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EFFECT OF CETYLPYRIDINIUM CHLORIDE ON PEROXIDE OXIDATION OF LIPIDS

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Annotation. The effect of cetylpyridinium chloride on lipid peroxidation was investigated in this paper. The intensity of lipid peroxidation was determined by reaction of thiobarbituric acid with malonic dialdehyde in human serum. It has been shown that cetylpyridinium chloride at concentrations of 25 μM-100 μM reduces the amount of lipid peroxidation products by 1.9-2.9 times. Cetylpyridinium chloride has antiseptic properties and is used for the treatment of oral cavity's infections of bacterial and mycological etiology. The results show that we can effectively use this compound in infectious diseases, accompanied by intensification of peroxidation.

Keywords: analysis, cetylpyridinium chloride, lipid peroxidation, surfactants, kinetics, reactive oxygen species.

Introduction. Cetylpyridinium chloride (CPC) is an effective ionogenic surfactant. Surfactants are compounds that reduce surface tension in aqueous solution. Surfactant molecules usually consist of two parts, opposite in nature and features. The hydrophobic part is non-polar and insoluble in water, but easily soluble

in non-polar liquids. The hydrophilic part is polar in nature and easily soluble in water [1, p. 68].

CPC is well soluble in water, has high antibacterial properties and high bactericidal and fungicidal activity, which are effective against simple viruses. But it should be remembered that this group of compounds of Quaternary ammonium salts has a number of disadvantages that complicate their use in pharmacy (chemical incompatibility with different groups of substances, high toxicity and tissue irritation) (fig. 1).

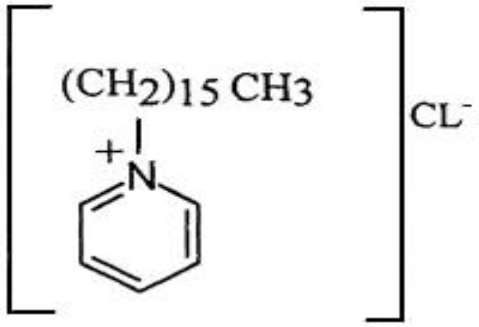


Fig.1. The structural formula of cetylpyridinium chloride

Cation active surfactants have high bactericidal activity, their action is almost independent of microbial inoculation and is manifested in a wide range of pH. It is these properties of cationic surfactants which determine their use as disinfectants and sterilizing agents in clinical surgery. In this group of compounds, cetylpyridinium chloride has found practical application in pharmacy.

The aim of the study is to study the effect of the surfactant cetylperidinium chloride on the kinetics of inhibition of lipid peroxidation.

Materials and methods of research. To study the level of lipid peroxidation the following reagents were used in this research: control serum LIONORM GUM N (Czech Republic, Erba Lachema s.r.o.); hydrogen peroxide solution, 3%; iron (II) sulfate solution; orthophosphoric acid solution, 2%, pH 1.3; thiobarbeturic acid solution (TBA), 0.8%; n-butanol; CPC in powder form (Chengdu Okay Pharmaceutical Co., LTD, China).

The following equipment and auxiliary materials were used within the present experiment: single-channel automatic dispensers 50, 200, 1000 μL; water thermostat;

water bath; laboratory centrifuge; cuvette with an optical layer thickness of 1 cm; Optizen POP UV spectrophotometer (Mesosys, South Korea); isopropyl alcohol; timer.

The study of the level of lipid peroxidation performed in the study was based on the method of determining the number of products that react with TBA [3, p. 11].

Research results and discussion. Lipid peroxidation (LPO) is the oxidative degradation of lipids that occurs under the action of free radicals and is one of the main causes of damage to cell membranes and subsequent cell death due to exposure to reactive oxygen species. This process regulates the lipid composition of biomembranes and membrane-associated enzymes, participates in the synthesis of leukotrienes, prostaglandins, metabolism of catecholamines and steroid hormones, affects the permeability of membranes and the transport of substances through them [5, p. 59].

The main initiator of free radical oxidation is reactive oxygen species (ROS), which can grow under the influence of adverse factors and cause oxidative stress.

Depending on the concentration of ROS in cells, they can be either physiological signals which are necessary for cell life, or toxic substances that damage the structure and function of cells. In particular, ROS cause the oxidation of polyunsaturated fatty acids in lipid membranes, which cause formation of aldehydes, which are considered toxic messengers of oxidative stress, capable of spreading and exacerbating oxidative damage [6, p. 85].

In the cell ROS are formed in the process of various redox reactions. ROS include superoxide anion radical ($O_2^{\bullet}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($^{\bullet}OH$), singlet oxygen ($^1O_2$), hypochlorite (HOCl). ROS also include nitric oxide (NO) and peroxynitrite (ONOO-), which have high oxidative activity.

As a result of oxidative stress in the organism there is an accumulation of toxic LPO products, which cause metabolic disorders in the organism, dysfunction of various systems and changes in immune status. However, the LPO process is inherent in normal organism tissues and occurs in the restoration of lipid and protein membrane structures, synthesis of many biologically active substances

(prostaglandins, thromboxanes, leukotrienes, glucocorticoids, progesterone, etc.). This process participates in the regulation of cell division, modulates apoptosis, modulates apoptosis action of phagocytes, prevents malignant transformation of cells. LPO is the main indicator of the body's resilience, its adaptive capacity to the effects of adverse conditions. In the body there is a dynamic balance between the formation of free radicals and their neutralization by the antioxidant system (AOS).

Reactive forms of oxygen, which are formed during oxidative stress, damage all biological structures. ROS stimulate direct Ca^{2+} – independent release of histamine and other specific reactions that cause the riddance of histamine. ROS from cells enter the extracellular space and plasma [7, p. 18]. The consequences of their harmful effects depend on the target molecules and the nature of ROS. Products formed due to LPO activation are unstable compounds that expose oxidative destruction and have cytotoxic and mutagenic effects, that cause disruption of cell metabolism, activation of cytosolic and membrane enzymes, and even cell death.

Oxidizing components include molecular oxygen, hydrogen peroxide, hydroperoxides, hydroxyl radical, super oxidation radical, free metal ions. LPO products include malonic dialdehyde (MDA), diene conjugates (DC) and Schiff bases.

MDA is an aldehyde that is formed in the organism during the degradation of polyunsaturated fats under the action of ROS (fig. 2).

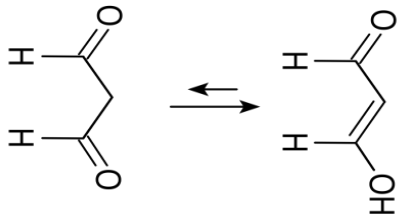


Fig. 2. Structural formula of malonic dialdehyde

There are two major classes of analytical methods for determining MDA: direct methods in which MDA is analysed by itself, and indirect methods for

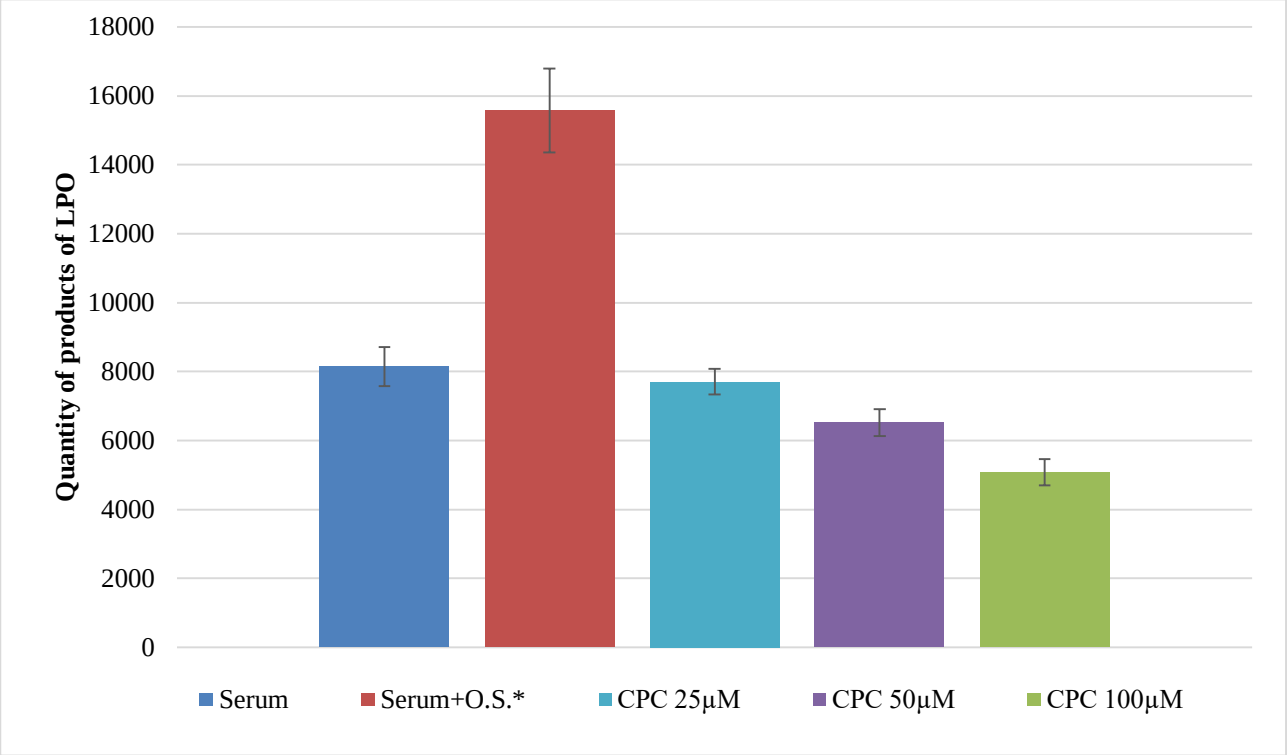
evaluating the product's reaction to MDA with other compounds having fluorescence, absorption, and other properties that can be revealed.

One of the indirect methods, which is the most common, was the TBA method. The difficulty of applying this method includes primarily its non-specificity. Even under ideal experimental conditions and analysis in the formation of MDA from lipid hydroperoxides, MDA can be used as a quantitative index with certain restrictions, because the source of MDA can be the products of DNA decay in its oxidative damage, and possibly other lipid molecules.

The TBA test is very sensitive. It can be used to capture nanomolar concentrations of pure MDA standard. Another complication is that TBA reacts with other compounds to form red pigment at high temperatures and low pH (some aldehydes, deoxysaccharides, sialic acids, glycosylated proteins). Spectrophotometrically it is impossible to determine the formation of the adduct 1:2 MDA:TBA. It is possible that under conditions of high temperature TBA reacts with MDA formed from hydroperoxides during the reaction. This is confirmed by experiments with the addition to the reaction medium of ions of variable metals (copper and iron). The formation of the complex increases due to the decomposition of hydroperoxides. The autoxidation of lipids can be limited by adding antioxidants to the reaction medium or by reacting in a stream of inert gas.

The testing of lipid peroxidation, which was performed, was based on the method of determining the quantity of products that react with TBA. The method is indirect and is based on the ability of TBA to react with malonic dialdehyde, an intermediate product of the stage of enzymatic oxidation of arachidonic acid and the final product of oxidative degradation of lipids. The principle of the method is to determine the intensity of the colour that is formed during the reaction between MDA and TBA. This reaction takes place in an acidic environment and at high temperature. As a result of the reaction, a trimethine complex with a maximum at $\lambda = 535$ nm characteristic absorption spectrum is formed.

The results of the obtained tests were evaluated by the quantity of formed products during the reaction between TBA and MDA (fig. 3).



***O.S. – oxidation system**

Fig. 3. Change in the amount of LPO products in human serum under the action of CPC (100, 50 and 25 μM)

According to the results of the obtained tests, it is clear that CPC at a concentration of 100, 50 and 25 μM, added to the biological model, which is represented by control serum, reduces the amount of formed trimethine complex, which is the result of interaction of TBA and MDA.

The obtained results suggest that *in vitro*:

– CPC at a concentration of 25 μM inhibits the formation of LPO products 1.9 times;

– CPC at a concentration of 50 μM inhibits the formation of LPO products by 2.3 times;

– CPC at a concentration of 100 μM inhibits the formation of LPO products 2.9 times.

Conclusions. Proceeding from the above experiment we can conclude that the CPC probably has antioxidant properties. Cetylpyridinium chloride inhibits the peroxidation of human serum lipids *in vitro* 1.9–2.9 times at concentrations of 25–100 μM, respectively. One of the most promising areas of scientific research in the

field of pharmacy are the experimental research about antioxidant properties of CPC in different environments.

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