Sirman Y. V., Savytskyi I. V., Blavatska O. M. Dynamic of blood glucose levels in the model of experimental diabetic retinopathy and different ways to correct it. Journal of Education, Health and Sport. 2021;11(04): 366-373. eISSN 2391-8306. DOI http://dx.doi.org/10.12775/JEHS.2021.11.07.036
https://apcz.umk.pl/czasopisma/index.php/JEHS/article/view/JEHS.2021.11.07.036
https://zenodo.org/record/7115363

The journal has had 5 points in Ministry of Science and Higher Education parametric evaluation. § 8. 2) and § 12. 1. 2) 22.02.2019.

Received: 06.07.2021. Revised: 11.07.2021. Accepted: 29.07.2021.

UDC:616-06:616-092.9

Dynamic of blood glucose levels in the model of experimental diabetic retinopathy and different ways to correct it

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Abstract

The aim of our study was to analyze the changes in the blood glucose level in experimental animals that simulated diabetic retinopathy and against the background of its correction. The obtained results show the development of experimental diabetes when streptozotocin is administered at a dose of 55 mg/kg of animal weight against the background of a high-fat diet. Correction with hypoglycemic agents in the 3rd group had a positive effect, but it was not able to completely normalize the level of glycemia, so the need arose for the use of additional agents. The use of aflibercept and a nitric oxide donor in the 4th group had a more pronounced positive effect compared to the 3rd group, but the result did not reach the control indicators. Combined administration of bromfenac and aflibercept in the 5th group showed itself to be less effective in the study of the blood glucose level at all stages in comparison with the data of the 4th group, in which the NO donor was involved in the

correction. Administation of aflibercept, L-carnitine and bromfenac to the animals of the 6th group had a more pronounced positive effect in comparison with the group No. 5, but did not reach the values of the 4th group of the experiment. The most effective correction was the combination of metformin, aflibercept, L-arginine and citicoline in rats of the 7th group, which is evidenced by the normalization of the level of the studied indicators on the 30th and 60th days of the experiment, and on the 180th, the most pronounced decrease was recorded hyperglycemia.

Key words: experimental diabetic retinopathy; streptozotocin diabetes; blood glucose; correction; metformin; aflibercept; bromfenac; L-carnitine; L-arginine; citicoline.

Introduction. Today, according to the World Health Organization, diabetes mellitus (DM) is a global problem in all countries and in all age groups of the population. Among endocrine diseases, DM reaches 70% [1, 2]. According to the WHO, diabetic retinopathy (DR) is the main cause of vision loss and blindness in this pathology [3].

It is generally accepted that chronic hyperglycemia underlies the development of complications of diabetes, both first and second types. A high level of glucose initiates a whole cascade of metabolic changes both inside the cell and in the extracellular space [4]. A high level of not only glucose, but also a number of metabolites of water and lipid metabolism is considered to be the initial mechanisms that lead to damage to vascular, nervous and other cells of the body. Such metabolites include acetone-acetate, diacylglycerol, deoxyglucose, methylglyoxal, sorbitol, etc. [5].

One of the main pathogenetic factors in the development of diabetic retinopathy is the activation of the sorbitol pathway of glucose utilization with the accumulation of sorbitol in the cell, the binding of a protein to a glucose molecule without the participation of enzymes with the subsequent formation of end products of accelerated glycosylation, increased synthesis of diacylglycerol, activation of lipid peroxidation and inactivation of glyceraldehyde- 3-phosphate dehydrogenase due to an increase in the level of superoxide in mitochondria [6, 7]. Aldose reductase, converting glucose into sorbitol, uses NADPH as a cofactor, thereby reducing the possibility of its use by glutathione reductase to restore oxidized glutathione to the main intracellular and antioxidant - reduced glutathione. In turn, the accumulation of inert sorbitol leads to hyperosmotic conditions inside cells, which leads to their death [4].

The aim of our study was to analyze the changes in the blood glucose level in experimental animals that simulated diabetic retinopathy and against the background of its correction.

Materials and methods. The study was conducted on white Wistar rats weighing 180-200 g. According to the tasks, the animals were divided into 7 groups:

1st group – intact animals;

2nd group – 60 animals with modelling of DR without correction (control pathology);

3rd group –60 animals with modelling of DR with correction of hyperglycemia;

4th group – 60 animals with modelling of DR with correction of hyperglycemia, administration of aflibercept and L-arginine solution;

5th group – 60 animals with modelling of DR with correction of hyperglycemia, administration of aflibercept and bromfenak;

6th group – 60 animals with modelling of DR with correction of hyperglycemia, administration of aflibercept, L-carnitine solution and bromfenak;

7th group – 60 animals with modelling of DR with correction of hyperglycemia, administration of aflibercept, L-arginine solution and citicoline.

Type 2 diabetes and DR were modeled by intraperitoneal administration of streptozotocin (Sigma, USA) dissolved in 0.1 M citrate buffer with pH 4.5 [14, 15]. Dose of streptozocin of 55 mg/kg of animal weight was divided into two administrations. Administration of streptozocin was preceded by a high-fat diet for 28 days [10].

Animals were subjected to research by decapitation in accordance with the "Rules for the performance of work using experimental animals", approved by the Order of the Ministry of Health of Ukraine No. 249 of 01.03.2012 and the Law of Ukraine No. 3447-IV "On the Protection of Animals from Cruelty" (as amended on 15.12.2009 and 16.10.2012).

The hypoglycemic medicine – metformin (Merck Sante, manufactured in France) - at at a dose of 300 mg/kg body weight [11] in a 0.9 % sodium chloride solution through a syringe with an intragastric probe daily, during the entire experiment.

The introduction of L-arginine solution, which is NO donor, (SIMESTA, manufactured in China, quality standard USP32) was carried out by intragastric administration of L-arginine solution in 0.9 % sodium chloride solution at a dose of 500 mg/kg [12] through a syringe with an intragastric probe.

Aflibercept (anti-VEGF therapy) was administered in the form of subconjunctival injections at a dose of 0.08 ml (25 mg/ml) [13] with an interval of 1 injection every 30 days.

Citicoline – 81.8 mg/kg (0.33 ml/kg) was administered intramuscularly once per a day [14].

Bromfenak – was introduced of 0,09 % eyes drop solution once per a day [15].

L-carnitine (manufacturing by “Sigma”, USA) was administrated in the form of an aqueous solution through a syringe with an intragastric probe at a dose of 25 mg/100 g of animal weight [16, 17, 18].

The glucose level in the blood of experimental animals was determined by the glucose oxidase method with the help of a set of reagents from the company Filisit-Diagnostika (Dnipro).

To identify changes in the studied indicators between different groups and at different stages, we used parametric statistical methods, which are based on operating with the parameters of the statistical distribution (mean and variance). The methods used are designed for normally distributed data, so we checked all data for normality using E.I. Pustilnyk's asymmetry and kurtosis criterion. All the data that we are considering turned out to be normally distributed, so we can pairwise compare the mean values of the samples. Note that in the following comparisons we perform comparisons in independent samples. These will be comparisons between different groups of animals or comparisons between the same group of animals (but since there is no correspondence between animals in the samples, they will also be independent). The value $p<0.05$ was chosen as the reliability criterion. An analysis was performed to see if the means differed. The results of determining the t-test give an answer about the equality or difference of the mean values, but they do not provide an opportunity to accurately measure the difference between the mean values. Note that this difference is quite conditional. This difference was calculated as a percentage. Thus, we demonstrated a comparison of mean values between different groups of animals.

Results of study and their discussion:

The obtained results obtained identified the development of experimental diabetes when streptozotocin is administered at a dose of 55 mg/kg of animal weight against the background of a high-fat diet. Correction with hypoglycemic agents in the 3rd group had a positive effect, but it was not able to completely normalize the level of glycemia that’s why the use of additional agents became necessary. The use of aflibercept and a nitric oxide donor in the 4th group had a more pronounced positive effect compared to the 3rd group, but the result did not reach the control indicators. Combined administration of bromfenac and aflibercept in the 5th group proved to be less effective in the study of the blood glucose level

at all stages in comparison with the data of the 4th group, in which the NO donor was involved in the correction.

Table 1 – Glucose level in the blood of experimental animals with simulated diabetic retinopathy and with different methods of its correction on the 30th, 60th, and 180th day (M±m) (mmol/l)

Stages / Groups	I stage(A)		2 stage (B)		3 stage (C)	
Group 1	3,5±0,06		3,74±0,07		3,46±0,06	
	-	-	1A-1B	p<0,05	1A-1C 1B-1C	p>0,05 p<0,01
Group 2	15,62±0,37		21,86±0,4		24,79±0,4	
	1A-2A	p<0,001	1B-2B 2A-2B	p<0,001 p<0,001	1C-2C 2A-2C 2B-2C	p<0,001 p<0,001 p<0,001
Group 3	9,63±0,28		8,63±0,28		6,99±0,28	
	1A-3A 2A-3A	p<0,001 p<0,001	1B-3B 2B-3B 3A-3B	p<0,001 p<0,001 p<0,05	1C-3C 2C-3C 3A-3C 3B-3C	p<0,001 p<0,001 p<0,001 p<0,001
Group 4	6,93±0,25		7,08±0,26		5,74±0,27	
	1A-4A 2A-4A 3A-4A	p<0,001 p<0,001 p<0,001	1B-4B 2B-4B 3B-4B 4A-4B	p<0,001 p<0,001 p<0,001 p>0,05	1C-4C 2C-4C 3C-4C 4A-4C 4B-4C	p<0,001 p<0,001 p<0,01 p<0,01 p<0,001
Group 5	10,12±0,3		8,74±0,24		6,8±0,3	
	1A-5A 2A-5A 3A-5A 4A-5A	p<0,001 p<0,001 p>0,05 p<0,001	1B-5B 2B-5B 3B-5B 4B-5B 5A-5B	p<0,001 p<0,001 p>0,05 p<0,001 p<0,001	1C-5C 2C-5C 3C-5C 4C-5C 5A-5C 5B-5C	p<0,001 p<0,001 p>0,05 p<0,05 p<0,001 p<0,001
Group 6	9,01±0,2		8,4±0,22		7±0,24	
	1A-6A 2A-6A 3A-6A 4A-6A 5A-6A	p<0,001 p<0,001 p>0,05 p<0,001 p<0,01	1B-6B 2B-6B 3B-6B 4B-6B 5B-6B 6A-6B	p<0,001 p<0,001 p>0,05 p<0,001 p>0,05 p<0,05	1C-6C 2C-6C 3C-6C 4C-6C 5C-6C 6A-6C 6B-6C	p<0,001 p<0,001 p>0,05 p<0,01 p>0,05 p<0,001 p<0,001
Group 7	7,52±0,24		6,22±0,31		5,39±0,25	
	1A-7A 2A-7A 3A-7A 4A-7A 5A-7A 6A-7A	p<0,001 p<0,001 p<0,001 p>0,05 p<0,001 p<0,001	1B-7B 2B-7B 3B-7B 4B-7B 5B-7B 6B-7B 7A-7B	p<0,001 p<0,001 p<0,001 p<0,05 p<0,001 p<0,001 p<0,01	1C-7C 2C-7C 3C-7C 4C-7C 5C-7C 6C-7C 7A-7C 7B-7C	p<0,001 p<0,001 p<0,001 p>0,05 p<0,001 p<0,001 p<0,001 p<0,05

It was proved that the introduction of aflibercept, L-carnitine and bromfenac to the animals of the 6th group had a more pronounced positive effect in comparison with the group No. 5, but did not reach the values of the 4th group of the experiment.

The combination of metformin, aflibercept, L-arginine and citicoline in rats of the 7th group proved to be the most effective correction, as evidenced by the normalization of the level of the studied indicators on the 30th and 60th days of the experiment, and on the 180th, the most pronounced reduction in hyperglycemia was recorded. The results of the study of the dynamics of the glucose level in the blood of experimental animals with simulated diabetic retinopathy and with various methods of its correction at all stages of the study are presented in Table 1.

The obtained data confirm the occurrence and progression of diabetes in the group without correction ($p<0.001$). A gradual reduction of hyperglycemia under the influence of metformin has been proven. It should be noted that in the groups where the nitrogen oxide donor was involved in the correction, the normalization of the glucose level is more pronounced and long-lasting.

Conclusions:

1. The obtained results shows the development of experimental diabetes when streptozotocin is administered at a dose of 55 mg/kg of animal weight against the background of a high-fat diet.

2. Correction with hypoglycemic agents in the 3rd group had a positive effect, but it was not able to completely normalize the level of glycemia, so the need arose for the use of additional agents.

3. The use of aflibercept and a nitric oxide donor in the 4th group had a more pronounced positive effect compared to the 3rd group, but the result did not reach the control indicators.

4. Combined administration of bromfenac and aflibercept in the 5th group showed itself to be less effective in the study of the blood glucose level at all stages in comparison with the data of the 4th group, in which the NO donor was involved in the correction.

5. Administation of aflibercept, L-carnitine and bromfenac to the animals of the 6th group had a more pronounced positive effect in comparison with the group No. 5, but did not reach the values of the 4th group of the experiment.

6. The most effective correction was the combination of metformin, aflibercept, L-arginine and citicoline in rats of the 7th group, which is evidenced by the normalization of the

level of the studied indicators on the 30th and 60th days of the experiment, and on the 180th, the most pronounced decrease was recorded hyperglycemia.

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