glycerate hydrolase) is a crucial enzyme that involved in glycolytic pathway for energy production. Therefore, it can be selected as a molecular target for novel antitheilerial therapy methods. In this study, steady state kinetic parameters of the enzyme were determined for the first time. Enzyme kinetic measurements using 2-PGA as substrate gave a specific activity of ~40 U/mg, K_m: 106 μM, k_{cat}: 37 s^{-1} and k_{cat}/K_m: 3.5 × 105 M^{-1} s^{-1}. Determination of kinetic properties of enolase from *Theileria annulata* enables application of further studies on new antitheilerial drug design.

Keywords: drug design, enolase, *Theileria annulata*.

TUE-443

Kinetic mechanism of smooth muscle cell plasma membrane Ca^{2+}, Mg^{2+}-ATPase selective inhibition by calixarene C-90

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Today a great attention of scientists is paid to calixarenes as original molecular platforms which are perspective for designing of biologically-active compounds. Calixarenes are macrocyclic oligophenolic compounds and some of them possess bactericidal, antiviral, antitumoral, antithrombotic activity, they almost have no toxic action on cells.

In our previous investigation carried out on the suspension of myometrium cell plasma membranes we found that calixarene C-90 efficiently inhibited Ca^{2+}, Mg^{2+}-ATPase ($I_{0.5}$ is about 20 μM) and did not influence on activity of other membrane-bound ATPases. Considering further development conception about mechanisms of inhibition of the plasma membrane Ca^{2+}, Mg^{2+}-ATPase of uterus cells by this calixarene we have more thoroughly investigated the influence of this compound on kinetic properties of the Ca^{2+}, Mg^{2+}-ATPase activity of myometrium plasma membrane.

We have shown that calixarene C-90, while inhibiting Ca^{2+}, Mg^{2+}-ATPase, did not influence on kinetic parameters (K_M, n_H) of reaction velocity dependence on substrate concentration. Changes of ATP concentration also did not effect on kinetic of Ca^{2+}, Mg^{2+}-ATPase inhibition by calixarene C-90 and its respective constants (K_i, n_H). The growth of calixarene concentration up to 100 μM caused the slight increase of enzyme activation constants by $MgCl_2$ (K_{Mg}) and by Ca^{2+} (K_{Ca}). The Hill cooperativity coefficients (n_H) of activation by both $MgCl_2$ and Ca^{2+} did almost not varied in the presence of mentioned calixarene. Both $MgCl_2$ and Ca^{2+} also slightly influenced on Ca^{2+}, Mg^{2+}-ATPase cooperativity coefficient n_H and coefficient of inhibition by calixarene C-90 (K_i). The most considerable impact of calixarene C-90 was observed to change the maximal velocity of enzyme reaction. In all cases calixarene C-90 proportionally itself concentration decreased mentioned kinetic parameter. Therefore, we can conclude that inhibitory action of calixarene C-90 on Ca^{2+}, Mg^{2+}-ATPase has mainly uncompetitive character because interaction of calixarene C-90 with enzyme leads to decrease of enzyme turnover number.

We consider that calixarene C-90 is perspective for creation of new pharmaceuticals in order to regulate intracellular Ca^{2+} concentration and therefore muscle tone and contractility.

We are thankful to professor V.I. Kalchenko for helpful discussion and scientific cooperation.

Keywords: Ca^{2+}, Mg^{2+}-ATPase, calixarene, plasma membrane.

TUE-444

Lack of association between genetic variation of -2548G/A of leptin gene polymorphismand hypertension in obese adult Saudi subjects

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Background: Leptin is a polypeptide hormone synthesized mainly by white adipose tissue. Common polymorphism -2548G/A of leptin (LEP) gene has been associated with obesity, but its association with cardiovascular diseases including hypertension has been little studied. Thereforeour study aimedto investigate whether the polymorphism -2548G/A of (LEP) gene is associated or not with hypertension in obesity in a sample of Saudi adult patients.

Patients and Methods: A total of 206 Saudi adult subjects (112 women and 94 men) their aged 40–60 years were recruited and subdivided into three groups: 50 normotensive ND controls (age: 47.9 ± 5.4 y; BMI 22.9 ± 2.1 kg/m^2), 80 normotensive obese ND (age: 47.7 ± 6.0 y; BMI 34.1 ± 4.2 kg/m^2) and 76 hypertensive obese patientswith T2DM (age: 49.4 ± 5.9 y; BMI: 35.1 ± 4.7 kg/m^2). Analyses of -2548G/A polymorphism of LEP gene were made by the polymerase chain reaction restriction-fragment length polymorphism technique (PCR-RFLP). Anthropometric data were collected from all subjects. Serum leptin and insulin concentrations were determined by Luminex as well as fasting blood glucose and serum lipids were determined by a chemical auto analyzer Konelab.

Results: Our study showed that the AA genotype of −2548G/A variant of LEP gene was significantly associated in individuals with higher fasting glucose levels ($p < 0.04$), and HOMA-IR ($p = 0.03$), as well as the A allele of LEP gene variant (−2548G/A) is more prevalent among the subjects with elevated fasting glucose [OR 1.9 (1.2, 3.0), $p = 0.006$], while GA genotype was more common in individuals with hyperleptinemia ($p = 0.04$). On the other hand no association was elicited with either systolic or diastolic blood pressure.

Conclusion: The study showed that the genotypes distribution of −2548G/A variant of LEP gene (GA and AA) are associated with plasma leptin, and glucose levels in set of Saudi individuals. Moreover, these genotypes as well as A allele of this gene might be an important risk factor predisposing healthy subjects to T2DM.

Keywords: gene polymorphism, leptin, obesity.

TUE-445

Lindane disturbs the capacity of *Saccharomyces cerevisiae* to scavenge lipid hydroperoxides via phospholipid hydroperoxide glutathione peroxidase causing cell death

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Reactive oxygen species (ROS) are by-products of aerobic metabolism in cells. Pollutants such as lindane may raise its production, causing cell damages in biomolecules as lipids. Cells possess defence systems to counter oxidative stress including glutathione and glutathione peroxidases (GPx). Phospholipid hydroperoxide glutathione peroxidase (PHGPx) is considered to be the main line of enzymatic defence against membrane damage. The experimental advantages of the yeast model *Saccharomyces cerevisiae* have been exploited extensively for advancing our understanding about

cell defences against ROS, because the yeast genome sequencing revealed genes that encode GPx which exhibit great homology with other eukaryotes, including man. Lindane has been used as pesticide in agricultural and human health applications. Several factors have contributed in concern over the use of lindane including its persistence, toxicity and bioaccumulation. Thus, the aim of this study was to evaluate the response to lindane mediated by yeast GSH and PHGPx. *Saccharomyces cerevisiae* UE-ME_3, a wild-type yeast deposited in the collection of laboratory of Enology, University of Évora, at mid-exponential phase, were inoculated in YEPD medium, 2% (w/v) glucose, at 28°C, and shaken 150 rpm for 72 h in presence of 5 μM or 50 μM lindane and compared with control. Cell viability was determined by cfu and the biomass by dry weight. Yeasts harvested were suspended in 10 mM phosphate buffer pH 7.0 and disrupted by sonication. The post-12000 *g* pellets were used for determination of malondialdheyde (MDA), glutathione (GSH) and glutathione disulfide (GSSG) by fluorescence and the post-12000 *g* supernatant for PHGPx determination by spectrometry. Statistics were performed by ANOVA I and Duncan ($p < 0.01$) using SPSS for Windows, version 22. The results showed that lindane, at 72 h of exposure, inhibited yeast growth decreasing biomass produced and cell viability. On the other hand, it was observed, for both levels of exposition, an increase in the GSH/GSSG ratio and in the level of proteins, total glutathione, GSH, and MDA of mitochondria as well as a decrease in the PHGPx. The increase in GSH/GSSG ratio of mitochondria probably resulted from the incapacity of the cell to scavenge lipid hydroperoxides, *via* PHGPx, preventing cell damages in mitochondrial membranes. This effect may have determined an increase in the mitochondrial MDA content. This response probably contributed to slowdown the energetic metabolism and over express mitochondrial proteins. So, lindane was toxic to *Saccharomyces cerevisiae* UE-ME_3, probably causing cell death by an active process.

Keywords: Antioxidant enzymes, organochlorine pesticides, yeast.

TUE-446
Lipid profile of the red blood cell membranes in acute pancreatitis: effects of traditional therapy

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The pancreatic acinar cell synthesizes, stores, and secretes digestive enzymes. Observing pancreatic cells is difficult due to time and physiological problems, however, by comparing the red blood cell membranes of people with acute pancreatitis (AP) and those without it is possible to see the damage caused by the activated enzymes to the blood cells and thus conclude that similar damage is taking place in the acinar cells themselves.

In this study the erythrocyte membranes of 110 AP patients were examined before and after traditional therapy. Two study groups comprising 58 patients with acute biliary pancreatitis (ABP) and 52 patients with acute non-biliary pancreatitis (ANBP) were compared. 26 age matched volunteers were used as a control group.

The lipid bilayer is composed of cholesterol and phospholipids in equal proportions by weight. These lipids were separated using the method of thin layer chromatography. Only cholesterol esters (CE), monoacyl glycerol (MAG), and diacyl glycerol (DAG) levels fall into the normal range in patients with ABP on admission. Elevated cholesterol (C) level indicates decreased mobility of fatty acids and deterioration of lateral diffusion; decreased sphyngomyelin (SM) concentration is a sign of impaired lipid phase micro viscosity; reduction in phosphatidyl choline (PC) indicates the decrease in membrane permeability and is a sign of impaired cholesterol metabolism. In contrast, patients with ANBP had normal values of C, TAG, and phospholipids. Decrease in CE, MAG and DAG and rise in FFA indicate the activation of the lipolytic processes.

There is strong correlation between ankyrin and PC levels and clinical symptoms in all the AP patients, whereas in ANBP there is an additional link between the clinical picture and MDA concentration.

In total, 80% of lipids of the RBC membranes were altered in ABP, whereas 50% of lipids were damaged in ANBP. In ABP, traditional therapy improved 25% of lipids, 37.5% fell into the normal range. In ANBP, traditional therapy was less effective and only improved and normalized up to 40% of lipid profile.

The present studies provide evidence that oxidative damage to both lipids and proteins of the RBC membrane occurs in acute pancreatitis. Traditional treatment corrects the structural aberrations in ABP more effectively than in ANBP. This suggests that there is much to be gained by introducing into the traditional therapy drugs with immunomodulating, antioxidant and membrane protective properties.

Keywords: membrane lipids, pancreatitis, red blood cell.

TUE-447
Localization of plasminogen-binding site in fibrin fragment DD

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Fibrinolytic system ensures the destruction of blood clots, but in addition perform other physiological and pathological functions and participates in the process remodulation tissues, reproduction, angiogenesis, inflammation, tumor cell invasion and others.

In order to investigate the mechanism of fibrinolysis and its regulation pathways we studied the potentiating effect of cross-linked fibrin fragments on Glu-plasminogen activation by tissue-type plasminogen activator (t-PA). It was found all plasmic degradation products of fibrin including DDE-polymers, DDE-complexes, fragments E_1E_2 (dissociation products of non-covalent DDE-complexes) and core product DD, apart from core fragment E_3, have potentiating effect on plasminogen activation by t-PA. These results indicate that plasminogen- and t-PA-binding sites on fibrin surface which is exposure under conversion of fibrinogen to fibrin remain on the fibrin fragments during fragmentation by plasmin. Thus, the process is irreversible.

Multifunctional fragment DD molecule is of particular interest because it inhibits platelet aggregation, slows fibrin polymerization, shows an affinity for fibrin clot and stimulates Glu-plasminogen activation.

Using method of izomolar-series, we have established the maximum potentiating effect of fragment DD on activation system plasminogen by t-PA. The effect is observed at a molar ratio of plasminogen to fragment DD 1.0–1.3. Considering this data and structure of fragment DD (identical D-domains of two neighboring covalently cross-linked by γ-chains fibrin molecules) we conclude that the plasminogen-binding site is located in region of γ-γ-cross-linking.

The results of this study can be the basis for the creation of new fibrinolytic drugs and the development of test systems to determine the fibrinolytic system parameters.

Keywords: fibrin fragments, fibrinolytic system, plasminogen activation.