APTAMERS AS A METHOD OF CORRECTION OF HEMOSTASIS DISORDERS UNDER THE INFLUENCE OF ANTHROPOGENIC FACTORS

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ABSTRACT — Currently, in the treatment of disorders of the hemostasis system, either anticoagulants, or thrombolytics, or antiaggregants, are used. They are used not only separately, but also in various combinations, depending on the patient's condition and clinical indications, which increases the burden on the body. Direct thrombin inhibitors are a promising class of antithrombotic drugs. A corrective effect on the hemostasis of the DNA aptamer, thrombin inhibitor, RE31 was studied after inhalation of natural gas on 62 white mongrel rats. The positive influence of the DNA-aptamer RE31 on the parameters of hemostasis was determined, and in the later periods of exposure to hydrogen sulfide-containing gas the changes after the use of the aptamer statistically significantly differed from those in the control groups. It was also determined that, in comparison with groups exposed to inhalation without subsequent correction, differences in parameters of haemostasis after passage through the vascular system of the lungs were more pronounced in groups of animals treated with the aptamer.

KEYWORDS — laptamer, thrombin, experiment, lungs, hemostasis, natural gas.

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Repeated attempts have been made to penetrate into the essence of the mechanisms of clotting of blood and to select the appropriate arsenal of drugs for the prevention and treatment of such complications. Currently, clinicians use either anticoagulants that inhibit the formation of fibrin (heparin and its derivatives, indirect anticoagulants, direct thrombin inhibitors) or thrombolytics, whose action is directed to the activation of the fibrinolytic blood system and the dissolution of the thrombus (streptokinase, urokinase, tissue plasminogen activator) or antiplatelet agents that inhibit platelet aggregation (aspirin, thienopyridines-ticlid and plavix, Ihib-IIIa glycoprotein antagonists). However, the available preparations for correction of the hemostasis process with a tendency (or presence) of thrombosis are not without drawbacks. The problem of hemostasis requires finding an inhibitor of thrombin, which would be specific in the process of blood clotting, would not cause an allergic reaction, through which it is possible to effectively control this process. The study of DNA-aptamers of thrombin inhibitors is very relevant and promising in terms of the possible use of research results in the development of a set of preventive and curative measures in environmentally harmful production, as well as in patients with bronchopulmonary pathology.

The purpose of the research was to determine the degree of the corrective effect of the DNA-aptamer on changes in the components of the hemostasis system against the background of chronic inhalation of hydrogen sulfide-containing gas.

MATERIALS AND METHODS

The experiment was performed on 62 white mongrel male rats in the autumn-winter period. The content of rats in the vivarium corresponded to international standards [6]. Animals were divided into control and four experimental groups, depending on the duration of the gas inhalation period. The model of gas intoxication was created by placing in the seed chambers, a volume of 200 liters with a controlled composition of an air-gas mixture of 3.4±0.3 mg/m^3 concentration by hydrogen sulfide. Animals were in these conditions for 4 hours, every 5 working days a week. The individuals of the control group were placed in the chamber in a similar time mode, but with the usual air composition. After preliminary narcotization with sodium thiopental (40 mg/kg), by opening the abdominal cavity, the blood for examination was taken from the inferior vena cava, that is, before entering the pulmonary system, and from the abdominal aorta, that is, after circulation in the pulmonary vascular system, volumes of 1.5 ml per disposable insulin syringes with sodium citrate (9:1). Manipulations with the blood being studied were performed according to the recommendations of Z.S. Barkagan and A.P. Momot [1]. Changes in the platelet and coagulation units of the hemostasis system, anticoagulant plasma activity and the state of the fibrinolytic system were recorded with the help of the "Technology – Standard" (Barnaul) kits. The number of platelets was calculated using a light microscope in the Goriaev chamber.

RESULTS OF THE RESEARCH

We have studied the effect on the hemostasis system of the DNA aptamer – RE31. When calculating the number of platelets, no special changes were detected. In the right department, their number was 782±16.7 · 10^9/l, in the left — 789±18.5 · 10^9/l. The induced platelet aggregation in the right part was 21.6±0.4 sec, in the left part — 21.0±0.7 sec. In the study of activated partial thromboplastin time (APTT) after the action of the aptamer, it reached a value in the right part of 29.2±0.9 sec, in the left part it increased to 29.6±0.78 sec, respectively. In the study of prothrombin time, the value in the right part was 28.6±1.5 sec, in the left part it was 29.2±1.7 sec, respectively. Corresponding changes were registered in relation to other studied indicators. Thrombin time in the right part was 36.7±1.0 sec, in the left — 37.4±1.4 sec. The concentration of fibrinogen after application of the aptamer in the right part was 1.58±0.06 g/l, in the left — 1.54±0.05 g/l. And the last parameter was the content of soluble fibrin-monomer complexes. In the right part, their amount reached 2.8±0.06 mg/dl, in the left — 2.7±0.11 mg/dl, respectively. When studying the properties of this aptamer in the control group of animals, we found its favorable pharmacological properties in relation to the parameters of hemostasis. When studying the effect on the hemostasis system of the DNA aptamer – RE31 after 30 days of experimental treatment: APTT in the right part reached a value of 27.4±1.25 sec, in the left part it was 27.6±1.6 sec, respectively. In the study of prothrombin time, the value in the right part was 29.6±1.4 sec, in the left part it was 30.3±1.5 sec. Corresponding changes were registered in relation to other studied indicators. The thrombin time in the right part was 33.9±1.2 sec, in the left 34.5±1.1 sec, respectively. The concentration of fibrinogen in the right part was 1.7±0.09 g/l, in the left — 1.66±0.07 g/l. Adhesive-aggregation function of platelets in the right part of 22.7±1.0 sec, in the left — 22.3±0.9 sec. The number of platelets in the right and left sections was 818±24.4 · 10^9/l and 831±22.6 · 10^9/l, respectively. The content of soluble fibrin-monomer complexes in the right part reached 2.4±0.07 mg/dl, in the left 2.5±0.08 mg/dl, respectively. When introducing the DNA aptamer — RE31 against the backdrop of a 60-day exposure to natural gas, the following changes were recorded. When studying APTT in the right part, it reached 22.7±1.4 sec, in the left part — 22.4±1.7 sec, respectively. In the study of prothrombin time, the value in the right part was 26.6±1.2 sec, in the left part it was 26.9±1.3 sec, respectively. The thrombin time in the right part was 31.2±0.9 sec, in the left part — 31.8±0.7 sec, respectively. The concentration of fibrinogen in the right ventricle was 1.74±0.13 g/l, in the aorta — 1.7±0.1 g/l. Adhesive-aggregation function of platelets in the right part was 22.9±1.1 sec, in the left section — 23.1±1.2 sec, their number in the right and left divisions was respectively 825±26.2 · 10^9/l and 826±20.7 · 10^9/l. The content of soluble fibrin-monomer complexes — in the right part reached 2.7±0.08 mg/dl, in the left — 2.7±0.13 mg/dl, respectively. When introducing the DNA aptamer (RE31) against the backdrop of 90-day exposure to natural gas, the following changes were recorded. In the right part, when studying APTT, it reached a value of 21.4±1.3 sec. In the study of prothrombin time, the value of 22.8±0.8 sec was determined, respectively. The thrombin time was 27.1±1.1 sec. The concentration of fibrinogen was 1.78±0.18 g/l. Adhesive-aggregation function of platelets was 23.4±1.3 sec, their number was 807±22.8 · 10^9/l. The content of soluble fibrin-monomer complexes reached 3.3±0.13 mg/dl, respectively. When studying blood taken from the aorta, after the introduction of RE31: APTT reached a value of 20.9±1.0 sec. In the study of prothrombin time, a value of 22.4±0.9 sec was determined, respectively. Corresponding changes were registered in

relation to other studied indicators. The thrombin time was 27.3±0.9 sec. The concentration of fibrinogen was 1.79±0.08 g/l. Adhesive-aggregation function of platelets was 23.8±1.0 sec, their number was 782±18.5 · 10^9/l. The content of soluble fibrin-monomer complexes reached 3.4±0.15 mg/dl, respectively. When introducing the DNA-aptamer (RE31) against the background of 120-day exposure to natural gas, the following changes in blood from the right ventricle were recorded. In determining the APTT, it reached a value of 17.6±0.9 sec. In the study of prothrombin time, a value of 19.3±0.9 sec was determined, respectively. The thrombin time was 24.4±1.3 sec. The concentration of fibrinogen was 1.8±0.14 g/l. Adhesive-aggregation function of platelets was 24.6±1.2 sec, their number was 757±16.8 · 10^9/l. The content of soluble fibrin-monomer complexes reached 3.8±0.1 mg/dl, respectively. In the study of blood taken from the aorta, the following indices were recorded: APTT after 120 days of experimental exposure reached a value of 17.2±0.7 sec. In the study of prothrombin time, a value of 18.8±0.7 sec was determined. Corresponding changes were registered in relation to other studied indicators. The thrombin time was -24.0±1.2 sec, respectively. The concentration of fibrinogen was 1.82±0.12 g/l. Adhesive-aggregation function of platelets was 25.2±1.4 sec, their number was 760±21.2 · 10^9/l. The content of soluble fibrin-monomer complexes reached 4.0±0.18 mg/dl, respectively.

Thus, the positive influence of the DNA-aptamer RE31 on the parameters of hemostasis was determined, and after 90 and 120 days of chronic exposure to hydrogen sulfide-containing gas, the changes after application of the aptamer were distinct and statistically significantly different from those in the control groups. It was also determined that in the groups of animals that received the aptamer, differences in hemostasis parameters after passing through the vascular system of the lungs were more pronounced compared to the groups subjected to inhalation without subsequent correction. This indicates a decrease in the load on the enzyme systems of the lungs in the background of the application of RE31 and the improvement of lung function for the purification and correction of components of the hemostasis system.

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