OBSERVATION OF BIOCHEMICAL RESULTS IN EXPERIMENTAL DIABETIC FOOT SYNDROME

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Annotation
Study of the effect of the new drug "Rheomannisol" on vital organs, taking into account of diagnostics and prevention of pathophysiological aspects in the complex treatment of experimental diabetic foot syndrome.

Keywords: experimental model of diabetic foot, experimental animals, diabetes mellitus, alloxan, surgical debridement, reomannisol.

Introduction

Currently, one of the leading places in terms of growth rates of morbidity, disability, and mortality was occupied by diabetes mellitus (DM) among the so-called "diseases of civilization" [1,4,5,17]. Today, over 460 million people are sufferingglobally from diabetes; according to the predicted facts announced by the International Diabetes Federation, by 2040 the number of patients will increase up to 642 million [11,12,18].

Diabetes mellitus is accompanied by the development of complications, including diabetic foot syndrome (DFS), one of the leading clinical symptoms of which is the persistence of an ulcer on the skin of the lower extremities [4,5].

Delayed wound healing is one of the complications of the disease due to multiple factors including poor circulation [4,5,12,17], prolonged inflammation, and hyperglycemia. It is a common cause of morbidity and mortality in patients with DM [2,5,15,16]. When the wound becomes chronic, it is prone to developing foot ulcers, including neuropathy and foot deformities [6,13,17,18]. Foot ulcers in DM are the cause of more than 50% of all non-traumatic leg amputations [3,7,15]. Evidence has shown that hyperglycemia is one of the main factors contributing to slow wound healing by increasing cell apoptosis and decreasing cell survival in diabetic wounds. It has been shown to inhibit endothelial cell and fibroblast proliferation in humans [9], up to 75% slower in adult mice with DM compared to control mice [10].

At the present stage in experimental diabetology, the most widespread chemical model of diabetes mellitus uses substances that destroy β-cells of the islets of Langerhans [1,2, 11,12]. This study describes a model of diabetes mellitus in rats,induced by the introduction of a reduced dose of alloxan, which significantly reduces the number of animal deaths.

The alloxan model of diabetes mellitus is one of the most widespread and studied. It is actively used by researchers around the world. Alloxan is a structural analog of glucose, due to which

it accumulates in pancreatic β-cells and leads to their death, followed by the development of diabetes. At the same time, damage to β-cells is accompanied by degenerative changes in the kidneys and liver, which leads to high mortality in laboratory animals on the first day after alloxan administration. The problem of violations of several types of metabolism with the introduction of alloxan, the prevalence of manifestations of oxidative stress as a typical pathological process in case of damage to the key organ involved in all types of metabolic processes (liver) dictate the need to prescribe pathogenetic drugs from the group of metabolic correctors with hepatoprotective and antioxidant orientation. One of the promising new drugs in this area is Rheomannisol (LLC "REKA-MED FARM" Republic of Uzbekistan) - a complex drug with antihypoxic, antioxidant, rheological, anti-shock, detoxifying, diuretic action. The main pharmacologically active substances are sodium succinate and mannitol.

Aim of the study. Study of the effect of the new drug "Rheomannisol" on endogenous intoxication and wound healing, taking morphological aspects into the complex treatment of experimental diabetic foot syndrome.

Materials and research methods. The work was done on experimental material. Healthy rats were selected for the experiment. Experimental studies were carried out on 140 outbred male rats weighing 220-250 g, kept in the Tashkent Medical Academy (TMA) vivarium. The rats were kept under optimal conditions, all rats lived in a room with a 12-hour light-dark cycle and a constant temperature of 22-25°C, with free access to water. All rats were given a sufficient amount of a normal rodent diet ad libitum. (diet for rodents, State standard No. GOST R50258–92) and tap water daily. Operations and all manipulations with animals were carried out using general anesthesia, in compliance with the principles of humanity outlined in the directives of the European Community (86/609/EEC) and the Declaration of Helsinki, by the "Rules for working with experimental animals". The experimental animals were divided into 4 groups: the 1st group was intact; 2nd group –the creation of an experimental model of alloxan diabetes mellitus; 3rd control group - against the background of alloxan diabetes, the creation of an experimental model of a diabetic foot using traditional complex treatment; 4th experimental group - on an experimental model of diabetic foot - traditional treatment and reomannisol.

After a 24-hour fast, the rats were weighed. A 2% solution of alloxan diluted in 0.9% saline was administered intraperitoneally as a single dose, corresponding to a dose of 20, 15, 12 mg of alloxan per 100 g of animal weight. Food and water were given to animals only 30 minutes after drug administration. On the 3rd day, the level of glucose in the blood was assessed.

Results

The body bodyweight before the experiment varied from 220 to 250 g. Group 1 - intact animals (10 rats each), served as controls for groups 3 and 4. 2nd group –the creation of an experimental model of a diabetic foot, against the background of alloxan diabetes; To do this, 10 rats were injected intraperitoneally with 2% alloxan in an amount of 20 mg / 100 g. In this experimental group, in the 2nd experimental group, 8 rats died in the first 3 days as a result of hyperglycemic and hypoglycemic coma, which amounted to 80%. When examining the level of glucose in the blood with a glucometer of the remaining rats, it was 33.3 mmol/l and may have been higher, since the maximum range of the glucometer is 33.3 mmol/l. The remaining 2 rats sat in the corner, there were no reactions to external stimuli, they were sedentary when picked up. The animals did not touch the food. On the next day 4, the remaining rats died.

The second series of the second group of the experimental model of diabetes mellitus was created based on alloxan at a dose of 15 mg per 100 g from 10 rats. In this series of experiments, in the first 3 days the lethality was 50% (5 rats). The glucose levels of the survivors (5 rats) ranged from 29.8 to 33.3 mmol/l. During the next 4 days, the remaining rats died.

3rd series of the experiment of the 2nd group - administration of alloxan intraperitoneally at a dose of 12 mg per 100 g per 100 rats. During the next 72 hours, no lethal outcome was observed in rats, the range of blood glucose levels in rats varied between 15.5 - 17 mmol/L. In rats on the skin of the footpad pad of the right hind paw, a full-thickness rectangular wound measuring 2 mm × 5 mm was created with a scalpel. Rats were randomly divided into 2 groups, each group of 50 rats. So it was created 3 –a control group on 50 rats and 4 experimental groups n=50 rats. In both groups, until the end of the experiment (17 days), no death was recorded.

Visual inspection. The first signs of diabetes were manifested in the form of a sharp increase in water consumption of 70-80 ml, polyphagia, polyuria, hyperglycemia. With alloxan-induced diabetes mellitus in animals during the experiment, lethargy, apathy, low activity, tarnishing and loss of coat, weight loss, clouding of the pupil and sclera, small-point erosion in the tails and limbs were noted. The wool of animals normally has a peculiar luster and is usually adjacent to the skin. In dynamic observation in rats of the experimental group, by the seventh day, the condition began to improve.

In the study of indicators characterizing the state of various organs, for example, indicators of the state of liver cells and the degree of their damage are the activity of AST and ALT enzymes. In our studies, the activity of these enzymes in the blood of animals in both groups was significantly higher than in intact animals (ALT-34U/l±1.ASTAsT-33.4 U/l±1.1), Although, it should be noted that already after three injections of the drug rheomannisol, in the animals of the experimental group, the ALT and AST enzymes recorded low numbers (52.6±1.6; 48.4±1.4, respectively) compared with the control group (82.8± 1.4, 86.3±1.5). By the 7th day in the control group ALT-79±0.ASTAsT-79±1.5, which is 1.8 times more than in the

experimental group - ALT-44.7±0.94 AST AST-43, 3±1.0. In our study, in animals, the degree of elevation of the levels of enzymes alanine transferase and aspartate transferase indicates a pronounced violation of the cellular structure of the liver. Recorded activation of transaminases may indicate a violation of the integrity of hepatocyte membranes, leading to an increase in their permeability, and subsequently to the death of liver cells. A decrease in the activities of both enzymes (ALT, AST) followed the injections of rheomannisol in the experimental gr, out and on the 10th and 14th days, the numbers (ALT-37.5±0.62, AST-37.1±0.69; ALT-35.3± 0.54, AST-34.9 ± 1.04, respectively) indicate the normalization of the functional capacity of the liver, while in the control group, the activity of ALT and AST enzymes even on days 10, 14 (ALT-75.5 ± 1.1, AST-74.4±1.6; ALT-57.2±1.2, AST-53.4±1.3, respectively) 2 times higher than in the experimental one, and remain at a high level until the end of the experiment (Table 1).

Table 1. Biochemical parameters of animal blood in an experimental model of diabetic foot.

Indexes / Group of animals	Glucose, mmol/l	ALT, U/l	AST, U/l	Urea, mmol/l	Creatinine, mkmol/l	Total protein, g/l
	Day					
Intact	5,8+0,19	34,0+1,0	33,4+1,1	5,1+0,20	61,5+2,0	74,1+1,2
Control	16,6±0,29***	75,1±1,8***	72,7±1,3***	13,8±0,27***	142,6±2,4***	58,4±1,0***
Main	15,2±0,43***^	70,4±1,0***^	66,7±1,1***^^	13,5±0,27***	141,0±3,3***	58,8±0,63***
3 days						
Control	15,4±0,28***	82,8±1,4***	86,3±1,5***	15,0±0,40***	145,7±1,8***	55,5±0,73***
Main	12,0±0,31***^^^	52,6±1,6***^^^	48,4±1,4***^^^	9,8±0,29***^^^	97,6±2,1***^^^	60,1±0,86***^^^
7 days						
Control	14,1±0,20***	79,0±0,93***	79,0±1,5***	12,8±0,20***	127,6±1,8***	59,4±0,51***
Main	9,0±0,37***^^^	44,7±0,94***^^^	43,3±1,0***^^^	7,2±0,30***^^^	78,2±2,6***^^^	66,0±1,4***^^^
10 days						
Control	13,8±0,16***	75,5±1,1***	74,4±1,6***	12,2±0,24***	113,2±2,4***	63,3±0,71***
Main	7,3±0,21***^^^	37,5±0,62*^^^	37,1±0,69*^^^	5,8±0,19*^^^	68,7±1,2*^^^	73,5±0,80^^^
14 days						
Control	12,9±0,19***	57,2±1,2***	53,4±1,3***	9,7±0,30***	96,7±1,6***	68,3±0,57***
Main	6,5±0,13*^^^	35,3±0,54^^^	34,9±1,04^^^	5,2±0,22^^^	63,8±1,3^^^	75,8±0,63^^^

Note: *- significantly compared with the intact group (*-P<0,05; **-P<0,01; ***-P<0,001)
^ - significantly compared with the control group (^-P<0,05; ^^-P<0,01; ^^^-P<0,001)

One of the laboratory signs of the development of renal dysfunction indicates the content of urea and creatinine in the blood plasma, in the first days of the experiments, in rats of the experimental and control groups were almost 2.5 times (without significant differences between these groups) higher than in the intact group (tables No. 1). After 3-fold intraperitoneal administration of the drug reomannisol, on the 3rd day, the experimental group showed a noticeable decrease in the values of urea (9.81±0.29) and creatinine (97.6±2.1) by 1.5 times relative to the values control group (urea-15.0±0.40; creatinine-145.7±1.8). On the 7th day in the groups of rats treated with reomannisol, the levels of urea and serum creatinine (7.2±0.30 and 78.2±2.6, respectively) were lower compared to control animals (12.8±0. 20 and 127.6±1.8, respectively) by almost 1.7 times. On days 10, 14, urea values and creatinine clearance (urin-5.8±0.19; creat-68.7±1.2; urine-5.2±0.22; creat-63.8±1.3 respectively) in the experimental group were close to those of the intact group of rats (urea-5.1±0.20; creatinine-61.5±2.0). However, in the control group, it was accompanied by a higher value of urea and creatinine clearance, and on the 14th day, they were urea-9.7±0.30 and creatinine-96.7±1.6 - an average of 1.7 times higher than the values of the experimental group (Table 1).

Conclusions

1. The best option for creating an experimental model of a diabetic foot is the introduction of alloxan intraperitoneally in a single dose of 12 mg per 100 g, in which moderate diabetes develops.
2. After using the drug reomannisol intraperitoneally at a dose of 1 ml / 100 g 1 time per day for 5 days, there was a sharp decline in EI numbers. On the 10th day, the EI values in the experimental group returned to normal, similar to those in the intact group. The drug reomannisol performs "biochemical rehabilitation", due to its inherent qualities: antioxidant, improves blood rheology, detoxification, and diuretic. In rats of the control group, the EI numbers remain at high levels until the end of the experiment.
3. The results of biochemical studies demonstrate positive dynamics in experimental animals with a diabetic foot model when using the drug reomannisol. This was manifested by the fact that by the 10th day there was a decrease and normalization of the level of glucose in the peripheral blood, indicators of renal clearance (urea, creatinine), liver (ALT, AST, albumins).
4. An open, full-thickness wound of the foot, in rats with DM, had low blood circulation, prolonged inflammation, and was characterized by a violation of the inflammatory and proliferative phases of the healing process, which is associated with hyperglycemia. Thus, this model of the open foot in rats provides a good approach for studying the process of wound

healing in DM, and this model can be regarded as creating an analog of the human diabetic foot syndrome in an experimental model of alloxan-induced diabetes mellitus.

Ethical approval. Operations and all manipulations with animals were carried out using general anesthesia, in compliance with the principles of humanity outlined in the directives of the European Community (86/609/EEC) and the Declaration of Helsinki, by the "Rules for working with experimental animals". The ethical approval for the study was granted by Tashkent Medical Academy and the Committee of Ethical Approval for Researches under the Ministry of Health of the Republic of Uzbekistan.

References.

1. Yusufjanovich E. U. et al. Evaluation of the lipid peroxidase index in diabetic complications //Conferencea. – 2023. – С. 68-73.
2. Yusufjanovich E. U. et al. Treatment of purulent-necrotic lesions of the lower extremities with modern drugs //Conferencea. – 2023. – С. 88-94.
3. Yusufjanovich E. U. et al. Assessment of the Process of Epithelialization After Complex Treatment of Diabetic Foot Syndrome //Texas Journal of Medical Science. – 2023. – Т. 16. – С. 19-23.
4. Ergashev U. Y., Zokhirov A. R., Minavarkhujaev R. R. Determination of changes in the lipid peroxidase index in purulent-necrotic lesions of the lower extremities. – 2022.
5. Ergashev U. Y., Zokhirov A. R., Minavarkhujaev R. R. Study and treatment of changes in biochemical processes in complications of diabetes mellitus. – 2023.
6. Ergashev U. Y., Zokhirov A. R., Minavarkhujaev R. R. The study of pathological physiology of indicators of endogenous intoxication in purulent-necrotic lesions of the lower extremities. – 2023.
7. Zohirov A. R., Ergashev U. Y., Ernazarov H. I. Qandli diabetda oyoqning yiringlinekrotik shikastlanishlarining patomorfologik jihatlarini kompleks davolashni o'rganish. International scientific forum-2022". June 2022. p132-136.
8. Ergashev U. Y. et al. The study of pathomorphological diagnosis of vital organs after modern treatment of diabetic foot syndrome. – 2022.
9. Ergashev U. Y. et al. The study of diagnostics and prevention of pathophysiological parameters after modern treatment of purulent-necrotic processes in diabetic. – 2022.
10. Ergashev U. Y. et al. Effective of Simultaneous Surgeries in Chronic Immune Thrombocytopenia //Jundishapur Journal of Microbiology. – 2022. – Т. 15. – №. 2. – С. 638-644.
11. Эрназаров Х. и др. Патоморфологическая картина жизненно важных органов при экспериментальной модели диабетической стопы. – 2022.

12. Каримов Ш. И. и др. Применение гибридных хирургических вмешательств у больных с критиче-ской ишемией нижних конечностей при мультифокальном атеросклерозе //Тиббиет янги куни. – 2019. – Т. 3. – С. 27.
13. Mustafakulov G. I. et al. Splenectomy for hairy cell leukemia //Central Asian Journal of Medicine. – 2021. – Т. 2021. – №. 4. – С. 160-167.
14. Ergashev U. Y. Ernazarov Kh. I., Zohirov AR, Alzabni ID 2022. Complex Treatment of Experimental Model of Diabetic Foot Syndrome //American Journal of Medicine and Medical Sciences. – 2022. – Т. 12. – №. 5. – С. 471-480.
15. Ergashev U. Y. et al. The role of minimally invasive technologies in the treatment of liver cavities //Frontiers in Bioscience-Landmark. – 2021. – Т. 8. – С. 82-89.
16. Зохиров А. Р., Абдусаломов Б. А., Моминов А. Т. Совершенствование комплексного лечения с учетом патофизиологических изменений гнойно-некротических поражений нижних конечностей при сахарном диабете. – 2022.
17. Зохиров А. Р., Эрназаров Х. И., Эргашев У. Ю. Патоморфологические особенности заживления ран при экспериментальной модели диабетической стопы. – 64-ой научно-практической конференции обучающихся «наука и здоровье» посвященная дню науки республики Казахстан с международным участием, 2022.
18. Эрназаров Х. и др. Патоморфологическая картина жизненно важных органов при экспериментальной модели диабетической стопы. – 2022.
19. Махмудова, Д. А., & Қарахонова СА, Х. К. (2015). Неврозларда фобия ва қўрқув. Психиатрия журнали, (1-Б), 82.
20. Karakhonova, S. A., & Ishanhodjaeva, G. T. (2016). Cognitive disorders in Parkinsonism. Parkinsonism & Related Disorders, 22, e59.
21. Karakhonova, S. A. (2022). The Significance of the Application of Psycho-Correction Methods in the Treatment of Psycho-Emotional Disorder. EUROPEAN JOURNAL OF MODERN MEDICINE AND PRACTICE, 2(12), 59-64.
22. Алишеровна, Қ. С., Сейткаримова, Г. С., & Юнусходжаева, Х. С. (2022). ЭФФЕКТИВНАЯ ПСИХОКОРРЕКЦИЯ ПРИ НЕВРОТИЧЕСКОМ РАССТРОЙСТВЕ. ЖУРНАЛ НЕВРОЛОГИИ И НЕЙРОХИРУРГИЧЕСКИХ ИССЛЕДОВАНИЙ, 3(1).
23. Ибодуллаев, З. (2022). EFFECTIVE PSYCHOCORRECTION IN NEUROTIC DISORDER.
24. Ибодуллаев, З. Р., Карахонова, С. А., & Сейткаримова, Г. С. (2021). Значение использования методов психокоррекции при лечении тревожно-фобического синдрома. ЖУРНАЛ НЕВРОЛОГИИ И НЕЙРОХИРУРГИЧЕСКИХ ИССЛЕДОВАНИЙ, (SPECIAL 1).
25. Karakhonova, S. (2019). Psychocorrection and optimal pharmacotherapy in anxiety-phobic syndrome. Psychosomatic Medicine and General Practice, 4(1), e0401175-e0401175.

26. Самигова, Н. Р. (2016). ИССЛЕДОВАНИЕ ФУНКЦИОНАЛЬНОГО СОСТОЯНИЯ СЕРДЕЧНО-СОСУДИСТОЙ СИСТЕМЫ У РАБОТАЮЩИХ ШВЕЙНОГО ПРОИЗВОДСТВА. ББК 28.903 я43, 203.
27. Шеркузиева, Г. Ф., Хегай, Л. Н., Самигова, Н. Р., Азизова, Ф. Л., & Курбанова, Ш. И. (2020). Результаты изучения острой токсичности пищевойсмеси “Мелла Круассан”. Журнал//Вестник, (1), 188-189.
28. Сушенок, М. В., & Замбржицкий, О. Н. (2018). Состояние профессионального здоровья медицинских работников. Молодежный сборник научных статей «Научные стремления», (24), 67-70.
29. Raimovna, S. N., Rikhsillayevna, M. M., & Ugli, B. F. I. (2017). Features of hygienic conditions of workers on poultry-farming complexes (Uzbekistan). European research, (4 (27)), 72-73.
30. Самигова, Н. Р., Шеркузиева, Г. Ф., Мусаев, Э. В., Рустамова, М. К. К., & Хаджаева, У. А. К. (2019). Особенности условий труда медицинских работников санитарно-гигиенических лабораторий. Academy, (2 (41)), 97-98.
31. Самигова, Н. Р., Мирсагатова, М. Р., & Нигматуллаева, Д. Ж. (2018). Экологические последствия урбанизации и индустриализации современности. In ДОСТИЖЕНИЯ ВУЗОВСКОЙ НАУКИ 2018 (pp. 249-252).
32. Самигова, Н. Р., & Мирсагатова, М. Р. (2017). Изучение динамики изменений в функциональном состоянии сердечно-сосудистой системы рабочих мебельного производства. Молодой ученый, (50), 126-129.
33. Самигова, Н. Р. (2005). Гигиено-физиологические основы оптимизации условий труда работников швейных производств Узбекистана (диссертация на соискание ученой степени кандидата медицинских наук).
34. Шеркузиева, Г. Ф., Самигова, Н. Р., Шайхова, Л. И., & Жалилов, А. А. (1999). Комплексная гигиено-токсикологическая оценка" Laktonorm-H". ИНФЕКЦИЯ, ИММУНИТЕТ и ФАРМАКОЛОГИЯ, 261.
35. Самигова, Н. Р., & Шеркузиева, Г. Ф. (2021). Использование модульной системы обучения как неотъемлемой части при подготовке клинических ординаторов профилактической медицины (Doctoral dissertation, Namangan).
36. Самигова, Н. Р., & Шеркузиева, Г. Ф. (2021). Использование модульной системы обучения как неотъемлемой части при подготовке клинических ординаторов профилактической медицины (Doctoral dissertation, Namangan).