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Endothelial dysfunction in experimental models of metabolic syndrome – effect of fructose
Endotelová dysfunkcia v experimentálnom modely metabolického syndrómu - význam fruktózy

Original Paper

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Abstract The aim of the work was to find an experimental model suitable for the study of endothelial dysfunction induced by MS. We used hypertriglyceridemic rats (HTG) that were fed a hypercholesterolemic diet of different composition and duration: a 6-week administration of standard diet with an addition of cholesterol and fat (HTGChol) and a three-month administration of the same diet with an addition of fructose (HTGCholF). We investigated the effect of different diets on aortic endothelial function. The standard diet fed Wistar (W) and HTG rats served as controls. Decision for addition of fructose to HTGChol was done based on in vitro experiments evaluating the effect of high concentration of saccharide in the incubation solution on aortic endothelial function. This intervention caused significant deterioration of relaxation induced by acetylcholine (ACh). While in HTGChol, we did not find significant differences in the function of the aorta compared to W or HTG rats, adding of fructose to high fat diet and prolonging its administration resulted in significantly impaired endothelium-dependent relaxation. It seems that such a model is suitable for the study of endothelial dysfunction in MS and the effect of substances that may protect the endothelium.

Slovak abstract Cieľom práce bolo nájsť experimentálny model, vhodný na štúdium endotelovej dysfunkcie počas MS. Použili sme hypertriglyceridemické potkany (HTG), ktorým sa podávala hypercholesterolemická diéta rôzneho zloženia a trvania. Jednalo sa o 6-týždňové podávanie štandardnej stravy s prídavkom cholesterolu a tuku (HTGChol) a 3-mesačné podávanie rovnakej diéty s prídavkom fruktózy (HTGCholF). Zisťoval sa vplyv jednotlivých diét na funkciu endotelu aorty. Wistar and HTG potkany kŕmené štandardnou diétou slúžili ako kontrola. Pre pridanie fruktózy k vysokotukovej diéte sme sa rozhodli na základe in vitro pokusov, kde sa hodnotila funkcia endotelu po inkubácii aorty v roztoku obsahujúcom vysokú koncentráciu cukru. Po takomto zásahu došlo k významnému poškodeniu relaxácie vyvolanej acetylcholínom v porovnaní s kontrolnou skupinou. Kým u zvierat kŕmených HTGChol sme nenašli významné rozdiely vo funkcii aorty v porovnaní s Wistar a HTG potkanmi, pridanie fruktózy k vysokotukovej diéte a predĺženie jej podávania malo za následok signifikantne poškodenú relaxáciu závislú od endotelu. Zdá sa, že tento model je vhodný na štúdium endotelovej dysfunkcie počas MS a látok, ktoré by mohli endotel ochrániť.

Keywords *Endothelium – Metabolic Syndrome – HTG*

Kľúčové slová: *Endotel – Metabolický syndróm – HTG*

INTRODUCTION

Metabolic syndrome (MS) is associated with serious metabolic abnormalities, which present a high risk for developing cardiovascular diseases. Vascular endothelial dysfunction, which can lead to atherosclerosis, belongs to the first signs of cardiovascular disorders (Oostrom et al., 2002). In our previous work (Kaprinay et al. 2016), we found that hypertriglyceridemic rats fed high fat, high cholesterol diet (HTGChol) are suitable non-obese models of MS, however, without significant changes in the vascular endothelium. The most important components of MS are hyperglycemia and glucose intolerance that are accompanied with oxidative stress – reduction of antioxidant activity and increased production of reactive oxygen species (Anderson et al. 2001, Bae et al. 2001). Oxidative stress has an important role in

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endothelial dysfunction (Sotníková & Bauer, 2010). Bartuš et al. (2008) found that a rich fructose diet induced serious abnormalities in the cardiovascular system. Therefore, the aim of the work was to evaluate the effect of fructose addition to high fat, high cholesterol diet on the aortic endothelial function during MS.

METHODS

The investigation conformed to the Guide for the Care and Use of Laboratory Animals. Male hypertriglyceridemic rats were divided into three groups: HTG control group fed standard diet, HTGChol group fed high fat, high cholesterol diet for 6 weeks and HTGCholF fed high fat, high cholesterol, fructose rich diet for 3 months. Wistar (W) rats fed standard diet served as control group. The composition of the modified diets was: standard pellets with 1% cholesterol, 7.5% lard, 10% fructose. After the decapitation of animals, function of the isolated thoracic aorta was tested under isometric conditions. Responses of the phenylephrine-precontracted (1µmol/l) arteries to cumulative acetylcholine (Ach) before and after NO-synthase (NOS) inhibition with N-nitro-L-arginine methyl ester hydrochloride (30 µmol/l; L-NAME) were investigated. Data are presented in percentage of phenylephrine-induced contraction and the results are statistically compared using the ANOVA t-test with Bonferroni post test.

RESULTS

Incubation of aorta with a medium containing 44 mmol/l glucose for 24 hours resulted in significantly smaller relaxation to Ach as compared to controls. Responses of aortas from HTG and HTGChol animals to Ach were not statistically different from those of W, while Ach-evoked relaxation of HTGCholF was damaged (Fig. 1). The responses to Ach after NOS-blockade were similar in W and HTG, and the biggest L-NAME-resistant component was found in HTGCholF. The L-NAME-resistant part of the endothelium-dependent relaxation was the smallest in HTGChol (Fig. 2).

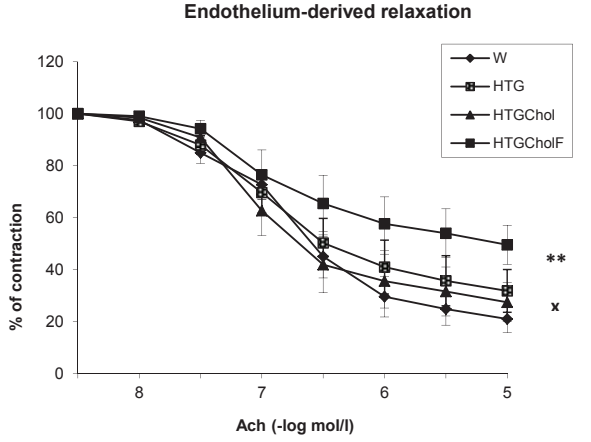

*Figure 1. Responses of the aortas of W, HTG, HTGChol and HTGCholF rats precontracted by phenylephrine (1 µmol/l) to acetylcholine (Ach). Data are means ± SEM obtained from 9-10 experiments. ** p <0.01 HTGCholF vs. W, x p <0.05 HTGCholF vs. HTGChol*

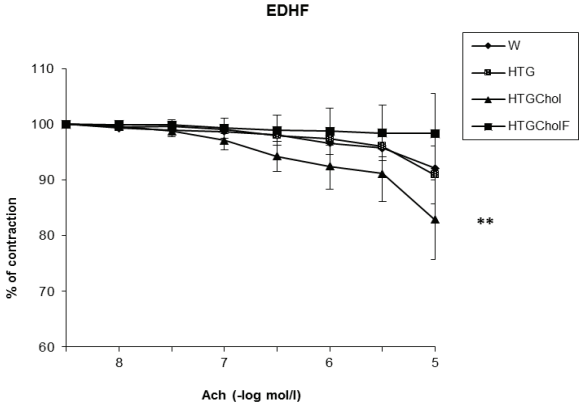

*Figure 2. Responses of the aortas of W, HTG, HTGChol and HTGCholF rats precontracted by phenylephrine (1 µmol/l) to acetylcholine (Ach) after the blockade of NOS by L-NAME. Data are means ± SEM obtained from 9-10 experiments. ** p <0.01 HTGCholF vs. W*

DISCUSSION

Our results showed endothelium dysfunction of the aorta of rats that were fed high cholesterol, high fat diet enriched with fructose. On difference, in HTGChol without fructose, the function of endothelium was preserved, although rats had signs of MS – impaired glucose and lipid metabolism, increased blood pressure and developing liver steatosis (Kaprinay et al., 2016). These results correspond with other authors (Bartuš et al., 2008; Sasaki et al., 1994; Kitagawa et al., 1995; Pisulewski et al., 2005), who did not find any modification of the endothelial function in HTGChol rats. Hyperglycemia and glucose intolerance are important components of human MS. Oxidative stress is a main reason of their pathological influence on the cardiovascular system, including the endothelium. The burst of ROS in the endothelium results in decrease of bioavailability of nitric oxide and subsequently to endothelial dysfunction. These facts supported our decision to add fructose to a high cholesterol, high fat diet. Indeed, the aortas of HTGCholF rats showed significantly lower response to Ach. In the aorta, the part of Ach-induced relaxation is assumed to be mediated by endothelium-derived hyperpolarizing factor (EDHF), which can be identified after the NOS-inhibition (McCulloh et al., 1997). From the group tested, HTGChol aortas showed the biggest part of L-NAME-resistant relaxation to Ach. It seems that HTGChol rats were able to maintain an unchanged endothelium-dependent relaxation by releasing EDHF. As the Ach-induced relaxation of HTGCholF aortas was completely inhibited by L-NAME, EDHF-participation was probably damaged. These results are in accordance with Miller et al.

(1998) and Young et al. (2008) who also found impaired EDHF response in rats administered fructose rich diet.

CONCLUSION

It seems that HTGCholF rats are closer models of MS than HTGChol, because they showed significant signs of endothelial dysfunction, which can lead to atherosclerosis and other cardiovascular diseases.

ACKNOWLEDGEMENT

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References

[1] Anderson RA, Evans ML, Ellis GR, et al. The relationships between post-prandial lipaemia, endothelial function and oxidative stress in healthy individuals and patients with type 2 diabetes. Atherosclerosis 2001;154:475–483.

[2] Bae JH, Bassenge E, Kim KB, et al. Postprandial hypertriglyceridemia impairs endothelial function by enhanced oxidant stress. Atherosclerosis 2001;155:517–523.

[3] Bartus M, Kostogrys RB, Kaÿmierczak P, et al. 1-Methylnicotinamide (MNA) prevents endothelial dysfunction in hypertriglyceridemic and diabetic rats. Pharmacol Rep. 2008;60:127–138.

[4] Kaprinay B, Lipták B, Slovák L, et al. Hypertriglyceridemic rats fed high fat diet as a model of metabolic syndrome. Physiol Res. 2016; 65:S515-S518.

[5] Kitagawa S, Sameshima E, Yamaguchi Y, Kwon Y, Shinozuka K, Kunitomo M. Comparison of the effects of hypercholesterolaemia on relaxation responses in aortas of spontaneously hypertensive rats and Dahl salt-sensitive rats. Clin Exp Pharmacol Physiol. 1995;22(suppl):S251-S253.

[6] McCulloch AI, Bottrill FE, Randall MD, Hiley CR. Characterization and modulation of EDHF-mediated relaxations in the rat isolated superior mesenteric arterial bed. Br J Pharmacol.1997;120:1431-1438.

[7] Miller AW, Hoenig ME, Ujhelyi MR. Mechanisms of Impaired Endothelial Function Associated with Insulin Resistance. J Cardiovasc Pharmacol Therapeut. 1998;3:125-133.

[8] Pisulewski PM, Kostogrys RB, Lorkowska B, Bartuś M, Chłopicki S. Critical evaluation of normotensive rats as models for hypercholesterolaemia-induced atherosclerosis. J Anim Feed Sci. 2005;14:339-351.

[9] Sasaki S, Yoneda Y, Fujita H, et al. Association of blood pressure variability with induction of atherosclerosis in cholesterol-fed rats. Am J Hypertens. 1994;7:453-459.

[10] Sotnikova R, Bauer V. Nitric oxide production and its changes in diabetic endothelial dysfunction : chapter 7. Advances in molecular mechanisms and pharmacology of diabetic complications. In: Trivandrum : Transworld Research Networ. India, Kerala; 2010.

[11] Van Oostrom AJ, Cabezas MC, Rabelink TJ. Insulin resistance and vessel endothelial function. J R Soc Med. 2002;95:54-61.

[12] Young EJ, Hill MA, Wiehler WB, Triggle CR, Reid JJ. Reduced EDHF responses and connexin activity in mesenteric arteries from the insulin-resistant obese Zucker rat. Diabetologia2008;51:872-881.