Biochemical and Biophysical Research Communications 496 (2018) 955–960

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Retina-derived endogenous sulfur dioxide might be a novel anti-apoptotic factor

Jiantong Du [a], Yaqian Huang [b], Kun Li [c], Xiaoqi Yu [c], Hongfang Jin [b], Liu Yang [a, *]

[a] Department of Ophthalmology, Peking University First Hospital, Beijing 100034, China
[b] Department of Pediatrics, Peking University First Hospital, Beijing 100034, China
[c] College of Chemistry, Sichuan University, Sichuan, Chengdu 610064, China

ARTICLE INFO

Article history:
Received 5 January 2018
Accepted 16 January 2018

Keywords:
Retina
Sulfur dioxide
Aspartate aminotransferase
Apoptosis
Oxidative stress

ABSTRACT

Endogenous sulfur dioxide (SO_2) was found to be generated from the enzymatic reaction catalysed by aspartate transference 1 (AAT1) in the mammals and play importantly biological effects. In the present study, we explored the existence of endogenous SO_2 pathway in mouse retinal tissues and 661w photoreceptor cell and investigated its possible pathophysiological role in the hydrogen peroxide (H_2O_2)-induced mouse photoreceptor cell apoptosis. The data showed that endogenous SO_2 pathway including AAT1 expression and SO_2 content was found to be presented in mouse photoreceptor cells. AAT1 protein and SO_2 were mainly distributed in the cytoplasm, while a small amount of AAT1 protein and SO_2 was found in the nucleus of 661W photoreceptor cells. H_2O_2 significantly decreased the SO_2 content and AAT1 expression, but increased the cleaved caspase-3 protein level and the apoptotic index, and the number of TUNEL-positive cells in the 661W photoreceptor cells. Moreover, an AAT inhibitor HDX treatment inhibited SO_2 synthesis and mimicked H_2O_2-induced apoptosis in 661W cells. In conclusion, the endogenous SO_2/AAT1 pathway is firstly found to be present in mouse photoreceptor cells, and might play an important role in the prevention from mouse photoreceptor cell apoptosis.

1. Introduction

Age-related macular degeneration (AMD) is a disease with irreversible damage to central vision as the main clinical manifestation. The pathological manifestations of AMD include drusen, degeneration of retinal pigment epithelium, photoreceptor cell atrophy, and choroidal neovascularization. In many previous studies several risk factors (e.g., age, race, and smoking) have been found to be correlated with the disease [1]. However, the pathogenesis of AMD remains unclear. Recently, many studies confirmed that excessive light exposure-induced oxidative stress could lead to photoreceptor cell apoptosis which was a critical phase in the pathogenesis of AMD [2–4]. Therefore, clarifying the mechanism underlying the apoptosis of retinal photoreceptor cells induced by oxidative stress is an important and meaningful scientific issue in the field of ophthalmology.

Sulfur dioxide (SO_2), previously considered as a toxic gas, is now recognized as a novel gaseous signalling molecule since it can be synthesized endogenously by aspartate aminotransferase (AAT) in the mammalian cardiovascular system and plays a critical role in physiological and pathophysiological regulation [5–7]. Our previous study demonstrated that endogenous SO_2 protected against isopropylarterenol-induced cardiomyocyte injury by inhibiting cardiomyocyte apoptosis [8]. Moreover, Chen et al. discovered that SO_2 donor prevented from oleic acid-induced lung injury by acting as an antioxidant [9]. Therefore, we put forward a novel hypothesis that there might be endogenous SO_2/AAT1 pathway in the retinal tissues and endogenous SO_2 might act as a protector suppressing oxidative stress-caused photoreceptor cell apoptosis. In the present study, we attempt to explore the possibility that there exists endogenous SO_2 pathway in the mouse retinal tissues and in 661W mouse photoreceptor cells. Furthermore, we plan to examine the pathophysiological significance of endogenous SO_2 in a cell model of H_2O_2-induced 661W cell apoptosis.

* Corresponding author.
E-mail address: liu.yang@pkufh.cn (L. Yang).

https://doi.org/10.1016/j.bbrc.2018.01.103
0006-291X/

2. Materials and methods

2.1. Cells and reagents

Mouse 661W photoreceptor cell line was purchased from American Type Culture Collection (ATCC, USA). Dulbecco's modified Eagle medium (DMEM), penicillin, and streptomycin were purchased from Invitrogen (USA). Foetal bovine serum (FBS) was purchased from Gibco (USA). AAT1 antibodies (SAB2500473), L-aspartic acid-β-hydroxamic acid (HDX), and horseradish peroxidase (HRP)-conjugated goat secondary antibody, rabbit secondary antibody, and rat secondary antibody were purchased from Sigma-Aldrich (USA). GAPDH antibodies (TA-08), rabbit anti-goat IgG (PV-9003), 5% bovine serum albumin (BSA), and 3,3-diaminobenzidine tetrahydrochloride (DAB) were purchased from ZSGB-BIO (Beijing). Alexa Fluor®594 donkey anti-goat IgG (A11058) was purchased from Molecular Probes (USA). Cleaved caspase-3 antibodies (9661L) were purchased from Cell Signal Technology (USA). Enhanced chemiluminescence (ECL) products were from Thermo Fisher Scientific. An *in situ* cell death detection kit was purchased from Roche (Germany). The SO_2 fluorescent probe was generously provided by Professor Kun Li, College of Chemistry, Sichuan University.

2.2. Immunohistochemical staining

The expression of AAT1 in the retinal tissues was detected by the immunohistochemical staining as previously described [10]. Briefly, the eyes of 8-week-old healthy male C57BL6/J mice were collected and fixed in 4% polyoxymethylene. The eyes were transferred to a 20% sucrose solution, subsequently embedded in paraffin, and prepared as 6 μm-thick sections. The sections were sequentially treated with xylene, an ethanol gradient, and phosphate-buffered saline (PBS) solution. The sections were sequentially treated with 3% hydrogen peroxide (H_2O_2) at room temperature for 10 min, pepsin at 37 °C for 30 min for antigen retrieval and 5% BSA at 37 °C for 20 min for blocking non-specific absorbance. Furthermore, goat *anti*-AAT1 antibody was added on the sections and incubated at 4 °C overnight. Subsequently, rabbit anti-goat IgG was incubated with the sections at room temperature for 1 h. Finally, DAB was added for staining and nuclear counterstaining was performed with haematoxylin. The positive signal representing AAT1 expression in retina tissues showed as brown colour examined by optical microscope (Olympus, Japan).

2.3. Cell culture and grouping

661W mouse photoreceptor cells were cultured in DMEM that contained 10% FBS, 100 U/mL penicillin, and 100 mg/L streptomycin. Cell cultures were kept in an incubator at 37 °C with 5% carbon dioxide (CO_2) and saturated humidity. H_2O_2 was used as a stimulant to induce the apoptosis of 661W cells. HDX, an AAT inhibitor, was used to mimic the effect of H_2O_2 on the 661W cells. Therefore, the 661W cells were randomly divided into three groups: control group, H_2O_2 group, and HDX group. The cells in the H_2O_2 group were treated with 750 μmol/L H_2O_2 for 24 h, while the cells in the HDX group were treated with 100 μmol/L HDX for 24 h.

2.4. Immunofluorescence staining

AAT1 expression in the 661W cells was observed by immunofluorescence staining according to the previous study [11]. Briefly, the photoreceptor cells were seeded onto an 8-well chamber cover glass. After the different treatment, the cells were rinsed 3 times with PBS. Subsequently, the cells were fixed using 4% polyoxymethylene at room temperature for 15 min and rinsed 3 times with PBS. The cells were sequentially treated with blocking solution 5% BSA at 37 °C for 30 min, goat *anti*-AAT1 antibody (1:100 dilution) at 4 °C overnight and Alexa Fluor®594 donkey anti-goat IgG at room temperature for 2 h under dark environment. The cells were then subjected to nuclear staining using 4′,6-diamidino-2-phenylindole (DAPI). The red fluorescence intensity indicating AAT1 expression was observed under Olympus laser confocal microscope and semi-quantitatively analyzed using Image J software.

2.5. Western blot

The expression of AAT1 and cleaved caspase-3 in 661W photoreceptor cells was examined by western blot. The 661W cells were seeded and cultured in a 6-well plate. After rinsing 3 times with precooled PBS, the 661W cells were lysed with lysis buffer at 37 °C for 30 min. The cell lysate was centrifuged and the supernatant was collected. The protein sample was mixed with loading buffer and boiled for 5 min to denature proteins. Equal protein samples were separated using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The protein bands were transferred from the gel to nitrocellulose blotting membranes and blocked using 5% milk at room temperature for 1 h. Specific primary antibody was then added to the samples (AAT1, 1:1000 dilution; cleaved caspase-3, 1:1000 dilution; GAPDH, 1:5000 dilution) and incubated at 4 °C overnight, followed by 3 rinses. The secondary antibody (1:5000 dilution) was added and incubated for 1 h before 3 rinses. Finally, ECL reagent was incubated at room temperature and exposed using a FluorChem™ M system (Protein Simple, USA). The optical density of the band representing the protein expression was measured by Image J software and GAPDH was used as internal reference.

2.6. In situ detection of endogenous SO_2

The SO_2 content in 661W mouse photoreceptor cells was analyzed with fluorescent staining with a specific SO_2 fluorescent probe as previous described [9,11]. The cells were cultured on an 8-well chamber cover glass. The SO_2 fluorescent probe was added to each well at 50 μmol/L, and incubated at 37 °C for 1 h. After rinsing 3 times with PBS, the cells were fixed using 4% polyoxymethylene at room temperature for 15 min and nuclear staining was performed using propidium iodide (PI). The blue fluorescence intensity indicating endogenous SO_2 content was observed using an Olympus laser confocal microscope analyzed using Image J software.

2.7. TdT-mediated dUTP nick-end labeling (TUNEL)

The apoptosis of 661W cells was determined by the TUNEL staining according the protocol provided by the manufacture. After 3 times of wash with PBS, the 661W cells were fixed using 4% polyoxymethylene at room temperature for 15 min following the treatment with blocking solution at 37 °C for 30 min. Then the cells were further incubated with freshly prepared reagent solution (50 μL of enzyme solution + 450 μL of labelling solution) in the dark at 37 °C for 60 min. DAPI dye was used for nuclear staining. The TUNEL positive signal indicating the apoptotic cell was observed under laser confocal microscope. The apoptotic index was calculated as the percentage of the number of TUNEL positive cells to the number of total DAPI positive cells.

2.8. Statistical analysis

The data are presented as mean ± standard deviation. Statistical analysis was performed using software SPSS 20.0 (SPSS Inc., USA). The comparison among groups was conducted using one-way