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General topics – various

species in RAW are hydrogen peroxide, nitrous acid and complex decaying into peroxynitrite and peroxynitrous acid during not less than 4 days. The reaction with active species in RAW lasts up to 4 days. Active species have both oxidizing and reducing properties, exhibit a strong antimicrobial effect. There is a delay in the action of radiation, since a significant concentration of active species is achieved in water not less than 1 min after switching on the radiation source. RAW characteristics are: pH \sim 3, ORP = 790 mV (SHE), conductivity \sim 1 mS/cm. The specific energy cost of producing RAW is about 10 times less than PAW. *The authors marked with an asterisk equally contributed to the work.

P-41-019

O-GlcNAcylation as marker of antioxidant effects of vitamin E-stabilized ultra high molecular weight polyethylene in human osteoblast

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High Crosslink process was introduced in the development of joint prosthetic devices, in order to decrease the wear rate of ultrahigh molecular weight polyethylene (UHMWPE) but it also triggers the formation of free radicals and oxidative stress (OS), which affects the physiological bone remodeling, leading to osteolysis. Vitamin E stabilization of UHMWPE was proposed to provide oxidation resistance without affecting mechanical properties and fatigue strength. The aim of this study is to evaluate the antioxidant effect of vitamin E added to UHMWPE on OS induced osteolysis, focusing in particular on the evaluation (by western blot analysis) of protein O-GlcNAcylation, OGA and OGT levels considered markers of cellular response to OS. O-GlcNAcylation levels increased in presence of vitamin E blended UHMWPE, in particular with not crosslinked vitamin E stabilized UHMWPE (P < 0.005) while, conversely, they fall in absence of vitamin E. Significant increase (P < 0.01) of OGT protein was found in presence of not crosslinked Vitamin E blended UHMWPE, whereas a significant increase (P < 0.05) of OGA enzyme was observed in Vitamin E absence. The OGT/OGA expression ratio show a behavior consistent with the observed O-GlcNAcylation levels. Our results suggest that the Vitamin E stabilization of UHMWPE: (i) seems to improve the ability of osteoblast to respond to oxidative stress, inducing cellular mechanism of defense, such as dynamic O-GlcNAcylation in order to promote cell survival; (ii) could contribute to reduce oxidation- induced osteolysis and the consequent loosening of the prosthetic device, therefore improving the longevity of total joint replacements.

P-41-020

Inhibition of adenosine deaminase activity by novel synthesized piperazine class compounds

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Adenosine deaminase (ADA) is an important enzyme in purine metabolism. The increase of the ADA activity is documented in such pathologies as diabetes, tuberculosis, cancer, rheumatoid arthritis, etc. The substrate of ADA, the adenosine, suppresses the inflammation and plays the role in the protection against injuries. The increasing of ADA activity in the extracellular medium or pathological effusions results in decrease of adenosine concentration and aggravation of inflammation. Therefore, the inhibition of ADA is considered as beneficial tool for regulation and remission of inflammation. This work describes the in vitro inhibition of purified from bovine lung ADA by novel synthesized tertiary amino alcohols substituted by piperazine ring. The screening of 15 compounds was carried out. Among them, the compounds, containing in the piperazine ring the phenyl and heliotropine substitutes were more effective. The IC₅₀ values for 6 potent compounds in inhibition of ADA were between 3.5-15.5 μg/mL. The average value of IC₅₀, for the compounds, in their turn, containing in the heliotropine ring different substituent groups was of 20 µg/mL. The inhibition of ADA by effective one - GGN 322*HCl the IC₅₀ value was equal to 15.2 μ g/mL. The inhibition was of competitive nature with $Ki = 2.5 \mu M$. For the compounds containing as a substitute phenyl group in the piperazine ring, the average IC50 value was of 10 µg/mL. The most significant results among them was registered for the PO191*2HCl, with benzhydryl (diphenylmethyl) as a substitute group. The IC₅₀ value for it in inhibition of ADA was a rather low 3.5 μ g/mL (6.5 μ M). The Ki for this compound was 1.5 μ M and the nature of inhibition was competitive. The constant of bimolecular interaction of PO191*2HCl with the tryptophan residues in ADA (K_{SV}) was evaluated from the fluorescence quenching, using Stern-Volmer equation as of 0.145 \pm 0.027.

P-41-021

Interaction of PCID2 and NudC proteins of Drosophila melanogaster in vitro

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There are many proteins and protein complexes involved in the mRNA transport from the nucleus to the cytoplasm in Drosophila melanogaster cells. PCID2 protein as one of the participants in this process binds mRNA in the nucleus and enters the cytoplasm, changing partners in the transport complex. In our laboratory we purified the PCID2 complex from cytoplasm and detected its interaction with the NudC (nuclear distribution protein). We investigated interaction between PCID2 and NudC proteins. First of all we generated antibodies to NudC and confirmed PCID2 and NudC proteins interaction by immunoprecipitation. We continued by testing whether PCID2 protein has a separate domain for interaction with NudC protein. We divided PCID2 into the following domains: N-terminal, C-terminal (WDdomain) and the "middle" domain (MID) located between them. Moreover, MID- and WD-domains together constitute a functional PCI-domain. We also divided NudC protein into N- and C-terminal domains in the same way. We expressed the 6xHistagged proteins related to said domains and full-size PCID2 and