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Db/db mice as diabetes genetic model, effect of trehalose, comparative study of liver, heart and brain

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Type 2 diabetes is a prevalent metabolic disorder, with steady increasing of numbers of cases [1, 2]. Several experimental models have been proposed for studies on Type 2 diabetes pathogenesis, the db/db mice are known genetic animal model of diabetes Type 2 [1–3] used for choice in experimental therapy. This phenotype includes severe obesity, hyperphagia, polydipsia, and polyuria, connected with a spontaneous mutation of leptin receptor [1, 2].

It was shown, that model of Type 2 diabetes in db/db mice was characterized also by intracellular lipid storage in hepatocytes and cardiomyocytes and some signs of inflammation (increased expression of TNF-α in the spleen, underexpression of IL-10 in the liver and spleen [3]. These changes can play role in development of early pathological changes of atherosclerosis (heart) and brain, the main complications of diabetes T2 in human.

The Aim of this study was evaluating the effect of autophagy inducer trehalose on liver, heart and brain ultrastructure, with concentration of attention at autophagy and intracellular lipids storage in cells.

Methods: 38 male db/db mice, aged 3 months at time 0 of acclimation (SPF vivarium of the Institute of Cytology and Genetics, Siberian Branch of Russian Academy of Sciences, Novosibirsk) and C57BL/6 mice (as a control) were used.

Experimental Design: The mice were subdivided into four groups (6–8 animals each):

1) "C57BL/6 mice", i. e., C57BL/6 mice drinking water ad libitum during the whole experiment (24 days);

2) "trehalose-treated C57BL/6 mice", i. e., C57BL/6 mice drinking a 2 % solution of trehalose (Trehalose dihydrate, Tokyo Chemical Industry, Japan) instead of water;

3) "db/db mice", i. e., db/db mice drinking water ad libitum during the whole experiment; and

4) "Trehalose-treated db/db mice", i. e., db/db mice drinking the 2 % trehalose aqueous solution. On the day of euthanasia (at day 25), the animals in each group were killed by decapitation.

Biochemical Assays: Murine blood was collected after decapitation, and serum was obtained by centrifuge Eppendorf 5415R Eppendorf AG, Hamburg, Germany) at

3000× g for 20 min. Fasting blood glucose, glycosylated hemoglobin HA1, and liver function (ALT activity) were assayed during the experiment [3].

Transmission Electron Microscopy: Myocardium, liver and brain (prefrontal cortex) samples for electron microscopy were fixed in 4 % paraformaldehyde in the Hanks medium and a 1 % OsO4 solution (Sigma, St. Louis, MO, USA) in phosphate buffer (pH 7.4) for 1 h, dehydrated in ethanol of ascending concentrations, and embedded in Epon (Serva) (3) and analyzed under a JEM 1400 electron microscope (JEOL Ltd., Tokyo, Japan) (Multiple-Access Centre for Microscopy of Biological Subjects, Institute of Cytology and Genetics, SB RAS, Novosibirsk, Russia).

Morphometric Electron-Microscopic Analysis: To determine volume density of autophagic structures in hepatocytes and cardiomyocytes, 30 cells in each group were randomly selected. Volume densities of autophagosomes, autolysosomes, and lysosomes were calculated using the ImageJ software (National Institutes of Health, Bethesda, MD, USA). Autophagic structures were identified according to the guidelines for monitoring autophagy.

Morphometric Analysis of Lipid Inclusions: Quantitative data were obtained using the iTEM software (Olympus, Tokyo, Japan). Results are presented as the mean ± SEM, with $p < 0.05$ were regarded as statistically significant.

Results: Trehalose treatment of C57L/6 mice significantly reduced blood glucose concentration and glycated hemoglobin. In comparison with C57BL/6 mice, blood glucose concentration was significantly ($p < 0.001$) higher in db/db mice, as was glycated hemoglobin ($p < 0.001$); trehalose treatment significantly ($p < 0.01$) reduced both parameters. Treatment of db/db mice by trehalose was followed by increased autophagy induction in the heart and liver.

Compared to the control (C57BL/6 mice), the weight of db/db mice (drinking plain water) was greater because of obesity ($p < 0.001$), whereas trehalose consumption decreased this parameter in comparison to untreated animals ($p < 0.01$). Heart weight decreased by trehalose consumption in db/db mice as compared to untreated mice ($p < 0.05$). Liver weight was greater both in untreated ($p < 0.001$) and trehalose-treated db/db mice ($p < 0.01$) vs respective controls. The consumption of trehalose with drinking water decreased the body weight of db/db mice compared to untreated db/db mice ($p < 0.05$). Spleen weight was ~2-fold greater in untreated db/db mice than in untreated C57BL/6 mice ($p < 0.001$), possibly attributable to enhanced functioning of the spleen in Type 2 diabetes as a consequence of liver steatosis.

In hepatocytes of db/db mice (compared to the control C57Bl/6 mice) there was a higher content of large lipid inclusions, a lower amount of glycogen, and a larger number of convoluted annular mitochondria. In the cytoplasm of hepatocytes from trehalose-treated db/db mice, we observed autophagosomes with cytoplasm fragments, and autolysosomes with visualized lipid inclusions.

In myocardium there was a moderate deficit of the sarcoplasm owing to lytic alterations. A distinctive feature of the cardiomyocyte structure in db/db mice was the presence of a large number of lipids droplets in their sarcoplasm, which were overall evenly distributed within a cell. Increased lipid droplets were observed also in some neurons of brain (perifrontal zone) of db/db mice.

Conclusion: The autophagy activation by trehalose revealed several positive effects on the heart and liver of db/db mice possibly via increased lipophagy (uptake of lipid droplets). Therefore, lipophagy activation seems to be a promising approach to prevent complications in diabetes.

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References

1. Zhang Y., Pan X.F., Chen J. et al. Combined lifestyle factors and risk of incident type 2 diabetes and prognosis among individuals with type 2 diabetes: A systematic review and meta-analysis of prospective cohort studies. *Diabetologia*. 2020;63:21-33
2. Banach M., Surma S., Reiner Z. et al. Personalized management of dyslipidemias in patients with diabetes – it is time for a new approach. *Cardiovasc Diabetol*. 2022;21(1):263. doi 10.1186/s12933-022-01684-5
3. Korolenko T.A., Ovsyukova M.V., Bgatova N.P. et al. Trehalose Activates Hepatic and Myocardial Autophagy and Has Anti-Inflammatory Effects in *db/db* Diabetic Mice. *Life*. 2022;12(3):442. doi 10.3390/life12030442