Research Results in Pharmacology

Research Results in Pharmacology 4(4): 41–52
UDC: 615.262
DOI 10.3897/rrpharmacology.4.31880

Research Article

Effects of Dimephosphone on skin survival in conditions of reduced blood circulation

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Academic editor: *Tatyana Pokrovskaya* ♦ **Received** 20 September 2018 ♦ **Accepted** 26 October 2018 ♦ **Published** 6 December 2018

Citation: Galenko-Yaroshevsky PA, Nefedov DA, Zelenskaya AV, Pavlyuchenko II, Chuyan EN, Ravaeva MY, Tkharkakhova NK (2018) Effects of Dimephosphone on skin survival in conditions of reduced blood circulation. Research Results in Pharmacology 4(4): 41–52. https://doi.org/10.3897/rrpharmacology.4.31880

Abstract

Introduction: The search for and creation of drugs with dermatoprotective and metabotropic activity is one of the priorities of modern diabetology. Synthetic organophosphorus compounds with no anticholinesterase activity, to which Dimephosphone belongs to, deserve great attention in this respect.

Materials and Methods: Experiments included 355 white non-linear male mice (18-34 g) and 799 male rats (150–305 g). The dermatoprotective activity (DPA) of Dimephosphone regarding the survival of a skin graft was studied against the background of normoglycemia, as well as against the background of experimental diabetes complicated by hypercholesterolemia. The study of microhemodynamics in the skin was performed using laser Doppler flowmetry. The effects on metabolic processes and the antioxidant system were studied by determining the levels of glucose, urea, creatinine, total bilirubin, total cholesterol, triglycerides, total protein, albumin, globulin, catalase, malondialdehyde, superoxide dismutase, glutathione reductase, glutathione and glutathione peroxidase.

Results: Dimephosphone has a pronounced DPA in conditions of reduced blood circulation against the background of normoglycemia and experimental (alloxan) diabetes complicated by exogenous hypercholesterolemia. By DPA, in most cases against the background of normoglycemia Dimephosphone exceeds Actovegine, is comparable to or inferior to Trental and Mexidol, and is more significant in terms of the therapeutic width than all the drugs taken for comparison.

Discussion: According to the obtained data , DPA of Dimephosphone may be due to its ability to exhibit significant vasodilating, antihypoxic, antioxidant, antiaggregant, membrane-stabilizing, anti-acidotic, antimicrobial and other properties and also to exert a normalizing effect on carbohydrate, protein, lipid and energy metabolism

Conclusion: Dimephosphone can be recommended for further preclinical and clinical studies in the form of various dosage forms, as well as in a combination therapy for metabolic disorders.

Keywords

dimephosphone, skin, reduced blood circulation, experimental (alloxan) diabetes, exogenous hypercholesterolemia.

Introduction

Diabetes mellitus (DM) is one of the serious socially significant diseases characterized by a high prevalence rate, a tendency to an increased frequency and severity of complications, and, thus, high disability and patient mortality rates (Vasan et al. 2012). To date, there are more than 175 million patients with diabetes in the world, and this number is expected to increase to 300 million by 2025.

Diabetes can cause a number of major complications, among which micro- and macro-angiopathies are quite frequent: changes in small vessels (capillaries, venules, arterioles) are specific for diabetes, and vascular lesions of medium and large vessels are regarded as manifestations of early and widespread atherosclerosis (Schlienger 2013). Microangiopathy is most frequently localized in eyes (retinopathy), kidneys (nephropathy) and peripheral nerves (neuropathy), whereas the macroangiopathies are localized in the heart (IHD, myocardial infarction), brain (acute and chronic impairment of cerebral circulation) and lower extremities (diabetic foot, gangrene) (Galenko-Yaroshevsky and Tegay 2016, Levy and Zeichner 2012, Roustit et al. 2016). In addition, diabetes often leads to the development of hypoxic states, impaired blood clotting and cellular immunity, aggravation of inflammatory processes, slow and complex wound healing (Domingueti et al. 2016)

It is generally recognized that the activation of free radical oxidation processes is important in the pathogenesis of diabetes (Faria and Persaud 2017, Shah and Brownlee 2016, Wada and Nakatsuka 2016). This fact served as the basis for the use of antioxidant drugs (Tocopherol, α-lipoic acid, Nicotinamide, Mexidol, etc.) in the complex treatment of the relevant category of patients (Gupta et al. 2012).

According to this, the most important task of modern pharmacology is the search for, the creation of and introduction into healthcare practice of highly active drugs that can selectively affect the main pathogenetic links of diabetes and ensure the prevention and correction of its vascular complications (Zimmet and Bloomgarden 2012).

Synthetic organophosphorus compounds with no anticholinesterase activity, capable of exhibiting pronounced metabotropic properties, deserve great attention in this respect. Dimephosphone (1,1-dimethyl-3-oxobutyl-phosphonic acid dimethyl ester), which has low toxicity and multifunctional therapeutic properties: antioxidant, antihypoxic, antiaggregant, anti-inflammatory, wound-healing, membrane-stabilizing, vasodilating, immunocorrective, bacteriostatic and others (Rodina and Moiseeva 2011, Valeeva et al. 2010, 2011) belong to this class. At present, the use of multi-target drugs (with several types of pharmacological activity) is quite relevant, as it makes it possible to achieve a higher level of clinical effect with fewer side effects, when compared with mono-target drugs (Lera and Ganesan 2016).

To date, there is a large arsenal of drugs for the treatment of diabetic foot syndrome (hypoglycemic drugs, B vitamins, antioxidants, antihypoxants, analgesics, antiplatelet drug, anticoagulants, antiseptics, broad-spectrum antibiotics, etc.), but many of them do not always meet the requirements imposed on them: they can cause various side effects, and sometimes do not show a proper therapeutic effect, which makes a surgical intervention necessary, including skin-plastic surgery (autodermoplasty, plastics with local tissues, combined skin plastics), up to a high limb amputation at the level of compensated blood flow.

Given the complexity and variety of complications arising from diabetes, it is very important to create new, more effective and less toxic drugs designed to improve the efficacy of skin-plastic surgery for DM. Based on this, it seemed appropriate to investigate the possible dermatoprotective activity (DPA) of Dimephosphone under conditions of experimental diabetes and exogenous hypercholesterolemia.

Materials and methods

The studies were carried out in accordance with the requirements of GOST ISO/IEC 1704-2009, GOST R ISO 5725-2002 and *The Rules of Laboratory Practice* approved by Order № 708n of the Ministry of Healthcare and Social Development of the Russian Federation of August 23, 2010, in compliance with *The European Convention for the Protection of Vertebrates Used for Experiments or Other Scientific Purposes* (Directive 2010/63/EU). The experiments were conducted in accordance with *The Guidelines for Pre-clinical Study of Medicinal Products* (2012).

The experiments included 355 white non-linear male mice (weighing 18–34 g) and 799 male rats (weighing 150–305 g) which had passed the quarantine and were kept in the standard vivarium conditions.

The effect of Dimephosphone and comparison drugs Actovegine, Trental and Mexidol with intravenous (iv) administration on the survival of the pedicle skin flap (SF) was studied on anesthetized (urethane 1100 and 1000 mg/kg, intraperitoneally, ip) white nonlinear male mice and male rats in a wide range of doses (Fig. 1).

Evaluation of the results of studying the effect of substances on the survival of SF was performed in mice 24 hours, and in rats – 72 hours after the surgery, using the following gradation: vastly increases (VsI; more than 50%), moderately increases (MI; from 18 to 50%), slightly increases (SI; from 15 to 18%).

The effect of Dimephosphone and comparison drugs Actovegine, Trental and Mexidol with their combined local and iv administration on the survival of pedicle SF was studied in the experiments on anesthetized (urethane 1100, ip) white non-linear male mice. SF was treated (by irrigation or lubrication) with Dimephosphone (15% solution for external use), Actovegine (5% officinal ointment), Trental (2% solution prepared on isotonic NaCl solution from an officinal concentrate) and Mexidol (5% solution).

Then, the test drugs (ampouled solutions) were administered at increasing doses twice iv (with a 4-hour interval): Dimephosphone – 12.5, 25, 50 and 75 mg/kg, Actovegine – 50, 100, 150 and 200 mg/kg, Trental – 2, 5, 5, 7.5 and 10 mg/kg, and Mexidol – 20, 30, 40 and 50 mg/kg. In the control experiments, an isotonic solution of NaCl was used at the rate of 10 ml/kg. Evaluation of the results of studying the effect of substances on SF survival was carried out 24 hours after modeling the SF using the following gradation: VsI (more than 50%), MI (from 18 to 50%), SI (from 15 to 18%), no increase, NI (±15%).

Investigation of the influence of Dimephosphone and comparison drugs Actovegin and Trental with their combined local and ip administration on SF engraftment was carried out in the experiments on anesthetized (urethane 1000, ip) white non-linear male rats. The test substances were used immediately after the SF simulation and over the next 19 days: initially SF was treated with Dimephosphone (irrigated with 15% solution for external use), Actovegine (smeared with 5% official ointment) and Trental (irrigated with 2% solution prepared with isotonic NaCl solution, from the official concentrate), and then the drug was administered (as ampouled solutions) at doses of 110, 410 and 25 mg/kg, corresponding to the effective average doses ($ED_{0.5}$) determined for rats when studying the effect of these drugs on pedicle SF survival. An isotonic solution of NaCl (control) was also applied locally (by irrigation) and ip (10 ml/kg/day). Each experimental group (3) of animals included 10 rats, control – 12. Evaluation of the SF survival rate was performed on the 5th, 10th, 15th and 20th day of the study. The area of SF necrosis (wound defect) after a scab coming off was measured planimetrically and expressed in %.

The effect of Dimephosphone on the pedicle SF survival against the background of experimental diabetes mellitus (EDM), complicated by exogenous hypercholesterolemia (EHC) was carried out in experiments on anesthetized (urethane 1000, ip) white non-linear male rats. The animals were randomized into 4 groups:

1 – intact rats – 50 individuals (outcome);

2 – animals in which type 1 EDM (Mozheiko 2013) and EHC were simulated, for which Alloxan tetrahydrate (Fluka-Sigma, Germany) was administered ip once at a dose of 135 mg/kg. After 2 weeks (after stable EDM was formed) and throughout the next 14 days, an oil cholesterol emulsion was injected at a dose of 40 mg/kg (in 0.5 ml of vegetable oil); In order to enhance peroxide stress, Ergocalciferol was added to it at the rate of 12,500 U/kg – 50 individuals (control);

3 – animals without EDM and EHC, which had received Dimephosphone at a dose of 100 mg/kg/day subcutaneously (sc) for 14 days – 40 individuals;

4 – rats with EDM and EHC, to which, simultaneously with the cholesterol emulsion, Dimephosphone was injected at a dose of 100 mg/kg/day sc – 50 individuals. During the preparation of the animals for the study of Dimephosphone DPA, their mortality in groups 1, 2, 3 and 4 was 0, 18, 2.5 and 8%, respectively.

To assess *the state of carbohydrate and lipid metabolism in EDM, complicated with EHC and the use of Dimephosphone*, one part of rats from groups 1, 2 and 4 (8 animals from each) on the 29th day of the experiment, after 16-17 hours of starvation, were euthanized by decapitation under ether anesthesia. Then, the animals' blood serum was used to determine the glucose content (by the glucose oxidase method using the standard Glyukoza-FKD reagents, Russia), total cholesterol (TC), triglycerides (TG), and high-density lipoprotein cholesterol (HDLC) (by means of standard reagents by Olvex on an analyzer OP-901, Finland). Very low density lipoprotein (VLDL) and low density lipoprotein (LDL) cholesterol, as well as atherogenic index (AI) was calculated by the generally accepted formulas.

The influence of Dimephosphone on microhemodynamics in the skin was carried out in the experiments on white non-linear male rats. The animals were divided into 2 groups. The rats of the *first group* served as a *control* (n=7); they were administered ip a 0.2 ml isotonic NaCl solution once a day. The animals of the *second group* (n=15) were ip administered with Dimephosphone at a dose of 100 mg/kg, which, according to the data obtained in the present research, is close to the average effective dose of this drug (113.8 mg/kg, iv), which causes a 50% dermatoprotective effect when simulating SF in the back of rats. The study of changes in the parameters of blood microcirculation (BM) in the skin was performed by laser Doppler flowmetry (LDF) by means of a laser blood flow analyzer LAKK-02 (Lazma, Russia) using the LDF 2.20.0.507WL software. The basal blood flow was determined within 6 minutes before and 2 hours after administration of isotonic NaCl solution (control) and Dimephosphone. As parameters analyzed by the LDF method, non-oscillatory indices of the basal blood flow were recorded: perfusion index (perfusion units; M – the steady component of perfusion), average standard deviation; coefficient of variation (%) (Krupatkin and Sidorov 2013). To identify the mechanisms underlying hemodynamic changes, the amplitude of blood flow oscillations of different frequency ranges was determined using the wavelet analysis of the LDF signal. Oscillations in the

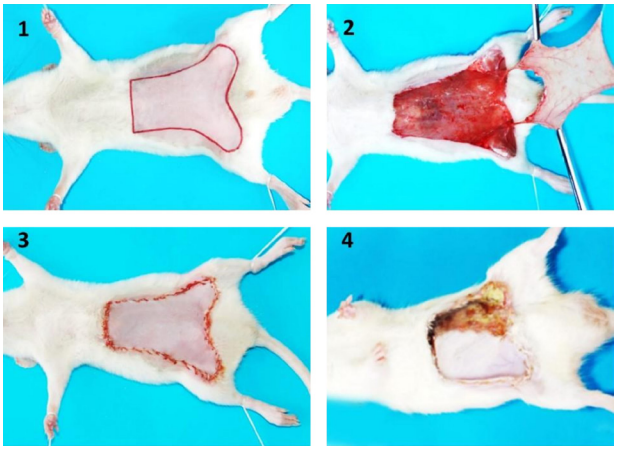

Figure 1. Scheme of skin flap modeling in rats.

range of 0.0095-0.02 Hz were taken as endothelial waves (Krupatkin and Sidorov 2013). Oscillations in the range of 0.07–0.15 Hz were interpreted as myogenic oscillations caused by the periodic activity of smooth muscle cells (vasomotion). Oscillations in the range of 0.02–0.046 Hz were considered as neurogenic oscillations, reflecting sympathetic regulatory activity (Hoffman et al. 1990). High-frequency oscillations included respiratory (0.15–0.4 Hz) and pulse (0.8–0.16 Hz) waves (Hoffman et al. 1990, Krupatkin and Sidorov 2013).

In-depth studies of the effect of Dimephosphone on the metabolic profile, indicators of oxidative stress and antioxidant systems under conditions of EDM complicated by EHC, and the presence of the pedicle SF was conducted in experiments on non-linear white male rat. EDM and EHC were created by daily intravenous administration of Alloxan tetrahydrate (Fluka-Sigma, Germany) to the animals at a dose of 50 mg/kg for 14 days and for the next 14 days by injecting oil cholesterol emulsion at a dose of 40 mg/kg (0.5 ml of vegetable oil). The studies were performed on 2 groups of animals, each of which included 3 subgroups consisting of *intact* (10 and 10 individuals), *control* (isotonic NaCl solution was injected; 8 and 9 individuals) and *experimental* (Dimephosphone 100 mg/kg/day was injected sc for 14 days; 9 and 10 individuals). In the *first group*, the indicators of glucose, urea, uric acid, creatinine, TC, TG, total protein, albumin, globulin, total bilirubin, and the activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined, with calculating the de Ritis ratio (AST/ALT), and in the second – the indicators of catalase, malonic dialdehyde (MDA), superoxide dismutase (SOD), glutathione reductase (GR), glutathione and glutathione peroxidase (GPO).

The effect of Dimephosphone on the rats' heparin plasma metabolic profile (concentration of glucose, urea, uric acid, creatinine, TC, TG, total protein, albumin, globulin, total bilirubin, ALT and AST activity with calculating the de Ritis ratio) was studied using Dia-Sys (Germany) and Cormay (Poland) standard commercial kits, and a Targa BT 3000 (Italy) automatic biochemical analyzer (when determining bilirubin).

The effect of Dimephosphone on indicators of oxidative stress (MDA) and antioxidant systems (SOD, catalase, GR, GPO, glutathione). The concentration of MDA was determined by the method of K. Yagi (1976), the activity of SOD – by a degree of inhibition of nitro-blue tetrazolium reduction by superoxide anion-radical generated in vitro in the xanthine-xanthine oxidase system, catalase on a Thermo Electron Evolution spectrophotometer (USA), while GR – by the Hosoda and Nakamura method (Hosoda and Nakamura 1970). The concentration of glutathione and its system components was also determined using a Thermo Electron Evolution spectrophotometer (USA).

Statistical processing of research results. The significance of differences in mean values was calculated using Student's t-test. The reliability of intergroup differences was assessed using the Mann-Whitney U-test at $p<0.05$. For all substances, $ED_{0.5}$ (Rajevski 1976) corresponding to an increase in the SF survival by 50% was determined graphically, and therapeutic indices, or the therapeutic width (TW – $LD_{50}/ED_{0.5}$), were calculated, using personal computer software developed at the Department of Pharmacology of Kuban State Medical University of the Ministry of Healthcare of the Russian Federation, licensed Microsoft® Office® Professional Plus 2013 and STATISTICA-8.0 software.

Results

Comparative DPA of Dimephosphone, Actovegine, Trental and Mexidol when they are administered iv.

Influence on the pedicle SF survival in experiments on mice. Dimephosphone (25, 50, and 75 mg/kg) on the 2nd day after a twofold (with an interval of 4 hours) iv injection shows a dose-dependent DPA. When using the drug at total doses of 50, 100 and 150 mg/kg, the length of the necrotic part (LNP) of SF was 55.0, 41.7 and 21.1% versus 69.4% in the control. The difference in the length of the necrotic parts (DLNP) of the control and experimental groups of animals was 14.1, 27.8 and 48.3%, the surviving part (SP; when comparing the necrotic parts of the SF in these groups) – 20.8, 40.0 and 69.6%, respectively. And at the first 2 doses (50 and 100 mg/kg), Dimephosphone caused an MI survival rate of SF, in the last (150 mg/kg) – a VsI survival rate (Fig. 2). The revealed regularity of Dimephosphone DPA made it possible to calculate $ED_{0.5}$, which amounted to 113.3 mg/kg.

Actovegine (100, 150, and 200 mg/kg), Trental (5, 10, and 15 mg/kg) and Mexidol (30, 40, and 50 mg/kg), taken as reference drugs, also showed a dose-dependent DPA in the accepted experimental conditions. When administering Actovegin at the total doses of 200, 300 and 400 mg/kg, the LNPs of SF amounted to 53.3, 35.0 and 17.0% versus 66.5% in the control. The DLNPs of SF in the control and experimental groups of animals were 13.2, 31.5 and 49.5%, SP – 16.8, 47.4 and 74.4%, respectively. At the same time, when administered at the first dose (200 mg/kg), Actovegin showed SI survival rates of SF, at the second (300 mg/kg) – MI, and at the third (400 mg/kg) – VsI; $ED_{0.5}$ of Actovegine was 313.2 mg/kg.

When Trental was used at the total doses of 10, 20 and 30 mg/kg, the LNPs of SF were 47.0, 36.7, and 21.0% versus 65.0% in the control. The DLNPs of SF in the control and experimental groups of animals were 18.0, 28.3 and 44.0%, SP – 27.7, 43.5 and 67.7%, respectively. At the same time, when administered at the first two doses (10 and 20 mg/kg), Trental caused MI of SF survival, at the last dose (30 mg/kg) – VsI; $ED_{0.5}$ was 21.9 mg/kg.

In cases of using Mexidol at the total doses of 60, 80 and 100 mg/kg, the LNPs of SF were 43.9, 32.5 and 25.0% versus 56.5% in the control. The DLNPs of SF in the control and experimental groups of animals were 12.6, 24.0 and 31.5%, SP – 22.3, 42.5 and 55.8%, respectively. When administered at the first two doses (60 and 80 mg/

kg), Mexidol induced MI for SF survival, at the latter (100 mg/kg) – VsI. However, it should be noted that the DPA of Mexidol at the doses of 80 and 100 mg/kg did not have statistically significant differences ($p>0.05$). On this basis, $ED_{0.5}$ Mexidol, equal to 91.7 mg/kg, is conditional.

When comparing the indicators of DPA ($ED_{0.5}$), acute toxicity (according to LD_{50} for mice in case of ip injection) and TW of the test drugs, it was found that by the first indicator Dimephosphone was 2.8 times superior to Actovegin, close to Mexidol, and 5.2 times inferior to Trental; by the second indicator – 9.5 and 7.0 times more significant (less toxic) than Trental and Mexidol, and almost comparable to Actovegin; by the third indicator – 5.6, 1.8 and 2.9 times superior to Mexidol, Trental and Actovegin, respectively (Table 1).

Thus, in experiments on mice, Dimephosphone by DPA is 2.8 times superior to Actovegine, close to Mexidol, and 5.2 times inferior to Trental; and by TW it is 5.6, 1.8 and 2.9 times more significant than Mexidol, Trental and Actovegine.

The effect on the pedicle SF survival in experiments on rats. Dimephosphone (25, 50 and 75 mg/kg), Actovegine (100, 150 and 200 mg/kg), Trental (5, 10 and 15 mg/kg) and Mexidol (30, 40 and 50 mg/kg) 3 days after a twofold (with an interval of 4 h) intravenous injections also showed a dose-dependent DPA. When using Dimephosphone at the total doses of 50, 100 and 150 mg/kg, LNPs of SF were equal to 48.2, 36.7 and 24.7% versus 66.5% in the control. DLNPs of SF in the control and experimental groups of animals were 18.3, 29.8 and 41.9, SP – 27.5, 44.8 and 62.9%, respectively; and at the first 2 doses (50 and 100 mg/kg) the drug induced MI for SF survival, and at the latter (150 mg/kg) – VsI (Fig. 3); $ED_{0.5}$ was 113.8 mg/kg.

Based on the data obtained, it was of interest to investigate DPA of Dimephosphone at higher doses of 200 and 250 mg/kg. It turned out that the drug at these doses also showed DPA, but it did not differ much from that in the study at a dose of 150 mg/kg: at doses of 200 and 250 mg/kg, LNPs of SF were 25.6 and 26.1% against 69.2% in

Table 1. DPA of Dimephosphone, Actovegine, Trental and Mexidol (1 day after double intravenous drug injection) when modeling SF in the mice back area

Drug	DPA		Acute toxicity (mice)[1]		TW	
	$ED_{0.5}$, mg/kg	Relative	LD_{50}, mg/kg	Relative	Absolute	Relative
Dimephosphone	113.3	0.8	3000.0[2]	7.0	26.5	5.6
Actovegine	313.2	0.3	2854.6[3]	6.6	9.1	1.9
Trental	21.9	4.2	316.0[4]	0.7	14.4	3.1
Mexidol	91.7	1.0	430.0[5]	1.0	4.7	1.0

Note: DPA – dermatoprotective activity; TW – therapeutic width
[1]ip injection. [2]Arbuzov et al. (1968). [3]Gorovoy (1991). [4]Tegay (2004). [5]Gavrilova (2001).

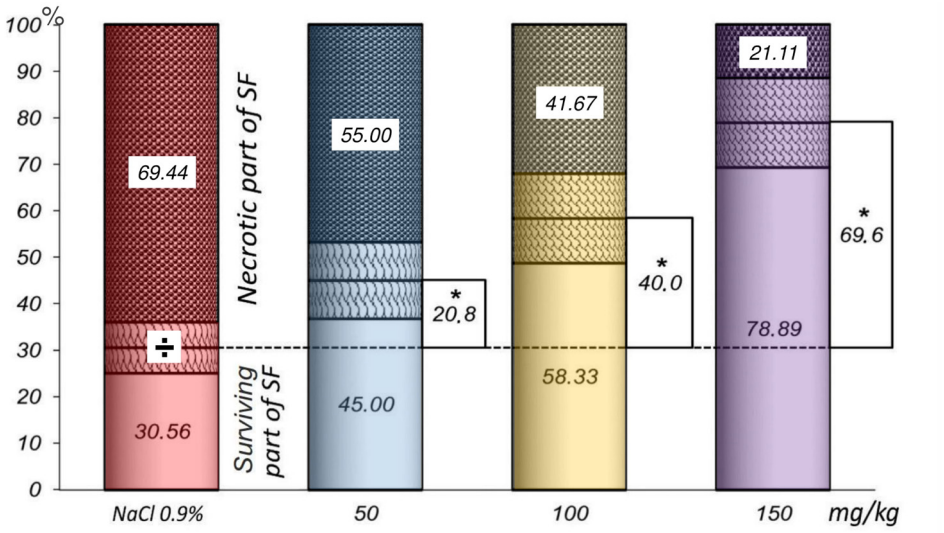

Figure 2. The ratio of the necrotic and surviving parts of the pedicle SF (in the back) in mice 1 day after a twofold intravenous injection of isotonic NaCl (control) and Dimephosphone with an interval of 4 h at total doses of 50, 100 and 150 mg/kg
Note. On the ordinate axis – the length of the SF, taken as 100%, on the abscissa axis – isotonic NaCl solution and the total dose of the drug. Bar graphs: shaded (dark) area – necrotic part of SF, light area – surviving part, wavy area – confidence boundaries (÷) at $p<0.05$; the numbers on the right side of the columns are the indices of the surviving part of SF when using drugs compared to the control. *($p<0.05$)

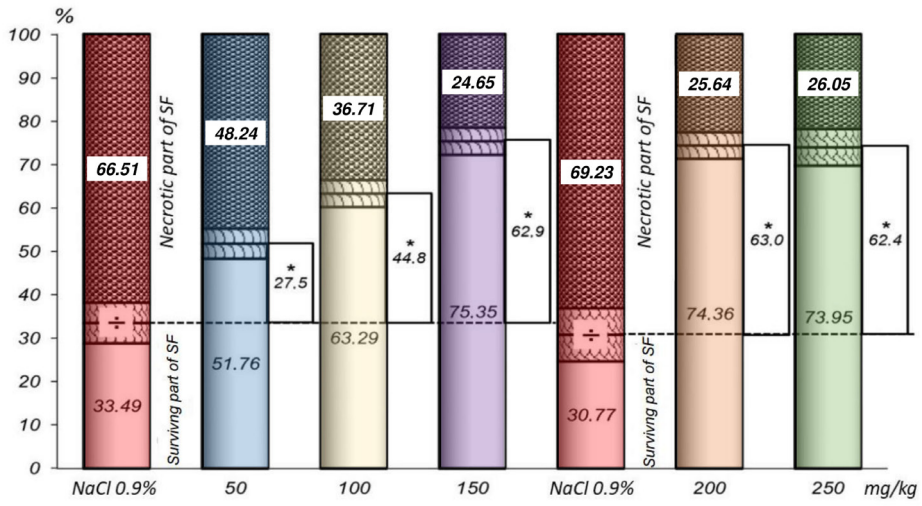

Figure 3. The ratio of the necrotic and surviving parts of the pedicle SF (in the back area) in rats 3 days after double iv administration of isotonic NaCl solution (control) and Dimephosphone with an interval of 4 hours at total doses of 50, 100, 150, 200 and 250 mg/kg
Note. On the ordinate axis – the length of the SF, taken as 100%, on the abscissa axis – isotonic NaCl solution and the total dose of the drug. Bar graphs: shaded (dark) area – necrotic part of SF, light – surviving part, wavy – confidence boundaries (÷) at $p<0.05$; the numbers on the right side of the columns are the indices of the surviving part of SF when using drugs compared to the control. * ($p<0.05$)

the control, DLNPs of SF in the control and experimental groups of animals and SP of SF were 43.6 and 63.0, 43.2 and 62.4%, respectively, and corresponded to VsI gradation.

In cases of applying Actovegine at the total doses of 200, 300 and 400 mg/kg, LNPs of SF were 46.0, 36.9 and 30.5% versus 62.9% in the control. DLNPs of SF in the control and experimental groups of animals were 17.0, 26.1 and 32.5%, SP – 27.0, 41.4 and 51.6%, respectively; and at the first 2 doses (200 and 300 mg/kg) the drug caused MI of the SF survival, and at the latter (400 mg/kg) – VsI; $ED_{0.5}$ was 408.2 mg/kg.

Like the case of Dimephosphone, it was of interest to investigate DPA of Actovegine at higher doses of 500 and 600 mg/kg. It is established that at these doses the drug also had DPA; however, it was close to that when using the drug at a dose of 400 mg/kg: at doses of 500 and 600 mg/kg, LNPs of SF were 27.8 and 25.0% versus 64.4 % in the control. DLNPs of SF in the control and experimental group and the SP of SF were 36.6 and 56.8, 39.4 and 60.3%, respectively, which corresponded to VsI gradation.

When using Trental at the total doses of 10, 20 and 30 mg/kg, LNPs of SF were 47.5, 35.8 and 30.2% versus 68.9% in the control. DLNPs of SF in the control and experimental groups of animals amounted to 21.4, 33.0 and 38.6%, and SP – 31.0, 47.9 and 56.1%. At the same time, when administered at the first 2 doses (10 and 20 mg/kg), the drug induced MI for SF survival, and at the last (30 mg/kg) – VsI; $ED_{0.5}$ was 24.0 mg/kg.

In cases of using Mexidol at the total doses of 60, 80 and 100 mg/kg, LNPs of SF were 46.7, 37.9 and 28.2% versus 66.0% in the control. At the same time, DLNPs of SF in the control and experimental groups of animals were 19.3, 28.2 and 37.8%, and SP – 29.2, 42.6 and 57.3%. At the first 2 doses (60 and 80 mg/kg), the drug caused MI for SF survival, and at the latter (100 mg/kg) – VsI; $ED_{0.5}$ was 90.0 mg/kg.

The comparison of the DPA of the test substances in the experiments on rats showed that Dimephosphone by its activity ($ED_{0.5}$) was 3.6 times superior to Actovegin, but 4.7 and 1.3 times inferior to Trental and Mexidol, and by TW it was 3.8, 2.0 and 5.5 times more significant than Actovegine, Trental and Mexidol, respectively (Table 2).

Thus, in the experiments on rats, Dimephosphone by DPA is 3.6 times superior to Actovegine, and 4.7 and 1.3 times inferior to Trental and Mexidol, and by TW it is 3.87, 2.0 and 5.5 times more significant than Actovegine, Trental and Mexidol, respectively.

Comparative DPA of Dimephosphone, Actovegine, Trental and Mexidol with their combined local and parenteral use.

The effect on the survival of pedicle SF in experiments on mice (iv injection). Studies of the effect of Dimephosphone, Actovegine, Trental and Mexidol on the survival rate of pedicle SF in mice 24 hours after combined local and iv application showed that all the drugs have a dose-dependent DPA. Thus, with irrigating SF with a 15% solution of Dimephosphone and its intravenous injection at the total doses of 25, 50, 100 and 150 mg/kg, LNPs of SF were 48.2, 42.5, 29.4 and 22.9% versus 59.0% in control. DLNPs of SF in the control and experimental groups of animals were 10.8, 16.5, 29.6 and 36.1%, SP of SF – 18.3, 28.0, 50.1 and 61.3 %. The drug in the first combined (local and iv) administrtion showed SI survival of SF, in the second – MI, and in the third and fourth –

Table 2. Dermatoprotecting activity of Dimephosphone, Actovegine, Trental and Mexidol (*1 day after double intravenous drug administration*) when modeling SF in the back area in rats

Drug	DPA		Acute toxicity (mice)[1]		TW	
	$ED_{0.5}$, mg/kg	Relative	LD_{50}, mg/kg	Relative	Absolute	Relative
Dimephosphone	113.8	0.8	3000.0[2]	7.0	26.4	5.5
Actovegine	408.2	0.2	2854.6[3]	6.6	7.0	1.5
Trental	24.0	3.8	316.0[4]	0.7	13.2	2.8
Mexidol	90.0	1.0	430.0[5]	1.0	4.8	1.0

Note: DPA - dermatoprotective activity – TW - therapeutic width
[1]ip injection. [2]B.A. Arbuzov et al. (1968). [3]Gorovoy (1991). [4]Tagay (2004). [5]Gavrilova (2001).

VsI. Based on the fact that the dermatoprotective effects of Dimephosphone in the last two combined applications did not have statistically significant differences ($p>0.05$), the data of the first three combined applications were used to calculate its $ED_{0.5}$ (Fig. 4); $ED_{0.5}$ was 100.1 mg/kg.

When 5% Actovegine ointment was used to irrigate SF and Actovegine was administered iv at the total doses of 100, 200, 300 and 400 mg/kg, LNPs of SF were 55.5, 44.6, 33.9 and 21.4% versus 61.1% in control. DLNPs of SF in the control and experimental groups of animals were 5.6, 16.6, 27.2 and 39.8%, and SP of SF – 9.2, 27.1, 44.5 and 65.1%. In its first combined use, the drug induced NI in the survival rate of SF, in the second and third – MI, in the fourth – VsI; $ED_{0.5}$ was 323.2 mg/kg.

In experiments with the use of Trental by irrigating SF with its 2% solution and its iv administration at the total doses of 5, 10, 15 and 20 mg/kg, LNPs of SF were 51.5, 37.2, 29.0 and 21.2% versus 58.3% in control. DLNPs of SF in the control and experimental groups of animals were 6.8, 21.2, 29.3 and 37.3%, and SP of SF – 11.7, 36.2, 50.3 and 63.9%, and in the first combined use, the drug had NI impact on the SF survival rate, in the second induced MI, in the third and fourth – VsI; $ED_{0.5}$ was 15 mg/kg.

When a 5% solution of Mexidol was used to irrigate SF and Mexidol was administered iv at the total doses of 40, 60, 80 and 100 mg/kg, LNPs of SF were 56.2, 42.3, 29.6 and 22.3% versus 51.5 % in control. DLNPs of SF in the control and experimental groups of animals were -5.1, 8.8, 21.5 and 28.7%, and SP of SF was -9.9, 17.2, 42.1 and 56.3%. In the first combined use, the drug had NI impact on the survival rate of SF, and in the second, third and fourth, respectively, – SI, MI and VsI; ED0.5 was 91.8 mg/kg.

Comparison of DPA (according to $ED_{0.5}$), acute toxicity (according to LD_{50} for mice with ip injection) and TW of the test drugs revealed that by the first indicator

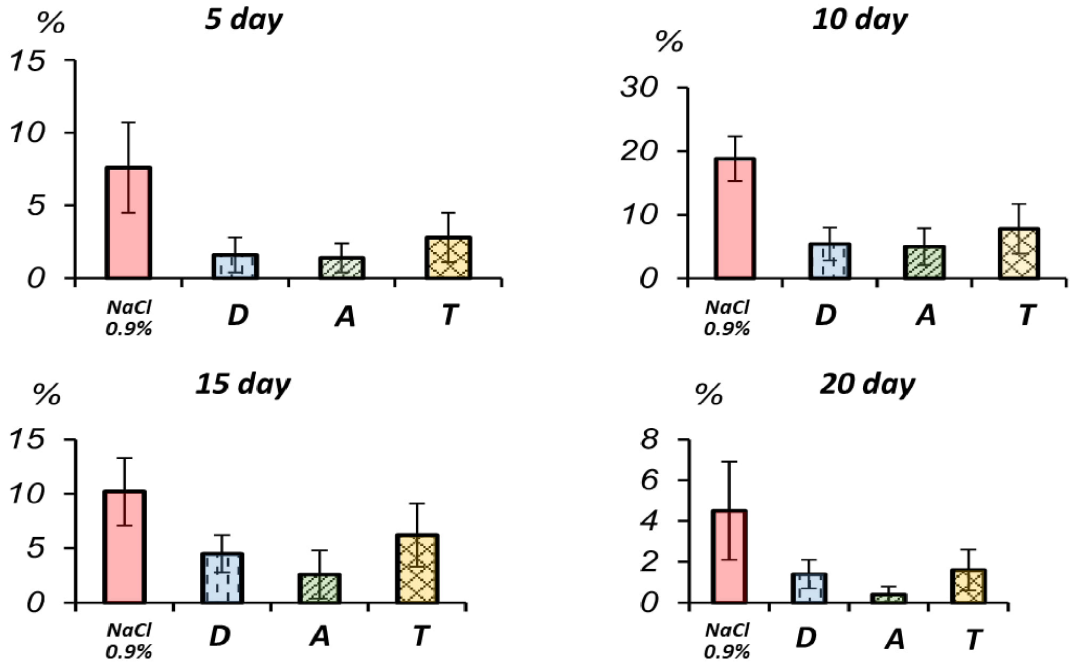

Figure 4. The effect of Dimephosphone, Actovegine and Trental on the engraftment of free SF in experiments on rats.
Note. On the ordinate axis – the area of SF necrosis on the 5th, 10th, 15th and 20th day after the operation; on the abscissa axis – isotonic NaCl solution, Dimephosphone (D), Actovegine (A), Trental (T).

Dimephosphone is 3.2 times superior to Actovegine, close to Mexidol and 6.7 times inferior to Trental; by the second indicator – 9.5 and 7.0 times more significant (less toxic) than Trental and Mexidol, and practically comparable to Actovegine; by the third – 6.4, 1.5 and 3.4 times superior to Mexidol, Trental and Actovegine, respectively (Table 3).

It was also important to compare DPA and TW of Dimephosphone, Actovegin, Trental and Mexidol (Table 3) with the same indicators presented in Table 1, when the test drugs were used parenterally (iv injection twice with an interval of 4 hours) in the experiments on mice. It turned out that only in the combined use of Trental, its $ED_{0.5}$ and TW significantly changed: the first indicator decreased 1.5 times, and the second increased 1.4 times. As for Dimephosphone, Actovegine and Mexidol, in case of their combined use, $ED_{0.5}$ and TW compared with those in case of iv injection did not undergo significant changes.

A statistically significant increase in DPA of Trental and practically absence of such in Dimephosphone, Actovegine and Mexidol in their combined local and iv administration, unlike in case of solely iv administration may be due to a higher rate of absorption and the lipophilicity of Trental when it is applied on SF in contrast to the other test drugs.

Thus, in the experiments on mice, Dimephosphone and comparison drugs Actovegine, Trental, and Mexidol, in their combined application – with a single local and double (with an interval of 4 hours) iv administration – 24 hours after the simulation of a pedicle SF increased SF survival. By their DPA, the test drugs can be arranged as follows: Trental>Dimephosphone=Mexidol>Actovegine, and by TW – Dimephosphone>Trental>Actovegin>Mexidol.

Trental, unlike Dimephosphone, Aktovegin and Mexidol, when combined, exhibits a more significant DPA than when administered solely iv.

Influence on engraftment of free SF in experiments on rats (with ip administration). The areas of SF necrosis (wound defect) which appeared after a scab coming off) on the 5th, 10th, 15th and 20th days of the study in the control (isotonic NaCl solution topically and ip) were 7.6, 18.8, 10.2 and 4.5%, and with a similar use of Dimephosphone (15% solution topically + ip 110 mg/kg/day), Actovegin (5% ointment topically + ip 410 mg/kg/day) and Trental (2% solution topically + ip 25 mg/kg/day) – 1.6, 5.4, 4.5 and 1.4%, 1.4, 5.0, 2.6 and 0.4%, 2.8, 7.8, 6.2 and 1.6 %, respectively, i.e. compared with the control, taken as 100% for all the study periods, SF necrosis under the influence of the drugs decreased by 21.1, 28.7, 44.1 and 31.1%, 18.4, 26.6, 25.5 and 8.9%, 36.8, 41.5, 60.8 and 35.6%, respectively; in all the study periods, the differences between the control and experimental indicators were statistically significant (Fig. 3).

When comparing Dimephosphone, Actovegine and Trental by their ability to increase the engraftment of free SF, it was found that on the 5th and 10th days of the study they had no statistically significant differences. On further study dates, Actovegine was the leader, which on the 15th day increased SF engraftment more significantly than Trental and was comparable to Dimephosphone, and on the 20th day exceeded both Trental and Dimephosphone in this regard (Fig. 4).

Thus, in the experiments on rats, Dimephosphone and reference drugs Actovegine and Trental, with local and ip administration for 20 days, increased engraftment of free SF and epithelization of the wound defect (after a scab coming off) on the 15th and 20th days of the study, while the drugs can be arranged in the following order: on the 15th day – Actovegine>Trental=Dimephosphone, on the 20th day – Actovegine>Dimephosphone=Trental. Dimephosphone, unlike Actovegine and Trental, prevents the development of suppuration in the area of free SF.

Investigation of the state of carbohydrate and lipid metabolism in EDM complicated by EHC and the use of Dimephosphone.

In the blood serum of the control rats, the levels of glucose, TC, TG, VLDL and LDL, compared with the corresponding indicators of the intact animals, increase by 226.7, 86.3, 35.6, 33.3 and 94.5%, respectively, and HDLC decreases by 57.3%, while AI increases by 356.3%.

The administration of Dimephosphone to rats (100 mg/kg/day for 14 days, sc) against the background of EDM and EHC had a normalizing effect on the state of carbohydrate and lipid metabolism. Thus, the indicator of glucose in the serum of rats did not differ much from that of the intact animals and was 67.8% lower than the

Table 3. Comparative DPA of Dimephosphone, Actovegine, Trental and Mexidol 24 hours after the combined single local and double (with an interval of 4 hours) iv administration

Drug	DPA		Acute Toxicity (mice)[1]		TW	
	$ED_{0.5}$, Mg/kg	Relative	LD_{50}, mg/kg	Relative	Absolute	Relative
Dimephosphone	100.1 (113.3)	0.9 (0.8)	3000.0[2]	7.0	30.0 (26.5)	6.4 (5.6)
Actovegine	323.2 (313.2)	0.3 (0.3)	2854.6[3]	6.6	8.8 (9.1)	1.9 (1.9)
Trental	15.0 (21.9)	6.0 (4.2)	316.0[3]	0.7	20.0 (14.4)	4.3 (3.1)
Mexidol	91.8 (91.7)	1.0 (1.0)	430.0[3]	1.0	4.7 (4.7)	1.0 (1.0)

Note: In brackets – DPA and TW of the test drugs 24 hours after the double dose (with an interval of 4 hours) iv administratino to the mice. [1]ip injection. [2]Arbuzov et al. (1968). [3]Galenko-Yaroshevsky et al. (2003).

corresponding indicator of the control rats. The indicators of lipid metabolism were characterized by the following changes; the levels of TC, TG, VLDL and LDL were close to those of the intact animals (2.29, 0.67, 0.31 and 1.31 mM/L versus 2.05, 0.59, 0.27 and 1.03 mM/L, respectively), although they remained statistically significantly elevated, and when compared with the corresponding indicators of the control rats significantly – decreased by 40.1, 16.3, 13.8 and 56.8%, respectively. The level of HDL in relation to that of the intact animals was statistically significantly reduced (0.68 mM/L versus 0.75 mM/L), and increased by 58.1% compared to that in the control rats. AI with respect to that of the intact animals remained statistically significantly elevated (2.38 vs. 1.74), and decreased by 70.0% compared with the corresponding index of the control rats.

Thus, in EDM complicated by ECH, considerable disturbances of carbohydrate and lipid metabolism are observed in rats, while Dimephosphone has a significant corrective effect on them.

Effect on microhemodynamics in rats' skin.

In the animals of the control group after the administration of an isotonic NaCl solution, no significant changes in the BM indices in the skin were observed. At the same time, with the administration of Dimephosphone, there were significant changes in both oscillatory and non-oscillatory indices of cutaneous microhemodynamics compared with those obtained in the control group before and after the administration of an isotonic NaCl solution to the experimental group of rats before the administration of Dimephosphone.

Among the oscillatory parameters, the amplitudes of endothelial (Ae; by 74%, $p<0.05$) and neurogenic (An; by 57%, $p<0.05$) genesis increased most significantly relative to the values of these indicators recorded in the animals of the control group after administration of isotonic NaCl solution. Since Ae is synchronized with periodic release of nitric oxide (NO) by the vascular endothelium, an increase in this indicator clearly shows an increase in the secretion of NO by the endothelium and, as a result, the development of endothelium-dependent vasodilation.

An increase in fluctuation amplitudes of LDF-grams in the neurogenic range (An), which are associated with adrenergic effects on the smooth muscle cells (SMC) of arterioles and arteriolar areas of arteriovenular anastomoses, reflects a decrease in peripheral resistance in these areas of the microbed, resulting in an improvement in nutritive blood flow.

In addition to Ae and An, after the administration of Dimephosphone, the amplitudes of myogenic rhythms (Am; by 24%, $p<0.05$) reliably increased in relation to the values of these indicators recorded in the animals of the control group, which reflects a decrease in the tone of precapillary sphincters and metarteriols (Stefanovska and Bracic 1999). It is known that the rhythms of this range are caused by fluctuations in the Ca^{2+} concentration through SMC membranes (Stefanovska and Bracic 1999); therefore, an increase in Am indicates a decrease in the tone of the precapillaries due to the development of Ca^{2+}-dependent muscular relaxation under the influence of Dimephosphone.

Against the background of the stimulation of the active components of regulating the skin microcirculation, an increase in the amplitudes of pulse oscillations (An, by 16%, $p<0.05$) occurred relative to the values of these indicators recorded in the control animals. This indicator reflects the perfusion pressure in microvessels, caused both by cardiac output, changes in systolic and diastolic pressure, and by the influence of postcapillary resistance (Stefanovska and Bracic1999). Consequently, an increase in this indicator proves that under the influence of Dimephosphone, the inflow of arterial blood into the micro-bed increases.

It is worth noting that Dimephosphone did not significantly influence the amplitude of the respiratory rhythms (Ar), which are associated with respiratory modulation of the venular blood flow and respiratory effects on the vegetative support of the heart (Stefanovska and Bracic1999). At the same time, the constant level of Ar against the background of decreased tone of resistive microvessels (increase in Ae, An, Am) and the corresponding increase in the blood inflow to the micro-bed (increase in An) reflects an increase in the arteriovenous pressure gradient in the micro-bed, which indirectly indicates the optimization of venular outflow.

These changes in microhemodynamics were reflected in the increased non-oscillatory indices of the basal blood flow: perfusion rate – by 42% ($p<0.05$), standard deviation – by 33% ($p<0.05$) and coefficient of variation – by 46% ($p<0.05$) relative to the values of these indicators recorded in the animals of the control group, which indicates an increase in blood perfusion and modulation of blood flow in all frequency ranges.

Thus, in the experiments on rats, Dimephosphone causes changes in the oscillatory and non-oscillatory indices of microhemodynamics in the skin, which indicates that it increases perfusion of peripheral tissues and modulation of blood flow in the microvessels by increasing the functional activity of the endothelium, reducing peripheral resistance, increasing blood flow to the nutritive microvascular system, and improving the venular outflow.

Studies of the effect of Dimephosphone on the metabolic profile, indicators of oxidative stress, antioxidant system in the blood of rats with pedicle SF in the back area and EDM complicated by EHC.

Effect on carbohydrate, lipid and protein exchanges, ALT and AST activity. In the blood of the control animals, which had been injected with isotonic NaCl solution (within 14 days, sc), the levels of glucose, urea, uric acid, creatinine, TC, TG, total bilirubin, ALT and AST increased statistically significantly compared to the intact animals by 390.3, 44.7, 10.3, 31.0, 91.1, 44.8, 92.9, 80.7 and 124.0%, respectively. The indicators of total protein, albumin and globulin decreased by 80.6, 81.0 and 80.1%, respectively.

The use of Dimephosphone (100 mg/kg/day for 14 days, sc) in accepted experimental conditions caused normalization of carbohydrate, lipid and protein metabolism in rats. Thus, the content of glucose, urea, uric acid and creatinine were almost the same as that of the intact animals and were by 76.8, 23.2, 7.0 and 19.4% lower than the corresponding indicators of the control rats.

The levels of TC and TG when using Dimephosphone compared with those of the intact rats were elevated (2.27 and 0.66 mm/L versus 2.02 and 0.58 mm/L, respectively), although in the case of TC, these differences were statistically insignificant, and in relation to the corresponding indicators of the control animals they were significantly lower by 41.2 and 21.4%, respectively. The idicators of total protein, albumin and globulin compared with those of the intact rats were almost comparable (65.36, 35.24 and 30.12 g/L versus 68.04, 35.23 and 32.80 g/L, respectively), and in relation to the indicators of the control animals, statistically significantly increased by 19.2, 23.4 and 14.6%. As for the total bilirubin, its level compared with that of the intact rats did not undergo significant changes (0.15 g/L versus 0.14 g/L), and in relation to the control data it significantly decreased by 44.4%.

The activity of ALT and AST in the blood serum of the intact animals was 32.4 and 146.5 U/L, and in the blood of control ones – 58.6 and 328.2 U/L, respectively, which may be due to significant differences (fluctuations) in individual indicators. This, obviously, affected the de Rittis Ratio, which in the first case was greater than 1 and amounted to 4.54, and in the second it turned out to be equal to 5.68, i.e. had increased.

After the use of Dimephosphone, the activity of ALT and AST compared to that of the intact rats was almost comparable (35.2 and 149.7 U/L versus 32.4 and 146.5 U/L, respectively), and when compared to the control indicators, they were statistically significantly lower – by 39.9 and 54.4%, respectively. At the same time, the de Rittis ratio compared with that of the intact animals did not undergo significant changes, while it was statistically significantly lower when compared to that in the control rats.

Thus, in the experiments on rats with pedicle SF in the back area, EDM and EHC, Dimephosphone has a normalizing effect on carbohydrate, lipid and protein exchanges, and also has (judging by the changes in ALT and AST) anti-inflammatory, antinecrotic, cardioprotective, and possibly hepatoprotective effects.

Discussion

Dimephosphone after double iv injection to mice and rats against the background of normoglycemia increases the survival rate of pedicle SF in the back area in a dose-dependent manner. In the experiments on mice, by its DPA, it exceeds Actovegin, is close to Mexidol, and is inferior to Trental; by its TW, it is more significant than Mexidol, Trental and Actovegin. In the experiments on rats, by its DPA, it exceeds Actovegine, is inferior to Trental and Mexidol; by its TW, it is more significant than Actovegine, Trental and Mexidol.

In case of the combined local (single) and iv (double) administration in the experiments on mice, Dimephosphone increases the survival rate of pedicle SF in the back area: by its DPA, it exceeds Actovegine, is comparable to Mexidol and inferior to Trental; by its TW, it is more significant than all the comparison drugs.

In the experiments on rats, Dimephosphone with repeated (20-day) local and ip administration promotes engraftment of free SF and wound epithelization. By its activity on the 15th and 20th days of the study, Dimephosphone is comparable to Trental and inferior to actovein. Unlike Actovegine and Trental, Dimephosphone, does not cause development of suppuration in the area of free SF.

With a single ip administration to rats, Dimephosphone increases tissue perfusion and modulation of blood flow in the skin microvasculature by increasing endothelial functional activity, reducing peripheral resistance, increasing blood inflow to the nutritive microvascular bed and improving venular outflow.

Taking into account its ability to promote engraftment of free SF under ESD conditions complicated by EHC, the DPA mechanism of Dimephosphone may be due to its inclusion in various homeostasis links and indirectly, at the level of the whole organism, to correct the functioning of disturbed local (myogenic and metabolic) and remote neurohumoral processes regulating the blood supply to the skin. An important role in promoting DPA in Dimephosphone against the background of EDM complicated by EHC is also played by its positive effect on carbohydrate, protein and lipid exchanges.

Conclusion

The results obtained suggest that Dimephosphone needs further studying as a drug that improves skin survival in conditions of reduced blood circulation against the background of diabetes mellitus, combined with atherosclerosis. Since the drug demonstrated a significant pharmacological activity in both systemic and topical administrations, the development of new dosage forms containing Dimephosphone is a promising direction. The high activity of the drug in case of metabolic disorders makes it possible to recommend it for preclinical studies in combination with insulin, oral hypoglycemic agents, statins, fibrates, etc.

Conflicts of interests

The authors state no conflict of interest concerning the present manuscript.

References

- Arbuzov BA, Vizel AO, Ivanovskaya KM (1968) Synthesis and new biological effects of organophosphorous compounds with low toxicity. Reports of the Academy of Sciences of the USSR [Doklady Akademii nauk SSSR] 182(1): 101-104. [in Russian]
- Domingueti CP, Dusse LMSA, Carvalho MdG, Sousa de LP, Gomes KB, Fernandes AP (2016) Diabetes mellitus: the linkage between oxidative stress, inflammation, hypercoagulability and vascular complications. Journal of Diabetes and its Complications 30(4): 738–745. https://doi.org/10.1016/j.jdiacomp.2015.12.018 [PubMed]
- Faria A, Persaud SJ (2017) Cardiac oxidative stress in diabetes: mechanisms and therapeutic potential. Pharmacology & Therapeutics 172:50–62. https://doi.org/10.1016/j.pharmthera.2016.11.013 [PubMed]
- Galenko-Yaroshevsky PA, Tegay AV (2016) The effect of diabenol on the survival of the skin graft under hyperglycemia. In: Spasov AA, Petrov VI (Eds) Antidiabetic Potential of Benzimidazoles: Chemistry, Pharmacology, Clinic. Publishing House of Volgograd State Medical Unievrsity, Volgograd, 334-354. [in Russian]
- Galenko-Yaroshevsky VP, Bagmetova EN, Filchukova IA (2003) Comparative research of influence of mexidol, emoxypine, α-tocopherol, pentoxyphillinum, actovegine and galidor on survival of skin in the conditions of the reduced blood circulation. Science of Kuban [Nauka Kubani] 3: 184-191. [in Russian]
- Gavrilova LV (2001) Influence of some antioxidants on hemostasis in experimental dislipidemiya, arterial hypertension and 2 type diabetes mellitus. PhD thesis. Saransk. Russia. University of Saransk, 48 pp. [in Russian]
- Gorovoy VI (1991) Influence of some derivatives of benzofuran, piperidine and carbonic acids on survival of skin in the conditions of the reduced blood circulation. PhD thesis. Rostov on Don, Russia. University of Rostov on Don, 45 pp. [in Russian]
- Grisolia S, Moore K, Luque J, Grady H (1969) Automatic procedure for the microestimation of 2,3-diphosphoglyceratr. Analytical Biochemistry 31(1): 235-245. https://doi.org/10.1016/0003-2697(69)90262-0
- Gupta R, Sharma AK, Mahesh C (2012) Antioxidant activity and protection of pancreatic β-cells by embelin in streptozotocine-induced diabetes. Journal of Diabetes 4(3): 248-256. https://doi.org/10.1111/j.1753-0407.2012.00187.x [PubMed]
- Hoffman U, Yanar A, Franzeck UK (1990) The freaquency histogram – a new method for the evaluation of laser Doppler flux motion. Microvascular Research 40(3): 293-301. https://doi.org/10.1016/0026-2862(90)90028-P [PubMed]
- Hosoda S, Nakamura W (1970) Role of glutathione in regulation of hexose monophosphate pathway in Ehrlich ascitec tumor cells. Diohimica et Biophysica Acta 222(1): 53-64. https://doi.org/10.1016/0304-4165(70)90350-8 [PubMed]
- Krupatkin AI, Sidorov VV (2013) Functional diagnostics of a condition of microcirculation and tissue systems. fluctuations, information, nonlinearity. Knizhnyy Dom Publishing House, Moscow, 496 pp. [in Russian]
- Lera de AR, Ganesan A (2016) Epigenetic polypharmacology: from combination therapy to multitargeted drugs. Clinical Epigenetics 8(1): 105. https://doi.org/10.1186/s13148-016-0271-9 [PubMed] [PMC]
- Levy L, Zeichner AJ (2012) Dermatologic manifestation of diabetes. Journal of Diabetes 4(1): 68-76. https://doi.org/10.1111/j.1753-0407.2011.00151.x [PubMed]
- Mozheyko LA (2013) Experimental models for studying diabetes mellitus. Part I. Alloxan diabetes. Magazine of the Grodno State Medical Universtity [Zhurnal Grodnenskogo Gosudarstvennogo Meditsinskogo Universiteta] 3: 26-29. [in Russian]
- Petrov VI, Spasov AA, Lenskaya KV, Chepelyaeva NI (2016) Scientific basis for the search for drugs to treat diabetes. In: Spasov AA, Petrov VI (Eds) Antidiabetogenic potential of benzimidazoles: chemistry, pharmacology, clinic. Publishing House of Volgograd State Medical Unievrsity, Volgograd. 40-57. [in Russian]
- Rajevski KS (1976) Pharmakologiya of neuroleptics. Medicine, Moscow, 272 pp. [in Russian]
- Rodina OP, Moiseeva IIa (2011) Comparison of stress protector activity of drugs with antioxidant activity (vitamin E, dimephosphone, reamberin) in rats. Russian Journal of Experimental and Clinical Pharmacology [Eksperimentalnaya i Klinicheskaya Farmakologiya] 74(7): 16-18. [in Russian] [PubMed]
- Roustit M, Loader J, Deusenbery C, Baltzis D, Veves A (2016) Endothelial dysfunction as a link between cardiovascular risk factors and peripheral neuropathy in diabetes. The Journal of Clinical Endocrinology & Metabolism 101(9): 3401-3408. https://doi.org/10.1210/jc.2016-2030 [PubMed] [PMC]
- Schlienger JL (2013) Type 2 diabetes complications. Presse Med 42: 839-848. https://doi.org/10.1016/j.lpm.2013.02.313 [PubMed]
- Schmid-Schonbein H, Ziege S, Grebe R (1997) Synergetic interpretation of patterned vasomotor activity in microvascular perfusion: descrete effects of myogenic and neurogenic vasoconstriction as well as arterial and venous pressure fluctuations. International journal of microcirculation, clinical and experimental 17(6): 346-359. https://doi.org/10.1159/000179251 [PubMed]
- Shah MS, Brownlee M (2016) Molecular and cellular mechanisms of cardiovascular disorders in diabetes. Circulation Research 118(11): 1808-1829. https://doi.org/10.1161/CIRCRESAHA.116.306923 [PubMed] [PMC]
- Sholokhov VM, Lyubimov BI, Samoylov NN(1986) Way of quantitative assessment of the impact of medicines and other factors on viability of an ischemic skin flap. Bulletin of Experimental and Biological Medicine [Vestnik Eksperimentalnoy i Biologicheskoy Meditsiny] 3: 375-376. [in Russian]
- Shumakov VI, Onishchenko NA, Kirpatovsky VI (1983) Pharmacological protection of a transplant. Medicine, Moscow, 230 pp. [in Russian]
- Stefanovska A, Bracic M (1999): Physics of the human cardiovascular system: Contemporary Physics 40(1): 31-35. https://doi.org/10.1080/001075199181693
- Tegay AV (2004) Dermatoprotective properties of derivatives of 2.3-di-gidroimidazo[1.2]benzimidazoles having hypoglycemic activity in the conditions of the reduced blood circulation. PhD thesis, Volgograd, Russia: University of Volgograd. [in Russian]
- Valeeva IKh, Titarenko AF, Ziganshina LE (2010) Comparative analysis of dimephosphone, ionol, and xydiphone effects on indomethacin-induced gastric lesions in rats. Russian Journal of Experimental and Clinical Pharmacology [Eksperimentalnaya i Klinicheskaya Farmakologiya] 73(12): 21-24. [in Russian] [PubMed]

- Valeeva IKh, Titarenko AF, Khaziakhmetova VN, Ziganshina LE (2011) Dimephosphone shows anti-inflammatory and anti-oxidative activity on chronic autoimmune inflammation model. Russian Journal of Experimental and Clinical Pharmacology [Eksperimentalnaya i Klinicheskaya Farmakologiya] 74(3): 13-16. [in Russian] [PubMed]
- Vasan SK, Pittard AEI, Abraham J (2012) Cause-specific mortality in diabetes. Retrospective hospital based data from south India. Journal of Diabetes 4(3): 47-54. https://doi.org/10.1111/j.1753-0407.2011.00165.x [PubMed]
- Wada J, Nakatsuka A (2016) Mitchondrial dynamics and mitochondrial dysfunction in diabetes. Acta Medica Okayama 70(3): 151-158. doi: 10.18926/AMO/54413. [PubMed]
- Yagi K (1976) Estimation of products of lipid peroxidation by malonyl diadehyde in biochemical systems: Biochem Med 15: 212-213. https://doi.org/10.1016/0006-2944(76)90049-1
- Zimmet P, Bloomgarden ZT (2012) How do we gauge the success of treatment of diabetes. Journal of Diabetes 4(3): 193-194. https://doi.org/10.1111/j.1753-0407.2012.00218.x [PubMed]

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