioxidant enzymes SOD, GSH-Px, catalase between all groups. Stronger active caspase 3 expression was found in group I compared to group II and III. Bax expression was significantly higher in group I compared to group II and III. Stronger Bcl2 expression in group II was observed compared to group I. In group III, weak to moderate Bcl2 expressions was detected. Histopathological findings supported other biochemical and ımmunohistochemical changes.

CONCLUSION

Methylprednisolone and heparin protect testis from oxidative stres and these two drugs have no superiority from each other in the testis ischemia-reperfusion model.

Oxidative stress

W388

PALM OIL AND GROUNDNUT OIL SUPPLEMENTATION EFFECT ON HYPERGLYCAEMIA AND ANTIOXIDANT STATUS OF ALLOXAN – INDUCED DIABETIC RATS

F.A. Olabiyi[2], O.I. Olatunji[1], O.O. Oguntibeju[3]

[1]*CHEMICAL PATHOLOGY UNIT, DEPARTMENT OF MEDICAL LABORATORY SCIENCE, COLLEGE OF NATURAL & APPLIED SCIENCES, ACHIEVERS UNIVERSITY, OWO, NIGERIA.*

[2]*CLINICAL CHEMISTRY UNIT, DEPARTMENT OF MEDICAL LABORATORY SCIENCE, COLLEGE OF MEDICINE & HEALTH SCIENCES, AFE BABALOLA UNIVERSITY, ADO-EKITI, NIGERIA.*

[3]*OXIDATIVE STRESS RESEARCH UNIT, DEPARTMENT OF BIOMEDICAL SCIENCE, FACULTY OF HEALTH & WELLNESS SCIENCES, CAPE PENINSULA UNIVERSITY OF TECHNOLOGY, BELLVILLE, SOUTH AFRICA.*

BACKGROUND-AIM

In this study, two common cooking oils (Palm oil, PO) and (Groundnut oil, GO) were examined and their supplementation effects on the antioxidant status and diabetic index in Alloxan (100mg/kg) induced diabetic Wistar rats was investigated.
The study was aimed at studying, comparatively the supplementation effects of two common cooking oils on some antioxidant parameters in diabetic induced wistar rats. Specific objectives includes, to measure fasting glucose levels before and after supplementation; also to estimate Vitamins C and E, superoxide dismutase, Total protein and albumin; Finally, to compare the effects the oils had on these parameters in diabetes mellitus.

METHODS

A total of forty-eight Wistar rats of both sexes were used. They were categorized as follows: control, diabetic non-supplemented, diabetic supplemented with PO (200mg/kg/day) and diabetic supplemented with GO (200mg/kg/day) rats. Blood glucose level was measured spectrophotometrically using glucose oxidase method. Simple spectrophotometric methods were employed for measuring both plasma vitamins C and E. Superoxide dismutase (SOD) activity was also assessed spectrophotometrically. Total protein and albumin levels were measured using the dye methods of biuret and bromocresol green, respectively.

RESULTS

After three weeks of supplementation, diabetic supplemented groups showed a reduction in blood glucose ($p<0.05$) compared with the diabetic non-supplemented group. Plasma Vitamins C and E, SOD and albumin levels were significantly increased ($p<0.05$) among the supplemented groups when compared with the diabetic non-supplemented group. However, the plasma levels of these parameters were found to be higher ($p<0.05$) among the GO supplemented rats when compared with the PO supplemented group. Plasma vitamin C levels in the diabetic groups were lower than in the control group while changes in levels of the plasma total protein were not significant. There was no significant difference in the measured parameters with regards to the gender of the animals.

CONCLUSION

It is concluded from this study that GO exhibited superior antioxidant capacity and that supplementation of red palm oil and ground nut oil as a source of antioxidant was beneficial in the diabetic state as it reduced blood glucose level and enhanced antioxidant status.

Oxidative stress

W389

CORRELATION OF CAROTID ARTERY INTIMA-MEDIA THICKNESS WITH OXIDATIVE STRESS IN DIABETIC PATIENTS ON HEMODIALYSIS

T. Ozben [1], B. Dursun [4], B. Ozben[2], E. Dursun[1], G. Suleymanlar[3], I. Capraz [5], A. Apaydin[5]
[1] *Department of Biochemistry, Akdeniz University Medical Faculty, Antalya, Turkey*
[2]*Department of Cardiology, Marmara University Medical Faculty, Istanbul, Turkey*
[3]*Department of Nephrology, Akdeniz University Medical Faculty, Antalya, Turkey*
[4]*Department of Nephrology, Pamukkale University Medical Faculty, Denizli, Turkey*
[5]*Department of Radiology, Akdeniz University Medical Faculty, Antalya, Turkey*

BACKGROUND-AIM

Both diabetes and hemodialysis (HD) are associated with increased oxidative stress. The aim of this study was to clarify the effect of HD on oxidative stress parameters in diabetic patients and to explore any relation between carotid artery intima-media thickness (CIMT) and oxidative stress markers.

METHODS

Twenty Type 2 diabetic patients undergoing HD, 20 type 2 diabetic patients with normal renal function, and 20 age- and sex-matched healthy subjects were included into the study. Serum thiobarbituric acid reactive substances (TBARS), protein carbonyl content (PCO), and nitrite/nitrate levels were determined as oxidative stress markers. Serum vitamin E, plasma sulfhydryl (P-SH), erythrocyte glutathione (GSH) levels, and superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) activities were measured as antioxidants. CIMT was assessed by carotid artery ultrasonography.

RESULTS

Both diabetic patient groups had enhanced oxidative stress indicated by higher levels of TBARS, PCO, nitrate/nitrite and lower activities of SOD, CAT, and GPx compared to controls. Diabetic patients undergoing HD had significantly higher CIMT (P=.001) and higher levels of nitrite/nitrate (P=.05), PCO (P=.03), and GSH (P=.04) but significantly lower levels of P-SH (P<.001), serum vitamin E (P=.04), SOD (P=.02), CAT (P=.001), and GPx (P=.006) compared to diabetic patients with normal renal functions. There were significant negative correlations between CIMT and SOD (r=-0.50, P<.001), CAT (r=-0.41, P=.003), and P-SH levels (r=-0.51, P<.001) and significant positive correlation between CIMT and nitrite/nitrate levels (r=0.41, P=.003) and TBARS (r=0.35, P=.02). Linear regression analysis showed TBARS was significantly and positively correlated with CIMT (P=.04), while SOD and P-SH were significantly and negatively correlated with CIMT (P=.05 and P=.02, respectively).

CONCLUSION

Hemodialysis exacerbates oxidative stress and disturbances in antioxidant enzymes in diabetic patients. Serum nitrite/nitrate and TBARS can be used as positive determinants, while erythrocyte SOD, CAT activities, and P-SH level can be used as negative determinants of atherosclerosis assessed by CIMT in diabetic patients.

Oxidative stress

W390

OXIDATIVE STRESS DURING NON-COMPLICATED PREGNANCY

M. Pijanović[3], A. Stefanović[1], M. Miljković[1], S. Marić-krejović [2], S. Spasić[1]
[1]*Department of Medical Biochemistry, Faculty of Pharmacy, University of Belgrade, Serbia*
[2]*Dom zdravlja, Arilje, Serbia*
[3]*Poliklinika Medilab, Čačak, Serbia*

BACKGROUND-AIM

Pregnancy is a physiological condition which is characterized by an increased susceptibility to oxidative stress (OS). Conditions restricted to pregnancy, such as gestational hypertension, insulin resistance and diabetes, exhibit exaggerated indications of OS. The aim of this study was to explore longitudinal changes of the parameters of oxidative stress status during a non-complicated pregnancy.

METHODS

We recruited 38 healthy pregnant women during their regular gynecological check-up. Parameters of oxidative stress status - total oxidative status (TOS), malondialdehyde (MDA), prooxidative-antioxidative balance (PAB) and parameters of antioxidative status - total antioxidant capacity (TAC) and sulphydrile (SH) groups were measured at the midpoint of the 1st, 2nd and 3rd trimester, before delivery (at the 36th gestational week) and more than 4 weeks postpartum.

RESULTS

Repeated measures analysis of variance with post hoc Bonferroni correction showed significant change of all the measured parameters during pregnancy. TOS showed a significant increase at the beginning of the 3rd trimester, and a decrease after delivery ($p<0.001$). MDA increased significantly before delivery, and decreased after delivery ($p<0.05$). Similarly, PAB increased significantly before delivery, and then showed a more pronounced decrease after delivery ($p<0.001$), compared to the 1st trimester value. TAC increased significantly after delivery ($p<0.05$) , and SH groups showed significant increase in the 3rd trimester and after delivery.

CONCLUSION

Results of our study showed that parameters of oxidative stress increase during a non-complicated pregnancy, especially in the late phase of pregnancy. Production of prooxidant species is counterbalanced by antioxidative mechanisms, which reach the peak after delivery, when the oxidative status returns to a normal, pre-pregnancy state.

Oxidative stress

W391

THE EFFECT OF AGMATINE ON OXIDATIVE DAMAGE IN EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

I. Stevanovic[2], M. Ninkovic[2], I. Stojanovic[4], S. Ljubisavljevic[1], B. Dejanovic[3]
[1]*Clinical Centre of Nis, Clinic of Neurology, Nis, Serbia*
[2]*Military Medical Academy, Institute for Medical Research, Belgrade, Serbia*
[3]*Military Medical Centre, Karaburma, Belgrade, Serbia*
[4]*University of Nis, Faculty of Medicine, Institute for Biochemistry, Nis, Serbia*

BACKGROUND-AIM

BACKGROUND: Experimental autoimmune encephalomyelitis (EAE) is the most frequently used animal model for studying the pathogenesis of multiple sclerosis. In the inflamatory conditions in CNS, high production of reactive oxygen species and altered antioxidant status can cause damage to nerve cells components and may lead to cell death. The aim of the present study was to examine a potential benefit effects of agmatine (AGM) on oxidative stress development and efficiency of antioxidant protection during EAE in mice.

METHODS

METHODS: Wild-type (WT) and knockout (KO) CBA/H iNOS-/- mice, 3 months old (15 ± 5 g) were used for EAE induction by myelin basic protein (MBP) disolved in Complete Freund's adjuvant (CFA). The animals were divided into five groups: control, EAE, CFA, EAE+AGM and AGM. Twenty-four days of EAE induction, animals were sacrificed and biochemical examination were performed in medulla oblongata.

RESULTS

RESULTS: We have demonstrated a significant elevation of both malondialdehyde concentration and superoxide anion production in WT and KO mice comparing with controls. Also, we have noticed a significant elevation of superoxide dismutase activity and reduction of cytochrome C oxidase activity of WT and KO EAE animals. The treatment with AGM significantly decreased malondialdehyde concentration, superoxide anion production, and superoxide dismutase activity, while increased cytochrome C oxidase activity in medulla oblongata of both study groups, compared to EAE groups.

CONCLUSION

CONCLUSIONS: The present study indicates an important role of oxidative stress in the pathogenesis of EAE, whereas AGM has protective effects on antioxidant defense system and restores antioxidant capacity in brain tissue.

Oxidative stress

W392

PRO- AND ANTIOXIDATIVE ACTIVITY OF ENZYMES CORRELATED WITH CYTOKINES IN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS

L. Zvezdanovic-celebic[1], V. Cosic[1], T. Cvetkovic[2], S. Kundalic[1], J. Lalic[1], D. Stankovic-ferlez[1], A. Marinkovic[1]
[1]*Centre of Medical Biochemistry, Clinical Centre, Nis*
[2]*Institute of Biochemistry, Faculty of Medicine, Nis*

BACKGROUND-AIM

In addition to cytokines, free radicals have a significant role in the regulation and induction of systemic lupus erythematosus (SLE) by way of their involvement in target organ damage. By the exchange of multidirectional messages assisted by tumor necrosis factor (TNF-α) there occurs the activation of various metabolic pathways with the production of potent free oxygen radical generators, such as the enzyme xanthine oxidase (XO). At the same time, the activity of catalase (CAT) as an antioxidant enzyme is induced.

METHODS

In the study, plasma samples from 55 SLE patients (47 women and 8 men) in acute disease exacerbation phase were used. The patients were divided into four groups: skin (S-SLE), neurological (N-SLE), joint (J-SLE), and vascular (V-SLE) disease. Twenty healthy blood donors made up our control group. XO activity was determined using modified spectrophotometric UV method by Kalckar, while CAT activity was measured in erythrocytes using the method by Beutler, and in serum using the method by Goth. TNF-α concentration was determined using the ELISA method.

RESULTS

The results showed that XO activity was significantly elevated in the plasma of patients with S-SLE (9,67±1,99U/l); N-SLE (9,36±1,75U/l); Z-SLE (9,32±1,13U/l), and V-SLE(9,78±1,81U/l) with an identical degree of significance of P<0,001 related to controls (6,44±1,40U/l). Catalase had marked effects in the reduction of creation of free radicals and there was increased activity of the enzyme in erythrocytes and plasma in all groups (P<0,001) related to controls. A positive correlation between TNF-α concentration and XO (r=0,61; P<0,001) and CAT (r=0,45; P<0,05) activity in the plasma was observed, indicating an association between proinflammatory cytokines and XO-prooxidant activity and CAT as an antioxidant enzyme.

CONCLUSION

Establishing circulation after antiinflammatory therapy in patients with acute disease exacerbation results in tissue reperfusion and release of free oxygen radicals, accompanied by elevated XO activity in the plasma. A positive correlation with TNF- α, a factor with possible protective role, is also significant. Increased CAT activity could be a compensatory mechanism or the result of induction of its synthesis by TNF- α.

Oxidative stress

W393

LIPOLYSIS AND LIPID PEROXIDATION IN 3T3-L1 ADIPOCYTES EXPOSED TO ENDOTHELIN-1

D. Monneret[1], A. Briançon-marjollet[1], P. Faure[1], D. Godin-ribuot[1]
[1]*INSERM U1042, HP2, University Grenoble Alpes, F-38000 Grenoble, France*

BACKGROUND-AIM

Lipid peroxidation is a deleterious process occurring in many cardiovascular diseases related to excess weight, atherosclerosis, hypertension and metabolic disturbances. Endothelin-1 (ET1), a vasoactive peptide secreted by endothelial cells, is known to stimulate lipolysis in adipocytes. We aimed to answer the question whether such increase of lipolysis by ET1 may produce lipid peroxidation.

METHODS

Differentiated 3T3-L1 adipocytes were exposed to ET1 during 24 hours (without ET1=Control), in presence or not of BQ123 (type A ET-receptor antagonist), BQ788 (type B ET-receptor antagonist) or bosentan (dual type A and B ET-receptors antagonist). After exposure, glycerol release was quantified in culture media as a lipolysis marker. Lipid peroxidation was assessed by quantification of 8-iso-prostaglandin F2α concentrations in culture media, using immunoaffinity extraction followed by liquid chromatography coupled to tandem mass spectrometry (IAE-LC-MSMS).

RESULTS

As expected, glycerol concentrations increased significantly in culture media after 24h ET1 exposure (+54% vs Control, P<0.001). This lipolytic effect was blocked by BQ123 and bosentan (−30.8%, P=0.003 and −29.7%, P=0.005 vs ET1 alone, respectively) but partially by BQ788 (−14.1% vs ET1 alone, P=0.075). 8-iso-PGF2α production tended to decrease slightly in culture media from adipocytes exposed to ET1 (−18.0% vs Control, P=0.17), but such effect was not significantly abolished by BQ123, BQ788 and bosentan (+15.2%, +10.0% and +24.3% vs ET1 alone, respectively).

CONCLUSION

ET1 increases lipolysis in 3T3-L1 adipocytes, seemingly through a mechanism involving type A receptors. 8-iso-PGF2α production slightly decreases; therefore the lipolytic effect of ET1 is not associated with significant lipid peroxidation.

Oxidative stress

W394

THE DIAGNOSTIC VALUE OF ISCHEMIA MODIFIED ALBUMIN IN FIBROMYALGIA

Z. Kusku-kiraz[1], C. Mertoglu[3], A. Habiboglu[5], E. Altuntas[4], F. Gurdol[2]
[1]*Department of Biochemistry, Bingol State Hospital*
[2]*Department of Biochemistry, Istanbul Faculty of Medicine, Istanbul University.*
[3]*Department of Biochemistry, Tokat State Hospital*
[4]*Department of Cardiology, Bingol State Hospital*
[5]*Department of Physical Therapy, Bingol State Hospital*

BACKGROUND-AIM

Fibromyalgia (FM) is a chronic pain syndrome with unknown etiology. Although several clinical symptoms have been shown to accompany with the disorder, results from routine laboratory tests remain unaffected. Previous studies revealed that oxidative stress and mitochondrial dysfunction are involved in pathophysiology of FM. Ischemia modified albumin (IMA) is a novel marker for various disorders that related to ischemia and oxidative stress. The aim of this study was to measure the IMA levels in patients with FM together with markers of inflammation, and to search for a possible relation between IMA and other measured parameters.

METHODS

58 newly-diagnosed fibromyalgia patients (women, median age 31.2 years) and 45 matched controls were included in the study. Smoking, drug intake and chronic diseases were the exclusion criteria. Serum IMA levels were measured using the method developed by Bar-Or et al. Albumin, AST, ALT, creatinine, CRP, TSH, vitamin B12, and rheumatoid factor (RF) were measured by routine laboratory techniques (BS-2000, Mindray, China and Immage 800, Beckman Coulter, USA). Complete blood counts were performed by Coulter LH 750 (Beckman, USA) and sedimentation rate was measured on Therma Ne (Linear C, Spain). Albumin-adjusted IMA (IMA index) was calculated to eliminate the effect of albumin concentration by using the equation: [IMA index=serum albumin conc. (g/dL)x23+ IMA(AbsU) -100].

RESULTS

IMA levels, IMA index and sedimentation rates were significantly higher in fibromyalgia patients ($p=0.006$, $p=0.014$ and $p<0.001$; respectively). CRP, TSH, B12, RF values and BMIs of patients did not differ significantly from the controls. Neutrophil/lymphocyte ratio, MPV, PDW and other hematological parameters were also similar in both groups. A positive correlation between IMA index and BMI was observed ($r=0.27$, $p=0.02$).

CONCLUSION

To our knowledge, this is the first study showing that IMA levels are elevated in patients with fibromyalgia. High IMA level supports the theory of oxidant/antioxidant imbalance in FM, as well as it indicates the involvement of ischemia in the pathogenesis of FM. Measurements of serum IMA levels may be of help for the differential diagnosis in patients suffering from musculoskeletal pain.

Oxidative stress

W395

EVALUATION OF HYDROGEN PEROXIDE POINT OF CARE TEST TO CLASSIFY PERITONEAL EFFUSIONS AS EXUDATE OR TRANSUDATE

E. Valencia Vera[1], A. Martínez-escribano García-ripoll[1], A.M. Fernández Ramos[1], R. Gallardo Magaña[1], M. Navarrete Carmona[1], A. Enguix Armada[1]
[1]*Laboratory Department, Virgen de la Victoria Hospital, Málaga*

BACKGROUND-AIM

Peritoneal and pleural effusions are classified as exudates or transudates according to some biochemistry criteria. Recently Subendu et al. have developed a new point of care test to classify effusions as exudate and transudate, using a drop of hydrogen peroxide. Some clinical decisions could be taken earlier with the application of this kind of tests. The test relies upon the presence of a significant amount of catalase activity in exudates which is observed to be negligible in transudate.
The aim of this study is to apply the drop of hydrogen peroxide as a method for the differentiation between transudates and exudates and to compare it with an accepted criterion.

METHODS

All visually bloodless peritoneal fluids which were received for diagnostic workup in the clinical biochemistry laboratory of our hospital between June 2014 and December 2014 were considered for the study. Peritoneal fluids were classified as exudate if they met at least one of the following criteria; otherwise they were designated as transudate. (1.-Peritoneal fluid proteins >3 g/dL. 2.-Peritoneal fluid protein divided by serum protein > 0.5. 3.-Peritoneal fluid LDH (Lactate dehydrogenase) > 200 UI/L. 4.-Peritoneal fluid LDH divided by serum LDH >0.6).
A drop of hydrogen peroxide produced profuse bubbles within 1 minute when added to exudative peritoneal effusions due to the presence of significant catalase activity. On the contrary, if that was not observed, it was classified as transudate. Sensitivity, specificity and ROC curves were determined using STATA 13.

RESULTS

A total of 37 (visually bloodless) peritoneal fluids were analyzed. According to the used criteria, 35 (94.6%) were classified as transudate and 2 (5.4%) as exudate. Liver disease was the cause in 70% of the cases of transudative fluid. The analysis of the data showed that the test sensitivity is 71.4% (CI 95% 29.0 to 96.3 %) and test specificity is 94.6% (CI 95% 81.8% to 99.3%). If more than 1100 erythrocytes per mm3 fluids were excluded from the analysis, the sensitivity and specificity were improved to 83.3% (CI 95% 35.9 to 99.6 %) and 97.1% (CI 95% 85.1 to 99.9 %), respectively. The ROC area value 0.9 showed the test to be a good one to distinguish between exudates and transudates. In this case, the values of sensitivity and specificity were acceptable.

CONCLUSION

The hydrogen peroxide bubbling can be used as point of care testing to distinguish between exudative and transudative bloodless peritoneal fluid samples.