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Alpha-lipoic acid reduces retinal cell death in diabetic mice

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ABSTRACT

Oxidative stress plays an important role in the development of diabetic retinopathy. Here, we examined whether α-lipoic acid (α-LA), a natural antioxidant, attenuated retinal injury in diabetic mice. The α-LA was orally administered to control mice or mice with streptozotocin-induced diabetes. We found that α-LA reduced oxidative stress, decreased and increased retinal 4-hydroxy-2-nonenal and glutathione peroxidase, respectively, and inhibited retinal cell death. Concomitantly, α-LA reversed the decreased activation of AMP-activated protein kinase (AMPK) and acetyl-CoA carboxylase, and increased the levels of peroxisome proliferator-activated receptor delta and sirtuin3 in diabetic mouse retinas, similar to results shown after metformin treatment of retinal pigment epithelial cells (RPE) exposed to high glucose. Moreover, α-LA lowered the levels of O-linked β-*N*-acetylglucosamine transferase (OGT) and thioredoxin-interacting protein (TXNIP) in diabetic retinas that were more pronounced after metformin treatment of RPE cells. Importantly, α-LA lowered interactions between AMPK and OGT as shown by co-immunoprecipitation analyses, and this was accompanied by less cell death as measured by double immunofluorescence staining by terminal deoxynucleotide transferase-mediated dUTP nick-end labelling and OGT or TXNIP in retinal ganglion cells. Consistently, α-LA lowered the levels of cleaved poly(ADP-ribose) polymerase and pro-apoptotic marker cleaved caspase-3 in diabetic retinas. Our results indicated that α-LA reduced retinal cell death partly through AMPK activation or OGT inhibition in diabetic mice.

1. Introduction

Production of mitochondrial reactive oxygen species (ROS) in response to hyperglycaemia may be an initiating cause in the pathogenesis of diabetes complications [1], including diabetic retinopathy, the leading cause of acquired blindness in developed countries [2]. In diabetic retinopathy, early neurodegeneration in the retinal ganglion cells via apoptosis is evident [3].

Alpha-lipoic acid (α-LA), a potent antioxidant, improves insulin sensitivity and skeletal muscle fatty acid oxidation by activating AMP-activated protein kinase (AMPK) in diabetic patients [4]. The α-LA acts in a hypoglycaemic manner that may lower O-GlcNAcylation [5], which influences the activities of several proteins, including AMPK [6]. Although α-LA beneficially affects obesity, type 2 diabetes and dyslipidaemia [7], the mechanism by which this occurs remains incompletely understood.

AMPK, a critical cellular energy sensor, affects metabolic stress via phosphorylation of downstream substrates, including acetyl-CoA carboxylase (ACC) [8]. Previous reports have shown possible anti-apoptotic effects of AMPK [9], consequently, AMPK may influence the pathophysiology and therapy of diabetes [10].

Hyperglycaemia elevates protein modification by O-linked *N*-acetylglucosamine (O-GlcNAc) [11]; reversible modification by O-GlcNAc might act in parallel with protein phosphorylation to modify protein-protein interactions [12]. Crosstalk between the O-GlcNAc and AMPK systems has been reported, suggesting O-GlcNAc transferase (OGT), the enzyme for this modification, and AMPK might affect each other in regulating nutrient sensitive intracellular processes [13].

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Apoptotic thioredoxin-interacting protein (TXNIP) might cause the development of diabetic retinopathy (DR) [14], and is indirectly regulated by AMPK [15], but no studies have shown its relationship to α-LA in DR.

The peroxisome proliferator-activated receptor family of nuclear receptors (PPAR) δ act as metabolic sensors, and prevent ER stress, inflammation, and insulin resistance in skeletal muscle cells by activating AMPK [16].

Sirtuin3 (SIRT3) regulates mitochondrial oxidative stress [17]. Manganese superoxide dismutase (MnSOD) is one of SIRT3 targets, which is the primary mitochondrial enzyme converting superoxide to water [18]. Therefore, decreased SIRT3 function due to hyperglycaemia could lead to reduced MnSOD activity [19], leading to retinal cell death in diabetic mice.

Here, we investigated whether α-LA was effective at reducing retinal cell death in the early stages of DR, using a streptozotocin-induced diabetic mouse model.

2. Materials and methods

2.1. Animals

Diabetes was induced in male C57BL/6 mice (KOATEC, Pyeongtaek, Republic of Korea), as previously described [20]. All animal experiments were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 8023). The α-LA was purchased from MP Biomedicals (101138; Burlingame, CA, USA) and orally administered to the mice at 200 mg/kg/day. All mice were sacrificed at 2 months after the final injection of 2-deoxy-2-(3-methyl-3-nitrosoureido)-D-glucopyranose (STZ) or saline. Blood was obtained by tail puncture, and diabetes induction was verified weekly after STZ injection by evaluating blood glucose concentrations using a Precision glucometer (Abbott Laboratories, Alameda, CA, USA). Mice with a blood glucose concentration ≥250 mg/dL were considered diabetic.

2.2. Cell culture and treatments

The ARPE-19 human retinal pigment epithelial (RPE) cell line was purchased from American Type Culture Collection (Manassas, VA, USA), and grown at 37 °C in Dulbecco's modified Eagle medium supplemented with 10% fetal bovine serum (Invitrogen, Carlsbad, CA, USA), 100 g/mL streptomycin, and 100 units/mL penicillin (Invitrogen). Cells were treated with low glucose (LG, 5 mM), high glucose (HG, 25 mM) ± α-LA (ALA, 300 μM; T1395; Sigma-Aldrich, St. Louis, MO, USA), HG ± metformin [MET, 80 mM; Enzo Life Sciences (ENZO-ALX-270-432, Farmingdale, NY, USA)] or HG ± Compound C (CC, 20 μM; Calbiochem, 171260; San Diego, CA, USA).

2.3. α-LA administration

The α-LA was orally administered to mice. When conventional antidiabetic doses were used in mice, the equivalent dose was 250 mg/kg/day; based on previous reports [4,7], we administered 200 mg/kg α-LA once daily for 8 weeks after the final STZ or saline injection. Control and diabetic mice were gavaged daily with saline. Blood glucose levels and body weights were measured weekly.

2.4. Antibodies

The following antibodies were used: 4-HNE (ab46545; Abcam, Cambridge, UK), GPx-1/2 (sc-133160; Santa Cruz Biotechnology, Santa Cruz, CA, USA), AMPK (#2532; Cell Signaling Technology, Beverly, MA, USA), phospho-AMPK (Thr172) (#2535; Cell Signaling Technology), OGT (ab96718; Abcam), TXNIP (sc-166234; Santa Cruz Biotechnology), ACC (#3662; Cell Signaling Technology), phospho-ACC (#3661; Cell Signaling Technology), PPARδ (ab23673; Abcam), SIRT3 (ab189860; Abcam), PARP (#9532; Cell Signaling Technology), cleaved PARP (#5625; Cell Signaling Technology), cleaved caspase-3 (#9664; Cell Signaling Technology), caspase-3 (#9662; Cell Signaling Technology), β-Actin (A5441; Sigma-Aldrich), secondary horseradish-peroxidase-conjugated goat anti-mouse IgG (#31430; Thermo Scientific/Pierce Biotechnology, Rockford, IL, USA), and goat anti-rabbit IgG (#31460; Thermo Scientific/Pierce Biotechnology).

2.5. Western blotting

Protein extraction and western blotting were performed as described previously [21].

2.6. Immunoprecipitation

Immunoprecipitation was performed as described previously [22].

2.7. Immunohistochemistry analysis

Immunohistochemistry was performed on frozen retinal sections (5-μm thick), as described previously [20].

2.8. Immunofluorescence analysis

Immunofluorescence analysis was performed as described previously [22].

2.9. Statistical analysis

Quantitative analyses were performed using ImageJ analysis software (National Institutes of Health, Bethesda, MD, USA) and GraphPad Prism 5 (GraphPad Software, San Diego, CA, USA). Data are representative of three independent experiments and are presented as the mean ± standard error of the mean (SEM). The statistical significance of differences was determined using one-way analysis of variance followed by Bonferroni's post hoc analysis to compare groups. Results were considered significant when P was <0.05.

3. Results

3.1. α-LA lowered blood glucose levels in diabetic mice

Blood glucose levels were significantly increased in diabetic mice compared to control mice (Fig. 1A, $P < 0.005$), whereas control mice showed normoglycemia throughout the study. However, α-LA administration significantly reversed these levels compared to those without α-LA (Fig. 1A, $P < 0.01$ or $P < 0.005$). Regarding body weight, diabetic mice showed significant weight loss compared to control mice (Fig. 1B, $P < 0.005$), whereas α-LA only marginally changed this loss (Fig. 1B, not significant).

3.2. α-LA reduced oxidative stress in diabetic mouse retina and retinal cells exposed to high glucose

To determine the α-LA's effects on oxidative stress [23], we examined whether α-LA affected 4-hydroxynonenal (4-HNE), an oxidative stress marker [24,25], and glutathione peroxidase (GPx), an antioxidant enzyme [26], in diabetic mice and retinal cells exposed to high glucose. Indeed, we found that 4-HNE or GPx were significantly increased or decreased, respectively, in diabetic retinas

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