VIOLATION OF THE TRANSMEMBRANE ASYMMETRY OF LIPIDES OF HEPATOCYTES AS THE INDEX OF APOPTOSIS IN THE ACTION OF XENOBIOTICS ON THE ORGANISM OF RATS

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The use of constantly growing assortment of cosmetics and detergents, washing powders and modern building materials for finishing apartments in the everyday life of Ukrainians determines the aggressive penetration of surfactants into all spheres of human habitation, and then into the human body [Francesco V., 2015; Shcherban M. G., 2018]. Xenobiotics are in close contact with the human body irrespective of sex, age, profession, state of health, etc. Experts have found that 42% of surfactants enter sewage waters, 22% in atmospheric air, 12% are dumped, 7% pollute settlements, 11% go to household plots, and 6% remain in living quarters [Laurier L.S., 2016]. Concerning the global techno-anthropogenic load of surfactants on environmental objects convincingly testify the official data of the State Statistics Service of Ukraine [Zubkova I. A., Kryvonos K. A., 2013]. It has been established that surfactants modulate radiomimetic effects in biological objects and stimulate the development of free radical pathology, which in turn leads to the development of membrane pathology [Nakonechna O.A., 2012; Marakushin D.I., 2013].

The aim is to estimate the distribution of phosphatidylserine in the phospholipid bilayer of hepatocyte membranes and stages of apoptosis in rats with the influence of surfactants: ethylene glycol (EG), polyethylene glycol 400 (PEG-400) and polypropylene glycol (PPG) at a dose of 1/10 DL50.

Materials and methods. The study was performed on 30 white rats of both sexes of the WAG line lasting 45 days. Animals were in the standard conditions of the vivarium. The content and monitoring of animals was carried out in accordance with the provisions of the "General principles of animal experiments", agreed upon by the First National Congress on Bioethics (Kiev, 2001), "European Convention for the Protection of Vertebrates used for experimental and scientific purposes" (Strasbourg, 1986).

The experiment was carried out in four groups of animals: a control group and three groups of experimental animals in a quantity of 10 animals each. Aqueous solutions of surfactants (ethylene glycol, polyethylene glycol and polypropylene glycol) were daily injected intragastrically at a dose of 1/10 DL50 using a metal gavage. After the end of the 45-day subacute toxicological experiment, rats were with drawn from it in accordance with the "International recommendations for conducting biomedical studies using laboratory animals" by decapitation. Liver perfusion and isolation of hepatocytes were performed according to A.Yu. Petrenko. The degree of lipid membrane asymmetry and evaluation of the apoptosis / necrosis stages were performed by flow cytometry on a FACS Calibur flow cytometer from Bector Dickinson (BD) (USA) using the AnnexinV-FITC detection KIT of BD Pharmingen (Becton, Dickinson and Company) in accordance with the AnnexinV-FITC staining protocol and 7-amino-actinomycin D (7 AAD) (BD Pharmingen). The study was conducted in the department of cryocytology of the Institute of Problems of Cryobiology and Cryomedicine of NAS of Ukraine.

Results. It was found that the investigated xenobiotics at a dose of 1/10 DL50 led to imbalance in the asymmetry of the distribution of phospholipids in the plasma membrane of hepatocytes, phosphatidylserine translocation from the inner layer into the outer layer. The most pronounced structural changes in the phospholipid bilayer of membranes were observed as a result of the action of polyethylene glycol. Phosphatidylserine is expressed in the outer layer in 24.87 $\pm$ 3.07% of hepatocytes (p0.05 in comparison with the control).

Almost the identical percentage of hepatocytes with phosphatidylserine in the outer membrane was observed for rats administered ethylene glycol and polypropylene glycol (15.21 ± 2.15% and 14.54 ± 2.93% respectively). In this study, we showed that after toxification of white rats with xenobiotics at a dose of 1/10 DL50, the rate of early apoptosis was higher compared with control animals.

Conclusion. It was found that in subacute toxicological experiment on rats the investigated xenobiotics caused a change in the plasma membrane of hepatocytes: phosphatidylserine translocation from the inner layer into the outer layer.

THE DYNAMICS OF PLASMA AMINO ACIDS CONTENT

IN THE SUBACUTE EXPERIMENT UNDER

ETHOXYALKYL PHENOLS ACTION

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The researches was devoted to the studding of the biochemical mechanisms of protein metabolism violations under sub-acute effects of detergents on rats.

The group of surfactants "neonols" of brands APh9-6, APh9-10 and APh9-12 was chosen for the research. The term of subacute experience was 45 days. The research program included a sub-acute toxicological experiment on mature white rat of WAG population, administered intragastrically of 1/10, 1/100, 1/1000 LD50 every morning before feeding. Average lethal doses (LD50) were determined at 4.2, 4.3, and 3.4(in g / kg body weight) levels respectively.

The obtained data proved that all types of neonols have a mediated action on protein metabolism dysfunction. The degree of influence depends on the dose of the substances. The most significant changes were observed in animals that were intoxicated by Neonol APh9-1/10 LD50. Dynamic changes of plasma amino acids pool are characterized by decreasing of Cys, Cystathionine, Gly, Thr, Ser, Ala, Met, Ile, Val, Tyr, Phe, Lys, Trp and Leu against increase of Asp, Asn, Glu, Gln, Arg, Pro, His and Hyp. The enhance of plasma free amino acids may indicate the prevalence of catabolic processes over anabolic ones, while reducing of their concentration may indicate the increasing in proteins synthesis. The results have showed that the neonol APh9- doses 1/10 and 1/100 LD50 reduced a content of glycine by 54% and 32%; cysteine by 47.3% and 21%, cystathionine by 47.3% and 27.4%, threonine by 45.7% and 24.2%, serine by 36.7% and 15%, alanine by 41.13% and 19.2% in serum respectively. All of these amino acids are capable to