h_{+1}/h_0 ratio of 5-DS. This effect was more pronounced for peptide:lipid molar ratio 1:100. It has also been shown for a 5-DSA labeled membranes containing $A\beta_{1-40}$ that membrane fluidity preferentially decreased for aggregated form of amyloid. The membranes containing 16-DS did not show statistically significant difference between control and $A\beta_{1-40}$ treated samples. But in the case of aggregated form of $A\beta_{1-40}$ (P:L ratio: 1:10) a decrease of membranes fluidity existed that suggests that aggregated form of AB were more active in comparison with non aggregated peptide fragments in both external and internal regions of liposomal membrane.

Obtained results show that the investigated peptides affect the bilayer order of liposomal membrane. The incorporation of Aβ fragments into the model lipid vesicles decreases the bilayer fluidity.

27. Poli(amido)amine dendrimer PAMAM G2.5 as a potential modulator of the intracellular calcium concentration

A. Jarosz, M. Łabieniec

University of Łódź, Poland

Mitochondria play a pivotal role in the intracellular Ca^{2+} signaling by taking up and releasing the ions upon specific conditions. The potential cytotoxicity of Ca^{2+} ions has been recognized for about three decades notwithstanding, the exact mechanisms of calcium cytotoxicity remain controversial. This cytotoxicity is believed to manifest itself via different mechanisms. In the present investigation, we attempted to study the protective activity of anionic PAMAM dendrimer G2.5 against deleterious effect of Ca^{2+} ions on isolated rat liver mitochondria. We hypothesized that low half-generation PAMAM dendrimers are less toxic than the higher and full-generation ones and hence they can be used as calcium ions chelators, thus protecting mitochondria against overload with Ca^{2+} ions.

Mitochondria isolated from Wistar rats were exposed to Ca^{2+} ions (at the concentration equal to its estimated IC_{50}) and to dendrimer G2.5 (at its neutral concentration) and several parameters of mitochondrial function were monitored with the use of the spectrofluorimetric technique. Briefly, the mobilization of calcium ions was detected using spectrofluorimetry with Calcium Green 5-N. Mitochondrial membrane fluidity and transmembrane potential were tracked using the staining with 1,6-diphenyl-1,3,5-hexatriene (DPH) and JC-1, respectively.

Obtained data revealed that PAMAM G2.5 used at the concentration of 5 μM is not able to effectively reduce the adverse effects of calcium ions on the mitochondria function. All the measured parameters that became reduced following the incubation with Ca^{2+}, were not improved in the presence of dendrimer G2.5. Therefore, based on these preliminary results, we conclude that calcium ions-induced mitochondrial damage and the subsequent liver dysfunction cannot be prevented by PAMAM G2.5.

28. Oxidative and nitrative modifications of plasma proteins isolated from breast cancer patients

M. Kędzierska[1], B. Olas[1], J. Kołodziejczyk[1], B. Wachowicz[1], A. Jeziorski[2], J. Piekarski[2], U. Czernek[2], P. Potemski[2]

[1]University of Łódź;
[2]Medical University of Łódź, Copernicus Memorial Hospital

Breast cancer is the most popular and very heterogenous malignant cancer in women in Poland and in the whole world at both the clinical and molecular levels. The process of cancer formation and development is associated with loss of redox balance in the cell and overproduction of reactive oxygen and nitrogen species. In breast cancer patients, oxidative stress and oxidative changes in the cells take place. Plasma proteins play an important role in the pathomechanism of altered hemostasis in cancers, therefore, the aim of our present study was to evaluate oxidative/nitrative modifications of plasma protein by measurement of the level of different biomarkers of oxidative/nitrative stress such as carbonyl group, 3-nitrotyrosine, total antioxidant status in plasma from patients with invasive breast cancer, patients with benign breast diseases and healthy volunteers.

Plasma was isolated from healthy subjects and patients, hospitalized in Department of Surgical Oncology, Copernicus Memorial Hospital, Medical University of Łódź, Poland. Identification of the level of 3-nitrotyrosine and carbonyl groups in plasma proteins was performed by ELISA tests. The level of total antioxidant status in plasma was measured spectrophotometrically by of inhibition of oxidation of 2,2-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS).

We observed a statistically significant increase of 3-nitrotyrosine and carbonyl groups level in plasma proteins; a decrease of total antioxidant status in plasma from patients with breast cancer (compared to the healthy group) was also found.

The different modifications (nitration or carbonylation) of plasma proteins might be partly associated with modulation of structure and function of proteins involved in hemostasis of breast cancer patients.

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29. Age-related endocrine changes in the blood of Polish elderly people – the PolSenior study

K. Kędziora-Kornatowska[1], J. Czuczejko[1], K. Szewczyk-Golec[1], M. Kozakiewicz[1], H. Pawluk[1], M. Mossakowska[2], B. Kłapcińska[3], J. Kędziora[1]

[1]Nicolaus Copernicus University, Collegium Medicum in Bydgoszcz
[2]International Institute of Molecular and Cell Biology, Warsaw,
[3]Academy of Physical Education, Katowice

One of the main problems of modern countries is the aging society. According to the projection of Polish Central Statistical Office (CSO), the life expectancy in Poland may increase from 70.0 years in 2007 to 77.1 yr in 2035 for males and from 79.7 to 82.9 for females, respectively. To approach to the solution of the problems of the aging society, the Polish Ministry of Science and Higher Education (MEIN) has supported a grant for a comprehensive research programme, named "PolSenior" ("Medical, psychological, sociological and economic aspects of aging in Poland"), for providing data on the health and socio-economic situation of the oldest group of Polish population. Specific endocrine changes occur with the aging process. Thus, one of the project's focus was to evaluate concentration of some endocrine factors in the blood of elderly Polish inhabitants.

The study was carried out on 158 Polish citizens divided into four age subgroups (55–60, 66–80, 81–90 and 90+ yrs). The participants were recruited according to a multi-stage procedure designed for PolSenior project. Blood samples were collected in the morning after overnight fasting from the cubital vein. Serum insulin-like growth factor-1 (IGF-1), insulin-like growth factor binding protein 3 (IGFBP3) and melatonin were determined in the study.

The decline of IGF-1 and IGFBP3 with age were observed. The lowest levels of these factors were noticed in the subgroup of the oldest people. Unchanged concentration of melatonin were found in the all examined subgroups.

These results give information about some hormonal profile of geriatric population in Poland. The decline in IGF-1 and IGFBP3 with age indicates that growth hormone-insulin-like growth factor axis is affected by ageing in this population.

30. Induction of autophagic cell death in MCF-7 breast cancer cells treated with docetaxel

K. Kochel, K. Matczak, A. Pieniążek, A. Koceva-Chyła

University of Łódź

Autophagy is a catabolic process by which damaged or long-lived cellular proteins and organelles are degraded. A range of stressors can induce autophagy, which is associated with the formation of an autophagosome, a double-membrane vesicle that engulfs organelles and cytoplasm, and subsequent lysosomal degradation of this structure. In some cancer cells, autophagy has been reported to play a protective role under conditions of stress. However, prolonged autophagy in cancer cells that lack the machinery to undergo apoptosis can result in a nonapoptotic programmed cell death. It is still unclear whether autophagy has primarily a protective or destructive effect on cancer cells.

The aim of our study was to examine weather docetaxel (DTX), a second-generation taxane anticancer drug, can induce autophagy of breast cancer cells. As a marker of autophagy, the volume of the cellular acidic compartments was visualized by acridine orange staining. Monolayer of MCF-7 breast cancer cells was treated with IC_{50} concentration of DTX (0.5 μM) for 2 h. After incubation the drug was removed and cells were grown in fresh medium for 0, 24, 48 and 72 h. At each of these time points 1 μg/ml of acridine orange was added directly to the medium for 15 min. Then the acridine orange was removed and cell morphology was analyzed under an inverted fluorescent microscope.

We observed appreciable level of cellular acidic compartments after treatment with DTX. A progressive increase in acidic vesicular organelles indicative of development of autophagy over time was found.

Our results show that breast cancer cells treated with DTX can die not only via the apoptotic or necrotic pathways, but also by autophagy.

31. Antioxidative properties of *Trifolium pallidum* extract

J. Kołodziejczyk, J. Malinowska, B. Olas

University of Łódź

The present study was designed to assess *in vitro* the antioxidative properties of *Trifolium pallidum* extract. *T. pallidum* is a clover, very similar to well known red clover (*Trifolium pratense*). Herbal medicines (including *Trifolium*) have been widely used for the treatment of various diseases; these beneficial properties are attributed to their biologically active substances such as saponins, isoflavones, clovamides, proanthocyanid-

ins and other polyphenolic compounds. However, most of clovers still remain poorly characterized. Therefore, in present experiments we studied the protective activity of *T. pallidum* against the oxidative/nitrative modifications of human plasma proteins and lipids, induced by peroxynitrite ($ONOO^-$), a strong oxidative and nitrative agent, which is formed *in vivo*.

The antioxidative effects of the tested extract were estimated by measurements of the level of different biomarkers of oxidative/nitrative stress such as carbonyl groups, 3-nitrotyrosine (3-NT), thiol groups and TBARS (thiobarbituric acid reactive substances). The effects of *T. pallidum* on plasma protein carbonylation and 3-NT formation were established by ELISA tests. Thiol groups in plasma proteins were determined with 5,5'-dithio-bis(2-nitro-benzoic acid, DTNB). Plasma lipids peroxidation was measured as the TBARS level.

Exposure of plasma to 0.1 mM $ONOO^-$ resulted in an increase of carbonyl groups and 3-NT formation; a significant decrease of reduced thiols was also observed. *T. pallidum* extract added to the final concentration of 12.5, 25 and 50 µg/ml, partly decreased the level of carbonyl groups (by about 30%) and effectively prevented the 3-NT formation in plasma proteins (at the higher concentration of the extract the 3-NT was comparable to the control plasma sample). In plasma incubated with $ONOO^-$ and the tested extract, the level of protein –SH groups was significantly higher in comparison to plasma samples treated with $ONOO^-$ only. The tested extract had also an inhibitory effect on the peroxynitrite-induced plasma lipids peroxidation.

The extract obtained from *Trifolium pallidum* possesses antioxidative properties and partly protects plasma proteins and lipids against peroxynitrite-induced damage.

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32. Effect of melatonin supplementation on the oxidative stress parameters under hyperbaric conditions

M. Kozakiewicz[1], K. Kędziora-Kornatowska[1], K. Szewczyk-Golec[1], P. Siermontowski[2], R. Olszański[2], J. Czuczejko[1], J. Szypuła[3], J. Kędziora[1]

[1]Nicolaus Copernicus University Collegium Medicum in Bydgoszcz;
[2]Military Institute of Maritime Medicine, Gdynia;
[3]Clinic of Traumatology and Orthopedics Surgery, Military Hospital, Olsztyn

The purpose of this study was to evaluate the influence of exposure in hyperbaric chambers on selected parameters of oxidative stress in divers blood and to determine the influence of melatonin supplementation on the oxidative stress parameters under hyperbaric conditions.

25 healthy men (non-smoking, experienced in diving at great depths), aged 18–40 yrs (average age 27 yrs), took part in the experiment. The subjects were submitted to hyperbaric conditions. The exposure simulated conditions in the hyperbaric chambers were similar to those at 60 m of depth while diving. A control group consisted of 20 healthy men who have never dived nor have been exposed to hyperbaric conditions. The blood samples were taken from the cubital vein after overnight fasting once from the control group and 4 times (before and after hyperbaric exposure at baseline and after 30 days of melatonin supplementation (5 mg daily)) from the divers group. Zn,Cu-superoxide dismutase (SOD-1) activities and malondialdehyde (MDA) concentrations were estimated in the red blood cells, whereas the carbonyl groups in proteins and melatonin levels were measured in blood serum.

The erythrocyte MDA level and carbonyl group content of serum proteins were significantly increased, whereas the melatonin level in serum and the activity of SOD-1 in the red blood cells were significantly decreased in the divers group in comparison with the control group. The considerable increases in the content of erythrocyte MDA and the carbonyl groups of serum proteins, as well as in the erythrocytic SOD-1 activities, and a significant decrease in serum melatonin were observed in the divers group after hyperbaric exposure. After 30 day treatment of melatonin the indoloamine concentrations increased in the divers blood to the levels similar to the control group. The activities of SOD-1 in the erythrocytes increased both before and after hyperbaric exposure. The observed changes in the MDA and the carbonyl group levels, as well as in the activities of SOD-1 during hyperbaric exposure were no statistically significant after melatonin supplementation.

Considerably weakened enzymatic antioxidative defense was observed in the red blood cells of people exposed to hyperbary in comparison with people in normobary. This issue indicates that a diver organism is more susceptible to the negative effects of oxidative stress. Moreover, the obtained results also indicatethat hyperbaric conditions can induce the intensification of the reactions with free radicals. Melatonin treatment may prevent some of long term negative effects of hyperbaric exposure, that involves the free radical generation.

33. Fullerenol $C_{60}OH_{20–33}$ protects human erythrocyte membrane proteins from damage during storage

A. Krokosz, J. Grębowski, A. Rodacka, M. Puchała

University of Łódź

Fullerenols, hydroxyl-containing derivatives of fullerene C_{60}, can be effective antioxidants and radical scavengers. They may protect cells from oxidative

stress induced by many factors e.g. storage (erythrocyte aging *in vitro*). The protein composition of the erythrocyte membrane undergoes some conspicuous changes during storage. These changes are connected with the damage of protein of band 3, associated frequently with increased membrane binding of modified hemoglobins.

We have studied the influence of fullerenol $C_{60}OH_{20–33}$ on the human erythrocyte membrane damage during storage.

Human erythrocyte membranes suspended in PBS (1.5 mg protein/ml) were incubated with fullerenol $C_{60}OH_{20–33}$ at a concentration range of 0–150 μg/ml at 37°C. After 3 or 48 hours of incubation SDS-PAGE was performed according to Laemmli using a Bio-Rad system. Proteins were stained with Coomassie Brilliant Blue. The gels were digitalized and analyzed with the GelScan software. The relative quantities of proteins in selected bands were expressed as percentage of the total protein quantity.

Our results showed that the incubation of erythrocyte membranes at 37°C for 48 hours resulted in a significant loss of band 3 protein. Fullerenol preserved the protein of band 3 at all the concentrations employed.

Changes in band 3, the main integral membrane protein and anion exchanger of the erythrocyte, trigger the binding of physiological autoantibodies to senescent erythrocytes leading to their degradation. Fullerenol protecting band 3 protein may help to maintain the erythrocyte structure and function.

34. Biophysical studies of the cap-binding ability and the structure stability of the ovary-specific *Xenopus* eIF4E1b

D. Kubacka[1], J. Stępiński[1], J. Jemielity[1], N. Minshall[2], N. Standart[2], E. Darżynkiewicz[1]

[1]University of Warsaw, Poland;
[2]University of Cambridge, UK

Eukaryotic initiation factor 4E, named eIF4E1b, is a close homologue of the canonical class I eIF4E cap-binding protein (eIF4E1a; 71% identity). As a component of the CPEB complex along with the eIF4E-binding protein 4E-T (eIF4E-Transporter), the Xp54 RNA helicase and other RNA-binding proteins, eIF4E1b is proposed to mediate silencing of translation during *Xenopus* oogenesis. All residues required to bind the mRNA 5'cap structure (W56/W102/W166/E103/R112/R157/K162) are present in its primary structure but eIF4E1b in oocyte lysates interacts only weakly with m^7GTP compared to eIF4E1a. Interestingly, several additional cap-proximal residues which may impact on cap-binding are found to systematically differ between vertebrate eIF4E1a and 1b proteins (1). Furthermore, other differences in the primary structure of eIF4E1b in relation to *Xenopus* eIF4E1a may determine the stability of its apo-form.

We have used emission spectroscopy and site-directed mutagenesis to investigate the influence of the systematic substitutions in eIF4E proteins on the binding of *Xenopus* eIF4E1b to the cap structure. As a solvent component, glycerol has emerged to be a good tertiary structure stabilizing agent which strengthens the protein internal hydrophobic interactions and enables long-term fluorescent measurements. While no single characteristic feature was found to be responsible for the weakened eIF4E1b-cap analogue interactions, these studies show that the cap-bound conformation of eIF4E1b is stable. Quantitative cap analogue-binding analyses of *Xenopus* eIF4E1a and 1b proteins and the mutant results from ongoing studies will be presented.

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35. Antioxidant activity of new Cu(II) complex: dichloro-(3,5-dimethyl-N1-pyrazol-1-yl) and dichloro-(3,4,5-trimethyl-N1-pyrazol-1-yl) in patients with colorectal cancer

K. Kubiak[1], K. Malinowska[1], Ł. Dziki[2], A. Dziki[2], I. Majsterek[1]

[1]Medical University of Łódź;
[2]Clinic of General and Colorectal Surgery, Łódź

Colorectal cancer is a serious medical and economic problem. Based on vast research performed, a conclusion was drawn that the disturbances of antioxidative mechanisms and big expansion of oxygen free radicals might participate in the development of colorectal cancer. The main aim of this work was an investigation of the influence of Cu(II) complexing compounds: dichloro-(3,5-dimethyl-N1-pyrazol-1-yl) and dichloro-(3,4,5-trimethyl-N1-pyrazol-1-yl**)** on the activities of superoxide dismutase (SOD-1) and catalase (CAT) in patients with colorectal cancer. The study were performed on erythrocytes from 30 subjects (23 men and 7 women mean age 66.5 ± 10.2) including patients with colorectal cancer and controls with minor alimentary tract disturbances (polyps, hernias) without neoplastic lesions. The activity of CAT was measured by the spectrophotometric method according to Beers et al. and SOD-1 according to Misra and Fridovich. It was found that the activities of CAT and SOD-1 were significantly lower in patients than in controls. Interestingly, our data showed an over 50% increase of CAT as well as SOD-1 activities induced by both Cu(II) complexing compounds in colorectal cancer patients according to controls. In conclusion, we suggested that Cu(II) chelators are effective antioxidant factors which could be useful in prevention and treatment of colorectal cancers.

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36. Antioxidative and biological activity of natural polyphenols from *Rosaceae* family

A. Z. Kucharska, A. Sokół-Łętowska, A. Bąkowska-Barczak, K. Pyrkosz-Biardzka, A. Dudra, J. Gabrielska

Wrocław University of Environmental and Life Sciences

The excessive production of oxygen species, such as $^{\bullet}OH$, $O_2^{\bullet-}$ and singlet oxygen, and other radicals is thought to cause damage to cells. The damage is believed to be strongly associated with carcinogenesis, mutagenesis, aging, chronic inflammation, cardiovascular disorder, atherosclerosis, and so on. Endogenous antioxidants from plants must play an important role in the antioxidant defense and are expected to protect the biological functions of cells and reduce the risk of many diseases. Great attention has been directed to fruits as natural sources rich in polyphenols compounds such as: flavones, flavanols, flavanones, anthocyanins, procyanidins and phenolic acids. We reported literature data for the biological and antioxidative activity of fruits from *Rosaceae* family such as: raspberry (*Rubus idaeus L.*), blackberry (*Rubus plicatus* W. et N.), chokeberry (*Aronia melanocarpa* (Michx.) Elliott), blackcurrant (*Ribes nigrum* L.), gooseberries (*Ribes uva-crispa* L.), quince japanica (*Chaenomeles speciosa* (Sweet) Nakai), quince (*Cydonia oblonga* Mill.), hawthorn (*Crataegus monogyna* Jacq), rose (*Rosa canina* L.), and cherry (*Prunus cerasus* L.). The results give evidence for the effectiveness of polyphenol extracts from *Rosaceae* in prevention of oxidative stress *in vitro*, which is in correlation with the ability to scavenge free radical (as $^{\bullet}$DPPH and $ABTS^{+\bullet}$) and with polyphenol content. We also report some data on micro- and nanoencapsulation of bioactive compounds that suggest a long-term stability of these compounds, which may be important for their applications both as potential diet supplements and to support the healing process.

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37. Comparison of selected parameters of oxidative stress in elderly patients with type 2 diabetes supplemented with prolonged-release melatonin.

D. Kupczyk, J. Rybka, K. Kędziora-Kornatowska, J. Motyl, J. Czuczejko, K. Szewczyk-Golec, M. Kozakiewicz, H. Pawluk, J. Kędziora

Nicolaus Copernicus University, Collegium Medicum in Bydgoszcz

An elevated oxidative stress and decreased antioxidant defense in type II diabetes mellitus have been reported. We aimed to study effect of prolonged-release melatonin (Circadin®) on parameters of oxidative stress in elderly patients with type II diabetes mellitus (DM).

The concentration of whole blood glutathione (GSH); Cu-Zn superoxide dismutase (SOD-1), catalase (CAT), glutathione peroxidase (GPx-1), glutathione reductase (GR) activities and level of malondialdehyde (MDA) in erythrocytes, as well as serum morning melatonin concentration, in 11 elderly patients (mean age 82) with type II DM at baseline and after the 30 days of melatonin supplementation (2 mg daily), were determined. The values of parameters were expressed as mean ± S.D. The effect of the treatment was assessed with t-test for dependent samples and the Wilcoxon signed-rank test for normally and non-normally distributes variables, respectively. P-values of 0.05 and less were considered to indicate statistical significance in all analyses performed.

After the treatment significant increase in activities of SOD-1 (2169.0 ± 161.48 vs. 2407.5 ± 129.26 U/g Hb, $p < 0.01$); CAT (20.5 ± 2.04 vs. 21.9 ± 2.01 BU/g Hb, $p = 0.01$); GPx-1 (12.9 ± 2.83 vs. 14.01 ± 3.47 U/g Hb, $p < 0.05$) and GR (61.9 ± 14.39 vs. 75.7 ± 13.57 U/g Hb, $p < 0.01$) have been observed. Melatonin administration in type II DM patients resulted in a significant decrease in the MDA level (0.223 ± 0.0337 vs. 0.195 ± 0.0142 μmol/g Hb, $p = 0.01$). No significant alterations in GSH concentration (2.73 ± 0.400 vs. 2.85 ± 0.477 mmol/L, $p = 0.27$ and morning melatonin level (7.3 ± 3.24 vs. 15.25 ± 9.88 pg/ML, $p = 0.07$), have been seen.

Altogether these results show enhanced antioxidant defense system, expressed as increased antioxidant enzyme activities, after melatonin supplementation in type 2 DM elderly patients. The results also suggest decreased level of systemic oxidative stress indicated by reduced lipid peroxidation after melatonin treatment.

38. Influence of ion channel inhibitors on circumnutations of *Helianthus annuus* stem

A. Kurenda

Maria Curie-Skłodowska University, Lublin

As early as in 19th century, biologists were aware of existence of autonomous movements of plant organs. These circle-, ellipse- or pendulum-like movements were called circumnutations by Darwin (Darwin and Darwin 1880). In spite of the long history of researches of circumnutations, the mechanism and source of these movements are still unknown. The existing theories postulate a gravitropic, growth or turgor mechanism of these phenomena and the circadian clock is indicated to be the reason for the cyclic changes in the position of organs. The experiment shown below is a complex approach to the investigation of the circumnutation mechanism, engaging techniques in the field of plant electrophysiology and time-lapse recording. Ion channel inhibitors e.g.: gadolinium chloride, anthracene-9-carboxylic acid, tetraethylammonium or dicyclohexylcarbodiimide were injected into the stems of 3-week old plants of *Helianthus annuus*. Plant reactions were determined on the basis of changes in the amplitude and period of circumnutations. The results of the experiment revealed a temporal cessation of circumnutations for about 8 hours. Inhibition of other types of ion channels either caused a decrease in the amplitude and an increase in the period of nutations or no changes were recorded.

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39. The effect of cadmium and lead on the membrane potential and photoelectric reaction of *Nitellopsis obtusa* cells

R. Kurtyka, Z. Burdach, W. Karcz

University of Silesia, Katowice

The effects of Cd and Pb ions on membrane potential (E_m) and photoelectric reaction of internodal cells of *Nitellopsis obtusa* were investigated. These metals are common pollutants which cause a number of toxic symptoms in plants. The standard electrophysiological technique was used for membrane potential measurements. It was found that Cd and Pb at 10^{-3} M caused a depolarization of the E_m, whereas both metals at lower concentrations changed the E_m in a different way. Pb at 10^{-4} M and 10^{-5} M hiperpolarized the E_m, whereas Cd at same concentrations depolarized and did not change the E_m, respectively. It was also showed that these toxic metals changed the light-induced electric reaction (photoelectric reaction) of *Nitellopsis obtusa* cells. In the presence of 10^{-5} M Pb the light-induced hyperpolarization of the E_m, was higher as compared to the control (medium without metal), whereas at 10^{-3} M Pb it was 2-fold lower. Pb at 10^{-4} M did not change the light-induced membrane hyperpolarization. Similar to the 10^{-4} M Pb, Cd at lower concentrations (10^{-5} M and 5×10^{-5} M), also did not change the light-induced membrane hyperpolarization. However, in the presence of Cd at 10^{-4} M and 10^{-3} M this hyperpolarization was 2-fold lower or was completely abolished, respectively.

These results suggest that a depolarization of the membrane potential of *Nitellopsis obtusa* cells incubated at high Cd and Pb concentrations is probably due to inhibition of the PM H^+-ATPase activity, whereas both metals at lower concentrations differed in the mechanism of induction of membrane potential changes. The possible ionic mechanisms of light-induced membrane potential changes of *Nitellopsis* cells treated with Cd and Pb are discussed.

40. Mechanism of acrolein toxicity in the yeast *Saccharomyces cerevisiae*

M. Kwolek-Mirek, R. Zadrąg-Tęcza, T. Biliński, G. Bartosz

University of Rzeszów

Acrolein is an ubiquitous environmental pollutant, which is formed during the incomplete combustion of petrol, coal, wood, plastic materials, and overheating frying oils. This aldehyde is a by-product in chemical industry and an important constituent of tobacco smoke. The most important endogenous source of aldehydes in cells of higher eukaryotes is the lipid peroxidation; however, aldehydes are also formed during metabolic processes including ethanol oxidation. The toxicity of acrolein involves inhibition of enzyme activities, DNA damage, disturbance of membrane fluidity or interaction with high- and low-density lipoproteins. It was found that during reaction with proteins acrolein shows the highest reactivity for the sulfhydryl group of cysteine, imidazole group of histidine, and amino group of lysine [1, 2, 3].

The yeast *Saccharomyces cerevisiae* are an useful simple eukaryotic model organism to study the biochemistry of cellular action of this aldehyde. Our results show that a yeast mutant lacking Cu,Zn-superoxide dismutase (Δ*sod1*) is hypersensitive to acrolein. Yeast was treated with allyl alcohol, which is oxidized intracellularly to acrolein by alcohol dehydrogenase (ADH). One-hour incubation with 0.4 mM allyl alcohol significantly increased superoxide and peroxide generation, the content of protein carbonyls group and

of lipid peroxidation products and the number of the TUNEL positive cells, decreased the level of reduced glutathione (GSH), and cell metabolic activity, increased redox potential and induced morphological changes in the cells including disintegration of mitochondria.

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41. Yeast *Saccharomyces cerevisiae* as a model to study acrylamide toxicity

M. Kwolek-Mirek, R. Zadrąg-Tęcza, S. Bednarska, G. Bartosz

University of Rzeszów

Acrylamide is a synthetic chemical used to form polyacrylamide and as a component of cosmetics, textiles, paper and many other products. Since acrylamide was found a common component of many heat-processed food products, it has become a subject of interest of many fields in biochemistry, toxicology and public health due to its toxicity and potential carcinogen activity for animals and humans. In the cells acrylamide is metabolized by conjugation with glutathione (GSH) either nonenzymatically or by glutathione-*S*-transferases and by the epoxidation reaction mediated by cytochrome P450 to form glycidamide, a reactive substance which may bind to DNA [1]. It was reported that the toxicity of acrylamide involves induction of cellular oxidative stress by depleting GSH, generation of reactive oxygen species and DNA damage [2]. The cellular effects of treatment with acrylamide have been assessed mainly in rodents or human cell lines but have never been established in microorganisms. We propose that yeast *Saccharomyces cerevisiae* may serve as a simple and useful model to study the mechanisms of acrylamide toxicity. Our study show that yeast deficient in Cu, Zn-superoxide dismutase (Sod1p), enzyme removing superoxide anion, is hypersensitive to acrylamide and may be used to elucidate further the cellular effects of action of this compound. We found that exposure of the yeast cell devoid of Sod1p to acrylamide in the concentration range between 10 and 40 mM induces a significant increase of superoxide generation, inhibition of growth which may be overcome by addition of low molecular antioxidants like ascorbate, cysteine or *N*-acetylocysteine and decrease in the glutathione level. No effect was observed in this concentration range for the wild type strain. Our findings demonstrate that the mechanism of acrylamide toxicity to the yeast cell involves oxidative stress.

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42. Mitochondria as target of toxic liver damage and pharmacological treatment

E. A. Lapshina[1], V. T. Cheshchevik[1], I. K. Dremza[1], S. V. Zabrodskaya[1], I. B. Zavodnik[1,2]

[1]National Academy of Sciences of Belarus, Grodno, Belarus;
[2]Yanka Kupala Grodno State University, Grodno, Belarus

Mitochondrial dysfunction is the first sign of toxic liver damage. We compared the changes in the functional activity of rat liver mitochondria under oxidative damages *in vitro* (HOCl treatment) and *in vivo* under toxic exposure of rats to tetrachloromethane (CCl_4) and acetaminophen (APAP). In our experiments, the exposure of isolated mitochondria to HOCl (50–300 μM), known cytotoxicity mediator, impaired mitochondrial functional activity, inhibiting respiration and decreasing the respiratory and acceptor control ratios, which may be a consequence of a damage of the mitochondrial membrane (and, consequently protone gradient dissipation) and the respiratory chain components. At the same time under toxic CCl_4 exposure (4 g/kg, intragastrically) of rats *in vivo*, the phosphorylation coefficient ADP/O declined, whereas under oxidative exposure to HOCl *in vitro*, it remained unchanged along with the drastically decreased substrate oxidation rate and respiratory control ratio during oxidative exposures of both types. The effects of the agents on the phosphorylating mitochondrial function are qualitatively different. HOCl significantly inhibited a key enzyme in the Krebs cycle, 2-oxoglutarate dehydrogenase. The mitochondrial alterations after 24 h of acute CCl_4-induced intoxication were associated with oxidation of intramitochondrial GSH by 25% ($p < 0.05$), oxidative damage of succinate dehydrogenase and the rise of blood plasma nitric oxide level by 45% ($p < 0.05$). Despite of significant mitochondrial GSH depletion during rat intoxication with APAP (500 –1500 mg/kg body weight, intragastrically) we did not observe any inactivation of the mitochondrial enzymes: succinate dehydrogenase, 2-oxoglutarate dehydrogenase, glutathione peroxidase, and also any decrease in the respiratory activity of liver mitochondria isolated from APAP-intoxicated rats. Taking into consideration the important role of mitochondrial damage in development of toxic liver injury and the mitochondrial ability to accumulate melatonin specifically, we considered the possibility of correction of disturbed mitochondrial functional activity by melatonin. Melatonin administration under CCl_4-induced intoxication (three times at doses of 10 mg/kg) increased the rate of succinate oxidation in mitochondrial state 3 by 30% ($p < 0.05$) and reversed the increase in

glutathione peroxidase activity. Melatonin prevented structural liver damage and an elevation of nitric oxide level in the blood plasma of intoxicated animals but it did not protect mitochondrial functions under acute CCl_4 intoxication and restored the level of protein-glutathione mixed disulfides under APAP-intoxication.

43. Influence of passage number effect on cellular response to damage induction by proton irradiation

E. Lipiec, A. Wiecheć, J. Dulińska-Litewka, J. Lekki, J. Wiltowska-Zuber, W. M. Kwiatek

[1]Polish Academy of Sciences, Kraków;
[2]Jagiellonian University, Kraków

Cancer cell lines are commonly used in biological research as relatively simple experimental models. However, growing number of publications demonstrate that cell culture may cause changes in cell lines properties [1–3].

The aim of this study was to find out whether the passage number may influence the response to proton damage induction in PC-3 cells. Human prostate adenocarcinoma PC-3 line derived from bone metastases was examined against non-tumorigenic prostate epithelial cells PZ-HPV-7 as a reference. Cells were kept in laboratory conditions for 2–50 weeks. 2 MeV horizontal focused proton microbeam from the Van de Graaff accelerator, ca. 16 μm in diameter at the irradiated spot, was used for irradiation. All single cells were treated with a number of counted H^+ ions (50 –8000), corresponding to doses of 1.3–209 Gy/cell. Then, necrotic and apoptotic cells were visualized under a fluorescence microscope after staining with Propidium Iodide and Hoechst 33342, respectively. For comparison, necrosis was also induced by UVA and UVC radiation, and apoptosis by incubation with 0.65–10 μM staurosporine. The LDH (lactate dehydrogenase) test was used to evaluate and compare cytotoxicity of the cell-damaging agents.

The results confirm that the laboratory conditions influenced cellular response of PC-3 cells. The „older" PC-3 cells (after about 50th passage) are much more sensitive to proton irradiation than „younger" ones (few passages only). Moreover, in PC-3 cells, cytotoxicity of 2 MeV protons was higher than UVA and UVB radiation and staurosporine at applied concentrations.

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44. The effects of homocysteine and homocysteine thiolactone on the haemostatic activity of fibrinogen and plasminogen

J. Malinowska, J. Kołodziejczyk, B. Olas

University of Łódź

Cardiovascular diseases are the most common cause of death and disability in developed countries. Numerous epidemiologic studies indicate that non-protein amino acid homocysteine (Hcys) may be an independent risk factor, involved in the pathology of these disorders. Homocysteinylation of protein lysine residues results in the incorporation of additional groups, leading to various effects on the biological properties of proteins. Therefore, the aim of our study was to examine *in vitro* the effect of homocysteine in the reduced form and its thiolactone on the haemostatic activity of fibrinogen and plasminogen.

Fibrinogen was isolated by the cold ethanol precipitation technique (according to the Doolittle method). For the isolation of plasminogen, the affinity chromatography technique was used. Fibrinogen (2 mg/ml) and plasminogen (5 μg/ml) were incubated with homocysteine (10 μM; 100 μM) and thiolactone homocysteine (0.1 μM; 1 μM) at 37°C for 30 minutes. Thrombin-catalyzed polymerization was monitored for 15 minutes as the change in turbidity at 595 nm. The efficiency of plasminogen binding was assessed by ELISA test.

Polymerization of human fibrinogen treated with Hcys or HTL was significantly accelerated in comparison to control fibrinogen. Both homocysteine and its thiolactone diminished the plasminogen binding to fibrinogen.

Both Hcys and HTL disturb the function of haemostatic proteins: plasminogen and fibrinogen. The elevated concentrations of Hcys and HTL may promote the prothrombotic state by the acceleration of fibrinogen clotting as well as by the impairment of plasminogen binding to fibrinogen.

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45. Melatonin supplementation and its role in antioxidant status of patients with open angle glaucoma

K. Malinowska[1], A. Kowalska-Bielecka[1], M. Kowalski[1], J. P. Szaflik[2], J. Szaflik[2], J. Kędziora[3], I. Majsterek[1]

[1]Medical University of Łódź;
[2]Medical University of Warsaw;
[3]Nicolaus Copernicus University, Collegium Medicum in Bydgoszcz

Glaucoma is one of the most important civilization diseases and leads to irreversible blindness. In spite of many years of research, the causes of this disorder remain unclear. This disease is extremely difficult to diagnose because its primary phase is asymptomatic. The progressive loss of trabecular cells in patients with glaucoma may be connected with a long-term action of oxidative stress caused by free radicals. One hundred subjects (20 men and 20 women of mean age 64 ± 11.48) including patients with primary open-angle glaucoma (POAG) and healthy controls were enrolled to our study. The main aim of the work was to evaluate the antioxidant status of patients with open-angle glaucoma and the role of their diet supplementation with melatonin. Peripheral blood samples of POAG patients and controls were used to determine catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase activity as well as the total antioxidant status (TAS) before and after melatonin supplementation. The data obtained indicated a significant drop of antioxidant activity in POAG with respect to healthy controls. Interestingly, melatonin supplementation recovered CAT, SOD and GPX activity in patients suggesting its important role in the antioxidant status of open-angle glaucoma. Therefore, we suggest that melatonin supplementation may increase the antioxidant capacity of POAG patients what in turn may improve the treatment of primary open-angle glaucoma.

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46. Fluidity of liposome membranes doped with lipopolysaccharide (LPS 144): An ESR study

A. Man[1], D. Man[2], M. Podolak[2], R. Słota[2], C. Ługowski[3]

[1]Wrocław University of Technology;
[2]Opole University;
[3]Polish Academy of Sciences, Wrocław

In this work, changes in liposome membrane fluidity induced by admixtures of lipopolysaccharide (LPS144) extracted from *Plesiomonas shigelloides* by means of a phenol-water mixture were investigated. The obtained fractions (phenol and water) were added to liposomes produced by sonication of egg lecithin (EYL). Concentrations of the studied compounds relative to EYL were in the range of 0–4%. Electron paramagnetic resonance (EPR) technique involving two spin labels of different location of the nitroxyl group within the bilayer was applied in the study. The TEMPO probe is soluble both in the hydrophobic part of the membrane and in the water medium, whereas the 16-doxylstearic acid locates itself in the center of the lipid bilayer. From the ESR spectra of the TEMPO probe the spectroscopic partition parameter (F) was determined. From the spectrum of the 16-doxylstearic acid spin label the spectroscopic parameter , rotation correlation time, (τ) was calculated. The values of these parameters provide information on the degree of membrane fluidization and on membrane dynamics in different regions. From the analysis of the results obtained, the following conclusions may be drawn: $LPS_{H2O}144$ obtained from the water fraction and $LPS_{phenol}144$ from the phenol fraction alter the membrane fluidity to a different degree. The dynamics of this process in the surface layer differed from that in the central part of the membrane. The F parameter indicated a slight stiffening of the interface layer under influence of the both LPSs studied. The membrane structure was somewhat stronger stiffened by the polysaccharide obtained from the water fraction (LPS_{H2O}). The τ parameter indicated a fluidization of the central part of the lipid bilayer due to the influence of LPS_{phenol}. LPS obtained in the phenol fraction, LPS_{phenyl} had a stronger effect on the bilayer center. The study demonstrates also that liposomes modified by the LPS do not undergo destruction in the course of time, for at least 200 h. After this point, investigations were not carried out.

47. Effect of selected phthalocyanines upon the dynamic properties of liposome membranes

D. Man[1], G. Engel[1], R. Słota[1], A. Man[2], A. Michalska[3], Ł. Mach[3]

[1]Opole University;
[2]Wrocław University of Technology;
[3]T. Koszarowski Oncology Center in Opole

In this work, the effect of selected phthalocyanines on the dynamic properties of liposome membranes obtained by sonication of egg yolk lecithin (EYL) was investigated. Concentration of the phthalocyanine admixtures relative to EYL were in the range of 0–7%. Electron paramagnetic resonance (EPR) technique involving two spin labels of different location of the nitroxyl group within the bilayer was applied in the study. The TEMPO probe is soluble both in the hydrophobic part of the membrane and in the water medium, whereas the 16-DOXYL-stearic acid locates itself in the center of the lipid bilayer. From the ESR spectra of

the TEMPO probe the spectroscopic partition parameter (F) was determined. From the spectrum of the 16-DOXYL-stearic acid spin label the spectroscopic parameter , rotation correlation time, (τ) was calculated. The values of these parameters provide information on the degree of membrane fluidization and on membrane dynamics in different regions. From the analysis of the results obtained, the following conclusions may be drawn: the phthalocyanines studied fluidized the liposome membranes in a different way. The dynamics of this process in the surface layer differed from that in the central part of the membrane. Changes of the F parameter shown by the TEMPO probe indicated a slight stiffening of the interface layer, proportional to the concentration of $NdPc_2$ and ZnPc, whereas in the case of H_2Pc for a concentration of ca. 3% the maximum stiffness was observed. In the case of the 16 DOXYL spin probe, changes of the τ parameter indicated a progressive increase in stiffness of the membrane center with the rise in $NdPc_2$ concentration and increase in membranes fluidity in proportion to the concentration of ZnPc.

For the H_2Pc admixture, similarly like in the case of the TEMPO spin label, a maximum in membrane stiffness was observed at concentration of ca. 3%. Above this concentration the fluidity of the membrane was increasing, however to a much smaller degree than for ZnPc.

48. Molecular modeling of dye-labeled DNA oligonucleotides

M. Markiewicz, A. Górka, P. Bonarek, A. Górecki

Jagiellonian University, Kraków

Fluorescent dyes are commonly used to stain molecules for imaging and detection applications. Many of dyes bind to DNA and act as labels in various fluorescence assays. Therefore, understanding DNA-dye intramolecular interactions is essential for interpretation of experiments where dynamics of dyes or distance between two of them are monitored. Dyes linked to DNA could freely rotate, interact with minor or major groves and could also be stacked on the top of DNA.

Models of two commonly used fluorescence dyes, carboxy-x-rhodamine and carboxyfluorescein, were constructed and parameterized in AMBER force-field. Models of dyes were linked to the 5' and 3' ends of the 20 base pairs long DNA. The model of DNA with labels was optimized in vacuum, solvated and re-optimized. The location and dynamics of dyes were studied using molecular dynamics simulations. The distance between the labels and other structural parameters were analyzed and compared with the results of fluorescence resonance energy transfer measurements. The results of molecular dynamics simulations are in agreement with those from the experiment.

49. Prooxidant activity of doxorubicin and paclitaxel in tumor cells

K. Matczak, A. Pieniążek, A. Koceva-Chyła

University of Łódź

Doxorubicin (DOX) is an anthracycline antibiotic, which has been in clinical use for more than 4 decades. A key factor of its cytotoxicity is the free radical formation due to metabolic activation and deleterious actions of free radicals on cell membrane and DNA. Paclitaxel (PTX) is a clinically effective antineoplastic agent, which mechanisms of cytotoxicity are still under extensive study. PTX direct interaction with microtubules and induction of apoptosis are commonly accepted but possible involvement of ROS in drug activity has also been considered.

Using fluorescent probes dihydroethidine and 2',7'-dichlorofluorescin diacetate (DCFH-DA) we measured the kinetics of formation of superoxide and H_2O_2, reactive oxygen species generated by DOX, PTX and their combination in MCF-7 breast cancer cells. The cells were treated with IC_{50} concentration of drugs for 2 hours, then washed out with PBS and further incubated with fresh medium for a period of 0–180 min. The amount of ROS was measured using a fluorescence microplate reader.

We have found that DOX generated higher level of superoxide than PTX. In cells treated simultaneously with both drugs, we observed the same level of ROS as in the cells treated with DOX. Appreciable level of deacetylation and oxidation of DCFH-DA to fluorescent DCF was found in cells treated with DOX, PTX and their combination. Surprisingly PTX produced considerably higher amount of ROS than DOX and combination of both drugs. In all cases a progressive increase in ROS production in time was found.

We can conclude that both drugs cause oxidative stress in breast cancer cells, and that doxorubicin generates mainly superoxide while paclitaxel enhances mainly H_2O_2 production.

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50. Oxidative stress induced by doxorubicin and paclitaxel in breast cancer cells

K. Matczak, A. Pieniążek, A. Koceva-Chyła

University of Łódź

Doxorubicin (DOX) is an effective anthracycline antibiotic against a wide spectrum of tumors. It mainly interacts with DNA, but can also generate reactive oxygen species that damage cell components. A taxane paclitaxel (PTX) is an inhibitor of microtubule depolimerization. Since in clinical practice both drugs

(DOX and PTX) are predominantly used in combination, it is important to investigate the effect of PTX on oxidative stress induced by DOX.

We measured total antioxidant capacity of MCF-7 breast cancer cells treated with DOX, PTX and their combination and the amount of hydroperoxides as a marker of lipid peroxidation. The cells was treated with IC_{50} concentrations of DOX or PTX and then incubated with fresh medium for 3, 12, 24, 48 and 72 h. Hydroperoxides were measured by the xylenol orange method and total antioxidant capacity was estimated with the FRAP method.

Our results demonstrated significant and progressive elevation in the hydroperoxide level during the first 24 h after the treatment, considerably greater in cells treated with DOX or with combination of both drugs. Maximal increase was observed at 24 h, which was followed by a steady-state decrease during the next 48 h. The highest level of hydroperoxides was found in cells incubated with DOX or with combination of both drugs. At the same time (24 h after the treatment) maximal decrease in total antioxidant capacity of cells was evident in the case of DOX and combination of DOX + PTX. During the next 48 h restoration of total antioxidant capacity occurred.

On the basis of these results we can conclude that both investigated drugs induce oxidative stress, which is caused rather by DOX than PTX activity.

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51. Spectroscopic studies of interaction of 1,3,4-thiadiazoles with lipid membranes

A. Matwijczuk[1], A. Niewiadomy[1], G. P. Karwasz[2], M. Gagoś[1]

[1]University of Life Sciences in Lublin;
[2]Nicolaus Copernicus University, Toruń

Spectroscopic studies of the new biologically active compound 2-(4-fluorophenylamine)-5-(2,4-dihydroxybenzene)-1,3,4-thiadiazol (FABT) derived from the 1,3,4-thiadiazol group have been conducted. These compounds are characterized by antimycosal, antibacterial, cytostatic, neuroprotective, antiproliferative and antitumor activity. Various compounds of this group acting on kidney and large intestine tumors are the object of our interest and their research poses an important challenge for modern science. A rise of keto forms of the compound in alkane solvents was observed on the basis of studies conducted with the application of UV-Vis spectroscopy and FTIR. The domination of the enol form over the keto form was confirmed with a distinct broader absorbance area at 340nm in polar solvents such as water or ethanol, whereas in non-polar solvents such as n-heptane or higher alkanes absorbance vanishes at 340 nm with a concurrent increase of the absorption band in the area related to the keto form (around 273 nm). The studies conducted showed also that tautomeric transformation in FABT is not associated with the permanent dipole moments of the solvents but rather with their dipole polarizability. In this way the solvent mediates the keto-enol equilibrium and thus intermolecular proton transfer. Studies were also conducted on the effects of FABT incorporation into the monolayer, which showed that the compound may also create keto forms in a lipid environment, especially in the hydrophobic milieu. This effect may impact the process of very efficient incorporation of FABT into the lipid membrane. The recognition of the molecular organization of the FABT in lipid membranes may also be of importance for its pharmaceutical applications.

52. Sensitization of glioma cells to etoposide and ionizing radiation by inhibitor of DNA-dependent protein kinase – NU7441

A. Merecz, E. Pastwa

Medical University of Łódź

DNA double strand breaks (DSBs) are considered the most lethal form of DNA damage for eukaryotic cells. A major pathway for the repair of DSBs is nonhomologous end joining (NHEJ), which requires DNA-dependent protein kinase (DNA-PK) activity. NHEJ modifies the broken DNA ends and ligates them together with no regard for homology. Catalytic subunit of DNA-PK (DNA-PKcs) promotes NHEJ and phosphorylates itself and other DNA damage response and repair proteins. We evaluated the role of DNA-PKcs inhibitor, NU7441 in sensitization to etoposide (topoisomerase II poison) and ionizing radiation of glioma cells proficient and deficient in DNA-PKcs (MO59K and MO59J), respectively.

The effect of NU7441 on cellular survival following exposure to etoposide and ionizing radiation was measured in MO59K and MO59J cells by XTT assay. Treatment with 1 μM of NU7441, which was non cytotoxic to both types of cells, significantly enhanced sensitivity of cells to etoposide and ionizing radiation. In MO59K cells, an inhibitor increased 11-fold the cytotoxicity of etoposide, 4-fold the cytotoxicity of ionizing radiation and 20-fold the cytotoxicity of combined treatment. In the case of MO59J cells, NU7441 increased 9-fold the growth inhibition of etoposide, 2-fold the growth inhibition of ionizing radiation and 5-fold the growth inhibition of combined treatment.

We conclude that etoposide, radiation and combination treatment-induced cell killing were greatly enhanced by NU7441 in MO59K and MO59J cells. Moreover, the MO59K cells were more sensitive to NU7441 than MO59J. Our data show that NU7441 inhibitor can sensitize of glioma cells and may improve anticancer chemotherapy and radiotherapy.

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53. Assessment of apoptotic changes in human lymphocytes provoked by selected chlorophenolic compounds

J. Michałowicz, K. Pawlicka, P. Sicińska

University of Łódź

Apoptosis is a mechanism, by which unwanted, defective, or damaged cells are rapidly and selectively eliminated from the body. It occurs during normal physiological processes, e.g. tissue remodeling, embryonic development, and immune response; however it may be enhanced by numerous factors including organic xenobiotics. Chlorophenols are environmental toxins that are introduced into the environment as a result of the activity of chemical industry and also because of usage and degradation of numerous pesticides such as phenoxyherbicides, chlorobenzenes, and chlorinated cyclohexanes. In this work the effect of 2,4,5-trichlorophenol (2,4,5-TCP), pentachlorophenol (PCP), 4,6-dichloroguaiacol (4,6-DCG), tetrachloroguaiacol (TeCG), 4,5-dichlorocatechol (4,5-DCC) and tetrachlorocatechol (TeCC) on apoptosis induction in human peripheral blood lymphocytes was examined. The analysis of the changes in mitochondrial transmembrane potential ($\Delta\Psi m$) was performed using JC-9 fluorescent probe. It was noted that all the compounds studied (excluding 4,6-DCG) in a dose-dependent manner (0.2–25 ppm) increased the number of cells, which were characterized by the $\Delta\Psi m$ reduction, with the strongest effect observed for TeCG and TeCC. Moreover, fluorimetric assay of the caspase-3 activity revealed that 2,4,5-TCP, chlorocatechols and TeCG in the range of the concentrations from 1 to 50 ppm increased the caspase-3 expression, whereas PCP in its highest concentration of 50 ppm decreased the activity of this enzyme. It was also noted that the caspase-3 activity was the most strongly induced by tetrachlorocatechols, particularly by TeCC. The analysis of YO-PRO-1 iodide/propidum iodide staining revealed that TeCC increased the most strongly the number of apoptotic cells at 5 and 25 ppm, whereas at a dose of 100 ppm the strongest effect on apoptosis induction was observed for 2,4,5-TCP.

54. Evaluation of oxidative DNA damage of human lymphocytes induced by chlorophenols and their derivatives

J. Michałowicz, A. Surlit, I. Majsterek

University of Łódź

Phenols are strong environmental toxins, which are found in food, drinking water as well as in the indoor and outdoor air environment. Exposure of a cell to chlorinated compounds usually results in an enhanced DNA damage such as double or/and single strand breaks or DNA base oxidation. Oxidative DNA bases damage is mainly related to the formation of the highly reactive hydroxyl radical (˙OH) that is produced in the Fenton reaction, in which hydrogen peroxide is converted to ˙OH by transition metal ions such as Fe^{2+} or Cu^{+}. In this work we investigated the effect of low concentrations of 0.2 μg/ml, 1 μg/ml and 5 μg/ml of 2,4,5-trichlorophenol (2,4,5-TCP), pentachlorophenol (PCP), 4,6-dichloroguaiacol (4,6-DCG), tetrachloroguaiacol (TeCG), 4,5-dichlorocatechol (4,5-DCC) and tetrachloro-catechol (TeCC) on DNA base oxidation in human peripheral blood lymphocytes. The analysis was performed using alkaline single cell gel electrophoresis (comet assay). Detection of oxidized pyrimidynes and purines we conducted by the use of the repair enzymes such as endonuclease III and form-amidopyrimidine-DNA glycosylase. DNA oxidation was expressed as a percentage of comet tail, which was formed after the xenobiotics treatment. The obtained results showed that all the compounds examined were able to oxidize DNA bases in human lymphocytes. It was also observed that pyrimidine bases were more strongly oxidized in comparison to purine ones. Finally, it was found that chlorinated catechols and TeCC in particular, revealed a higher oxidative potential in comparison to chlorophenols and chloroguaiacols, and a rise in the number of chlorine atoms in the compound from each group examined led to an increase in DNA base damage.

55. Interaction between phosphorus dendrimers and α-synuclein

K. Miłowska, M. Małachowska, S. Sękowski, T. Gabryelak

University of Łódź

α-Synuklein (ASN) is a cytosol proteins found predominantly in the central nervous system, particularly in parts of presynaptic terminals. ASN properties depend on the adopted conformation and degree of aggregation. Under physiological condition, ASN is involved in the regulation of dopaminergic system and several reports suggest its potential role in the regulation of synaptic function and neuronal plasticity.

Physiological functions of this protein are disturbed by its aggregation. In the body, posttranslational modification, oxidative stress, catabolism defects and genetic factors (mutation) can promote ASN aggregation. This process is accompanied by a conformational transition from random coil or α-helical structure to β-sheet structure.

Protein aggregation reduces its bioavailability, which interferes with its physiological function, and aggregates may have toxic effects on neurons. Disorders of the ASN structure play a key role in the pathogenesis of Parkinson's disease, Alzheimer's disease with Lewy bodies, dementia with Lewy bodies, multiple system atrophy and other neurodegenerative diseases collectively called synucleinopathies.

The interaction between phosphorus dendrimers (generations G3 and G4) and ASN has been studied by fluorescence spectroscopy. The decrease in the fluorescence intensity was the most marked change in the fluorescence intensity observed upon addition of dendrimers. For both generation of dendrimers, their increasing concentrations caused a linear reduction in the fluorescence of tyrosine residues. Conformational changes in ASN, which results in β structures formation were investigated by CD spectroscopy in the absence or presence of phosphorus dendrimers. After 48 h of incubation ASN the changes in CD spectrum to β structures was observed, but in the presence of dendrimers (2 μM), this process has been inhibited.

56. Finding the relationship between micellization parameters and microscopic molecular properties using QSPR-like methods

P. Misiak, B. Różycka-Roszak

Wrocław University of Environmental and Life Sciences

Micellization is one of the most frequently studied process of self-association of surfactant molecules or ions in aqueous solution. In general, the process is governed by two opposing forces, related to the hydrophobic tail and polar head group of the molecules or ions. Calorimetric experiments allow to determine two macroscopic quantities characterizing micellization process i.e. the critical micelle concentration (CMC) and the enthalpy of micelle formation (ΔH_{mic}). On the other hand, the contemporary theoretical tools enable to calculate many quantitative characteristics of the molecules or ions on the microscopic level, both static and dynamic, using classical and quantum modeling.

The aim of the present work is to relate the experimentally measured micellization characteristics (CMC and ΔH_{mic}) of the selected cationic surfactants (quaternary ammonium salts) to the theoretically obtained descriptors connected with both molecular properties and behavior in aqueous solvent. Mainly static and dynamic (obtained from molecular dynamics simulations) 3D descriptors are taken into account. The statistical methods used in the quantitative structure-property relationship (QSPR) approach are applied to reveal the most important microscopic factors influencing the micellization processes in surfactant solutions.

57. Influence of the lead compounds o the long range correlations in the current of ion channels

J. Miśkiewicz, Z. Trela, S. Przestalski

Wrocław University of Environmental and Life Sciences

In this study, the influence of the organolead compounds i.e. the trimethyllead chloride (Met_3PbCl) on the long range correlations in the current of ion channels of the SV cation channels of vacuolar membrane of *Beta vulgaris* was investigated. The long range correlation of ion channels exhibits interesting properties such as fat tails of the opening time distribution or power laws of correlations of the opening time. In the investigations the detrended fluctuation analysis and random matrix method is applied. The results are compared with numerical simulations of an ion channel model.

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58. Electrical and thermal properties of lyophilized and irradiated human bone grafts

J. Młynarski[1], J. Dec[2], S. Miga[2], S. Grzegorczyn[1]

[1]Medical University of Silesia, Zabrze
[2]University of Silesia, Zabrze

The determination of some thermal and electrical properties of lyophilized and irradiated human compact bone grafts such as electrical complex permittivity as a function of temperature and electrical field frequency was the aim of this study. The compact bone is the tissue of lamellar structure, composed of collagen fibers connected to hydroxyapatite. Such structure gives the compact bone high strength and elasticity. Compact bone contains up to 20% of water. Part of the water fills canals in the bone and the rest is combined with collagen. The lyophilization process removes the free water from bone canals. Besides, the irradiation can influence the collagen and the hydroxyapatite net. In that case the temperature characteristics of electrical permittivity, measured for different frequencies of electrical field, can give information about the structure of compact bone grafts without free water in bone canals. The complex permittivity was measured by means of impedance meter (Precision LCR Meter Agilent E4980A, Temperature controller LakeShore

340) in the rage of temperature from 200 to 400 K and in the range of frequencies of electrical field from 100 to 106 Hz. It was found that complex part of electrical permittivity has a local maximum at a temperature (about 220 K) dependent on the frequency of used electrical field. Besides, at temperatures lower than room temperature the complex permittivity did not demonstrate hysteresis for measurement with cooling and subsequent heating of the bone graft sample. The hysteresis of complex permittivity during heating and subsequent cooling of bone samples at temperatures higher than 310K was observed. The areas of hysteresis in the determined range of temperatures were dependent on frequencies of electrical field and for higher frequencies the area of hysteresis was lower. The values of real and complex permittivity observed at room temperatures for greater frequencies were lower.

59. Temperature dependence of the activation energy of viscous flow for hen egg-white lysozyme in aqueous solutions

K. Monkos

Medical University of Silesia, Zabrze

The activation energy of viscous flow ΔE depends on temperature when the Arrhenius plot, i.e. the plot of liquid viscosity η (in logarithmic scale) versus a reciprocal of the absolute temperature (T^{-1}) is non-linear. This is the case for hen egg-white lysozyme in aqueous solutions, when the viscosity is measured in a wide range of temperature: from the neighborhood of the freezing point of a solution up to the vicinity of temperature of denaturation. In this case, the activation energy at the individual temperature can be defined in the following way: $\Delta E = R[d\ln\eta/d(T^{-1})]$, where R is the gas constant. To obtain an analytical dependence of ΔE on T, the functional dependence of viscosity on temperature is needed. Such functional dependences have been taken from three models of viscosity for glass-forming systems: the Avramov's model, the Adam-Gibbs model and the power-law model. Each model gives three parameters for the dependence of viscosity on temperature. These parameters have been obtained from the fit of the temperature dependence of viscosity to the experimental values. For hen egg-white lysozyme in aqueous solutions, the viscosity measurements were made at temperatures ranging from 5°C to 55°C by steps of 5°C and at a wide range of concentrations: from 25 kg/m^3 up to 343 kg/m^3. The obtained results show that – at a given temperature – the activation energy of a solution increases mononously with increasing concentration. In the function which describes this dependence, the activation energy of viscous flow of dissolved proteins is a parameter. It appears that activation energy obtained from the Avramov's model decreases from 1.9×10^7 J/mol (5°C) to 3.62×10^6 J/mol (55°C), that obtained from the Adam-Gibbs model decreases from 1.98×10^7 J/mol (5°C) to 4.86×10^6 J/mol (55°C) and that obtained from the power-law model decreases from 1.92×10^7 J/mol (5°C) to 6.66×10^6 J/mol (55°C). The discussed models give very similar values of the activation energy of viscous flow only up to the temperature of about 35°C.

60. Translational diffusion coefficient for some mammalian serum albumins

K. Monkos

Medical University of Silesia, Zabrze

Translational diffusion coefficient of a protein at infinitely dilute solutions can be calculated from the generalized Stokes-Einstein formula:

$$D_o(T) = kT/6\pi\eta_o(T)R_h,$$

where k is the Boltzmann's constant, $\eta_o(T)$ denotes the viscosity of water and R_h is the hydrodynamic radius of the dissolved protein. R_h depends on the main semi-axes of a protein, modeled as an ellipsoid of revolution, and can be obtained from the Perrin's relation. For hydrated bovine serum albumin (BSA), equine serum albumin (ESA), ovine serum albumin (OSA) and rabbit serum albumin (RSA) R_h is equal to 3.83 nm, 3.74 nm, 3.92 nm and 4 nm, respectively. It gives $D_o(T)$ in the range from 3.5×10^{-11} m^2/s (at 5°C) to 10.2×10^{-11} m^2/s (at 55°C) for BSA, from 3.59×10^{-11} m^2/s (at 5°C) to 10.4×10^{-11} m^2/s (at 45°C) for ESA, from 3.42×10^{-11} m^2/s (at 5°C) to 9.92×10^{-11} m^2/s (at 55°C) for OSA and from 3.36×10^{-11} m^2/s (at 5°C) to 9.74×10^{-11} m^2/s (at 55°C) for RSA. Translational diffusion coefficient of a protein in a solution with the protein's volume fraction Φ at temperature T can be obtained from the relation:

$$D(T,\Phi) = D_o(T)\eta_o(T)/\eta(T,\Phi),$$

where $\eta(T,\Phi)$ is the viscosity of the solution at temperature T. To obtain $D(T,\Phi)$ the viscosity of aqueous solutions of the albumins studied has been measured at temperatures ranging from 5°C to 45^{0}C and at a wide range of concentrations. The measurements were conducted using an Ubbelohde-type capillary microviscometer. The dependence of $D(T,\Phi)$ on Φ in the range from diluted solutions up to concentrated ones is non-linear and (at fixed temperature) can be described by a stretched exponential function:

$$D(T,\Phi) = D_o(T)\exp(-\beta\Phi^{\nu}),$$

where β and ν are scaling parameters. The parameter ν is – for a given albumin – independent of temperature and its mean value is equal to 1.43 for BSA, 1.3 for ESA, 1.26 for OSA and 1.21 for RSA. The parameter

β, in turn, slowly decreases with increasing temperature: from 16.3 (at 5°C) to 14.3 (at 45°C) for BSA, from 11.6 (at 5°C) to 10.9 (at 45°C) for ESA, from 8.91 (at 5°C) to 8.31 (at 45°C) for OSA and from 8.52 (at 5°C) to 7.75 (at 45°C) for RSA.

61. A glass-transition temperature for ovalbumin obtained from viscosity measurements and the Avramov's model

K. Monkos

Medical University of Silesia, Zabrze

All fully hydrated proteins undergo a distinct change in their dynamical properties at temperatures between 180 and 230 K. For temperatures above this range, anharmonic motions of bonded and nonbonded groups of atoms dominate, as in a liquid state. At lower temperatures, harmonic motions predominate, as in a solid state. For a given protein, the temperature at which its properties change from liquid-like to solid-like is called glass-transition temperature T_g. In the present paper T_g for ovalbumin has been obtained from viscosity measurements of aqueous solutions of ovalbumin and from the Avramov's model. According to the model, molecules in a flowing liquid jump from one equilibrium state to the other with different activation energy they have to overcome, and the frequency of those jumps follows a Poisson distribution. The model gives three-parameter dependence of liquid viscosity on temperature, and one of those parameters is T_g. The viscosity of ovalbumin aqueous solutions was measured at temperatures ranging from 5°C to 55°C and in the range of concentrations from 6 kg/m^3 to 430 kg/m^3. The measurements were performed with an Ubbelohde-type capillary microviscometer. Glass-transition temperature of a solution has been then obtained as one of the fitting parameters of the Avramov's relation to the experimental values of viscosity. Such obtained T_g of ovalbumin solutions increases with increasing concentration from about 127 K (c = 6 kg/m^3) to about 180 K (c = 430 kg/m^3).

The glass-transition temperature of a solution depends both on T_g for a dissolved protein $T_{g,p}$ and water $T_{g,w}$. To obtain $T_{g,p}$ for ovalbumin the modified empirical Gordon-Taylor equation was used. It gives the concentration dependence of T_g of a solution, and $T_{g,p}$, $T_{g,w}$ and a parameter describing the strength of the protein-solvent interaction (K) are fitting parameters. The numerical values of the parameters are as follows: $T_{g,p}$ = (220 ± 10) K, $T_{g,w}$ = (125 ± 1) K and K = (1.88 ± 0.39).

62. Molecular interactions between dl-α-tocopherol glycosidic derivative and DPPC in Langmuir monolayers

G. Neunert[1], J. Makowiecki[2], R. Hertmanowski[2], T. Martyński[2], K. Polewski[1]

[1]Poznań University of Life Sciences;
[2]Poznań University of Technology

The present work investigates the molecular behavior of dl-α-tocopheryl β-D-glucopyranoside (BG) at the air-water interface and the interaction between BG and a model lipid membrane (monolayer) composed of dipalmitoyl phosphatidylcholine (DPPC). The behavior of pure BG, DPPC and mixed BG/DPPC Langmuir monolayers was characterized by surface pressure-mean molecular area (π–A) isotherms.

The isotherm of BG monolayer showed surface pressure onset at ca. 60 Å^2/molecule and collapse at 26 Å^2 with surface pressure value of 58 mN/m. Inclination angle of the isotherm changed at mean molecular area of 40 Å^2, what corresponds to a phase transition from the liquid to the liquid condensed state. For the DPPC isotherm, a characteristic for this phospholipid plateau region appeared at about 5 mN/m, what is ascribed to a phase transition between the liquid-expanded (LE) and the liquid-condensed (LC) state. The isotherm collapse point occurred at 70 mN/m.

On the basis of the π–A isotherms registered for binary BG/DPPC monolayers, the plots of the mean area occupied by a single molecule (A_{12}) as a function of the BG molar fraction (X_{BG}) were drawn up. The study of A_{12}–X_{BG} dependences at surface pressures below the plateau region (~5 mN/m) indicates the presence of a negative deviation, corresponding to the existence of attractive interactions between BG and DPPC and only partial miscibility. The positive deviation observed for π above 30 mN/m indicates the repulsive interactions between the two compounds. Only for $X_{BG} < 0.3$ small negative deviations of A_{12} were found. This suggests that BG and DPPC are almost completely miscible in the monolayer at low BG concentration and the surface pressures above the plateau region.

The presented results may be used to describe and explain the phase transition phenomena in biological membranes where the surface pressure is in the range of 30–35 mN/m.

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