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MANIFESTATIONS OF PHYSIOLOGICAL HEMOSTASIS DYSFUNCTIONS

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Abstract. *The study is devoted to the study of hemocoagulation factors in white rats under the action of pyrogenal. It was found that administration of pyrogenal 50 μg/kg increased active recalcification time, active relative thromboplastin time, prothrombin and thrombin time, as well as fibrinogen content. The observed changes indicate an increase in the protective properties of the organism that promote the acceleration of hemocoagulation processes. It is assumed that the administration of this dose of pyrogenal increases the specific and nonspecific resistance of the organism.*

Keywords**: *hemostasis, fibrinogen, pyrogenal, trombin, hemocoagulation processes*.**

INTRODUCTION

In the human body, the morpho-functional integrity of blood vessels is maintained under normal conditions due to the action of complex enzymatic systems.

The production of a vascular lesion, no matter how small, triggers a well-determined sequence of reactions that ensure:

- formation of an obstacle that opposes blood loss;
- resumption of normal circulatory flow after the integrity of the damaged vessel has been restored;
- stimulation of tissue repair mechanisms.

The term hemostasis defines precisely this sequence of biological events, in which plasma proteins, cellular components, and humoral components participate in a conjugated manner. Two enzymatic systems with antagonistic action essentially contribute to hemostasis: the coagulation system and the fibrinolytic system. They are normally in a dynamic equilibrium, which is why the term hemostasis is increasingly replaced by the term coagulolytic equilibrium.

Hemostasis is a complex physiological phenomenon, an autocatalytic phenomenon that opposes hemorrhage, ensuring a normal coagulability state through the integrity of vascular walls. Blood coagulation is a process in which blood changes its fluidity due to the transformation of a soluble plasma protein, fibrinogen, into a three-dimensional, insoluble network. This transformation occurs locally, under the action of specific stimuli, and aims to stop or limit blood loss at the level of vascular lesions, as a result of their sealing.

Blood coagulation involves the interaction of several components: vascular endothelium, platelets, and plasma glycoproteins. It is tightly controlled through positive and negative feedback mechanisms and counterbalanced by the fibrinolytic system.

The endothelium plays a crucial role in maintaining the fluid state of blood and limiting clot formation only at the local level. After producing a vascular lesion, endothelial cells express procoagulant factors such as tissue factor (TF), plasminogen activator inhibitor (PAI), von Willebrand factor (vWF), and protease-activated receptors (PAR), while to inhibit clot formation, they express tissue factor pathway inhibitor (TFPI), heparan sulfate, thrombomodulin, endothelial protein C receptor, tissue plasminogen activator (t-PA), ecto ADP-ase, prostacyclin, nitric oxide, and ADAMTS, a metalloprotease that limits the procoagulant activity of vWF (1,2,3).

Platelets have multiple roles during hemostasis: adhesion, degranulation, aggregation, fusion, and procoagulant (2). When the endothelium is damaged, the subendothelial matrix, rich in vWF and collagen, is exposed, which favors platelet adhesion through glycoprotein receptors (GP) Ib/IX/V and Ia/IIa and initiates activation (3). During adhesion, platelet receptors GP IIb/IIIa are activated, which can bind to fibrinogen and vWF, resulting in platelet aggregation. Activated platelets release the contents of alpha and dense granules into the extracellular space. The degranulation process is dependent on prostaglandin synthesis. Following degranulation, platelets release procoagulant material into the extracellular space such as: vWF, factor V, beta-thromboglobulin, platelet factor 4, fibrinogen, adenosine diphosphate (ADP), serotonin, calcium, etc. The released substances, especially ADP, facilitate a positive feedback loop that enhances ADP and thromboxane A2 release, resulting in secondary aggregation (2). Platelets have a procoagulant role also by providing the phospholipid membrane surface (platelet factor 3-PF3) for the development of coagulation protein reactions.

In the classic model of coagulation, plasma glycoproteins act in a cascade, on two pathways, intrinsic and extrinsic, which meet at the level of factor X activation (common pathway), resulting in the formation of thrombin which cleaves fibrinogen into fibrin monomers that are polymerized and stabilize the platelet clot. This cascade model is useful for understanding laboratory tests but does not reflect the coagulation process in vivo, which is more complex. The current model of coagulation is based on only one common pathway, in which the interaction between TF and factor VII (FVII) has an essential role and unfolds in 3 overlapping phases: initiation, amplification, and propagation (4).

Blood coagulation is a very important mechanism for defending the body against hemorrhages, involving numerous substances found or formed in plasma and platelets. But sometimes it is influenced by certain factors that trigger major cellular disorders. The interaction between coagulation factors and some stressful states, such as the toxic effect of certain compounds, is extremely complex and still incompletely known. Depending on their origin, toxins can be exogenous or endogenous. Exogenous toxins are: 1. non-biological - exogenous chemical substances that cannot be tolerated at any concentration and chemical substances that become toxic when exceeding the maximum allowed dose 2. biological - toxins produced by living organisms.

Exotoxins cause: ribosylation of intracellular proteins, damage to cell membranes through enzymatic effect, interference with certain neurotransmitters, and endotoxins (lipopolysaccharides) cause the generation of mediators (cytokines, arachidonic acid derivatives, etc.) that act directly on the cell. All of these are pyrogenic substances.

In general, exogenous pyrogens act mainly by inducing the formation of endogenous pyrogens through stimulation of host cells - usually monocytes and macrophages. However, the distinction between exogenous and endogenous pyrogens is sometimes unclear. For example, lipopolysaccharides can act directly on brain endothelial cells to generate fever, while many exogenous products lead to the release of endogenous pyrogens, thus producing fever. Such endogenous substances include antigen-antibody complexes with complement, complement cleavage products, steroid hormone metabolites, bile acids, and some cytokines. [1]

Exogenous pyrogens are represented by: pathogenic microorganisms and their toxins: Gram-negative bacterial endotoxins (LPS), Gram-positive bacterial exotoxins (lipoteichoic acid, peptidoglycans), viruses, fungi, toxic substances - antigen (Ag) - antibody (Ab) complexes that fix Complement, etc. Endogenous pyrogens: are cytokines (IL-1, TNF alpha, IL-6) produced by numerous cells (monocytes, macrophages, tumor cells) and released into circulation, which trigger fever through prostaglandin release. The biological effects of endogenous pyrogens have central action: ↑ threshold of thermoregulatory center regulation with role in triggering the febrile reaction and peripheral actions: - proinflammatory effect, increasing the body's defense capacity: - ↑ leukocyte mobilization at the hematopoietic marrow level ⇒ leukocytosis - induce synthesis of chemotactic factors for monocytes and neutrophils - activation of phagocytosis - ↑ hepatic synthesis of acute phase proteins (IL-6): fibrinogen, C-reactive protein, antiproteases, haptoglobin, complement system - ↓ serum Fe and Zn (trace elements necessary for bacterial multiplication) - activation of B and T lymphocytes - acceleration of muscle catabolism + negative nitrogen balance ⇒ weight loss in prolonged febrile states - synergistic toxic effects ⇒ toxic shock when released in very large quantities. [8,9,10]

The thermoregulatory system is able to maintain the central temperature relatively constant, accepting a variation of approximately 0.2° C. When it rises above the permissible limit, the body's defense mechanisms are also deregulated. [10, 11, 12]

MATERIALS AND METHODS

The purpose of this work was to study the action of the biogenic preparation (complex of lipopolysaccharides extracted from cell membranes of gram-negative bacteria) pyrogenal in doses of 50µg/kg for 3 days on coagulation factors in laboratory white rats. Before being subjected to the action throughout the experiment, the rats were weighed, body temperature was measured, as well as blood glucose concentration.

After 1, 2, 3 days of experiment, blood samples were collected from laboratory white rats for the determination of 5 coagulation factors: activated recalcification time, activated partial thromboplastin time, prothrombin time, thrombin time, fibrinogen quantity.

To study the action of the preparation on coagulation factors, a set of reagents was used, intended for the investigation of the "plasma chain" of the blood coagulation system, by the method of modeling the blood coagulation process "in vitro", which allows obtaining data regarding the stages of blood coagulation.

RESULTS AND DISCUSSIONS

Analyzing the obtained data, in the control group of rats we observe that they coordinate with the results obtained by other scientists (Cosmulescu I., 1985; Niguleanu V.N., 1990; Paladi E., Budeanu L., 2004).

In control rats, the activated recalcification time was 12.53 ± 0.65 s., activated partial thromboplastin time - 11.48 ± 0.42 s., prothrombin time - 13.36 ± 0.32s., thrombin time - 11.78 ± 0.18 s., and fibrinogen quantity - 1.71 ±0.08 g/l.

In rats subjected to the action for 1 day, it was found that all the indices investigated by us practically did not change compared to those of the control rats. The activated recalcification time was 13.98 ± 0.33 s., activated partial thromboplastin time - 12.18 ± 0.12 s., prothrombin time - 14.15 ± 0.29s., thrombin time - 11.68 ± 0.8 s., and fibrinogen quantity - 1.75 ±0.09 g/l.

Increasing the duration of administration to 2 days, we found that the changes are still non-essential in the coagulation indices investigated by us compared to the data obtained from control rats and the previous group.

From the obtained results, we found that the activated recalcification time in rats subjected to the biogenic preparation for 2 days remained almost unchanged compared to the previous group (13.98 ± 0.33 s compared to 14.4 ± 0.46 s), but comparing the results with those from control animals, we detect a small insignificant increase.

Essential changes were obtained in the determination of activated partial thromboplastin time compared to the same index in rats of group 3 (14.98 ± 0.16 s, compared to 12.18 ± 0.12 s).

Analyzing the data obtained in this group for activated partial thromboplastin time in control rats, we observed that this index has changed, constituting an essential difference. In determining prothrombin time in rats of this group, we found almost constant data compared to those of the previous group (13.74 ± 0.26 s, compared to 13.36 ± 0.32 s).

In investigating thrombin time in rats of this group, a small increase in this index was observed compared to that of control animals, which constitutes (12.03 ± 0.17 s compared to 11.68 ± 0.8 s).

The fibrinogen quantity in rats subjected to biogenic preparation administration for 2 days increased compared to that of control rats (1.88±0.03 g/l compared to 1.75±0.09 g/l).

Increasing the duration of action on experimental rats to 3 days, we found that changes in the investigated indices continue to appear (1.97 ±0.13 g/l compared to 1.75±0.09 g/l), but which are not so significant.

CONCLUSIONS

From the above, we observe a non-significant increase in the 5 investigated indices (activated recalcification time, activated partial thromboplastin time, prothrombin and thrombin time, fibrinogen quantity), which indicates that the rats are subjected to a stressful state, increasing the body's defense capacity when required.

Under the influence of the preparation, essential changes occur in the sympathetic nervous system, which lead to the acceleration of blood coagulability. It is likely that the lipopolysaccharide complex increases the immune response in doses of 50µg/kg as well as the specific and non-specific resistance of the body.

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