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II. ISSUES OF THE MANIFESTATIONS AND TREATMENT OF COLD TRAUMA

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ACTION OF COLD ON THE ORGANISM. CRYOPROTECTORS AND MEANS OF ANTI-ISCHEMIC TISSUE PROTECTION

ABSTRACT

Temperature is the most important environmental factor affecting humans and animals. The effect of low temperatures on biological objects depends on the degree of phylogenetic maturity of the organism and is realized through various mechanisms including in vitro and in vivo conditions. The report discusses the mechanisms of cold and ischemic damage to biological objects, examines the mechanisms of damage to tissues and organs after heating, and cryoprotection products of biological objects. Particular attention is paid to preparations of pharmacological protection of tissues with antioxidant properties from cold ischemia.

Keywords: mechanisms of cold action on the body, preparations preventing cryo-damage of biological objects.

1.1 Mechanisms of cryo-damage of biological objects

During action of a low temperature on biological objects, including the organism as a whole, there are two main mechanisms of the damaging effect of cold [1, 22, 25, 28, 28, 29, 30].The first, most obvious mechanism, is a direct cryo-damage. Cold impact is crucial when a low temperature leads to frostbite or is used to conserve cells, cell suspensions and tissues.

Damage to biological objects develops both during cooling and during heating. Even before the transformation of water into a solid phase, the rate of metabolic processes slows down, and the cells undergo deep changes in the activity of enzymes. In the development of cryo-damages, the formation of ice is of great importance, which occurs in different ways depending on the freezing rate [12]. In the case of slow cooling, crystallization of water first occurs in extracellular fluids, since they have a higher freezing point than protoplasm and the nucleus. This process leads to an increase in the concentration of salts and other substances in the extracellular space and the disturbance of osmotic equilibrium. The release of water from the cells begins, the mass of ice outside the cells gradually grows, and cells lose water and undergo osmotic compression. This process up to a certain limit contributes to the preservation of cells' life, as the loss of water, in turn, increases the concentration of salts and colloids in the protoplasm, preventing its freezing.However, the continuation of the process leads to an «osmotic shock», disrupting the permeability of the membranes. After reaching the temperature of tissues -21.2 ° C, salts begin to crystallize, membranes break and the death of cells occurs.

In conditions of rapid cooling, the dehydration processes do not have time to develop, small crystals are formed both inside and outside the cells. Microscopic examination reveals minimal changes, however, the protoplasm of cellular elements is already in a state of severe disorganization, leading to cell death, mainly due to damage to membrane structures.

Thus, generalizing the factors that cause cell damage in the direct effect of cold (freezing), we can distinguish the following:

1) cell compression by extracellular ice;

2) increase in the concentration of extra- and intracellular electrolytes;

3) disturbanceof membrane permeability as a result of changes in cell lipoproteins (phase transition and separation of lipids and proteins in the plane of the membrane);

4) rupture of membranes due to rapid loss of water;

5) abnormal pH shifts;

6) mechanical rupture of membrane structures by intracellular ice;

7) thermal shock upon warming.

The second, the most probable mechanism is when at the same low temperature action on the body as a whole, only at a temperature 30 ° Cbelow zero, frostbites may appear on exposed parts of the body, which are caused by the damaging effect of the cold directly on the tissue [18]. In most cases, frostbite develops when the level of local hypothermia of tissues is clearly insufficient to immediately cause their death. It occurs as a result of disturbances in metabolic processes developing against the background of progressive circulatory disorders.

Speaking about the mechanisms of peripheral blood flow disorder during frostbite, a number of authors consider the start-up moment to be a persistent and prolonged spasm of peripheral vasculature, which occurs under the influence of the stimulating action of the sympathetic nervous system that activates under cold stress [18, 26]. At a temperature of +12 to +8 ° C, the dissociation of oxyhemoglobin ceases and the blood does not give oxygen to the tissues. Further decrease in temperature leads to a complete disruption of blood circulation. Erythrocytes are glued together in «coin pillars» and occlude capillaries, a progressive occlusion of the microcirculatory system occurs [6,7]. In this case, the fact that the cold is a specific activator of the internal mechanism of hemocoagulation plays its role, which leads to intense intravascular thrombosis [20].

Thus, local cold trauma should be considered as acute ischemia (complete or incomplete) followed by the development of early and late postischemic disorders [11].

1.2 Mechanisms of ischemic damage to biological objects

Normally, the cells of functioning organs receive energy by oxidizing substrata brought by the blood (glucose, fatty and amino acids, and ketones). In the case of liver cells and muscle cells, energy can also be obtained by cleavage of endogenous glycogen. Within the cells, energy is stored in the form of cyclic phosphates: ATP, creatine-phosphate and other nucleotides. The constancy of the composition of the cellular environment is supported by numerous chemical reactions catalyzed by enzymes, the consistency of which is ensured by the humoral mechanisms of regulation.

Ischemia leads to a cessation or a significant restriction of the influx of nutrients and oxygen into the cell. Metabolism becomes anaerobic; glycolysis is not a method to maintain a normal level of high-energy phosphates in the cell. The decomposition of ATP causes an increaseof adenosine, inosine and hypoxanthine in the cellular levels. The exhaustion of cellular energy reserves inactivates K-Na-dependent ATP, an enzyme that feeds the membrane K-Na pump. Sodium and chloride, freely hydrated ions, normally actively excluded from the cell, begin to penetrate the membrane through a concentration gradient. Since the osmotic power of the non-hydratable cellular proteins and anions is no longer balanced by the displacement of sodium, water penetrates the cell, enlarging it more and more. Mitochondrial oxidation is impossible and calcium penetrates into the cytosol and mitochondria. Experimental reproduction of cytosol overload by calcium ions showed that damage to biomembranes developing in this case is accompanied by dissociation of oxidation and phosphorylation processes, however, the penetration of calcium into the cell is not the only mechanism of influence of cold ischemia on the functions of mitochondria [32].

Anaerobic metabolism temporarily uses glucose reserves for the production of ATP, but lactic acid also forms, which leads to progressive intracellular acidosis, and subsequently to the activation of lysosomal enzymes and cell death.

Most cells and organs are able to withstand acute ischemic hypoxia within 30-60 minutes without irreversible damage [10,11], but the sensitivity of different tissues to ischemia is not the same.

Endothelial vascular cells are more sensitive to hypoxia, and microcirculation disorders that result from the damage to these cells lead to aggravation of ischemic disorders. Normally, blood cells, due to their ability to deform, freely pass through capillaries, the size of which can be less than the diameter of erythrocytes and especially leukocytes, and transcapillary exchange is normal. This is due to the functioning of the system of autoregulation of microcirculation, including, among others, the normal function of the endothelium, in particular the production of prostacyclin and nitric oxide (NO). NO is continuously synthesized from L-arginine with the participation of the NO synthetase enzyme. The main target for NO is the smooth muscle cells of arteries and arterioles: by stimulating guanylate cyclase, NO increases the concentration of cyclic guanosine monophosphate in cells, which leads to relaxation of contractile elements and vasodilation. Like prostacyclin, NO inhibits adhesion and aggregation of platelets. In physiological conditions, the endothelium carries out active transport of metabolites between the blood and perivascular tissues. It synthesizes and destroys metabolites, which regulate the interaction of blood components with the vessel wall [24].

With the fall of tissue temperature, the ability of erythrocytes to deformation decreases and their aggregation capacity increases [41]).

In the conditions of ischemia, the regulatory function of a number of vasoactive substances, primarily prostaglandins, which under normal conditions have an anti-thrombotic action and stimulate the function of leukocytes and endothelium, isdisrupted. As a result, the microcirculation protection system begins to be activated, which is designed to ensure adequate interaction of platelets, leukocytes and endothelium. Activation of blood cells under conditions of ischemia is accompanied by the release of a number of vasoactive substances (cytokines) consisting of small polypeptides. Leukocytes produce the following cytokines: tumor necrosis factor (TNF), interleukins (1L-1, IL-6, IL-8, IL-12, IL-15), platelet activating factor (PAF), proteolytic enzymes, oxygen radicals. Platelets produce serotonin, thromboxane A2, plasminogen activator inhibitor, PAF. The listed products of cytokine activity in turn activate granulocytes, platelets, endothelial cells, fibroblasts, etc.

The damaged endothelium releases the von Willebrand factor and inhibitors of the plasminogen activator. In the endothelium, as well as in platelets and leukocytes, adhesion receptors are formed. Neutrophils induced by IL-1 and TNF secrete lysosomal proteases - elastase, collagenase, toxic metabolites of oxygen, which damage the microcirculatory bed, causing increased vascular permeability, hemorrhage and microthrombosis. The action of hydrolases is realized in the development of endothelial necrosis, destruction of the basal membrane, facilitating the emigration of granulocytes. Along with interleukins, the above-mentioned PAF, which is secreted by various cells of the body, plays an important role in the pathogenesis of tissue damage. Sources of its origin are the phospholipids of cell membranes. Hyperproduction of PAF affects mainly the development of microcirculatory disorders due to activation and hyperaggregation of platelets [24].

These processes are accompanied by local hypoxia and metabolic acidosis, the

development of endothelial and tissue edema, the formation of platelet and leukocyte «plugs» in microvessels, leading to capillary obstruction and post-capillary adhesion of blood cells [20], which leads to increased ischemic disorders.

The principal difference between cold ischemia and ischemia of cardiovascular genesis is that the processes of cellular degradation in the first case are slowed down by cooling, but they do not cease altogether; therefore, ischemic disorders in the freezing of limbs are reversible only to some extent [10,11]. The simple cooling from + 37 ° C to 0 ° C increases the tolerance of most organs to ischemia from 1-2 to 12 hours [20].

The experience of cold preservation of the liver indicates that the degree of damage to the cellular organ membranes significantly increases with the duration of the cold ischemia period [18]. Similar data were obtained when studying the effect of the duration of cold ischemia on the preservation of endothelial cells of arterial vessels [23]. This circumstance, according to The authors, is a prerequisite for the occurrence of post-ischemic disorders after warming.

1.3 Mechanisms of damage to tissues and organs after warming

Not less, but no more destructive processes develop after the restoration of tissue temperature. At the cellular level during the period of cold ischemia, toxic end products of anaerobic metabolism are accumulated; after the restoration of adequate blood flow, they enter the blood, causing the development of a reperfusion syndrome.

Free radicals accumulating in cells in the form of oxygen singlets to toxic concentrations lead to secondary damage to cell membrane structures. Lipid peroxidation (LPO) of cell membranes is one of the types of normal metabolic process and proceeds continuously with low efficiency in all tissues of the body. Enzyme systems (dysmutaz, catalase, glutathione system), which under normal conditions inactivate free radicals that are formed, are mostly damaged by low temperature; endogenous cellular antioxidants (non-enzymatic part of the antioxidant system), including vitamin E, ascorbic acid, selenium salts, are rapidly depleted during cold ischemia [12].

Damage of biomembranes, primarily the walls of the endothelial vessels by free radicals , leads to increased aggregation of platelets and erythrocytes, thrombus formation with the beginning of the restoration of blood flow, increased permeability of the vascular wall, accompanied by a thickening of blood. Thus, the restoration of tissue temperature after a short period of the resumption of blood flow leads to the progression of ischemia with the only difference that in this case the processes of cellular autolysis are no longer suppressed. Cellular elements perish, in the surrounding necrosis of tissues the inflammatory process begins [23].

1.4 Mechanisms of cryoprotection of biological objects

To protect cells from the direct damaging effects of cold (usually in order to preserve them), various liquid media and chemical compounds, cryo-protectors, are used. The study of Polge C.et al. was fundamental in this regard [38,39], which showed that with deep cooling the spermatozoa can be protected from damage with the help of glycerin, which actually became the first known cryo-protectors.

In addition to the glycerol mentioned, conventional materials include such widely known substances as dimethylsulfoxide (DMSO, dimexide), polyvinylpyrrolidone, polyethylene glycol with various molecular weights.

Some of these cryoprotectants (glycerol, DMSO, polyethylene glycol) penetrate well into the cells during their freezing and balance the osmotic gradient during warming and modify the ice crystals in such a way that they become loose and do not cause large damage to the cell.

Experience in the use of polyethylene glycol with a molecular weight of 20,000 daltons (PEG-20) in the conservation of organs taken for transplantation shows that it reduces the degree of inflammatory response in the donor after the transplantation performed [34]. The authors believe that this effect is explained by the ability of PEG-20 to reduce the degree of ischemic and reperfusion injury of tissues. The disadvantage of this group of compounds is that solutions of a sufficiently high concentration (for example a 5% solution of dimethylsulfoxide (DMSO) in Ringer's solution) should be used for effective cryoprotection of biological objects, which causes their cytotoxic effect. Dimexide is also able to enhance the activity and toxicity of most drugs [2].Another group of cryoprotectants (polyvinylpyrrolidone, polyethylene oxide, dextran, oxyethylated starch) penetrates into the cell not enough. These substances are fixed mainly on the outer membrane of cells, stabilizing it both in the process of freezing and heating. These substances are low-toxic, but their effectiveness in terms of cryoprotection is still lower than that of penetrating cells [12].

On the basis of simple cryoprotectants, complex preservative solutions are created. The main requirement for a solution intended for cold preservation is the inclusion of compounds possessing osmotic activity in its composition, which makes it possible to perform effective prevention of cellular edema. Such substances include large anions of the lactate type (molecular weight 358 Da), as well as non-electrolytes for example, polysaccharides (molecular weight 505 Da) or citrate magnesium chelates (molecular weight - 1000 Da). Glucose (molecular weight - 180 Da) for these purposes is unsuitable, because it is able to penetrate slowly into cells, stimulating the production of lactic acid and hydrogen cations by anaerobic glycolysis. Therefore, glucose in complex canned solutions should be replaced with sucrose.

Another important requirement for a complex preservative solution is an effective buffer (phosphate or citrate) for arresting cellular acidosis. The importance of the two above-mentioned cryoprotection mechanisms can be illustrated by the fact that a two-component solution consisting only of sucrose and phosphate buffer has been successfully used for cold preservation of the kidney and is comparable in effectiveness to a much more complex «solution of the University of Wisconsin» [40].

The electrolyte composition of complex preservative solutions can be quite variable. The chloride-anion freely circulating through the membrane is usually replaced with lactate, gluconate or chelate citrate complex. Preservative solutions usually have a high concentration of potassium (130 mmol / L) and a low concentration of sodium (30 mmol / l). Since such a high concentration of potassium is capable of causing bradycardia, solutions of this composition can not be used for early systemic intravenous infusion. A high concentration of potassium in solutions also has a vasospastic effect, which significantly reduces the resistance of tissues to cold ischemia. In connection with the foregoing, the most effective in practice are preserving solutions with a Na / K ratio of 130/30 mmol / l, provided that buffer systems, osmoactive substances and a low concentration of chlorides are present in them.

Many preservative solutions contain magnesium ions, since they participate in the formation of chelates with citrate, making the cell membrane impenetrable to the latter. On the contrary, the content of calcium in most solutions is very low,

or this ion is excluded altogether, since cell damage in the process of cold ischemia is associated with the penetration of calcium inside it.

Among the complex solutions used for the cold preservation of organs and tissues, the most well-known solution was developed by Southard et al. [41] as a result of an analysis of a variety of factors affecting the efficiency of conservation. This solution was called «a solution of the University of Wisconsin» (UW-solution). It contains osmoactive substances (D-lactate, raffinose), phosphate buffer, free radical oxidation inhibitors, glutathione, allopurinol, precursors of energy metabolites (adenosine), vasoactive agents, hormones (steroids, insulin), and colloid (hydroxyethyl starch). This solution has proven to be clinically effective in storing entire organs used in transplantation [32,41], since in addition to cryoprotectants it includes pharmacological preparations that protect the tissues of the canned organ from cold ischemia, as it will be discussed below.

Prospects for improving the cold storage conditions of biological objects are associated with the introduction of such anti-ischemic tissue protection agents as calcium channel blockers (verapamil, diltiazem, trifluoperazine) into complex preservative solutions, as well as stable prostacyclin analogues.

1.5 Mechanisms of pharmacological protection of tissues from cold ischemia

Studies of recent decades in the field of molecular biology have shown that the pathological processes in acute ischemia of cold and cardiovascular genesis are developed according to the same laws. It was established that under these conditions there is an activation of free radical and peroxide oxidation of lipids, mediators of inflammation, adhesion molecules, etc., which take an active part in the damage of cellular and subcellular structures with the development of microthrombosis at the microcirculatory level [22, 25, 28, 28, 29, 30] .

In this connection, it is expedient to single out a special group of pharmacological preparations used to protect tissues from cold ischemia. Since the changes developing in tissues under the influence of cold ischemia are the basis for the development of postischemic disorders, the same drugs can also be considered as a means of preventing reperfusion syndrome that occurs after tissue temperature recovery and adequate blood flow.

Cold is a specific activator of the initial mechanisms of hemocoagulation [36]. However, the use of anticoagulants for the prevention and treatment of arterial thrombosis is ineffective (as opposed to venous thrombosis). This is due to the fact that there is a great difference in the mechanisms of blood clots in arteries and veins, which is largely due to the different blood flow velocity, which determines the structural features and composition of thrombi. The arterial thrombus consists mainly of platelets with a small amount of fibrinogen, since a large part of the procoagulant material is removed from the thrombotic focus by rapid blood flow even before the activation of the coagulation mechanism has occurred. In the venous thrombus, primarily fibrin is accumulated, platelets are retained in places of turbulent blood flow, in the area of valves [24]. Therefore, the most effective for the prevention of arterial thrombosis, which develops in cold ischaemia, are platelet function inhibitors, which include [4]:

1) inhibitors of cyclooxygenase (nonsteroidal anti-inflammatory drugs, acetylsalicylic acid);

2) inhibitors of phosphodiesterase and adenylatecyclase (dipyridamole, ticlopidine, pentoxifylline);

3) selective thromboxantinesynthetase inhibitors (imidazole derivatives);

4) stimulators of prostacyclin synthesis (pyrazoline derivatives, pentoxifylline, coumarin and nicotinic acid derivatives);

5) antagonists of calcium ions;

6) Prostanoids;

7) inhibitors of the release of platelet components (suloktidil, piracetam).

Thus, the main target of the action of platelet antiaggregantsare cyclooxygenase, thromboxane and prostacyclinsynthetases of platelets and vascular walls.

Among these drugs, the most popular and affordable is acetylsalicylic acid. Under its influence, on the one hand, blockade of the cyclooxygenase of platelets leading to inhibition of their aggregation occurs, on the other hand, a reduction in the synthesis of the prostacyclin of the vascular wall, a powerful antiaggregational and antithrombotic factor. The degree of oppression of prostacyclin synthesis depends on the dose of acetylsalicylic acid: small doses (3.5 mg / kg) inhibit platelet cyclooxygenase and platelet aggregation with a slight inhibition of prostacyclin synthesis, with an increase in dose (5-10 mg / kg), the antiaggregation effect of the drug rises slightly , but there is a complete loss of antiadhesive properties of the vascular endothelium [3].

For the prevention of arterial and venous thrombosis, agents that have less pronounced effects on the synthesis of prostacyclin, in particular ticlopidine or ticlid, which is the most effective antiaggregant agent, are used [5]. The drug inhibits ADP-induced platelet aggregation and aggregation, caused by collagen, reduces the adhesion of platelets to the vascular wall, normalizes erythrocyte deformability. The drug in a dose of 250 mg 2 times a day is used. However, a pronounced antiplatelet effect occurs from the 10th day of treatment, in contrast to acetylsalicylic acid, which significantly decreases the activity of platelets from the first days of application. Therefore, ticlid can be used only as a means of preventing ischemic damage in case of an expected cold exposure.

As it was noted, platelet antiaggregants are many medicinal agents of different mechanism of action, some of them have a more multiprofile effect and will be described below.

With the purpose of prevention and treatment of post-ischemic disorders, in particular, thrombo-formation, patients should use anticoagulants, the most common of which is heparin. In particular, in patients with limb frostbites, heparin is administered at a dose of up to 60,000 - 80,000 units per day for 7-10 days [7,8].

In recent years, low-molecular heparins (Fraxiparin), which have a pronounced antithrombotic activity and have a rapid and prolonged effect, have been actively used in anthological practice.

The most effective from the point of view of anti-ischemic protection of tissues are the so-called vasoactive drugs of complex action. Among the preparations of this group, pentoxifylpin (trental) is often used, which, in addition to the expressed influence on platelet hemostasis, helps to reduce aggregation of thrombocytes and increase their plastic properties, increases the content of c-AMP in tissues, and also possesses prostacyclin-stimulating action [19]. It inhibits the anti-inflammatory effect of various cytokines (IL-1 and TNF), as well as their superoxide production [24]. Pentoxifylline is most effective when applied at a dose of 1200 mg per day.

The most «old» vasoactive drug is nicotinic acid and its derivatives. It plays an essential role in the life of the organism, participating in oxidation-reduction processes, improves carbohydrate metabolism. Nicotinic acid is used in the composition of intravenous infusats for the prevention and treatment of microcirculation disorders as a 1% solution [21].

Long enough as a vasoactive agent

of complex action for the prevention and treatment of ischemic disorders curantil, which reduces the aggregation of platelets due to its ability to block phosphodiesterase is used [24, 37]. It also has a pronounced vasodilating effect, although a decrease in systemic blood pressure when it is taken, as a rule, does not occur. It is possible to use the drug in large doses (75-150 mg 3 times a day), but only in case of oral administration, since intravenous injection develops the syndrome of «intercoronary stealing».

Among non-steroidal anti-inflammatory drugs, which have anti-aggregation properties, mefenamic acid has been used in the treatment of cold ischemia and post-ischemic disorders. The drug is a derivative of anthranilic acid, which has elements of structural similarity with salicylic acid and its derivatives. According to the mechanism of action, mefenamic acid is close to other non-steroidal anti-inflammatory drugs, the ability to inhibit the synthesis of prostaglandins occupies an intermediate position between butadion and indomethacin. The anti-inflammatory effect is associated with a normalizing effect on increased permeability of capillaries and improvement of microcirculation processes; a decrease in the activity of enzymes involved in the synthesis of «mediators of inflammation» (histamine, bradykinin, serotonin, etc.); decrease in the formation of ATP and a decrease in the energy supply of biochemical processes; presence of fibrinolytic activity. For the prevention and treatment of frostbites, mefenamic acid is administered orally at a dosage of 50 mg / kg [1].

Antagonists of calcium ions (verapamil, diltiazem, corinfar) reduce the total peripheral resistance, blocking the pathologically increasing the transmembrane current of calcium ions into the cells of the smooth muscles of the vessels in ischemia. Calcium channel blockers also increase myocardial perfusion and improve its contractile function [26]. These drugs can prevent the development of distant postischemic disorders, inhibiting the proliferation of smooth muscle cells in blood vessels.

Taking into account the peculiarities of the pathogenesis of cold ischemia, drugs that have antioxidant activity play an important role in the treatment of its consequences. Among the antioxidants that have found wide application in medical practice in our country is emoxipine, a water-soluble compound from the class of 3-hydroxypyridines. Participation in various molecular reactions and influence on a number of enzyme systems of the organism cause a wide range of pharmacological activity of emoxipin [13, 14].

As an antioxidant, emoxipin inhibits the formation of hydroperoxides of phospholipids - the precursors of prostaglandins and leukotrienes. The latter can cause hemodynamic disorders that develop with cold trauma, and in addition, the inflammatory reaction caused by leukotrienes is one of the causes of microcirculatory disorders in the reactive period of acute cold trauma [40].

The effect of emoxipin on the blood coagulation systemis not associated with its antioxidant properties. At a dose of 20 mg / kg, the drug causes an increase in clotting time. This effect depends on the ability of emoxipin to form a complex with heparin, and is also the result of inhibition of fibrin polymerization [13,14]. The decrease under the influence of emoxipin both spontaneous and induced platelet aggregation is, on the contrary, due to the nonspecific antioxidant action of the drug, which inhibits the activity of platelet phosphodiesterase [17].

But the main property of emoxipin is the ability to inhibit lipid peroxidation when exposed to low temperatures, proven by the in vivo experiment, increasing the activity of the components of the antioxidant system to a greater extent than tocopherol acetate under similar conditions, which allows emoxipine to go to the number of cryoprotectants .

Among the promising antioxidant drugs used to prevent the harmful effects of cold on the body isotiorbamine (TB-6) has proved to bewell-established. It was synthesized at the OdessaStateUniversity by Candidate of Chemical Sciences S.G. Soboleva, under the leadership of Academician A.V. Bogotsky, with the participation of the Doctor of Chemical Sciences L.A. Litvinova. Isothirobamine is a pyrimidine derivative with pronounced antihypoxic properties and is a white powder with a weak specific odor, highly soluble in water and 95% ethyl alcohol [43,44].

In the course of a comprehensive study of the effect of the drug, it was found that due to its membrane-stabilizing action under conditions of general body cooling, isothiobamine prevents damage to ultrastructural elements of cardio-myocytes, reducing the cost of adaptation to cooling and thus preventing hemodynamic disturbances. In addition, changes in the morphology of the nervous system of the lungs under the influence of cold stress with simultaneous administration of TB-6 were less expressed [17].

It was established that the introduction of TB-6 in the experiment reliably prevents the development of hypercoagulability as a result of cold stress in experimental animals [17]. The obtained results indicate that TB-6 inhibits the blood coagulation, causes hypofibrinogenemia, activates the fibrinolysis system, increases the anti-coagulant activity of the blood. In the study of platelets under the influence of TB-6, antiadherent, antiaggregational and disaggregating effects of the drug were revealed. In some cases, in comparison with heparin, TB-6 causes less coarse changes in the coagulation system. Another advantage of the anticoagulant effect of TB-6 is a longer duration of action (6-fold) and the possibility of using different ways of administration. An increase in the dose from 10 to 50 mg / kg does not lead to an increase in hypocoagulation [13,14].

In addition, the use of TB-6 as a means of preventing cold stress increases the level of regenerative processes in bone tissue, reduces the risk of development of periosteal reactions with cold bone damage in the experiment [9, 15, 16].

Very promising means of anti-ischemic tissue protection are prostaglandin preparations, such as vasaprostan and iloprost [24]. Iloprost has already proved itself to be quite well in the treatment of freezing [32, 42].

The most promising means are bioflavonoid compounds. AmetisOOO (Blagoveshchensk, the Amur Region) receives dihydroquercetin (taxifolin) from Amur larch. The abbreviation is DQU. This compound according to the world literature has a powerful antioxidant effect. For the first time, information on the preparation of dihydroquercetin dates back to 1948, when Pew J.C. [60] published data that from the bark of trees belonging to the hardwood, it is possible to obtain compounds called flavonoids (dihydroquercetins). In Russia, Professor N.A. Tyukavkina in the 60's in the Irkutsk Institute of Chemistry namedafter A.E.Favorsky SB AS received dihydroquercetin (DQU) from Daurian larch.

In addition to the powerful antioxidant effect, dihydroquercetin has a rather pronounced effect in the inactivation of cytotoxic substances. [43, 44].

I would like to emphasize the report on the increase in the effect of antitoxic action of DQU in relation to acetaldehyde (the first product in the chain of processing the ethyl alcohol) by the body [31].

In addition to these effects, dihydroquercetins have an antidiabetic effect - they reduce the sugar content in the blood. [31].

Dihydroquercetins according to the literature have the ability to reduce the content of low density lipoproteins (forerunners of the atherosclerotic process) in the blood plasma and in the liver. [43].

It was noted that DQU have antitumor effect [33,34].

Flavonoid dihydroquercetin obtained from Daurian larch has anti-radiation, antiviral and immunoregulatory effects.

As it can be seen from the literature data, the application points of dihydroquercetins (DQU) are diverse. This information allows to use DQU as a basis for the creation of food additives (FA), as a material for the production of pharmaceuticals with a wide range of preventive and curative properties.

Preliminary experimental studies at the Department of Histology and Biology of the AmurMedicalAcademy have shown that dihydroquercetin obtained from Amur larch really has powerful antioxidant activity (AOA) in cold damage [28-49].

Taking into account the literature data, including the works carried out to determine AOA of DQU it is necessary to conduct research on further studying the properties of DQU received from Amur larch.

From the experimental directions it would be desirable to evaluate the effect of DQU on coagulation and aggregation hemostasis, on the thrombocytopoietic activity of the bone marrow under various models of induction of lipid peroxidation.

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