Water and effect of antioxidants in ultra-low doses on biological membranes.

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Solutions of many biologically active low concentration compounds (antioxidants, phorbol esters, peptides) are known to exhibit bioeffects correlating with the influence of the solutions of the same compounds in their usual concentrations (Burlakova et al., 2003). This phenomenon called the effect of ultra-low doses (ULD) has not received any adequate physicochemical substantiation as yet. There is a suggestion that biological membranes could be the target of this effect. Our studies showed that the introduction of different antioxidants (natural - α-tocopherol and artificial- potassium β- (4-hydroxy-3,5-di-*tert*-butylphenyl)-propionate,potassium phenosan, PP, in model system containing different membrane suspension (plasmatic membranes, microsomes, synaptosomes) was always accompanied by changes in lipid peroxidation process and structural characteristics of lipid bilayer of membranes (microviscosity and order parameter). We have found that there were three areas of concentrations for all of the investigated antioxidants (AO):1) the area of physiological concentrations (10^{-3}-10^{-8}M); 2) the area of AO interaction with cell membranes in ULD (10^{-9}-10^{-15}M); 3) the area of apparent concentrations (10^{-16}-10^{-25} M).

To reveal the mechanism of action of AO, including PP, at ultra- low doses, it is a significant question about the target of this agent, in particular, whether PP acts directly to the lipid bilayer or it affects indirectly via proteins and receptors. Therefore, the aim of this work is to study the effect of PP in a wide concentration range on the structural state and size of liposomes prepared from lipids extracted from plasmatic membranes (PM) of mouse liver cells. Carrying out the same type of experiments with spine-probes (5- and 16-doxyl-stearic acids) localized in different lipid regions of membranes and liposomes have been prepared from the lipid extracts of these membranes we have found that the dose dependences of PP effect on cell membranes and liposomes show the same regularities in both cases; the maxima of microviscosity and order parameter variations in ULD range coincide for both objects. Thus, these results convincingly indicate that precisely the lipids are the targets of the PP effect on biological membranes. The changes in the structural state of liposomal lipids affect their size. We have studied variations in the average hydrodynamic diameter of liposomes exposed to PP in a wide concentration range by dynamic light scattering (DLS) and have found that that PP increases (10^{-4}–10^{-7})M and (10^{-13}–10^{-15} M) or does not change (10^{-9}–10^{-11}) M and (10^{-17}–10^{-20} M) the average diameter of liposomes, and the growth is 12–20% and statistically significant as compared with control for 98% confidence interval. These data were confirmed by experiments with atomic force spectroscopy technique.

It was established by A. Konovalov et al. (2009-2012) that nanoassociates are formed in the low concentration solutions of AO. These nanoassociates contribute to the formation of anomalous physicochemical properties of the low concentration solutions. In our articles certain reliable correlations between the nanoassociate parameters and the structural changes in the biological membranes have been found. If the low concentration solutions are kept in a shielded permalloy container protecting its contents from external electromagnetic field (EMF) neither the nanoassociates nor any anomalous physicochemical properties of the solutions have been observed. The low concentration solutions (10^{-16} - 10^{-7} M) of the (PP) exhibit their ability to appear anomalous physicochemical properties and to influence the microviscosity of the lipid membranes only when the nanoassociates are formed in the solutions. Thus, it has been first demonstrated that nanoassociates are material carriers of the unique ability of the low concentration solutions of biologically active compounds to exhibit bioeffects.