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Alpha-lipoic acid regulates the autophagy of vascular smooth muscle cells in diabetes by elevating hydrogen sulfide level

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ABSTRACT

Dysfunctional vascular smooth muscle (VSM) plays a vital role in the process of atherosclerosis in patients with type 2 diabetes mellitus (T2DM). Alpha-lipoic acid (ALA) can prevent the altered VSM induced by diabetes. However, the precise mechanism underlying the beneficial effect of ALA is not well understood. This study aimed to determine whether ALA ameliorates VSM function by elevating hydrogen sulfide (H_2S) level in diabetes and whether this effect is associated with regulation of autophagy of VSM cells (VSMCs). We found decreased serum H_2S levels in Chinese patients and rats with type 2 diabetes mellitus (T2DM). ALA treatment could increase H_2S level, which reduced the autophagy-related index and activation of the 5′-monophosphate-activated protein kinase (AMPK)/mammalian target of rapamycin (mTOR) pathway, thereby protecting vascular function in rats with T2DM. Propargylglycine (PPG), a cystathionine-γ-lyase inhibitor, could weaken the ALA effect. In cultured VSMCs, high glucose level also reduced H_2S level, upregulated the autophagy-related index and activated the AMPK/mTOR pathway, which were reversed by concomitant application of sodium hydrosulfide (NaHS, an H_2S donor) or ALA. The protective effect of NaHS or ALA was attenuated by rapamycin (an autophagy activator), 5-amino-1-β-d-ribofuranosyl-imidazole-4-carboxamide (an AMPK activator) or PPG. In contrast, Compound C (an AMPK inhibitor) enhanced the effect of ALA or NaHS. ALA may have a protective effect on VSMCs in T2DM by elevating H_2S level and downregulating autophagy via the AMPK/mTOR pathway. This study provides a new target for addressing diabetic macroangiopathy.

1. Introduction

Globally, approximately 382 million people had diabetes mellitus (DM) in 2013, and type 2 DM (T2DM) represented approximately 90% of these cases [1]. Diabetic macroangiopathy can cause cerebro-cardiovascular diseases and constitutes one of the major causes of death in patients with T2DM. Endothelial dysfunction has long been identified as the key factor in diabetic macroangiopathy, and emerging data implicate dysfunctional vascular smooth muscle (VSM) in the pathogenesis [2,3]. Moreover, increasing evidence indicates that VSM dysfunction plays a vital role in atherosclerotic lesion growth and plaque rupture [4].

Autophagy, an important biological process for maintaining cellular homeostasis, affects the survival and function of vascular cells, including VSM cells (VSMCs) [5]. Moderate activation of autophagy is considered to protect VSMCs against cell death. By contrast, overloaded

Abbreviations: 3-MST, 3-mercaptopyruvate sulfur-transferase; Ach, acetylcholine; AICAR, 5-amino-1-β-d-ribofuranosyl-imidazole-4-carboxamide; ALA, alpha-lipoic acid; AMPK, adenosine 5′-monophosphate-activated protein kinase; Ang II, angiotensin II; CBS, cystathionine-β-synthase; CCK-8, cell counting kit-8; CSE, cystathionine-γ-lyase; DHLA, Dihydrolipoic acid; DM, diabetes mellitus; DMEM, dulbecco's modified Eagle's medium; FBG, fasting blood glucose; FBS, fetal bovine serum; H_2S, hydrogen sulfide; HG, high glucose; ip, intraperitoneal; mTOR, mammalian target of rapamycin; NaHS, sodium hydrosulfide; Phe, phenylephrine; PPG, propargylglycine; SNP, sodium nitroprusside; T2DM, type 2 diabetes mellitus; VSM, vascular smooth muscle; VSMCs, vascular smooth muscle cells

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autophagy may be deleterious because of excessive self-digestion [5]. Autophagy plays a crucial role in regulating VSMC phenotypes and inducing VSMC migration [5,6]. It is also involved in the proliferation of VSMCs that leads to diabetic atherosclerosis [7].

Alpha-lipoic acid (1,2-dithiolane-3-pentanoic acid, ALA), a natural dithiol compound synthesized from octanoic acid, can regulate mitochondrial energy metabolism [8]. ALA acts as an essential biological antioxidant and possesses many biochemical functions. For example, ALA could protect against diabetic neuropathy, kidney injury and retinopathy [9–12]. It also prevents the altered vascular morphology in diabetic rats [13]. Furthermore, recent studies demonstrated that ALA inhibits proliferation and induces apoptosis in VSMCs via the pathways Ras/mitogen-activated protein kinase (MAPK) kinase/extracellular-signal-regulated kinase (Ras/MEK/ERK) and p38 mitogen-activated protein kinase/Nur77, respectively [14,15]. ALA also plays a vital role in regulating autophagy, with ALA having protective effects on cardiomyocytes, injured retinal pigment epithelial cells and human umbilical vein endothelial cells [16–18]. However, whether and how ALA regulates autophagy in VSMCs under diabetic conditions remains to be elucidated.

Hydrogen sulfide (H_2S), a gasotransmitter, functions in various physiological and pathophysiological conditions, including DM [19]. The blood level of H_2S is significantly lower in African American patients with T2DM than age-matched normal controls [20]. In animal experiments, H_2S has many beneficial roles in protecting against diabetes-induced alterations in kidney, heart and blood vessels, such as diabetic renal fibrosis, palmitic acid-induced myocardial injury and nephropathy, proliferation of VSMCs and decreased thickness of VSM [21–25]. H_2S can also regulate autophagy in liver cells, H9c2 cells and colon epithelial cells [26–28]. However, whether H_2S protects VSMCs in DM by regulating autophagy is unknown.

Dihydrolipoic acid (DHLA), which is unstable without enzymes in in vitro conditions, is reduced enzymatically from ALA and exists in cells after the administration of lipoic acid [29,30]. As a reduced form of ALA, DHLA could release H_2S from endogenous sulfane sulfur compounds [30]. Accordingly, from the relation between ALA and H_2S and their effects on vascular function and autophagy, we hypothesized that ALA might modulate the autophagy of VSMCs in DM by elevating H_2S level. To confirm this hypothesis, we investigated patients with T2DM, the rat model of DM induced by a high-fat diet and low-dose streptozotocin, and cultured VSMCs with high glucose (HG) treatment to determine the H_2S level in DM and the effect of ALA on H_2S level, autophagy and vascular function.

2. Materials and methods

2.1. Patients and controls

This observational study complied with the principles established by the Declaration of Helsinki and was approved by the ethics committee of Hebei Medical University. We recruited 101 adults (aged ≥18 years) with a diagnosis of T2DM from August 2016 to December 2016. Exclusion criteria were (1) ketosis or diabetic coma; (2) serious circulatory system disease (New York Heart Association functional class III or IV heart failure), nephropathy (chronic kidney disease stage 4 or 5), hepatopathy (alanine transaminase or aspartate transaminase level > three times the upper limit of normal) or hematopathy (lymphadenoma, leucocythemia, multiple myeloma and other hematological diseases that seriously affect quality of life); (3) acute or chronic inflammation; (4) hypertension; and (5) women during gestation and lactation.

Patients with T2DM were divided into two groups — with ALA treatment or not. One group received routine hypoglycemic treatment, and the other group received ALA (Yabao Pharmaceutical Co., Taiyuan, Shanxi, China) at a dose of 0.6 g/day, in addition to routine hypoglycemic treatment for 14 days. An age- and sex-matched group of participants without DM (n = 20) were recruited from volunteers as a control group. Participants with hypertension and dyslipidemia were excluded from the control group. All participants signed informed consent forms for the study.

2.2. Animals

We purchased 40 adult male Sprague-Dawley (SD) rats weighing 200 to 220 g from the Animal Research Center of Hebei Medical University and housed them four per cage with a light cycle of 12 h per day in a temperature-controlled room (25 ± 1 °C). All animal procedures complied with the US National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Animal Care Committee of Hebei Medical University.

2.3. Induction of T2DM in rats

After feeding for 2-week acclimatization, rats were randomly divided into control and treatment groups, with normal chow diet and high-fat diet (fat 34.5%, protein 17.5% and carbohydrate 48%, as a percentage of total kcal), respectively, for 4 weeks. Then diabetes was induced in treatment rats by a single intraperitoneal (ip) injection of streptozotocin (STZ; Sigma, St. Louis, MO, USA; 27.5 mg/kg in 0.1 mol/L citrate buffer, pH 4.5) after overnight fasting [31] and the control group was given an ip injection of isopyknic citrate buffer. At the end of the fifth week, rats with fasting blood glucose (FBG) level > 11.1 mmol/L (T2DM) were used in the study. T2DM rats were divided into four groups (n = 8 each) for treatment for 8 weeks: (1) DM (0.9% saline, ip), (2) DM + ALA (100 mg/kg/day, ip), (3) DM + propargylglycine (PPG, 37.5 mg/kg/day, ip; Sigma Chemical, St. Louis, MO, USA), and (4) DM + PPG (37.5 mg/kg/day, ip) + ALA (100 mg/kg/day, ip). At the end of 13 weeks, all rats fasted overnight and were sacrificed with CO_2 asphyxiation.

2.4. Cell culture

VSMCs were isolated from the thoracic aorta of male SD rats (80–100 g) after anesthetization with 20% urethane (4 mL/kg, ip, another 2 mL/kg was added if anesthetization was not satisfactory; Sigma-Aldrich Chemical, St. Louis, MO, USA) [32] and cultured in Dulbecco's modified Eagle's medium (Life Technologies, Gaithersburg, MD, USA) with 5.5 mmol/L glucose, 10% fetal bovine serum (FBS, Gemini Biologicals, Calabasas, CA, USA) and 1% penicillin/streptomycin in a humidified atmosphere with 5% CO_2 at 37 °C. Cells at passages 5 to 8 were used in this experiment. For HG, serum-starved VSMCs were stimulated with 30 mmol/L glucose. In some experiments, VSMCs were primarily cultured in HG for 12 h, then co-treated with 500 μmol/L ALA or 100 μmol/L sodium hydrosulfide (NaHS, Sigma-Aldrich Chemical, St. Louis, MO, USA) for 36 h. Cells were pre-treated with PPG (400 μmol/L) and rapamycin (250 nmol/L; Biovision, Mountain View, CA, USA), 5-amino-1-β-d-ribofuranosyl-imidazole-4-carboxamide (AICAR, 0.5 mmol/L; MedChemExpress, Princeton, MA, USA), compound C (10 μmol/L; Sigma-Aldrich Chemical, St. Louis, MO, USA), or nothing for 30 min before ALA or NaHS treatment according to the requirements.

2.5. H_2S measurement

Blood samples were collected from overnight-fasted participants and rats. Of note, participant diets contained no allicin in the previous 2 days. The blood samples were centrifuged at 3500 rpm for 10 min, and serum was used for detecting H_2S. Cultured VSMCs were lysed with tris-HCl (pH 8.5) and centrifuged at 12,000 rpm for 15 min. The cell suspension was used for H_2S level analysis. H_2S level was measured by liquid chromatography-mass spectrometry (LC-MS/MS) as described [33]. This validated LC-MS/MS method with high sensitivity and specificity can robustly measure H_2S level in various biological samples.