EFFECT OF POLYPHENOLS ON Fe^{2+}/CITRATE-INDUCED MITOCHONDRIAL VOLUME CHANGE IN TETRACHLOROMETHANE-INDUCED TOXIC HEPATITIS

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Formation of H_2O_2 free radicals as a result of lipid peroxide oxidation (LPO) in the membrane is of great importance in pathological processes. Increased production of free radicals in the mitochondrial respiratory complex weakens the antioxidant defense system and damages membrane lipoprotein components. In toxic hepatitis caused by tetrachloromethane (CCl_4) and many other pathologies, the intensity of LPO increases based on the development of oxidative stress. In conditions of CCl_4-induced toxic hepatitis, biologically active substances are widely used to reduce membrane LPO [1; 2].

Until now, the effect of polyphenolic compounds gossitan and getasan on Fe^{2+}/citrate-induced lipid peroxyoxidation (LPO) of liver mitochondria in toxic hepatitis has not been studied in vivo. Membrane LPO can be determined in experiments by staining mitochondria.

Research materials and methods. Currently, there are many types of models of toxic hepatitis in laboratory animals. One such method used in many experiments is the CCl_4-induced toxic hepatitis model. To evaluate the hepatoprotective properties of many researched drugs and herbal substances, toxic hepatitis models are administered to animals. We also used a model of toxic hepatitis called CCl_4, which is widely used in our experiments. White male rats with a healthy weight of 180-200 g separated for the experiment were divided into groups.

I group. Control (healthy n=4)
II group. Experience (CCl_4 0,5 ml/kg n=5)
III group. CCl_4 + quercetin (50 mg/kg n=5)
IV group. CCl_4 + gossitan (50 mg/kg n=5)
V group. CCl_4 + getasan (50 mg/kg n=5)

In order to induce experimental toxic hepatitis in II, III, IV and V rats, animals were injected subcutaneously with 50% SSl4 (0.5 ml/kg) once every 3 days. 21 days after the administration of CCl_4 to rats, after the increase of ALT (60 Ud/l) and AST (120 Ud/l) enzymes in the blood, purified vegetable oil (0.5 ml/kg) was given once a day to animals of group II, and quercetin to group III of the experiment. flavonoid, gossitan to group IV and polyphenol getasan to group

V were administered per os once a day for 20 days . Blood was taken from the animals with experimental toxic hepatitis every 3 days, and the amount of ALT, AST enzymes was determined. Rat liver mitochondria were isolated using Schneider's method of differential centrifugation W.C.Schneider [3]. Fe^{2}+/ascorbate and Fe^{2}+/citrate are widely used as inducers to study LPO process in mitochondrial membrane. In the presence of 50 µM Fe^{2}+ and 2 mM citrate in the incubation medium, the LPO process is dramatically enhanced, resulting in a disruption of the barrier function of mitochondria and an increase in their size compared to the control [4]. Membrane LPO was studied using Fe^{2}+/citrate in CCl_4-induced toxic hepatitis and liver mitochondria of corrected rats.

The obtained results and their discussion. According to the obtained results, in the standard incubation medium containing 125 mM sucrose, 65 mM KCl, 10 mM HEPES, 1 mM EGTA and pH 7.2, there was no swelling in liver mitochondria of intact animals. In order to determine the inhibition of intact mitochondria, acute inhibition occurred when we added 2 mM citrate to the incubation medium and exposed to 50 µM Fe^{2}+ 2 min after the initiation of LPO, and this was recorded as a control. Under the same conditions, it was found that the amount of mitochondria isolated from the liver of group II rats with toxic hepatitis was increased by 45.4±3.6% compared to the control group (group I) under the influence of Fe^{2}+/citrate. Therefore, in the presence of Fe^{2}+/citrate in the incubation medium, the mitochondria swelling increases, and this indicates that the membrane LPO process has been completed. Compared to healthy liver mitochondria, liver mitochondria of animals with hepatitis were significantly increased by Fe^{2}+/citrate. Rats with toxic hepatitis showed increased Fe^{2}+/citrate-induced suppression of liver mitochondria, indicating that membrane LPO was accelerated compared to controls. Continuing our experiment, when we pharmacotherapy animals of group III with toxic hepatitis with standard quercetin, it was found that liver mitochondria Fe^{2}+/citrate was inhibited by 33.0±2.6% compared to group II, and the intensity of LPO decreased. . The antioxidant activity of the flavonoid quercetin is more effective than other bioactive compounds and neutralizes the formation of free radicals [5]. In addition, quercetin increases the activity of antioxidant enzymes in toxic hepatitis, reduces the amount of LPO product MDA [6]. In our experience, quercetin may have antiradical activity by inhibiting ROS generation in liver mitochondria in CCl_4-induced hepatitis.

Continuing our experiment, IV and V groups of rats were injected with 50% tetrachloromethane dissolved in vegetable oil (0.5 ml/kg) once every 3 days. 21 days after challenge of the hepatitis model, group IV and group V rats were pharmacotreated by oral administration of gossitan (50 mg/kg) and getasan (50 mg/kg) for 20 days, respectively. When the liver mitochondria of IV and V rats with pharmacotreated toxic hepatitis were studied by Fe^{2}+/citrate-induced membrane LPO, their mitochondrial function was inhibited by

25.9±1.8% and 19.5±1.6%, respectively, compared to pathological group II values. was determined.

Iron compounds induce LPO in the mitochondrial membrane. LPO induced in the presence of Fe^{2}+/citrate in the mitochondrial membrane causes a sharp increase in permeability dependent on Ca^{2}+ ions [7]. In such conditions, the mitochondrial membrane potential decreases, free radicals increase, swelling and loss of matrix components [7; 8]. It can be seen that gossitan and getasan inhibit LPO of the rat liver mitochondria membrane in the conditions of toxic hepatitis, and the stabilizing effect on the membrane can be explained by the antioxidant property. Gossitan and hetasan polyphenols may also cause increased ATF synthesis and decreased LPO process by reducing free radical generation from the liver mitochondrial respiratory chain. This, in turn, allows the use of gossitan and getasan in physiological studies as a corrective agent in the development of toxic hepatitis. But their pharmacological effect in toxic hepatitis requires further research.

Literature

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