ROLE OF ALDH2 IN PROTECTION OF NG108-15 NEURAL CELLS AGAINST HYPOXIA/REOXYGENATION-INDUCED INJURY

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ABSTRACT

Introduction*: Recent studies uncovered that toxic aldehydes accumulation under neurological diseases such as ischemic stroke was involved in neural cell death and that mitochondrial aldehyde dehydrogenase (ALDH2) plays a key role in clearance of toxic aldehydes. This study aims to explore whether ALDH2 is able to protect NG108-15 neural cells from hypoxia/reoxygenation (H/R) - induced injury through prevention of toxic aldehydes accumulation.*

Materials and methods*: NG108-15 cells were subjected to 5h of hypoxia (H) followed by 20h of reoxygenation (R) to establish H/R injury model. The cells were randomly divided into 6 groups as follows: the control group; the H/R group; the Alda-1 (5μM), the Alda-1 (10μM) or the Alda-1 (30μM) plus H/R group; and the vehicle plus H/R group. To verify the function of ALDH2, the NG108-15 cells were subjected to 4-HNE (50 μM) for 24 h. At the end, culture medium and cells were collected for analysis of cellular apoptosis, lactate dehydrogenase (LDH) release, reactive oxygen species (ROS), 4-hydroxynonenal (4-HNE) and malondialdehye(MDA) levels, and ALDH2 expression, respectively.*

Results*: It showed an increase in cell necrosis and apoptosis concomitant with down-regulation of ALDH2 expression and activity while accumulation of toxic aldehydes including 4-Hydroxynonenal (4-HNE) and malondialdehyde (MDA) in the H/R group. Administration of ALDH2 activator (Alda-1) reduced H/R-induced NG108-15 cells injury accompanied by a reverse in ALDH2 activation and toxic aldehydes accumulation. 4-HNE treatment increased cell necrosis and apoptosis concomitant with down-regulation of ALDH2 expression and activity while an increase in ROS production, all these effects were reversed in the presence of ALDH2 activator.*

Conclusions*: ALDH2 is able to protect neural cells from H/R-induced injury through prevention of toxic aldehydes accumulation and it might serve as a molecular target for the development of novel drugs to treat neurological diseases such as stroke.*

Keywords*: mitochondrial aldehyde dehydrogenase (ALDH2), NG108-15 cells, hypoxia/reoxygenation, 4-Hydroxynonenal.*

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Introduction

Neural cell death, including necrosis and apoptosis, is a frequent event under multiple neurological diseases such as ischemic stroke or Parkinson's Disease[1,2]. Although many pathological processes, such as excitotoxicity, inflammatory reaction and calcium overload, are involved in neural cell death under such conditions[1], oxidative stress is believed to play a key role in mediation of cellular necrosis and apoptosis[3,4].

Oxidative stress refers to cell, tissue or organ damage caused by overproduction of reactive oxygen species (ROS), such as hydrogen peroxide, superoxide anion and peroxynitrite. Lipid peroxidation is a common phenomenon for ROS to attack lipids within cell membranes[5]. The main end products of lipid peroxidation are toxic aldehydes

including 4-Hydroxynonenal (4-HNE) and malondialdehyde (MDA)[6,7]. Toxic aldehydes, particularly 4-HNE, are extremely toxic because they can form various adducts with lipids, proteins and DNA, resulting in them dysfunction and cellular damage[8]. It has been shown that toxic aldehydes are accumulated under conditions of many diseases, such as myocardial infarction or ischemic stroke[9-11], indicating a key role for toxic aldehydes in apoptotic and necrotic mechanisms leading to myocardial or brain cell death.

The detoxification of toxic aldehydes mainly depends on the activity of mitochondrial isoform aldehyde dehydrogenase (ALDH), also known as ALDH2, which catalyzes the conversion of aldehydes to carboxylic acids and thus significantly reduces their toxicity[9,12]. Although accumulating evidence indicates that ALDH2-mediated detoxification of toxic aldehydes represents an endogenous protective mechanism for myocardial or cerebral ischemia/reperfusion injury[10,11,13], there is still no direct evidence to show the protective effect of ALDH2 on cell injury in vitro. In the present study, by using neural cell model of hypoxia/reoxygenation (H/R)-induced injury (necrosis and apoptosis), we investigated whether ALDH2 was able to protect the neural cells from H/R injury. Since 4-HNE is a substrate of ALDH2 and it is highly toxic, we thus also investigated whether ALDH2 could protect the neural cells from 4-HNE-induced injury.

Materials and methods

Cell model of H/R

NG108-15 neural cells were purchased from the Chinese Academy of Sciences (Shanghai, China). Following the instruction provided by the supplier, NG108-15 cells were seeded at a density of 104 cells/cm2 and cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS, penicillin (100 IU/ml), streptomycin (100 mg/mL) and L-glutamine (2 mmol/L). The cells were incubated at 37 °C in 95% air/5% CO2 in a humidified incubator. Two days later, the cells were washed with PBS and cultured in serum-free DMEM for rendering them quiescent for 24 h prior to experiments. To establish the H/R model, NG108-15 cells were exposed to 5 h of hypoxia (O2/N2/CO2, 1:94:5) in a pre-conditioned hypoxic medium (serum-free DMEM without glucose and sodium pyruvate), which was incubated under hypoxic conditions for 2 h.

And then the NG108-15 cells were moved out of hypoxia work station and incubated in fresh medium followed by 20 h of reoxygenation.

Protocols

The first set of experiments was designed to evaluate the role of ALDH2 in H/R-induced NG108-15 neural cells injury. The cells were randomly divided into 6 groups as follows (8 individual experiments per group): the control group, cells were cultured under normal condition; the H/R group, cells were subjected to 5h-hypoxia followed by 20h-reoxygenation; the Alda-1 (L), the Alda-1 (M) or the Alda-1 (H) plus H/R group, cells were treated with ALDH2 activator Alda-1 (Daxing Biotechnology, Dongwan, China) at 5, 10 or 30μM before H/R; and the vehicle plus H/R group, cells were treated with equal volume of Alda-1 vehicle (DMSO, 0.5 %, final concentration) before H/R.

At the end of experiments, culture medium and cells were collected for analysis of cellular apoptosis, lactate dehydrogenase (LDH) release, ROS, 4-hydroxynonenal (4-HNE) and malondialdehye(MDA) levels, and ALDH2 expression (mRNA) and protein), respectively.

To verify the mechanisms of ALDH2 in the protection of NG108-15 cells, the second set of experiments was performed. The protocols for these set of experiments were same as the one above-mentioned except the H/R treatment was replaced with 4-HNE treatment, in which cells were subjected to 50 μM 4-HNE for 24 h. At the end of experiments, culture medium and cells were collected for the measurements same as before.

Determination of ROS and H2O2 levels

The measurement of intracellular ROS levels dependents on a fluorescent probe, 2',7-dichlorodihydrofluorescein diacetate (DCFH-DA). It is a cell-permeable indicator of ROS and displays nonfluorescent until the acetate groups are removed by intracellular ROS. The procedures for ROS measurement were carried out following instructions provided by the kits (Beyotime, Jiangsu, China). Briefly, NG108-15 cells were incubated with DCFH-DA (10 μM) at 37 °C for 20 min. Then, the ROS-mediated fluorescence was determined under a fluorescent microscope with excitation at 502 nm and emission at 523 nm. Fluorescent intensity were normalized by the control and expressed as fold of change.

The detection of H_2O_2 is based on the capability of H_2O_2 oxidizing ferrous (Fe^{2+}) to ferric ion (Fe^{3+}). In a sulfuric acid solution, the Fe3+ complexes with the xylenolorange dye yield a purple product with maximum absorbance at 560 nm. For measurement of H_2O_2 level, the mixture of 50 μL cell supernatants and 100 μL work solution (0.25 mM ammonium ferrous II sulfate, 25 mM H_2SO_4, 100 mM sorbitol, 125 μM xylenol orange) were incubated at room temperature for 20 min. The changes of absorbance at 560 nm were recorded, and the H_2O_2 level was calculated through a standard curve, which was made from the standard solutions provided by the supplier (Beyotime, Jiangsu, China).

LDH release assay

LDH release in culture medium, an indicator of cellular damage, was measured based on a coupled enzymatic reaction that leads to the conversion of a tetrazolium salt into a red color formazan by diaphorase. Acolorimetric assay kit (Beyotime, Jiangsu, China) was used for measurement of LDH release. Following the manufacturer's instructions, 20 μL of culture medium and 60 μL of LDH work solution were mixed and incubated at 30 °C for 25 min. Absorbance was monitored at 490 nm. Percentage of cell damage was calculated according to a formula provided by the kit supplier.

Measurement of 4-HNE and MDA contents

4-HNE is able to form stable adducts through binding to proteins, which is generally used to represent the content of 4-HNE. A commercially available ELISA kit (R&D, Minneapolis, USA) was used to measure HNE-protein adducts content. Briefly, 100 μL of cell lysates was added to a 96-well plate and incubated at 37 °C for 2 h. Then, a probe of an anti-HNE-His antibody was used for detection of the 4-HNE-protein adducts, followed by a horseradish peroxidase (HRP)-conjugated secondary antibody. The absorbance of each well was recorded at 450 nm immediately after adding the stop solution. The HNE-protein adduct content was calculated according to a standard curve prepared from predetermined HNE-bovine serum albumin (BSA) standards. The 4-HNE content was expressed as nanograms per gram protein.

The MDA contents were examined by thiobarbituric acid (TBA) method with slight modification (Beyotime, Shanghai, China). Briefly, 0.5 mL of cell lysates was mixed with 1 mL of TBA (0.67%) and 3 mL of phosphoric acid (1%). The mixture was incubated for 60 min at 95 °C and cooled down subsequently. To extract the MDA, 375 μL of N-butanol was added followed by vigorous vortex for 10 s. The upper N-butanol layer was transferred to a glass tube after centrifugation. The absorbance of the butanol phase was detected at 532 nm. The MDA content was presented as micromole per milligram protein.

Measurement of ALDH2 activity and expression

A microplate assay is used to determine ALDH2 activity in NG108-15 cells (Abcam, Cambridge, USA). Briefly, the enzyme is captured within the wells of the microplate and activity is determined by the production of NADH catalyzed by ALDH2. The microplate immunocaptures only native ALDH2 from the sample, which ensures all other enzymes, including unrelated ALDHs, can be removed. The generation of NADH is coupled to the 1:1 reduction of a reporter dye to yield a colored reaction product whose concentration can be monitored by measuring the increase in absorbance at 450 nm for 30-120 m. ALDH2 activity is expressed as μmol NADH/min/mg protein.

ALDH2 mRNA level was measured by Real-time PCR. Briefly, total RNA was isolated from NG108-15 cells by using TRIzol reagent (TakaRa, Dalian, China), and the concentration and purity of RNA were examined spectrophotometrically. Five hundreds ng RNA from each sample were used for reverse transcription reaction via a commercial available kit (TakaRa, Dalian, China). Quantitative analysis of mRNA expression was carried out by using the ABI7300 real-time PCR system (Applied Biosystems, Foster City, CA, USA) with SYBR® Premix ExTaq™ (TakaRa, Dalian, China). Briefly, a 10-μL reaction mixture containing 2μL cDNA template, 5μL SYBR Master Mix, 0.20μL ROX, 0.20μL of each primer and 2.4 μL H_2O was amplified by the following thermal parameters: an initial incubation for 15s at 95 °C, followed by 40 cycles of denaturation for 5s at 95 °C, annealing and extension for 30s at 60 °C. The PCR primers for ALDH2 were 5'- GCAGGAGGATGTGTATGATGAA -3'(Forward) and 5'- GGGCTGGATGAAGTAACCAC -3 (Reverse) with a product size of 268 bp, for β-actin were 5'- ATGTACGTAGCCATCCAGGC -3' (Forward) and 5'- AGGAAGGAAGGCTGGAAGAG -3' (Reverse) with a product size of 415 bp.

Data analysis was performed by comparative Ct method using the ABI software. Expression of ALDH2 was normalized by β-Actin.

ALDH2 protein level was determined by Western blot. Cell lysates were centrifuged at 12,000g for 15min, and the protein concentration in supernatants was determined by a BCA Protein Assay Kit (Beyotime, Jiangsu, China). Samples containing 40-80μg of proteins were resolved by 8% SDS PAGE gel and transferred on to polyvinylidene fluoride (PVDF) membranes, which were incubated with 1% BSA for 1h at room temperature and then immunoblotted with the primary antibodies against ALDH2 (Santa Cruz, CA, USA) or β-actin (Beyotime, Jiangsu, China) followed by HRP-conjugated secondary antibodies. To make sure equal loading, blots were incubated with an antibody of mouse anti-β-actin as a control (Millipore, Billerica, MA, USA). The signals of bands were measured by enhanced chemiluminescence (ECL kit, Amersham Biosciences, Piscataway, NJ, USA) through Molecular Imager ChemiDoc XRS System (Bio-Rad, Philadelphia, USA). The densitometric analysis was conducted with Image J 1.43 (National Institutes of Health).

Analysis of cellular apoptosis

Cellular apoptosis was measured by hoechst staining according to the manufacturer's instruction (Beyotime, Shanghai, China). Briefly, the NG108-15 cells were fixed for 15 min in 4% paraformaldehyde, and then washed by PBS and incubated at room temperature for 5 min with 1g mL^{-1} of Hoechst 33258, a bisbenzimide cell-permeant dye which fluorensces bright blue on binding to DNA. After washing twice with PBS, stained cells were imaged under a fluorescent microscope (excitation, 350 nm; emission, 460 nm). Twenty random high-power fields from each sample were blindly quantitated. The number of apoptotic cells was presented as percent of the total cells.

Statistical analysis

SPSS software (version 19, SPSS, Inc., Chicago, IL, USA) was chosen for statistical analysis. The data were presented as mean ± SEM. Statistical analysis was carried out by one-way analysis of variance (ANOVA) followed by the Newman Student-Keuls test for multiple comparisons. Differences were considered significant when $P < 0.05$.

Results

Activation of ALDH2 reduces H/R-induced NG108-15 cell injury

To examine H/R-induced NG108-15 cell injury, cellular apoptosis (Hoechst staining) and necrosis (LDH release) were assessed. Compared with the control group, the cellular apoptosis and necrosis were significantly elevated in the H/R group. Administration of ALDH2 activator (Alda-1) significantly attenuated H/R-induced cell apoptosis or necrosis in a dose-dependent manner. The protective effects of ALDH2 activator on H/R-induced cell injury were significantly attenuated by daidzin, a specific antagonist of ALDH2 (Figure 1). The vehicle of Alda-1 had no such effect.

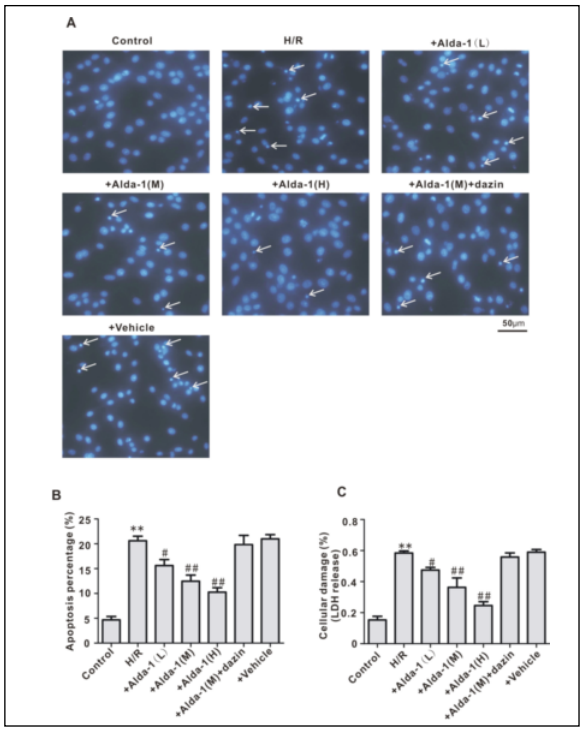

Figure 1: ALDH2 activator reduces hypoxia/reoxygenation-induced NG108-15 cell injury. **A**. Representative image of Hoechst staining from each group. The apoptotic cells are indicated by arrows. **B**. Percentage of apoptotic cells per total number of NG108-15 cells in each group. **C**. LDH release in NG108-15 cells from each group. All values were expressed as means ±S.E.M. (n = 8 in each group). H/R: Hypoxia/reoxygenation; +Alda-1 (L): H/R+Alda-1 (5 μM); +Alda-1(M): H/R+Alda-1(10 μM); +Alda-1 (H): H/R+Alda-1(30 μM); +Vehicle: H/R+DMSO; **P<0.01 vs Control, #P<0.05, ##P<0.01 vs H/R.

Activation of ALDH2 attenuates the inhibitory effects of H/R on ALDH2 expression and activity in NG108-15 cells

As displayed in Figure 2, in comparison to the control group, the ALDH2 expression (mRNA and protein) and activity in NG108-15 cells were significantly decreased in the H/R group. ALDH2 activator (Alda-1) treatment at three doses remarkably attenuated the inhibitory effect of H/R on ALDH2 expression and activity in a dose-dependent manner. The vehicle of Alda-1 had no such effect. The effects of Alda-1 on ALDH2 expression and activity was disappeared in the presence of ALDH2 antagonist daidzin.

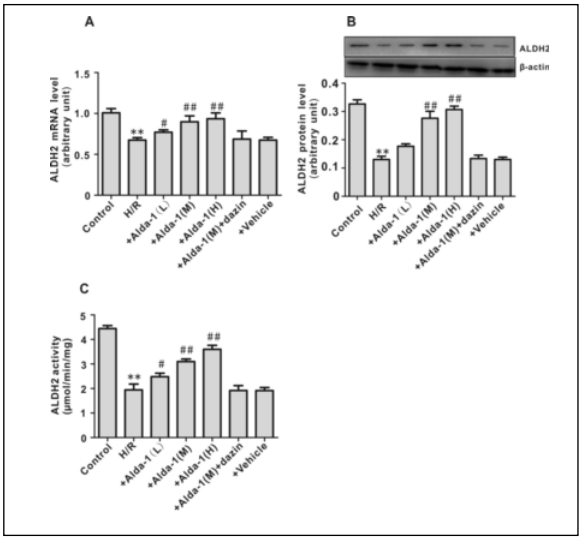

Figure 2: ALDH2 expression and activity in NG108-15 cells following hypoxia/reoxygenation. **A**. ALDH2 mRNA expression in NG108-15 cells from each group. **B**. ALDH2 protein expression in NG108-15 cells from each group. Top, representative images of Western blot results from each group; Bottom, ratio of optical density between ALdH2 and β-actin. **C**. ALDH2 activity in NG108-15 cells from each group. All values were expressed as means ±S.E.M. (n = 8 in each group). H/R: Hypoxia/reoxygenation; +Alda-1 (L): H/R+Alda-1 (5 μM); +Alda-1(M): H/R+Alda-1(10 μM); +Alda-1 (H): H/R+Alda-1(30 μM); +Vehicle: H/R+DMSO; **P<0.01 vs Control, #P<0.05, ##P<0.01 vs H/R.

Activation of ALDH2 reduces toxic aldehydes accumulation in NG108-15 cells caused by H/R

Both 4-HNE and MDA, well-known toxic aldehydes, are products from lipid peroxidation resulting from ROS attack. As showed in Figure 3, comparing with the control group, 4-HNE as well as MDA contents in the H/R group was remarkably increased. Alda-1 treatment significantly reduced 4-HNE and MDA accumulation in NG108-15 cells in a dose-dependent manner, and the reduction was abolished by ALDH2 antagonist daidzin. The vehicle of Alda-1 had no such effect.

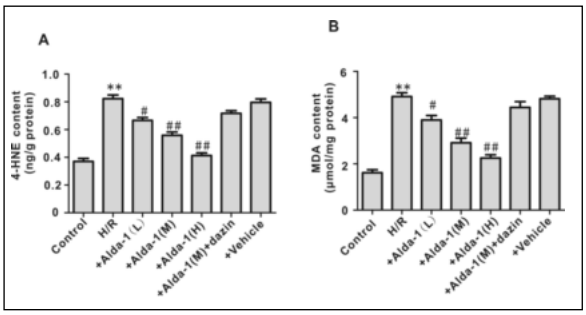

Figure 3: ALDH2 activator reduces toxic aldehydes accumulation in NG108-15 cells following hypoxia/reoxygenation. **A**. 4-HNE content in NG108-15 cells from each group. **B**. MDA content in NG108-15 cells from each group. All values were expressed as means ±S.E.M. (n = 8 in each group). H/R: Hypoxia/reoxygenation; +Alda-1 (L): H/R+Alda-1 (5 μM); +Alda-1(M): H/R+Alda-1(10 μM); +Alda-1 (H): H/R+Alda-1(30 μM); +Vehicle: H/R+DMSO; **P<0.01 vs Control, #P<0.05, ##P<0.01 vs H/R.

Activation of ALDH2 reduces 4-HNE-induced NG108-15 cell injury

To seek the direct evidence that ALDH2 protects NG108-15 cells from H/R injury via clearance of toxic aldehydes, a model of 4-HNE-induced NG108-15 cell injury was established. Compared with the control group, the cellular apoptosis and necrosis were significantly elevated in the 4-HNE group. Administration of Alda-1 significantly attenuated 4-HNE-induced cell apoptosis or necrosis in a dose-dependent manner. The protective effects of Alda-1 on 4-HNE-induced cell injury were significantly attenuated by ALDH2 antagonist daidzin (Figure 4). The vehicle of Alda-1 did not show no such effect.

Activation of ALDH2 reverses the inhibitory effect of 4-HNE on ALDH2 expression and activity in NG108-15 cells

Comparing with the control group, the ALDH2 expression (mRNA and protein) and activity in NG108-15 cells were significantly decreased in the 4-HNE group. ALDH2 activator (Alda-1) at three doses remarkably reverses the inhibitory effect of 4-HNE on ALDH2 expression and activity in a dose-dependent manner. The effects of Alda-1 on ALDH2 expression and activity were blocked in the presence of ALDH2 antagonist daidzin. The vehicle of Alda-1 did not display such effect.

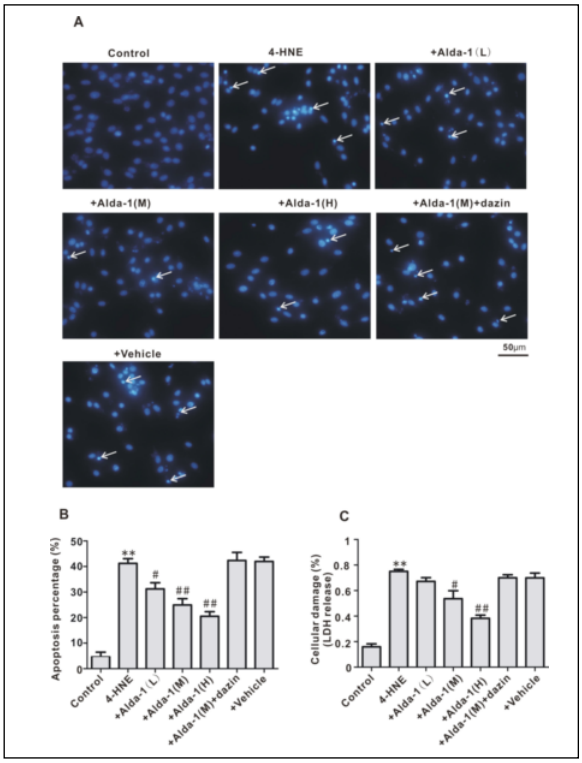

Figure 4: ALDH2 activator reduces 4-HNE-induced NG108-15 cell injury. **A**. Representative image of Hoechst staining from each group. The apoptotic cells are indicated by arrows. **B**. Percentage of apoptotic cells per total number of NG108-15 cells in each group. **C**. LDH release in NG108-15 cells from each group. All values were expressed as means ±S.E.M. (n = 8 in each group). 4-HNE: 4-Hydroxynonenal (50 μM); +Alda-1 (L): 4-HNE+Alda-1 (5 μM); +Alda-1(M): 4-HNE+Alda-1(10 μM); +Alda-1 (H): 4-HNE+Alda-1(30 μM); +Vehicle: 4-HNE+DMSO; **P<0.01 vs Control, #P<0.05, ##P<0.01 vs 4-HNE.

Activation of ALDH2 suppresses the 4-HNE-induced ROS production in NG108-15 cells

Compared to the control group, there was a dramatic increase in the fluorescent signal for ROS in the 4-HNE group, which was attenuated in the presence of Alda-1 in a dose-dependent manner (Figure 6A and B). Agreement with the results of total ROS level, H_2O_2 production in NG108-15cells was significantly enhanced in the 4-HNE group, which was suppressed in the presence of ALDH2 activator (Figure 6C). The vehicle of Alda-1 had no such effect. Treatment with daidzin blocked the effect of Alda-1 on both ROS and H_2O_2 generation in NG108-15 cells.

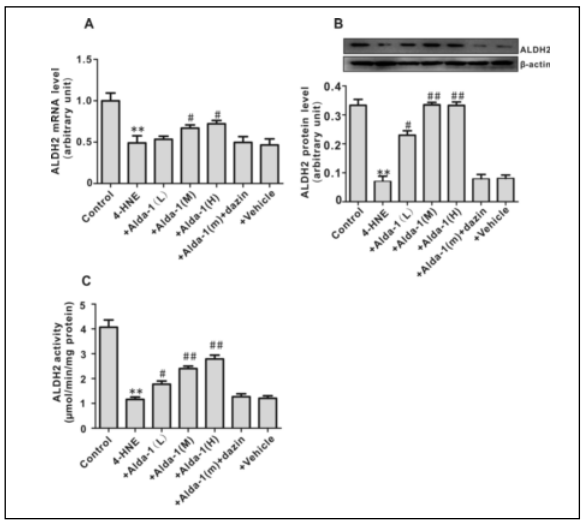

Figure 5: ALDH2 expression and activity in NG108-15 cells in the presence of 4-HNE A. ALDH2 mRNA expression in NG108-15 cells from each group. B. ALDH2 protein expression in NG108-15 cells from each group. Top, representative images of Western blot results from each group; Bottom, ratio of optical density between ALdH2 and β-actin. C. ALDH2 activity in NG108-15 cells from each group. All values were expressed as means ±S.E.M. (n = 8 in each group). 4-HNE: 4-Hydroxynonenal (50 μM); +Alda-1 (L): 4-HNE+Alda-1 (5 μM); +Alda-1(M): 4-HNE+Alda-1(10 μM); +Alda-1 (H): 4-HNE+Alda-1(30 μM); +Vehicle: 4-HNE+DMSO; **P<0.01 vs Control, #P<0.05, ##P<0.01 vs 4-HNE.

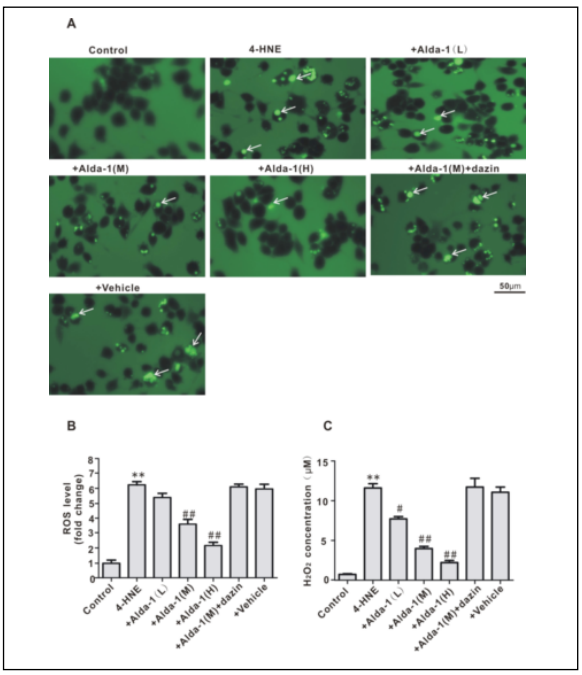

Figure 6: ALDH2 activator suppresses the 4-HNE-induced ROS production in NG108-15 cells. **A**. Representative image of fluorescent signals (indicated by arrows) of DCFH-D for total ROS in NG108-15 cells from each group. **B**. Statistic value for fluorescent density in each group.

C. H_2O_2 concentration in culture medium from each group. All values were expressed as means ±S.E.M. (n = 8 in each group). 4-HNE: 4-Hydroxynonenal (50 μM); +Alda-1 (L): 4-HNE+Alda-1 (5 μM); +Alda-1(M): 4-HNE+Alda-1(10 μM); +Alda-1 (H): 4-HNE+Alda-1(30 μM); +Vehicle: 4-HNE+DMSO; **P<0.01 vs Control, #P<0.05, ##P<0.01 vs 4-HNE.

Discussion

In the present study, by using the NG108-15 cell model of H/R or 4-HNE-induced injury, we explored the role of ALDH2 in protection of neural cells and the underlying mechanisms. We have found that under the condition of H/R, ALDH2 expression and activity in NG108-15 cells were markedly reduced accompanied by accumulation of toxic aldehydes (such as 4-HNE and MDA) and increase in cell death (necrosis and apoptosis), and that these phenomena were reversed by Alda-1, a specific activator of ALDH2. Furthermore, we have also found that in 4-HNE-treated NG108-15 cells, ALDH2 expression and activity were dramatically decreased concomitant with elevation of ROS levels and cell death, which were attenuated in the presence of ALDH2 activator. To the best of our knowledge, this is the first study to provide evidence that ALDH2 is able to protect neural cells from H/R injury through prevention of toxic aldehydes accumulation.

It is well known that over production of ROS under neurological diseases including ischemic stroke is one of the major causative factors for neural cell damage. So far, most of studies focus on the direct oxidative effect of ROS on all components of the cell including proteins, lipids and DNA, the so-called oxidative injury[14]. Actually, products, particularly toxic aldehydes, generated from the oxidative process can act as 'toxic second messengers' that extend the injurious potential of ROS[15]. Toxic aldehydes are the most common by-products of lipid peroxidation. Some of them, such as 4-HNE and MDA, are highly toxic and can form protein adducts with the amino acid residues of histidine, cysteine or lysine, which cause further damage of cells[15,16].

Expectedly, the results from our study showed that H/R treatment dramatically increased the contents of 4-HNE and MDA concomitant with dramatic cell injury (increase in cellular necrosis and apoptosis), suggesting a positive correlation between toxic aldehydes and H/R injury. To further verify the correlation between toxic aldehydes and cell injury, we successfully established the 4-HNE-induced NG108-15 cell injury model. We have found that incubation of NG108-15 cells with 4-HNE (50 μM) for 24 h significantly increased LDH release and cellular apoptosis, confirming the detrimental effect of toxic aldehydes on neural cells.

In human and mammalian animals, there exist the endogenous systems responsible for the clearance of toxic aldehydes. Among them, aldehyde dehydrogenases (ALDHs) are believed to play a major role in detoxification of toxic aldehydes. To date, there are 19 identified members in ALDH family, and the ALDH2 isoform is particularly attractive because it localizes within the mitochondria, a major site for ROS and toxic aldehydes generation[12,17]. As we mentioned earlier, accumulation of toxic aldehydes is an important mechanism responsible for H/R-induced NG108-15 cell injury, it is not known, however, whether there is a change in ALDH2 expression or activity under such condition.

In this study, our results clearly showed that both ALDH2 mRNA and protein levels were significantly down-regulated in NG108-15 cells subjected to H/R treatment accompanied by a decrease in ALDH2 activity, which might account for, at least partially, the accumulation of toxic aldehydes (4-HNE and MDA) under such condition. In the presence of ALDH2 activator (Alda-1), the down-regulation of ALDH2 expression and activity were markedly reversed in a dose-dependent manner, with a reduction in toxic aldehydes accumulation. These results supported the role of ALDH2 in clearance of toxic aldehydes as well as its protective effect on H/R-induced neural cell injury.

Moreover, a model of 4-HNE-induced NG108-15 cell injury was chosen to seek direct evidence for the toxic effect of aldehydes on neural cells and the function of ALDH2 in clearance of toxic aldehydes. In the presence of 4-HNE, both ALDH2 mRNA and protein levels in NG108-15 cells were significantly down-regulated concomitant with a decrease in ALDH2 activity and an increase in cell injury. These effects were significantly attenuated by ALDH2 activator in a dose-dependent manner, further confirming the role of ALDH2 in protection of neural cells against injury caused by toxic aldehydes. As one of the toxic aldehydes, 4-HNE is able to react with proteins to form various adduct, resulting in protein dysfunction. Thus, it is easy to explain the phenomenon for the decrease of ALDH2 activity in the presence of 4-HNE.

However, it remained unclear why 4-HNE was able to down-regulate ALDH2 mRNA and protein expression. Actually, in addition to its toxic effect, 4-HNE is also able to function as signaling molecule in stimulating/inhibiting gene expression and cell survival/death[18,19]. Therefore, it is likely that 4-HNE inhibited ALDH2 expression through activation of certain signal pathways. In the present study, we have found that ROS production in NG108-15 cell were dramatically elevated following 4-HNE treatment, which may suggest that the inhibitory effect of 4-HNE on ALDH2 gene expression might involve, at least in part, redox signaling pathway[19,20]. However, further studies are needed before making this issue clear.

In summary, our data demonstrate for the first time that ALDH2 is able to protect neural cells from H/R-induced injury through clearance of toxic aldehydes. Thus, ALDH2 might serve as a molecular target for the development of novel drugs to treat neurological diseases such as stroke.

References

1) Puyal J, Ginet V, Clarke PG. Multiple interacting cell death mechanisms in the mediation of excitotoxicity and ischemic brain damage: a challenge for neuroprotection. Prog Neurobiol 2013; 105: 24-48.
2) Venderova K, Park DS. Programmed cell death in Parkinson's disease. Cold Spring Harb Perspect Med 2012; 2 (8):
3) Rodrigo R, Fernandez-Gajardo R, Gutierrez R, Matamala JM, Carrasco R, Miranda-Merchak A, Feuerhake W. Oxidative stress and pathophysiology of ischemic stroke: novel therapeutic opportunities. CNS Neurol Disord Drug Targets 2013; 12 (5): 698-714.
4) Manzanero S, Santro T, Arumugam TV. Neuronal oxidative stress in acute ischemic stroke: sources and contribution to cell injury. Neurochem Int 2013; 62 (5): 712-718.
5) Arai H. Oxidative modification of lipoproteins. Subcell Biochem 2014; 77: 103-114.
6) Zhong H, Yin H. Role of lipid peroxidation derived 4-hydroxynonenal (4-HNE) in cancer: focusing on mitochondria. Redox Biol 2015; 4 193-199.
7) Sultana R, Perluigi M, Allan Butterfield D. Lipid peroxidation triggers neurodegeneration: a redox proteomics view into the Alzheimer disease brain. Free Radic Biol Med 2013; 62: 157-169.
8) Calamaras TD, Lee C, Lan F, Ido Y, Siwik DA, Colucci WS. The lipid peroxidation product 4-hydroxy-trans-2-nonenal causes protein synthesis in cardiac myocytes via activated mTORC1-p70S6K-RPS6 signaling. Free Radic Biol Med 2015; 82: 137-146.
9) Luo XJ, Liu B, Ma QL, Peng J. Mitochondrial aldehyde dehydrogenase, a potential drug target for protection of heart and brain from ischemia/reperfusion injury. Curr Drug Targets 2014; 15 (10): 948-955.
10) Chen CH, Budas GR, Churchill EN, Disatnik MH, Hurley TD, Mochly-Rosen D. Activation of aldehyde dehydrogenase-2 reduces ischemic damage to the heart. Science 2008; 321 (5895): 1493-1495.
11) Fu SH, Zhang HF, Yang ZB, Li TB, Liu B, Lou Z, Ma QL, Luo XJ, Peng J. Alda-1 reduces cerebral ischemia/reperfusion injury in rat through clearance of reactive aldehydes. Naunyn Schmiedebergs Arch Pharmacol 2014; 387 (1): 87-94.
12) Chen CH, Ferreira JC, Gross ER, Mochly-Rosen D. Targeting aldehyde dehydrogenase 2: new therapeutic opportunities. Physiol Rev 2014; 94 (1): 1-34.
13) He L, Liu B, Dai Z, Zhang HF, Zhang YS, Luo XJ, Ma QL, Peng J. Alpha lipoic acid protects heart against myocardial ischemia-reperfusion injury through a mechanism involving aldehyde dehydrogenase 2 activation. Eur J Pharmacol 2012; 678 (1-3): 32-38.
14) Pisoschi AM, Pop A. The role of antioxidants in the chemistry of oxidative stress: A review. Eur J Med Chem 2015; 97: 55-74.
15) Shoeb M, Ansari NH, Srivastava SK, Ramana KV. 4-Hydroxynonenal in the pathogenesis and progression of human diseases. Curr Med Chem 2014; 21 (2): 230-237.
16) Mali VR, Palaniyandi SS. Regulation and therapeutic strategies of 4-hydroxy-2-nonenal metabolism in heart disease. Free Radic Res 2014; 48 (3): 251-263.
17) Chen CH, Sun L, Mochly-Rosen D. Mitochondrial aldehyde dehydrogenase and cardiac diseases. Cardiovasc Res 2010; 88 (1): 51-57.
18) Ayala A, Munoz MF, Arguelles S. Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. Oxid Med Cell Longev 2014; 2014 360438.
19) Thompson JW, Narayanan SV, Perez-Pinzon MA. Redox signaling pathways involved in neuronal ischemic preconditioning. Curr Neuropharmacol 2012; 10 (4): 354-369.
20) Brown DI, Griendling KK. Regulation of signal transduction by reactive oxygen species in the cardiovascular system. Circ Res 2015; 116 (3): 531-549.

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