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New arguments for beneficial effects of alpha-lipoic acid on the cardiovascular system in the course of type 2 diabetes

Marzena Dworacka[a,*], Galina Chukanova[b], Saule Iskakova[b], Yergen Kurmambayev[b], Anna Wesołowska[a], Bartosz A. Frycz[c], Paweł P. Jagodziński[c], Grzegorz Dworacki[d]

[a] Department of Pharmacology, Poznan University of Medical Sciences, Rokietnicka 5d, 60-805 Poznań, Poland
[b] Department of Pharmacology, West Kazakhstan Marat Ospanov State Medical University, Mareshev str. 68, Aktobe 030019, Kazakhstan
[c] Department of Biochemistry and Molecular Biology, Poznań University of Medical Sciences, Święcickiego 6, 60-781 Poznań, Poland
[d] Department of Clinical Immunology, Poznań University of Medical Sciences, Rokietnicka 5a, 60-805 Poznań,Poland

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ABSTRACT

Purpose: Alpha-lipoic acid (ALA), widely known as an antioxidant, modifies also serum levels of angiogenic factors in type 2 diabetic patients. These pharmacological activities may influence the status of the cardiovascular system. Taking into consideration that diabetes is related to the increased cardiovascular risk we investigated several effects of ALA on angiogenic factors in the myocardium and in the aortal wall using a rat model of type 2 diabetes.

Methods: Diabetes was induced in Wistar rats by a fat-rich diet and by intraperitoneal injection of a small dose of streptozotocin (30 mg/kg). Animals were divided into 3 groups: ALA-treated type 2 diabetes rat model, placebo-treated type 2 diabetes rat model and placebo-treated non-diabetic rats. ALA was administered orally once a day, 20 mg/kg, for 8 consecutive weeks. mRNA VEGF, VEGF-R1 and VEGF-R2 expression was measured in the myocardium and the aortal wall, simultaneously with circulating VEGF and circulating endothelial cells (cEC) and endothelial progenitor cells (cEPC).

Results: ALA induced pro-angiogenic effect in the myocardium of rats with diabetes increasing mRNA VEGF expression and decreasing mRNA VEGFR-1 expression, while in the aortal wall ALA increased mRNA VEGFR-2 and VEGFR-1 expression. cVEGF in the ALA-treated group was higher comparing to both control groups. It was revealed that cEC percentage in the ALA-treated group was decreased with no effect on the percentage of cEPC.

Conclusions: In summary, the current data provide novel findings about potential beneficial effects of ALA on angiogenic factors in the cardiovascular system, especially on myocardium, in the course of type 2 diabetes.

1. Introduction

Diabetes mellitus and its complications are closely related to the aberrant angiogenesis. As far as the cardiovascular system is concerned, it was observed that in the course of diabetes, angiogenesis is attenuated in the myocardium which limits collaterals formation, while in the arterial walls angiogenesis is overexpressed which leads to the progression of atherosclerosis (Waltenberger, 2007).

Alpha-lipoic acid (ALA) or thioctacid acid is the substance which is naturally found in human mitochondria where it plays the role of the coenzyme for pyruvate dehydrogenase and α-ketoglutarate. Most of the biological effects of ALA are usually attributed to its potent antioxidant properties, including reactive oxygen species quenching and transition metal chelation (Li et al., 2012; Park et al., 2014). These properties drew attention to the potential use of ALA in the therapy of atherosclerosis and cardiovascular disease covering cardiovascular complications (Ghibu et al., 2009; Gomes and Negrato, 2014; Wollin and Jones, 2001). On the basis of recent publications, ALA should be considered as the substance with pleiotropic activity, useful in therapy of many disorders (Gomes and Negrato, 2014). Nevertheless, effects of ALA on angiogenesis in cardiovascular system, which may cause or contribute to vascular diabetes complications, are not clear. However, it has been suggested that ALA may induce beneficial effects on aberrant angiogenesis in terms of hyperglycaemia (Li et al., 2012). Moreover, in our previous study we documented that ALA may influence angiogenesis in type 2 diabetic patients mainly by modulation of VEGF, bFGF, MCP-1 and Il-10 levels in the serum (Dworacka et al., 2015).

VEGF-A (Vascular Endothelial Growth Factor-A), commonly known

* Corresponding author at: Department of Pharmacology, Poznan University of Medical Sciences, Rokietnicka 5a, 60-805 Poznań, Poland.
E-mail addresses: mdworac@ump.edu.pl (M. Dworacka), kazga57@mail.ru (G. Chukanova), saule1@inbox.ru (S. Iskakova), ergenkurma1984@gmail.com (Y. Kurmambayev), wesolowska_a@o2.pl (A. Wesołowska), bartekfrycz@gmail.com (B.A. Frycz), pjagodzi@amp.edu.pl (P.P. Jagodziński), gdwrck@ump.edu.pl (G. Dworacki).

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as VEGF, the main pro-angiogenic factor, has a role in the formation of intercellular gaps, vesico-vascular organelles, vacuoles and fenestrations in adult organisms, and in the vasodilatation through the increase in the nitric oxide production. This potent pro-angiogenic factor also stimulates the mobilization of hematopoetic stem cells from the bone marrow, acts as chemoatractant for monocytes, influences osteoblasts-mediated bones formation and neuronal protection, stimulates inflammatory cells recruitment and activates metalloproteinases (Tammela et al., 2005). VEGF interacts with tyrosine kinase receptors, among which the interaction with VEGF-R2 (KDR/Flk-1) and VEGFR-1(Flt-1) plays a especial role in angiogenesis. VEGF-R2 regulates endothelial cell proliferation, migration, vascular permeability and secretion. VEGF-R1 stimulates monocytes/macrophages migration. VEGF-R1, as opposed to VEGF-R2, is expressed not only in endothelial cells, but also on the membrane of macrophages/monocytes and transduces an important signal for the migration and cytokine/chemokine production by these cells (Olsson et al., 2006; Shibuya, 2014).

Endothelial progenitor cells (EPC) are bone marrow-derived cell populations which are mobilized to the peripheral blood in response to tissue injury, and contribute to vascular homoeostasis. It was documented that EPCs participate in endothelium repair and contribute to angiogenesis by the propagation of new vessels from the pre-existing ones and directly influencing the process of mature endothelial cells (ECs) migration and tube formation (Erdbruegger et al., 2006). In the course of diabetes, the function and number of EPC circulating in peripheral blood (cEPC) is reduced which facilitates the development of vascular complications (Li et al., 2012). Damage to endothelium resulting from oxidative and shear stress is an early step in vascular dysfunction and atherosclerosis development. It has been shown that circulating endothelial cells (cEC) are mature endothelial cells that are derived from the impaired vascular intima. The number of cEC in peripheral blood increases several-fold in association with vascular damage (Erdbruegger et al., 2006). The hypothesis that ALA may improve the endothelial function was documented earlier but mostly it was revealed that ALA treatment improves endothelium-dependent vasodilatation, however there is no data about direct ALA influence on circulating endothelial cells in vivo (Coletta et al., 2015; Zou et al., 2017).

In the present study we have investigated the effect of ALA on VEGF and its receptors VEGF-R1 and VEGF-R2 expression in the myocardium and in the aorta, as well as on the cEPC and cEC percentage in peripheral blood of rats with induced well-controlled diabetes which is the animal model of diabetes corresponding to diabetes type 2 in humans.

2. Material and methods

2.1. Animals

Experiments were performed using 43 male 8-week-old Wistar rats weighting 227.2 ± 13.0 g. The animals were housed in individual cages in the Department of Pharmacology's Animal House and allowed to acclimatize for 7 days in an environmentally controlled room at 22 °C under an alternating 12–12 h light-dark cycle. All animals received water ad libitum.

Rats were divided into 3 groups fed with the commercially available normal pellet Labofeed B diet: 1) ALA-treated rats with type 2 diabetes (ALA-T2DM), $n = 14$; 2) placebo-treated type 2 diabetes rat model (T2DM), $n = 14$; 3) placebo-treated non-diabetic animals (ND), $n = 15$. All procedures and protocols were in accordance with the Polish governmental regulations (2005.01.21) and were approved by the Local Ethics Committee on the Use of Laboratory Animals in Poznan, Poland (54-55/2013).

2.2. Induction of diabetes

To develop a rat model of early phase of diabetes type 2 that replicates the natural history and metabolic characteristics of human type 2 diabetes, we used the previously described model - low-dose-STZ-treated/fat-fed rats (Srinivasan et al., 2005). The animals from the ALA-T2DM and from the T2DM group were put on the commercial rich-fat Labofeed B diet containing 61% of fat and were fed with it for 4 consecutive weeks. The animals from the ND group were fed with the standard Labofeed B diet during the whole experiment. Animals from the ALA-T2DM and from the T2DM group were injected intraperitoneally with 30 mg/kg STZ dissolved in 0.1 M citrate buffer, while rats from the ND group were given citrate buffer as a vehicle.

2.2.1. Metabolic characterization

The low-dose-STZ-treated/fat-fed model was documented to simulate natural disease progression and metabolic characteristics typical of individuals with type 2 diabetes, such as insulin resistance, obesity and mildly impaired insulin secretion (Srinivasan et al., 2005).

The oral glucose tolerance test was performed to confirm or to exclude diabetes in animals. Rats were fasted overnight. Blood samples of each group were initially collected via tail vein and designated as the control base line of blood glucose. Glucose soluted in sterile water in the dose of 1 g/kg was given by oral gavage and blood glucose samples were collected 60 min after glucose administration.

The animals with fasting blood glucose > 7.0 mmol/l and/or with blood glucose 60 min after glucose load between 7.8 and 11.0 mmol/l were considered as having the initial phase of diabetes reflecting diabetes type 2 in humans. The animals with blood glucose at fasting or postprandially > 11.1 mmol/l or with clinical symptoms of hyperglycaemia were excluded from the study.

2.3. Study design

Within 8 weeks (day 0 – day 56) of the main experiment, the rats from the ALA-T2DM group were treated with alpha-lipoic acid (Neurolipon-MIP 600, MIP-Pharma Polska, Poland,) 20 mg/kg suspensed in methylcellulose by oral gavage once a day, while the animals from other groups received an equivalent volume of methylcellulose. The duration of the experiment was scheduled according to previous observations (Lee et al., 2012). Blood glucose concentration was measured in all animals twice a week three times a day: at fasting at 10.00 a.m., and postprandially at 12.00 at noon and at 7.00 p.m. All rats were treated similarly in terms of daily manipulation. At the end of the study, the animals were sacrificed and blood and tissue samples were collected.

2.4. Laboratory methods

Blood glucose was measured using a portable device (Diagnostics Gold System, Diagnosis S.A., Poland), HbA_1c was assayed by immunoturbidimetric method (COBAS Integra 400/700/800) standardized according to IFCC (Weykamp et al., 2008). Insulin, C-peptide and VEGF in the serum were measured with rat specific ELISA (Insulin rat Elisa, Demeditec Diagnostics; C-peptide rat Elisa, Quantikine Elisa Rat VEGF Immunoassay, R&D Systems). The concentration of 1,5-AG (1,5-anhydro-D-glucitol) – the marker of acute hyperglycaemia (Dungan, 2008; Dworacka and Winiarska, 2005) was analyzed by enzymatic colorimetric method (Chusney et al., 1995,Dworacka and Winiarska, 2005).

Insulin sensitivity of individual animals was evaluated using the homeostasis model assessment $HOMA_{IR}$ (homeostatic model assessment insulin resistance) index (Matthews et al., 1985).

2.5. Reverse transcription and real-time quantitative polymerase chain reaction (RQ-PCR) analysis

Total RNA from the rats' heart and aorta tissues were isolated according to the method of Chomczynski (Chomczynski and Sacchi, 1987)