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Effect of substances obtained from grape seed on nitrogen oxide system under experimental diabetes

Wpływ substancji uzyskiwanych z pestek winogron na system tlenku azotu w cukrzycy eksperymentalnej

INTRODUCTION

Pathogenesis of diabetes mellitus (DM) vascular complications is still an unsolved problem. Endothelium dysfunction and derangements of vascular tonus regulation are considered to play a key role in diabetic angiopathy development. Compensatory formation of vasoconstrictive (endothelin-1, thromboxan A_2, prostaglandin H_2) and vasodilating (nitric oxide (NO), prostacyclin, endothelial factor of polarization etc.) compounds is typical of the natural functioning of endothelium [6].

NO – a compound that has most apparent vasodilating ability – inhibits thrombocyte aggregation, regulates microcirculation and processes of filtration in renal canaliculus [3]. NO metabolism imbalance is considered to be one of the causes of type 1 diabetes mellitus genesis (DM1). These abnormalities can be induced by unfavorable ecological conditions that lead to inpour in organism NO, nitrites, nitrates and different N-nitrosocompounds, as well as conditions that intensify NO formation is in the organism [2, 13]. In this case NO has the evident selectivity with respect to pancreatic β-cells and its action is realized via nitric oxide toxic forms. The information concerning nitric oxide blood level under DM2 is rather contradictory [7, 15]. Chein et al. [7] found out the NO level augmentation and increase of NO-synthase activity in endotheliocyte in the patients with DM2, whereas other authors informed about NO synthesis suppression under DM2 [15].

The conventional therapy of diabetes mellitus is based on application of hypoglycemic preparations and insulin. At the same time, the intake of xenobiotics in the human organism is a powerful activator of processes of microsomal oxidation, and consequently free-radical oxidation. Increased production of the active forms of oxygen, activation of peroxidation processes are now considered as a probable cause of DM genesis [2]. The positive effect of antioxidant administration was shown under the correction of atherosclerosis and proatherogenic states [4]. Plant polyphenols

normalize the content of glucose, insulin, fatty acids and thriacylglycerols in rats with experimental 1 type diabetes. The role of phelolic compounds in the NO formation regulation is still unexplained.

The aim of the present research was to study the influence of polyphenolic concentrates "Merlo" and "Rkacitelli" on NO-synthase system in control animals and animals with experimental DM1 and DM2.

MATERIAL AND METHODS

The experiments were carried out on Wistar strain rats weighing between 140–200 g, kept on a standard vivarium diet. The effect of polyphenolic concentrates "Merlo-10" (containing up to 10% of sugar), "Merlo-30" (containing up to 30% of sugar), "Rkacitelly-10" (containing up to 10% of sugar), "Rkacitelly-30" (contains up to 30% of sugar) on the contents of nitrites, arginine and citrulline in blood of control rats and animals with DM1 and DM2 was studied.

DM1 was induced by intraperitoneal injection of a streptozotocin (STZ) solution ("Sigma", USA) [11]. Diabetes development was monitored by measuring glucose and insuline levels in rats blood serum. Diabetes was diagnosed after the glucose level in blood serum taken from overnight fasting rats was over than 14 mmol/l. DM2 was induced by keeping animals on a high-fructose diet (60 g/ on 100 g of food) during 60 days [9]. Polyphenol extracts were administred intragastrically. The doze of phenolic compounds was calculated according to the published works [9].

The glucose level was measured by the glucose oxidase method using standard assay kits. The insulin level in blood serum was measured using radioimmunoassay technique. Arginine and citrulline levels were measured by photometric methods using standard assay kits. The nitrite content which indicates the endogenous NO level was estimated spectrophotometricaly using Griss reactive [8].

Statistical analysis was carried out using one-way analysis of variance (ANOVA). $P < 0.05$ statistically significant difference.

RESULTS

It was shown that NO and citrulline levels significantly reduced and arginine level increased in peripheral blood in animals with DM1 caused by STZ introduction (Fig. 1-3). The polyphenolic concentrates administration significantly reduced NO level and normalized the arginine content (Fig. 1, 2). Thus, polyphenolic complex "Merlo-10" developed the greatest efficiency in relation to NO, arginine and citrulline – the second product of NO-synthase reaction.

In the present study the increase of NO and citrulline content in blood of rats kept on fructose diet was observed. At the same time the arginine content was significantly reduced (Fig. 1-3). The polyphenolic concentrates administration to experimental animals normalized the mentioned above indices. Thus, concentrate "Merlo-10" showed a greater activity in relation to NO-synthase system.

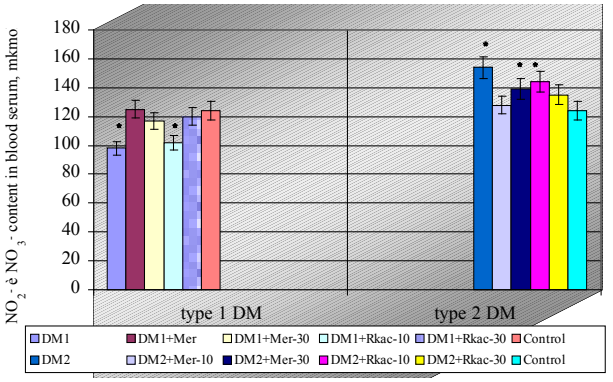

Fig. 1. Effect of grape polyphenols on nitrates+nitrites content in blood serum of animals with experimental DM (mmol/l, M±m, n=7); * – P control-experiment<0,05

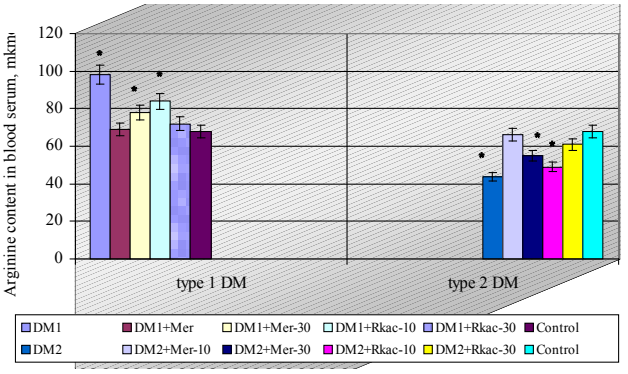

Fig. 2. Effect of grape polyphenols on arginine content in blood serum of animals with experimental DM (mmol/l, M±m, n=7); * – P control-experiment<0,05

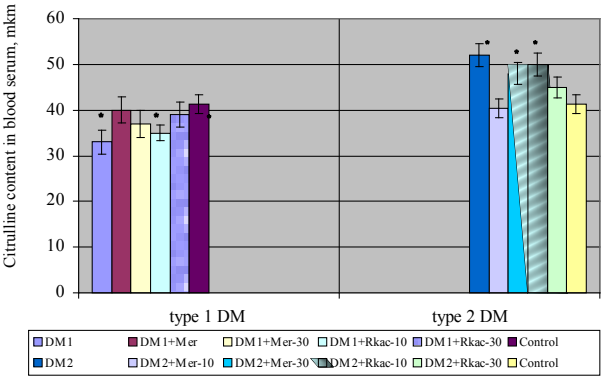

Fig. 3. Effect of grape polyphenols on citrulline content in blood serum of animals with experimental DM (mmol/l, M±m, n=7); * – P control-experiment<0,05

DISCUSSION

According to the literature data the activation of free radical oxidation processes can be accompanied by the reduction of the content of NO-syntase BH4 – the coenzyme which takes part in interaction between NO-syntase and substrate and, as a consequence, decreases enzyme affinity to substrate – arginine [14]. The increase of arginine content in blood serum, which we observed in our experiment, was accompanied by a decrease of NO. This effect can be caused by the afore mentioned interaction and also by a decrease of arginine transport into cells which developed under DM1 [1]. Normalization of the NO, arginine and citrulline levels in rats blood serum after polyphenol concentrates administration apparently and most likely depends on their high antioxidant activity (Fig. 1-3).

It is also necessary to note that individual components of polyphenol concentrates, specifically quercetin, increase the endothelial NOS expression, NO formation and release from endotheliocytes [10]. This process is mediated by activation of signaling pathway that involves Akt. This effect has dose-dependent character and develops under the long-term influence of poliphenol complexes components.

Taking into consideration the above mentioned data we can explain more effective influence of "Merlo-10" on NO system action under experimental DM1, which was described in the present work (Fig. 1-3).

According to the published data, polymeric procyanidins, whose contents is essential in the examined concentrates, suppress NOS formation by inhibiting specific mRNA iNOS synthesis in macrophage [5]. It is necessary to note that the observable effect of procyanidins depends on molecules polymerization degree. Quercetin also reduces COX-2 activity and iNOS expression by macrophage under experimental DM2 [12].

Thus, the presented results indicate that poliphenol concentrates has a normalizing effect on insuline production, glucose level and NO-system under experimental diabetes.

CONCLUSIONS

1. The positive influence of polyphenol concentrates "Merlo-10", "Merlo-30", "Rkacitelli-10" and "Rkacitelli-30" on nitric oxide generation system under DM1 and DM2 was determined.

2. The polyphenol complex "Merlo-10" revealed most evident normalizing action on nitric oxide generation system under DM1 and DM2.

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SUMMARY

The aim of the present research was to study influence polyphenolic concentrates "Merlo", "Rkacitelli" on NO-synthase system at control animals and animals with experimental DM1 and DM2. It has been shown that the increase of arginine content in blood serum, which we observed in our experiment, was accompanied by decrease of NO. This effect can be caused by decrease of enzyme affinity to substrate and also by decrease of arginine transport into cells taking place under DM1. The normalization of the NO, arginine and citrulline levels in rats blood serum after polyphenol concentrates administration apparently and most likely depends on their high antioxidant activity.

Key words: diabetes, arginine, citrulline, arginase, grape seed

STRESZCZENIE

Celem badań była ocena wpływu koncentratów polifenolowych „Merlo", „Rkacitelli" na system syntazy NO u zwierząt kontrolnych i z cukrzycą doświadczalną typu 1 oraz typu 2. Wykazano, że wzrostowi zawartości argininy w surowicy krwi, który stwierdzono w trakcie badań, towarzyszył spadek NO. Mogło to być efektem spadku powinowactwa enzymu do substratu jak też zmniejszenia transportu argininy do komórek w cukrzycy typu 1. Normalizacja poziomów NO, argininy i cytruliny w surowicy krwi szczurów po podaniu koncentratów polifenoli najprawdopodobniej zależy od ich wysokiej aktywności antyoksydacyjnej.

Słowa kluczowe: cukrzyca, arginina, cytrulina, arginaza, pestki winogron