European Journal of Pharmacology 810 (2017) 1–8

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Molecular and cellular pharmacology

The impact of α-Lipoic acid on cell viability and expression of nephrin and ZNF580 in normal human podocytes

Ulrike Leppert[a], Allan Gillespie[b], Miriam Orphal[b], Karen Böhme[b], Claudia Plum[b], Kaj Nagorsen[a], Janine Berkholz[a], Reinhold Kreutz[b], Andreas Eisenreich[b,*]

[a] Charité-Universitätsmedizin Berlin, CC02, Institut für Physiologie, Berlin, Germany
[b] Charité-Universitätsmedizin Berlin, CC04, Institut für Klinische Pharmakologie und Toxikologie, Berlin, Germany

ARTICLE INFO

Keywords:
α-lipoic acid
Human podocytes
ZNF580
Nephrin

ABSTRACT

Human podocytes (hPC) are essential for maintaining normal kidney function and dysfunction or loss of hPC play a pivotal role in the manifestation and progression of chronic kidney diseases including diabetic nephropathy. Previously, α-Lipoic acid (α-LA), a licensed drug for treatment of diabetic neuropathy, was shown to exhibit protective effects on diabetic nephropathy in vivo. However, the effect of α-LA on hPC under non-diabetic conditions is unknown. Therefore, we analyzed the impact of α-LA on cell viability and expression of nephrin and zinc finger protein 580 (ZNF580) in normal hPC in vitro.

Protein analyses were done via Western blot techniques. Cell viability was determined using a functional assay. hPC viability was dynamically modulated via α-LA stimulation in a concentration-dependent manner. This was associated with reduced nephrin and ZNF580 expression and increased nephrin phosphorylation in normal hPC. Moreover, α-LA reduced nephrin and ZNF580 protein expression via 'kappa-light-chain-enhancer' of activated B-cells (NF-κB) inhibition. These data demonstrate that low α-LA had no negative influence on hPC viability, whereas, high α-LA concentrations induced cytotoxic effects on normal hPC and reduced nephrin and ZNF580 expression via NF-κB inhibition. These data provide first novel information about potential cytotoxic effects of α-LA on hPC under non-diabetic conditions.

1. Introduction

Diabetes mellitus is a common metabolic disease and becomes a growing problem worldwide (Martinez-Castelao et al., 2015). It is associated with important clinical sequelae, such as diabetic nephropathy or diabetes-associated neuropathy (Lawson et al., 2005). Diabetic nephropathy is a fundamental problem in diabetes (Martinez-Castelao et al., 2015) that involves several patterns of organ damage among which changes of podocytes play a crucial role (Carney, 2016). Dysfunction and loss of human podocytes (hPC) play a substantial role in the progression of diabetic nephropathy (Carney, 2016). The biological functions of hPC are controlled via the expression of structural proteins, such as nephrin or transcription factors, e.g. nuclear factor 'kappa-light-chain-enhancer' of activated B-cells (NF-κB) and zinc finger proteins (Beltcheva et al., 2010; Ghiggeri et al., 2013; Li and He, 2015).

Nephrin is expressed and secreted by hPC and key compound of the slit diaphragm (Kindt et al., 2017; Li and He, 2015). Phosphorylation of nephrin plays an important role in maintenance of hPC integrity and glomerular function, and is considered as an indicator for hPC health (Carney, 2016). Nephrin expression and phosphorylation was shown to be decreased in different renal diseases (Carney, 2016; Li and He, 2015).

Zinc finger protein 580 (ZNF580) is a putative transcription factor (Hoffmann et al., 2011; Sun et al., 2010). This protein was found to be regulated by NF-κB (DangLi et al., 2012). The ZNF580 expression can be modulated by different stimuli, such as reactive oxygen species (DangLi et al., 2012) or high oxidized low-density lipoprotein ratios (Hoffmann et al., 2011).

α-Lipoic acid (α-LA) is a natural compound generated by plants, animals, and humans and exhibits antioxidant properties (Dozio et al., 2010; Ziegler et al., 1999). This compound is licensed for the treatment of diabetes-associated neuropathy (Chong and Hester, 2007). In this context, α-LA was shown to mediate protective effects in diabetic patients (Borcea et al., 1999) and to be effective and safe in patients with symptomatic diabetic polyneuropathy (Ziegler et al., 1999). Moreover, increased amounts of α-LA were shown to mediate reno-protective effects and to abolish the progression of diabetic nephro-

* Correspondence to: Charité-Universitätsmedizin Berlin, CC04, Institut für Klinische Pharmakologie und Toxikologie, Charitéplatz 1, 10117 Berlin, Germany.
E-mail address: andreas.eisenreich@charite.de (A. Eisenreich).

http://dx.doi.org/10.1016/j.ejphar.2017.06.013
Received 30 March 2017; Received in revised form 7 June 2017; Accepted 8 June 2017
Available online 10 June 2017
0014-2999/

pathy in a mouse model of diabetes mellitus (Yi et al., 2012). α-LA was demonstrated to be a negative regulator for NF-κB. In 1998, Hofmann et al. found that α-LA is capable of reducing NF-κB binding activity in monocytes of patients with type 1 diabetes (Hofmann et al., 1998). Moreover, α-LA inhibited tumor necrosis factor-alpha (TNF-α) -induced activation of NF-κB in human endothelial cells and monocytes (Ying et al., 2011; Zhang and Frei, 2001). No data have been reported on the effects of α-LA on normal podocytes biology and function so far. Therefore, we analyzed the impact of α-LA on cell viability of normal hPC. Moreover, we characterized the influence of this compound on protein expression of the NF-κB-regulated hPC proteins nephrin and ZNF580 as well as on expression of protein kinase B (Akt) and NF-κB p65 subunit (RelA). Here, we showed for the first time that α-LA negatively affects the expression of the NF-κB regulated proteins, nephrin and ZNF580 in hPC. Moreover, we demonstrate that treatment with α-LA leads to dynamic and concentration-dependent changes of hPC viability under non-diabetic conditions.

2. Materials and methods

2.1. Cell culture

Immortalized hPC were obtained from Dr. M. Saleem (University of Bristol, Bristol, UK). Cells were cultured and differentiated as described before (Langer et al., 2016). For stimulation and inhibition experiments, hPC were starved in fetal bovine serum-free RPMI 1640 medium (Life Technologies, Carlsbad, USA) for 12 h. After that, cells were incubated with different concentrations of α-LA (Sigma-Aldrich Chemie GmbH, Munich, Germany) ranging from 0 μM – 1000 μM for 4 h or 24 h, respectively. Controls were treated with corresponding amounts of ethanol (solvent). Inhibition of NF-κB and Akt was done by treatment of cells for 24 h with BAY 11–7082 (10 μM or 20 μM; Adipogen AG, Liestal, Switzerland) or triciribine (10 μM; Selleck Chemicals, Houston, TX, USA), respectively.

2.2. Real-Time polymerase chain reaction (PCR)

Analyses of mRNA expression was done via Real-Time PCR as previously described (Eisenreich et al., 2016). Total RNA was isolated using the Universal RNA Purification Kit (Roboklon GmbH, Berlin, Germany) following the manufacturer's protocol. Total RNA (1 μg) was reverse transcribed via High-Capacity cDNA Reverse Transcription Kit (Life Technologies GmbH, Darmstadt, Germany). For Real-Time PCR a 7500 Fast Real-Time PCR System (Applied Biosystems, Carlsbad, CA, USA) was used. Conditions: 50 °C, 2 min; 95 °C, 20 s; 45 cycles 95 °C, 3 s; 60 °C, 30 s. Expression analysis of RelA, Akt, and glyceraldehyde 3-phosphate dehydrogenase (GAPDH, for normalization) was performed using TaqMan® Gene Expression Assays (Life Technologies GmbH, Darmstadt, Germany) following the manufacturer's instructions.

2.3. Western blotting

Western blot analyses were performed as described previously (Eisenreich et al., 2008b, 2016). The following specific antibodies were used: anti-nephrin (1:1000; Thermo Fisher Scientific, Waltham, MA, USA), anti-phospho (p)-nephrin (phospho Y1176 + Y1193; 1:1000; Abcam, Cambridge, UK), anti-ZNF580 (1:500, Novus Biologicals, Littleton; USA), anti-RelA (1:500, Aviva Systems Biology, Corp., San Diego, CA, USA) anti-Akt (1:500, Merck Chemicals GmbH, Schwalbach, Germany), anti-GAPDH (1:20000, Calbiochem, Darmstadt, Germany), goat anti-rabbit (1:2000, DAKO, Glostrup, Denmark) and rabbit anti-mouse (1:2000, DAKO, Glostrup, Denmark). Quantification of Western blots was performed via Gel-Pro AnalyzerTM software (4.0.00.001; Media Cybernetics, Bethesda, MD, USA) as described before (Eisenreich et al., 2008a, 2016).

2.4. Cell viability assay

Cell viability assay was performed as described before (Chen et al., 2012; Eisenreich et al., 2016). In Brief, 1×10^5 hPC were cultured in 24-well plates and treated with or without α-LA for 24 h. After that, cells were removed from wells using trypsin-EDTA and resuspended in fresh RPMI 1640 medium mixed 1:2 with trypan blue solution (Biochrom, Berlin, Germany). Finally, trypan blue-stained living and dead cells were comparatively counted using a C-Chip Neubauer improved hemocytometer chamber (Carl Roth GmbH & Co. KG., Karlsruhe, Germany) as previously done by others (Chen et al., 2012; Chou et al., 2000).

2.5. Immunofluorescence

For immunofluorescence analyses 1×10^5 hPC were cultured in 24-well plates and stimulated for 24 h with or without α-LA. Thereafter, culture medium was aspirated from the wells. Cells were washed in phosphate buffered saline (PBS) and then fixated in 4% Formaldehyd + 1 mM $MgCl_2$ + 0.5% Triton X100 for 10 min at room temperature. After fixation, samples were washed with PBS and blocked in PBS + 5% fetal bovine serum + 0,1% Triton X100 for 20 min at room temperature. After that, samples were incubated for 1 h with the first antibody (anti-nephrin, 1:100, Thermo Fisher Scientific, Waltham, MA, USA) at room temperature. Then, cells were washed in PBS and incubated for 1 h at room temperature in the dark with the secondary antibody (FITC-conjugated goat anti-rabbit IgG antibody, 1:200, Merck Chemicals GmbH, Darmstadt, Germany). After final washing in PBS, cells were overlayed with Vectashield Mounting Medium (Vector Laboratories, Burlingame, CA, USA). Fluorescence microscopy was performed with EVOS FL Microscope (Life Technologies, Carlsbad, USA).

2.6. Statistical analysis

All data were expressed as mean ± standard error the mean (S.E.M.). Data were analyzed by Student's *t*-test or 1-way ANOVA. A probability value $P < 0.05$ was considered significant.

3. Results

3.1. Cell viability of hPC was affected by α-LA treatment

We characterized the influence of different concentrations of α-LA on cell viability in hPC after 24 h. Stimulation of hPC with 50 μM and 100 μM resulted in slightly increased hPC viability after 24 h (Fig. 1). In contrast to stimulation of cells with low α-LA concentrations, treatment of hPC with 500 μM or 1000 μM of α-LA, respectively, significantly decreased cell viability (Fig. 1). These data suggest that high α-LA concentrations reduced cell viability of hPC.

3.2. Treatment of hPC with α-LA reduced protein expression of nephrin and ZNF580

Nephrin expression plays an important role for maintenance of hPC function and viability (Eisenreich et al., 2016). Therefore, we next analyzed the impact of α-LA on nephrin expression in hPC. Treatment of cells with 50 μM, 100 μM, and 250 μM α-LA exhibited no significant impact on nephrin protein expression after 24 h (Fig. 2A). Whereas, stimulation of hPC with 500 μM or 1000 μM α-LA, respectively, resulted in a significant reduction of nephrin compared to controls (Fig. 2A). This was confirmed by immunofluorescence analyses, showing that application of 1000 μM α-LA (Fig. 2D) led to reduced nephrin exposition within the hPC-surrounding extracellular matrix (lower picture, black arrows), compared to control cells (Fig. 2C, lower picture, white arrows). Further, this was associated with the loss of a