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Original Article

The Effect of Alloxan-Induced Hyperglycemia on the Myocardium of Experimental Animals

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Abstract

Introduction: Diabetes mellitus is a current problem because the number of deaths from its complications is higher than the total number of people who died of AIDS, tuberculosis, and malaria. Nowadays, chemical models are the most widespread experimental models of diabetes mellitus. As inducers of diabetes, streptozotocin is used in 69% of experimental studies and alloxan in 31%. The dose and route of alloxan administration, duration and severity of diabetes induced by alloxan are debatable. Our study evaluated the effectiveness of the animal model of alloxan-induced hyperglycemia and determined the features of heart remodeling in rats of different ages. **Material and Methods:** The experiment was conducted on 28 laboratory male rats of two age groups: young (3 months old) and mature (8 months old). Alloxan-induced hyperglycemia involves the intraperitoneal injection of alloxan, pre-dissolved in 0.9% solution of sodium chloride, once at a dose of 170 mg/kg on an empty stomach. The animals also received a 10% glucose solution 24 hours after alloxan administration and a 5% glucose solution during the experiment. The glucose level was measured using Accu-Chek Advantage (Boehringer, Germany) after 2, 12, and 24 hours after alloxan injection, and then weekly. The average level of glucose in the blood remained at 11 mmol/L ± 2 mmol/L. The experiment lasted 45 days. We analyzed heart remodeling by organometry and light microscopy. Results: The mortality of 3 months old rats was 12.5%, and 8 months old rats' mortality was 25%. The organometric study indicated the weight increase of both ventricles, which was more pronounced in 8-month old rats. We also revealed the dilation of the left ventricle in young rats and the dilation of both ventricles in mature ones. Light microscopy showed microcirculation disorders, polymorphisms of cardiomyocytes nuclei, the phenomenon of cytolysis, disorientation, wavy deformation and fragmentation of cardiomyocytes fibers, swallowing of myocardial stroma, and local fibrosis with focal cell infiltration. **Conclusions:** The obtained results suggest that alloxan can be used for further experiments on laboratory animals to model type 1 diabetes mellitus and find ways to correct the detected changes. The features of myocardial remodeling under the influence of alloxan-induced hyperglycemia include the tendency for hypertrophy and ventricular dilatation, disturbance of myocardial microcirculation, its contractile dysfunction, and local fibrosis.

Keywords: heart remodeling, alloxan, hyperglicemia, age

Introduction

According to the International Diabetic Federation, in 2016, there were 425 million people in the world suffering from diabetes mellitus. Two-thirds of these are working-age people living in urban areas [1]. The leading causes of a significant increase in the incidence of diabetes in urban areas are the change in diet (fast food) and decreased physical activity[2].

If the number of deaths from the complications of diabetes amounted to 1.5 million people in 2012, the number was already 5 million in 2015. This is more than the total number of people who died of AIDS, tuberculosis, and malaria. For every 11 people, one person is suffering from diabetes mellitus. About 1 million children and adolescents in the world suffer from type 1 diabetes mellitus. It is predicted that in 2045, the number of diabetes patients will reach 629 million worldwide [1].

Diabetes mellitus, especially the second type, often contributes to the development of cardiovascular diseases, or, conversely, is found in patients who are being examined and treated for the already existing cardiovascular disease [3-7]. Type 1 diabetes mellitus

also causes the development of cardiovascular diseases, which often are subclinical in children and adolescents within the first decade of the diabetes mellitus diagnosis [8]. It is known that overweight is one of the cardiovascular disease risk factors [9]. Usually, it is considered an independent risk factor, but the prevalence of overweight and obesity in type 1 diabetes mellitus has increased significantly [8].

Nowadays, such experimental models as surgical, chemical, endocrine, immune, genetic, and virus-induced diabetes are used for the study of diabetes mellitus [10-14]. The most widespread model is the experimental chemical model. As inducers of diabetes, streptozotocin is used in 69% of experimental studies and alloxan in 31% [15]. When used, both substances cause the necrosis of the β-cells of the pancreas.

Over the past decade, the model of streptozotocin-induced diabetes has been actively used. The dose and route of administration of streptozotocin have been clearly defined [16, 17]. Another chemical substance, alloxan, is used to simulate diabetes mellitus since 1943. Despite this fact, scientists discuss the optimal dose, method of administration, duration, and severity of diabetes induced by alloxan even nowadays [18, 19].

Therefore, our experiment was designed to evaluate the effectiveness of the animal model of alloxan-induced hyperglycemia in order to study the features of heart remodeling in young and mature rats.
Our objectives were to investigate and compare changes in organometric indices of young and mature rats and to identify and compare myocardial changes in young and mature rats at the tissue level.

Material and Methods

The experiment was conducted in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes [20]. Fourteen rats at the age of 3 months (young rats) and 14 rats at the age of 8 months (mature rats) were divided into two groups: experimental and control. Control series included 6 young and 6 mature intact rats. Eight young and 8 mature experimental rats served as an animal model of alloxan hyperglycemia. To do this, we injected alloxan, pre-dissolved in 0.9% sodium chloride solution, intraperitoneally once at a dose of 170 mg/kg on an empty stomach. The dose and route of administration of alloxan were based on the results of recent studies [21-23]. Taking into account that alloxan has a toxic effect on the cells of the tubules of the nephron immediately after the injection, causing acute hypoglycemia, the animals additionally received a 10% glucose solution for 24 hours after the alloxan injection [19] and a 5% glucose solution during the experiment. The glucose level was measured using Accu-Chek Advantage (Boehringer, Germany) 2, 12 and 24 hours after alloxan injection, and then weekly. The average level of glucose in the blood remained at 11 mmol/L ± 2 mmol/L. The experiment lasted 45 days. The subject of the investigation was the heart of the experimental and control animals for correct comparative analysis.

To study the morphological alteration of the heart under the influence of alloxan-induced hyperglycemia, we used the following methods:

1. Organometry. Rats' hearts were dissected according to Avtandilov [24] and divided into four parts: the free wall of the left ventricle (LV), the free wall of the right ventricle (RV), interventricular septum and atria. We weighted the wall of the LV (LVW) and the RV (RVW) with the proportional mass part of the interventricular septum. We measured the endocardial surface area of the LV (LVSA) and the RV (RVSA) using the indirect planimetry method and calculated the planimetric index (PI) as the ratio of RVSA to LVSA.

2. Histological examination. The histological samples, prepared by the standard method, were stained with hematoxylin and eosin and investigated using the OLIMPUS BH-2 light microscope.

3. A statistical method. Obtained data were processed on a personal computer using the "GraphPad" software [http://graphpad.com]. Data were analyzed by unpaired t-test. P values ≤ 0.05 were considered statistically significant.

This study was approved by the Ethics Committee of the Sumy State University, Ukraine.

Results

The obtained data showed that alloxan-induced hyperglycemia causes a mass increase of the heart. Thus, in 3-month experimental rats, the LVW was 33.48% (p=0.0327), higher than in intact animals. The RVW increased by 20% (p=0.0287) during the experiment. Planimetric data show the increase of LVSA by 18.22% (p=0.0061); other organometric indices were not

significantly different from the corresponding indices of the 3-month intact rats.

In mature, 8-month-old rats, we observed a more pronounced remodeling process of the heart ventricles (Figure 1). All researched organometric indicators changed significantly. LVW increased by 56.29% ($p < 0.0001$). RVW was 42.4% ($p < 0.0001$), higher than the corresponding index of control rats. Besides hypertrophy, the dilation of both ventricles was observed in mature rats. LVSA increased by 31.77% ($p < 0.0001$), and RVSA by 45.89% ($p=0.0008$). PI changed unreliable, indicating a uniform dilatation of the heart ventricles.

Myocardial remodeling at the tissue level was observed in both age groups under the influence of alloxan-induced hyperglycemia. First of all, it is characterized by microcirculation disorders and is manifested by uneven vascular filling. In some visual fields, the vessels are empty; in others, we observed erythrocyte aggregation. Moreover, there are some signs of perivascular edema in mature rats.

The nuclei of cardiomyocytes are different in shape. Most of them are located in the cell's center, but some nuclei reside at the periphery of the cardiomyocytes (Figure 2). The areas around some cardiomyocytes' nuclei are enlightened (the phenomenon of

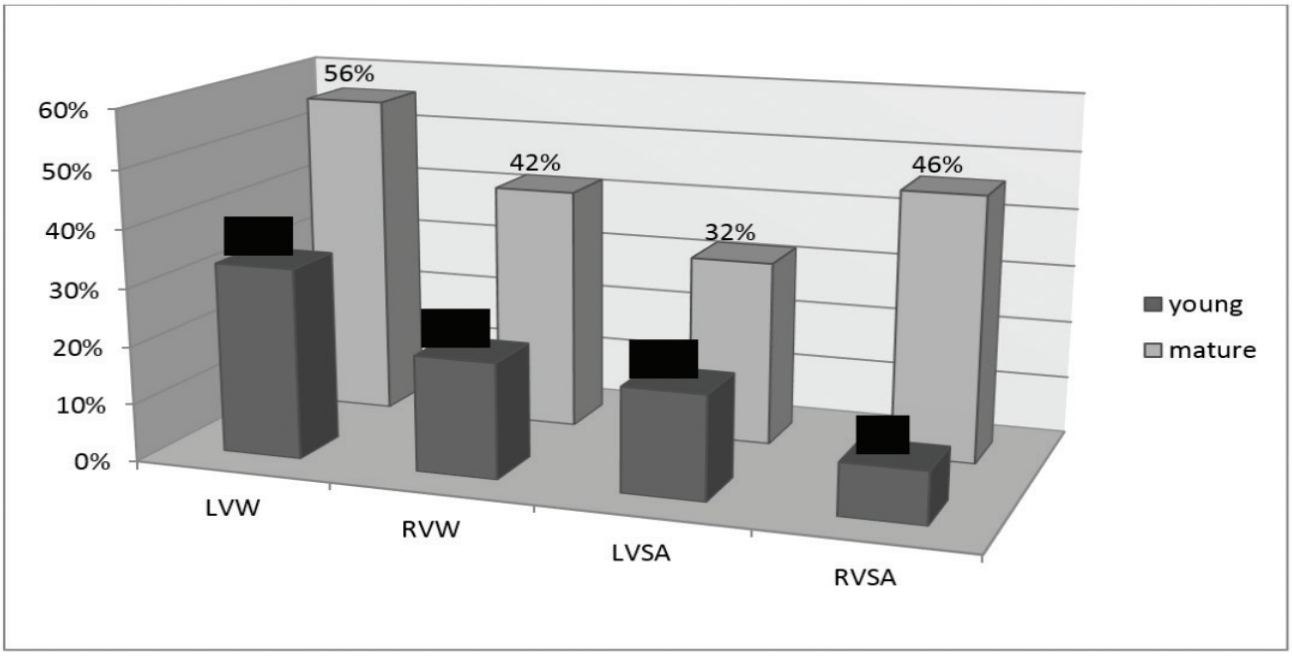

Figure 1: Changes in the organometric data of rat hearts under the alloxan-induced hyperglycemia.

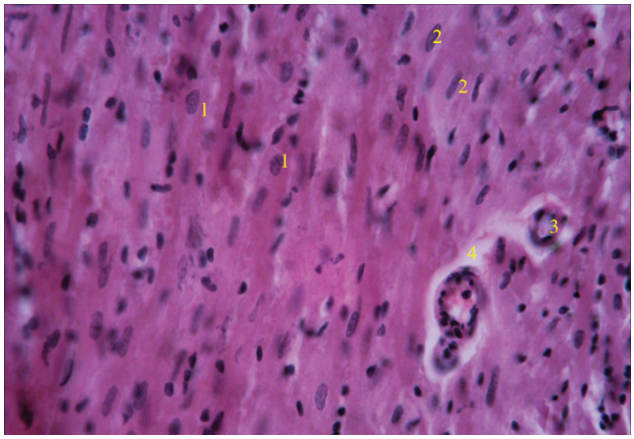

Figure 2: The myocardium of 8-month experimental rats x 800. 1 – rounded nuclei of cardiomyocytes, located in the center of the cell, 2 – elongated nuclei of cardiomyocytes, with marginal location, 3 – lumen of arteriola, 4 – perivascular edema.

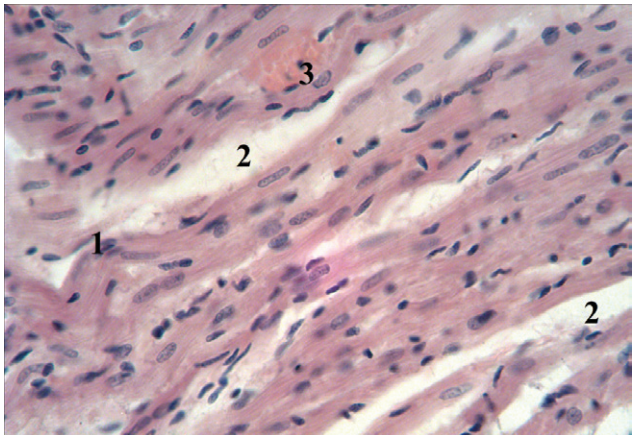

Figure 3: The myocardium of 8-month experimental rats x 800. 1 – wavy deformation of muscle fibers, 2 – stromal edema, 3 – capillary hyperemia

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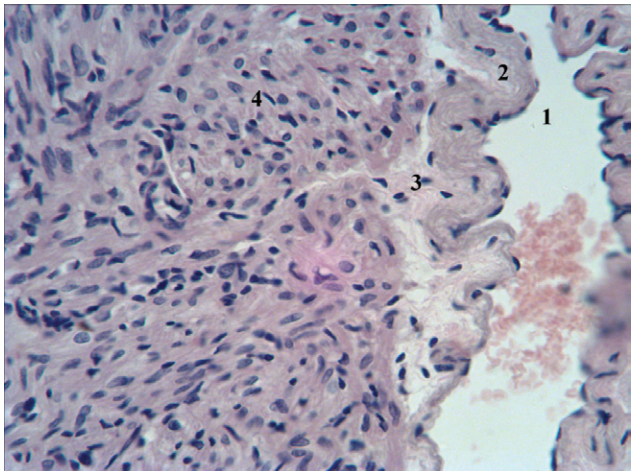

Figure 4: The myocardium of 3-month experimental rats x 800. 1– LV cavity, 2 – papillary muscle, 3 – stromal fibrosis, 4 – cell infiltration.

cytolysis). The light microscopy determined that fibers of cardiomyocytes can lose their orderliness; sometimes, they are wavy deformed or fragmented. Also, muscle fibers are irregularly colored due to their overcontraction (the phenomenon of contractional damage). The myocardial stroma is swollen and infiltrated by the blood cells (Figure 3).

We reveal a local substitution of muscle tissue with connective tissue (fibrosis), with focal cell infiltration. Such a process is mostly observed in the area of the papillary muscles (Figure 4).

Discussion

In the present study, we used young (3-month), and mature (8-month) animals and have not involved senile rats. This choice is based on the fact that alloxan causes type 1 diabetes mellitus, which is typical for the respective groups of the population, while type 2 diabetes mellitus dominates in the case of older people.

We found the increase of mass parameters in both age groups. The ventricular hypertrophy was more significant in experimental animals of 8 months of age. The same trend was observed with respect to the planimetric parameters: there was a dilatation of the LV in the 3-month-old animals, and a dilation of both ventricles in animals of 8-month-old age. The fact that young animals are less susceptible to alloxan is confirmed by recent studies [18] and might be explained by the fact that the antioxidant protection system is decreasing with age [23].

Light microscopy revealed nonspecific myocardial changes in both age groups of the experimental animals. These changes indicate the disturbance of microcirculation and are represented by uneven filling of vessels and perivascular edema. In addition, the shape of cardiomyocytes' nuclei changes; in some places, they are located marginally, which is a sign of cell hypertrophy. Disturbances in the direction of fibers of cardiomyocytes, their fragmentation, and contracture damage can occur as a result of electrolyte imbalance. Moreover, we observed stromal edema and local myocardial fibrosis in young and mature rats.

It is known that the leading pathogenetic link in diabetes is a disorder of the microcirculation in the glomeruli, retina, myocardium, skin and muscles, which leads to the development of diabetic microangiopathy. Furthermore, the restructuring of vasa vasorum can interconnect macro- and microangiopathy [25]. Furthermore, impaired renal function, as a result of diabetic nephropathy, leads to disorders of water-electrolyte balance [26, 27]. Therefore, we can interpret our findings as myocardial remodeling under the influence of prolonged hyperglycemia. We did not observe the normalization of blood glucose levels in rats during our study. The mortality of 3 months of age rats was 12.5%, and 25% in the case rats at 8 months of age.

Conclusion

The obtained results suggest that alloxan can be used for further experiments on laboratory animals to model type 1 diabetes mellitus and find ways to correct the detected changes.

The features of myocardial remodeling under the influence of alloxan-induced hyperglycemia are the tendency for hypertrophy and ventricular dilatation, disturbance of myocardial microcirculation, its contractile dysfunction, and local fibrosis.

In this case, more of our future studies need to investigate the remodeling of the heart and vessels in more prolonged terms.

Conflict of Interest

The authors declare that there is no conflict of interest.

References

1. IDF Diabetes Atlas, Eighth edition, 2017. Online version of IDF Diabetes Atlas: www.diabetesatlas.org
2. Siddiqui A, Siddiqui S, Ahmad S et al. Diabetes: Mechanism, Pathophysiology and Management A Review International Journal of Drug Development & Research 5(2): 1-23, 2013
3. Chernatska O, Demikhova N Improvement of treatment in persons with arterial hypertension and type 2 diabetes mellitus Georgian Medical News 11(284): 47-51, 2018
4. Sattar N Revisiting the links between glycaemia, diabetes and cardiovascular disease Diabetologia 56: 686-695, 2013
5. Demikhova N, Chernatska O, Mazur T et al. Markers of cardiovascular complications in patients with type 2 diabetes mellitus and arterial hypertension Bangladesh Journal of Medical Science 17(2): 319-322, 2018
6. Marushchak M, Krynytska I, Mikolenko A et al. Chronic heart failure causes osteopathy or is osteopathy a factor in development of chronic heart failure? Asian Journal of Pharmaceutical and Clinical Research 11(1): 111-115, 2018
7. Smiyan OI, Loboda AM, Manko YA et al. Dynamics of content of some minerals in teenagers with cardiovascular system pathology against the background of chronic tonsillitis Wiadomosci lekarskie 71(4): 861-864, 2018
8. de Ferranti SD, de Boer IH, Fonseca V et al. Type 1 Diabetes Mellitus and Cardiovascular Disease: A Scientific Statement From the American Heart Association and American Diabetes Association Diabetes Care 37(10): 2843-2863, 2014
9. Yarmolenko O, Sikora V, Bumeister V et al. Age-dependent cardioprotective action of meldonium on heart remodeling under the experimental hypoosmolar hyperhydration Bangladesh Journal of Medical Science 18 (2): 435-444, 2019
10. Grytsiuk MI, Bojchyk TM, Petryshen OI Comparative characteristics of experimental models of diabetes mellitus World of Medicine and Biology 2(44): 199-203, 2014
11. Mozheyko LA Experimental models for studying diabetes mellitus Part I. Alloxan diabetes Journal of the Grodno State Medical University 3: 26-29, 2013
12. Sakata N, Yoshimatsu G, Tsuchiya H et al. Animal Models of Diabetes Mellitus for Islet Transplantation Journal of Diabetes Research 2012(1): 256-277, 2012
13. Zaragoza C, Gomez-Guerrero C, Martin-Ventura JL et al. Animal Models of Cardiovascular Diseases Journal of Biomedicine and Biotechnology 2011: 497841, 2011
14. Prykhodko OA, Gulaya VI, Yarmolenko OS et al. Microscopic changes in the organs of rats under conditions of general dehydration of the organism Azerbaijan Medical Journal 4: 95-100, 2016
15. Etuk EU Animals models for studying diabetes mellitus Agriculture and Biology Journal of North America 1(2): 130-134, 2010
16. Guzyk MM, Dyakun KO, Yanytska LV et al. Inhibitors of Poly(ADP-Ribose) Polymerase-1 as Agents Providing Correction of Brain Dysfunctions Induced by Experimental Diabetes Neurophysiology 49(3): 183-193, 2017
17. Krynytska I, Marushchak M The Indices of Nitric Oxide System in Rats with Carrageenan-Induced Enterocolitis Combined with Diabetes Mellitus Romanian Journal of Diabetes, Nutrition and Metabolic Diseases 25(3): 283-288, 2018
18. Jain D, Arya R Anomalies in alloxan-induced diabetic model: It is better to standardize it first Indian Jornal of Pharmacology 43(1): 91, 2011
19. Misra M, Aiman U Alloxan: An unpredictable drug for diabetes induction? Indian Jornal of Pharmacology 44(4): 538-539, 2012
20. Council of Europe Strasbourg. European convention for the protection of vertebrate animals used for experimental and other scientific purposes 123: 52, 1986
21. Chougale AD, Panaskar SN, Gurao PM et al. Optimization of Alloxan Dose is Essential to Induce Stable Diabetes for Prolonged Period Asian Journal of Biochemistry 2 (6): 402-408, 2007
22. Mostafavinia A, Amini A, Kamran SG et al. The effects of dosage and the routes of administrations of streptozotocin and alloxan on induction rate of typel diabetes mellitus and mortality rate in rats Laboratary Animals Research 32(3): 160–165, 2016
23. Ighodaro OM, Adeosun AM, Akinloye OA. Alloxan-induced diabetes, a common model for evaluating the glycemic-control potential of therapeutic compounds and plants extracts in experimental studies Medicina 53: 365-374, 2017
24. Avtandilov GG. Moscow, Meditsina Publ., 2002, 240 p.
25. Chawla A, Chawla R, Jaggi Sh. Microvasular and macrovascular complications in diabetes mellitus: Distinct or continuum? Indian Jornal of Endocrinology and Metabolism 20(4): 546–551, 2016
26. Teslyk T, Yarmolenko O, Bumeister V et al. The remodeling of lungs under the influence of alloxan-induced hyperglycemia Romanian Journal of Diabetes, Nutrition and Metabolic Diseases 27(1): 51-56, 2020.
27. Popov S, Demikhova N, Melekhovets O et al. Application of "reyt-oil" in prevention of atherosclerosis in diabetes patients Likars`-ka sprava 8: 119-126, 2012.

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