ANNOTATION

dissertation work on the topic: **«Influence of β1-adrenergic blockers and metabolites of purine nucleotides on the immune status, activity of enzymes of the purine cycle and antioxidant system in sympathetic hyperactivation»,** for the degree of Doctor of Philosophy (PhD) in the specialty 6D110100 - Medicine

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Relevance of the research topic:

Currently, in cardiology, for the development of adequate methods of treating cardiovascular diseases, it is very important to establish the mechanisms of disturbance of the adaptive processes observed in sympathetic hyperactivation. As many studies show, myocardial ischemia is accompanied by a significant increase in the content of adrenaline in the heart. In the zone of myocardial ischemia, its concentration increases by more than 1.5-2 times compared with the background data; in distant parts of the heart - 1.4-1.6 times. At the same time, there is a progressive decrease in the adrenaline content in the adrenal glands. This indicates mainly the adrenal origin of adrenaline in the muscles of the heart [Makarova N.A., 2013].

Research in recent decades shows that adrenaline is a potent activator of lipid peroxidation in the myocardium [Rahman W, et al., 2016; Chinkin A.S., 2014].

However, the accumulation of adrenaline in the heart, on the one hand, potentiates the process of lipid peroxidation, while on the other hand it suppresses the activity of antioxidant enzymes. It is known that catecholamines promote lipid peroxidation, leading to an increase in ATP consumption. The latter leads to the accumulation of products of its hydrolysis - xanthine. In turn, the metabolism of xanthine is accompanied by the formation of reactive oxygen species [Karpishenko A.I., 2013].

According to the literature, activation of the sympathoadrenal system aggravates the course of coronary heart disease. An increase in the level of catecholamines is a risk factor for the development of repeated myocardial infarction and sudden death syndrome. The most common manifestations of sympathetic hyperactivation are sinus tachycardia, functional extrasystole, cardialgia, episodic increases in blood pressure, hyperhidrosis, and other autonomic disorders. In cardiological practice, in order to cure the above symptoms, the use of cardioselective β-adrenergic blockers, which do not have internal symptomatic activity, is preferred. Metoprolol meets these requirements, first of all. [Mittal N, et al., 2017].

Metaprolol, blocking B1-adrenergic receptors of the heart in low doses, reduces the formation of cyclic adenosine monophosphate (3`5`AMP) from adenosine triphosphate (ATP) stimulated by catecholamine's, and as a result, the intracellular current of Ca2 + ions decreases. As a result, metoprolol is recommended as the drug of choice in the treatment of coronary artery disease and heart rhythm disorders [Morris J, et al., 2020].

By its nature, sympathetic hyperactivation is accompanied by increased production of non-enzymatic adrenaline oxidation products, which accelerate the use of ATP by cells, contributing to an increase in the level of adenosine monophosphate (AMP) and adenosine (AD) in the human body.

Numerous studies have shown the importance of enzymes of the exchange of purine nucleotides in the formation of mechanisms of the general adaptation syndrome and some functions of the immune system. The relationship between the activity of enzymes of purine nucleotides and the function of lymphocytes responsible for cellular and humoral immunity has been established, and an imbalance in the activity of these enzymes leads to dysfunction of immunocompetent cells and to the subsequent development of immunodeficiency in the human body [Tapbergenov S.O. et all 2009].

These data show that the control of the metabolism and physiological activity of all cells of the body is carried out with the help of a complexly organized specific multifunctional ensemble. Analysis of literature sources allows us to conclude that for a more meaningful use of adenosine and its analogs in clinical practice, a more detailed comparative study of the effect of adrenaline, adenosine, AMR and β1-blocker metoprolol on the antioxidant defense system, on enzymes of purine nucleotide metabolism and immune status is necessary.

The purpose of the dissertation research: To study the effect of purine nucleotide metabolites (AMP and adenosine) and selective β1-adrenergic blocker metoprolol on the immune status and on the activity of enzymes of purine nucleotide metabolism and the antioxidant system in blood plasma and in heart and liver cells in normal conditions and in sympathetic hyperactivation.

Research objectives:

1. To study the effect of sympathetic hyperactivity caused by the administration of adrenaline on the antioxidant defense system, the activity of enzymes of the metabolism of purine nucleotides and the immune system.
2. To determine the peculiarities of the metabolic effects of adenosine and AMP, β1-blocker metoprolol, on the immune system, enzymes of exchange of purine nucleotides and the antioxidant defense system.
3. To give a comparative assessment of the features of the metabolic effects of adenosine and AMP, β1-blocker metoprolol in sympathetic hyperactivity.
4. To study the possibility of using a complex of adenosine and AMP to correct changes in the activity of the antioxidant defense system, enzymes of purine metabolism and immune status observed in sympathetic hyperactivity.

Research methods

Main research methods: The activity of glutathione reductase (GR) and glutathione peroxidase (GPO) was determined by the method of S.N. Vlasova and co-authors. Biochemical research methods were used. The amount of protein was determined by the Lowry method. The amount of phosphoric acid was calculated according to the calibration schedule. The activity of adenosine monophosphate deaminase (AMP-deaminase) and adenosine deaminase was determined by the method of S.O. Tapbergenov. Determination of the amount of MDA was carried

out by the method of Uchiyama M., Mihara M., diene conjugates by the method of V.B. Gavrilov and co-authors.

To assess the immunological status in peripheral blood, the total number of leukocytes and lymphocytes was calculated. The number of CD3+, CD4+, CD8+, CD19+ cells was determined by immunofluorescence analysis using FIT with conjugated monoclonal antibodies.

The reaction of inhibition of leukocyte migration (RTML) was determined by the method of Clausen J.E. NST-the test was carried out by the method of B.S. Nagoeva, M.G. Shubich.

The object of the study - 190 white male rats aged 3-3.5 months, weighing 225 (95% CI: 203-238) grams.

The subject of the study is homogenates from the liver and heart of experimental animals of the studied groups. Peripheral blood of experimental animals was used to assess the immunological status and biochemical parameters.

The study was conducted on 190 white male rats aged 3-3.5 months with a body weight of 225 (95% CA: 203-238) grams. The experimental animals were kept in the same conditions of the vivarium of the Center of the Research Laboratory of the Non-Profit Joint-Stock Company "Semey Medical University", in accordance with the basic therapeutic and nutritional regime. The experiment was approved by the Ethics Committee of the State Medical University of Moscow. Families in accordance with the Directive of the European Parliament on the Protection of animals used for scientific purposes (No. 6.1 Protocol of 02/14/2020).

The experiment was divided into 3 series: 1 series – intact animals of the 1st group (control); intact animals of the 2nd group were injected with 0.1 mg of AMP for 10 days (total dose of 1 mg); intact animals of the 3rd group were injected with 0.1 mg of adenosine for 10 days (total dose of 1 mg); intact animals of the 4th group were injected with epinephrine intraperitoneally at a dose of 4 mg / kg 60 minutes before the study; series 2 - – intact animals of the 1st group (control); intact animals of the 2nd group were injected with epinephrine intraperitoneally at a dose of 4 mg / kg 60 minutes before the study; The 3rd group of animals was injected with AMP and adenosine at a dose of 0.1 mg (total dose of 1 mg), the 4th group was injected with adrenaline for 10 days and on the last day of the experiment intraperitoneally at a dose of 4 mg / kg 60 minutes before the study. Series 3 - intact animals of group 1 (control); intact animals of group 2 were injected with epinephrine intraperitoneally at a dose of 4 mg / kg 60 minutes before the study; intact animals of group 3 were injected with metoprolol per os at a dose of 25 mg/kg for 2 days, the next day they were injected intraperitoneally epinephrine at a dose of 4 mg / kg 60 minutes before the study.

Homogenates were obtained from the liver and heart of experimental animals of the studied groups.

The main provisions submitted for protection

1. Epinephrine at a dose of 4 mg/kg allows us to study the effect of sympathetic hyperactivation in experimental animals on the antioxidant defense

system, the activity of purine nucleotide metabolism enzymes and the immune system.

2.The use of a complex of AMP and Adenosine, a β1-adrenoblocker (Metoprolol) is characterized by a decrease in peroxidation processes, a decrease in the activity of antioxidant defense enzymes, a deterioration in the immune status and changes in the activity of purine nucleotide metabolism enzymes. The effects caused by the introduction of AMP and Adenosine into the heart and liver are characterized by a focus on preserving the antioxidant defense system and ensuring the equilibrium of the oxidative homeostasis system.

3. AMP and adenosine, as a β1-blocker, metoprolol can be used to correct the dysfunction of antioxidant protection and enzymes of purine nucleotide metabolism observed in hyperadrenalinemia and oxidative stress of various origins.

4.With sympathetic hyperactivation and oxidative stress of various genesis, the use of AMP and Adenosine complex allows for correction of dysfunctions of antioxidant protection and enzymes of purine nucleotide metabolism.

Description of the main results of the study

1. Sympathetic hyperactivity caused by the administration of adrenaline at a dose of 4 mg / kg for 60 minutes in the heart with the activation of BP, AMPD, catalase, an increase in the level of MDA, a decrease in the activity of 5'H and the ratio of AD + AMPD / 5'H. The activity of the enzyme / 5 'N in the direction of increased deamination of adenosine and AMP is characterized by an increase.

The introduction of adrenaline into the liver increased the level of MDA ($p=0.05$) and DC ($p=0.05$), activation of catalase ($p= 0.05$) and enzymes of purine metabolism of AD, AMPD and 5'H ($p= 0.05$). reasons. With sympathetic hyperactivity, shifts close to the state of oxidative stress are observed in the blood, which is due to the activation of GPO ($p=0.05$), catalase ($p=0.05$) and purine nucleotide exchange enzymes AD, AMPD, 5'H ($p=0.05$), Characterized by an increase in the level of MDA ($p=0.05$) and PK ($p=0.05$), the functional relationship of the T- and B-links of immunity increases.

2. The effect of AMP and adenosine at a dose of 1000 mcg on intact animals is aimed at maintaining the antioxidant defense system and maintaining the balance of the oxidant homeostasis system. In particular, the activity of GR ($p=0.05$), GPO ($p=0.05$), catalase ($p=0.01$) decreases in the heart, the level of MDA decreases ($p=0.05$), the activity of AD and AMPD increases ($p=0.05$). The introduction of AMP and adenosine into the liver leads to a decrease in the activity of GR ($p= 0.05$), GPO ($p= 0.05$), a weakening of the formation of MDA ($p= 0.05$), an increase in the activity of AD and AMPD ($p = 0.01$). The level of MDA ($p=0.05$), RTML ($p=0.05$) and CD8+ ($p= 0.05$) in the blood decreases, the activity ratio of AD / AMPD ($p=0.01$) increases, the functional relationship of T- and B-immunity increases.

The features of the metabolic effects of the b1-adrenoblocker metoprolol were determined on intact animals. Metoprolol administered to intact animals at a dose of 25 mg / kg for two days increases the activity level of purine metabolism enzymes AD ($p= 0.05$), AMPD ($p= 0.05$) and 5'N ($p= 0.05$) in the blood, MDA.

(p=0.05) decreases and, accordingly, the activity of antioxidant defense enzymes (GPO, catalase) decreases (p=0.01). In the heart, metoprolol reduces the level of DC (p=0.05) and MDA (p=0.05) as an integral indicator of the propensity to free radical oxidation and, accordingly, reduces the activity of GPO and catalase (p= 0.05).

3. Adrenaline at a dose of 25 mg / kg weakens the level of severity of the reaction of the animal body to oxidative stress when administered adrenaline. The B1-adrenergic receptor blocker metoprolol can be used to correct the dysfunction of antioxidant defense enzymes and purine nucleotide metabolism observed in hyperadrenalinemia and various oxidative stresses.

In particular, in the heart with hyperadrenalinemia, metoprolol activates AD (p=0.01), AMPD (p=0.01) and 5 'N (p=0.01), MDA (p=0.01) and DC (p=0.01) and for the first time it has been proven that the activity of GR (p=0.01), GPO also decreases and catalase (p =0.01), which causes a decrease in the processes of peroxidation in the heart.

With hyperadrenalinemia in the liver, metoprolol reduces the amount of MDA and PC (p= 0.01), reduces the oxidative stress reaction caused by adrenaline, adequately reduces the activity of GPO and catalase (p= 0.01). At this time, there was a slight increase in the activity of AD (p=0.01), AMPD (p=0.01) and the ratio of the activity of enzymes AD + AMPD/5'H (p=0.01) towards adenosine catabolism. .

In hyperadrenalinemia, a decrease in the activity ratio of the enzymes AD + AMPD /5'H is aimed at maintaining the necessary level of adenosine, which is known to have antiarrhythmic, vasodilating and other effects on cardiomyocytes and vascular smooth muscle.

4. It has been established that adenosine and AMP complex can be used to correct changes in the antioxidant defense system, changes in purine metabolism enzymes and the activity of immune reactions observed in hyperadrenalinemia.

In particular, the introduction of a complex of adenosine and AMP in sympathetic hyperactivity at a combined dose of 1000 mcg leads to the activation of AD and AMP (p= 0.01) in the heart, the ratio of AD+AMPD /5'N activity increases towards catabolism of adenosine and AMP, MDA and DC (p=0.01) and for the first time it was found that accordingly, the activity of GR, GPO and catalase decreases (p = 0.01), which indicates a decrease in the processes of peroxidation in this organ.

With hyperadrenalinemia in the liver, the complex of adenosine and AMP reduces the level of MDA and DC (p= 0.01) and, accordingly, the activity of GPO and catalase (p= 0.01), thereby reducing the level of oxidative stress caused by adrenaline.

The introduction of a complex of adenosine and AMP in hyperadrenalinemia in the blood increases the activity of the enzymes of purine metabolism AD, AMPD, 5 'N and GR (p= 0.01), reduces the activity of GPO, catalase and MDA (p= 0.01).

With sympathetic hyperactivity, the β1-adrenoblocker, like metoprolol, reduces the processes of adenosine and AMP peroxidation, adequately reduces the

activity of antioxidant defense enzymes and changes the activity of purine metabolism enzymes.

Justification of scientific novelty

1. The influence of sympathetic hyperactivity on the antioxidant defense system, the activity of purine nucleotide metabolism enzymes and the immune system was studied for the first time: the strengthening of the functional relationship of T- and B-links of immunity was proved; changes corresponding to the state of oxidative stress, which is manifested by the activation of GPO, catalase and enzymes of purine nucleotide metabolism AD, AMPD, 5'H, an increase in the level of MDA and DC.

2. For the first time, a comparative analysis of the effect of purine nucleotide metabolites (AMP and adenosine) and selective β1-adrenoblocker (Metoprolol) on the immune status, antioxidant system, activity of purine nucleotide metabolism enzymes in blood plasma, in myocardiocytes and liver during sympathetic hyperactivation was carried out.

3. For the first time, the possibility of using the Adenosine and AMP complex to correct changes in the antioxidant defense system, the activity of purine metabolism enzymes and immune responses observed in hyperadrenalemia and oxidative stress of various genesis has been substantiated.

Practical significance of the results obtained

1. Determination of the activity of enzymes of metabolism of purine nucleotides AD, AMPD, 5'H and enzymes of antioxidant defense of GPO and catalase in blood can be recommended for diagnostics of diseases accompanied by hyperadrenalinemia.

2. To correct dysfunctions of the body's antioxidant defense and metabolic disorders of purine nucleotides, with severe sympathetic hyperactivity and oxidative stress of various origins, along with β1-adrenergic blockers, it is recommended to use a complex of adenosine and AMP.

3. The results of the dissertation were introduced in research laboratories, as well as in the educational and methodological process of the Department of Biochemistry and Chemical Disciplines, the Department of Physiological Disciplines for the implementation of the bachelor's educational program in the specialties "General Medicine", "Dentistry", "Pharmacy". The author's research paper was obtained on the topic:" the possibility of using adenosine and AMPH complexes to correct changes in the immune status and activity of purine nucleotide metabolism enzymes, an antioxidant defense system observed during sympathetic hyperactivation".(№ 14613 dated 22.01.2021)

Personal contribution of a doctoral student The author personally collected materials, statistical processing, systematization and scientific analysis of the results, as well as the formulation of conclusions and main provisions submitted for defense.

Conclusions

1. With sympathetic hyperactivation caused by the introduction of adrenaline and with the introduction of AMP and adenosine, there is an activation of the

enzymes of metabolism of purine nucleotides and an increase in the functional relationship of the T- and B-links of immunity.

2. The administration of AMR and adenosine to healthy animals, in contrast to adrenaline, does not cause a stress response. The effects of AMR and adenosine in the heart and liver are aimed at maintaining the antioxidant defense system and ensuring the balance of the oxidative homeostasis system.

3. Adenosine and AMP, as β1-adrenergic blocker metoprolol in sympathetic hyperactivation, reduce the peroxidation process and equivalently reduce the activity of antioxidant defense enzymes, cause changes in the state of the immune status and the activity of enzymes of purine nucleotide metabolism.

4. Adenosine and AMP can be used to correct controlled antioxidant defenses, immune system functions, and purine nucleotide metabolism in cases associated with severe sympathetic hyperactivity and oxidative stress of various origins.

Approbation of the results of the dissertation

The main conclusions of the work were discussed at the XII-th international scientific-practical conference “Ecology. Radiation. Health” named after B. Atchabarov, dedicated to the 25th anniversary of the closure of the Semey nuclear test site (Semey, August 28-29, 2016).

It was discussed at the VIII International Congress of the Physiological Society of the Republic of Kazakhstan by the Kazakh Physiological Society "Institute of Human and Animal Physiology" (Almaty, 2018), and an article was published in the journal "Physiology".

Publication

18 scientific papers were published, including 1 monograph, 5 articles in a journal included in the international Scopus database, including 3 articles in the journal "Biomedical Chemistry", 1 article in the journal "Open Access Macedonian Journal of Medical Sciences" and 1 article in journal "Bulletin of Experimental Biology and Medicine", 6 articles in journals recommended by the Committee for Quality Assurance in Education and Science of the Ministry of Education and Science of the Republic of Kazakhstan; 6 theses in the materials of international scientific and practical conferences.

Conclusions Volume and structure of the dissertation

The dissertation is written in computer text with a volume of 126 pages and consists of an introduction, a literature review, a description of materials and research methods, a chapter with research results, conclusions, practical recommendations and a list of references. The bibliographic list consists of a list of scientific works by 142 domestic and near and far authors. Illustrated with 28 tables.