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Paraoxonase-1 Mediates Hydrogen Sulfide-induced Protection Against Formaldehyde Neurotoxicity*

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Abstract We have previously demonstrated the protective function of hydrogen sulfide (H_2S) against the neurotoxicity and oxidative stress of formaldehyde (FA). Paraoxonase-1 (PON-1) is a pivotal endogenous antioxidant. The aim of this present study is to investigate whether PON-1 mediates the protection of H_2S against FA-induced neurotoxicity. In the present work, PC12 cells treated with FA were established to the model of FA-induced neurotoxicity. Treatment of PC12 cells with NaHS (a donor of H_2S) not only upregulates the activity of PON-1，but also significantly restores FA-induced downregulation of PON-1 activity and expression. A selective inhibitor of PON-1, 2-hydroxyquinoline (2-HQ), markedly attenuated H_2S-induced neuroprotection against FA-induced cytotoxicity, apoptosis, and accumulation of intracellular reactive oxygen species (ROS) in PC12 cells. Furthermore, 2-HQ blocks H_2S to reverse FA-caused activation of caspase-3 and downregulation of bcl-2 expression in PC12 cells. These results indicate that H_2S protects PC12 cells against FA-induced neurotoxicity in a PON-1-dependant manner. Our findings suggest a promising role of PON-1 as a novel therapeutic target for neuronal damage after FA exposure.

Key words formaldehyde, hydrogen sulfide, paraoxonase-1, apoptosis, reactive oxygen species
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Formaldehyde (FA) is a well-known indoor and outdoor pollutant [1]. FA is extremely reactive, cross-linking with protein, DNA, RNA and unsaturated fatty acids through nonenzymic means, which leads to cytotoxicity, genotoxicity, mutagenic and cancerogenic actions[2–4]. The central nervous system (CNS) is one of the most important systems affected by FA. Many studies have revealed that excessive FA can cause toxic effects to neuron and impair memory [5–13]. Interestingly, endogenous FA has been determined to be 0.5～4.28 mg/L (0.017～0.143 mmol/L) in human blood under normal conditions [14]. FA can be endogenously produced from methylamine, catalyzed by semicarbazide-sensitive amine oxidase (SSAO)[15–16]. Formaldehyde triggers the hyperphosphorylation and polymerization of Tau[17]. Increasing evidence indicates that excess endogenous FA by up-regulation of SSAO is a pathogenic factor involved in memory decline and Alzheimer′s disease (AD) [18–24]. Everyone living in society may be exposed to FA because of its wide usage in industrial and medical settings. Therefore, it is of utmost importance to develop new therapeutic approaches to prevent the neurotoxicity of FA.

Hydrogen sulfide (H_2S), which can be endogenously generated and widely distributed in mammalian tissues, plays signaling roles, modulating physiological functions in the cardiovascular and

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nervous systems like nitric oxide and carbon monoxide [25–26]. Desulfuration of cysteine constitutes the main source of H_2S in mammals, catalyzed mainly by two key pyridoxal 5′-phosphate-dependent enzymes: cystathionine-β-synthase (CBS) and cystathionine-γ-lyase (CES)[27–29]. Emerging evidence confirms that H_2S can exert cytoprotective and neuroprotective actions[30–32]. Remarkably, H_2S has also been revealed to have an antioxidant role, protecting the cardiovascular and neuronal systems from oxidative damage [33–34]. Interestingly, increasing evidence demonstrated that oxidative damage is one of the most critical effects of FA exposure [10, 35–38]. The most recent studies by our group has demonstrated that FA inhibites the endogenous H_2S generation [39] and that H_2S prevents the neurotoxicity of FA by its antioxidant action [40], suggesting a promising role of H_2S as a novel antioxidant for FA-induced neurotoxicity. Although the scavenging of reactive oxygen and nitrogen species by H_2S in biological systems is suggested by several studies [41–43], direct reactions with oxidants probably cannot completely account for the protective effects of H_2S[44]. Therefore, it needs to be further investigated the molecular mechanisms underlying the protection of H_2S against the neurotoxicity of FA.

Paraoxonase-1 (PON-1) is an enzyme predominantly associated with high-density lipoprotein and has been shown to possess antioxidative properties [45]. The level of PON-1 is an index of antioxidant defense [46]. It has been shown that PON-1 offers protection against homocysteine-induced neurotoxicity and CCl_4-caused oxidation [47–49]. Therefore, the purpose of the current study was to assess the potential role of PON-1 in H_2S-exerted protection against the neurotoxicity of FA. We demonstrated for the first time that H_2S-triggered upregulation of PON-1 plays an important role in its neuroprotective action against the toxicity of FA.

1 Materials and methods

1.1 Materials

Sodium hydrosulfide (NaHS), formaldehyde, 2-hydroxyquinoline (2-HQ), trypan blue, propidium iodide (PI), RNase, and 2′, 7′-dichlorfluorescein-diacetate (DCFH-DA) were bought from Sigma-Aldrich Co. (st.Louis, MO, USA). Specific monoclonal antibodies against Bcl-2 or cleaved caspase-3 were obtained from Cell Signaling Technology, Inc (Beverly, MA, USA). PON-1antibody was purchased from Abcam Technology (Cambridge, CB, UK). RPMI-1640 medium, horse serum and fetal bovine serum were supplied by Gibico BRL (Ground Island, NY, USA).

1.2 Cell culture

PC12 cells, a rat cell line derived from a pheochromocytoma cells, were supplied from Sun Yat-sen University Experimental Animal Center (Guangzhou, China), and maintained in RPMI-1640 medium supplemented with 10% heat-inactivated horse serum and 5% fetal bovine serum (FBS) at 37℃ under an atmosphere of 5% CO_2 and 95% air. The cells were passaged about 3 times per week.

1.3 Determination of cell viability

The viability of PC12 cell line was measured by trypan blue exclusion analysis. After the indicated treatments, PC12 cells were digested with 2.5 g/L trypsin and centrifuged at 1 500 r/min for 5 min, and the supernatant was discarded. One milliliter serum-free medium was added and 0.2 ml cell suspension was transferred to test tubes with 0.5 ml of 0.4% (*w*/*v*) trypan blue solution and 0.3 ml of serum-free complete medium and mixed thoroughly at room temperature for 5 to 15 min. The dead cells with a damaged cell membrane are permeable to trypan blue. The numbers of trypan blue-permeable blue cells and viable white cells were counted under a phase contrast microscope (BX50-FLA; Olympus, Tokyo, Japan). The percentage of viable cells was evaluated according to the following formula: The percentage of viable cells = the numbers of viable white cells / (The numbers of trypan blue-permeable blue cells + the numbers of viable white cells) ×100%.

1.4 Flow cytometry analysis of apoptosis

Treated PC12 cells were digested with 2.5 g/L trypsin and centrifuged at 1 500 r/min for 5 min and the supernatant was removed. After washed twice with PBS, the cells were fixed in 70% ethanol. The cells were then centrifuged at 1 500 r/min for 5 min, washed in PBS twice and adjusted to a concentration of 1×10^6 cells/ml. To a 0.5 ml cell sample, 0.5 ml RNase (1 g/L in PBS) was added. After gentle mixing with PI (at a final concentration of 50 mg/L), mixed cells were filtered and incubated in the dark at 4℃ for 30 min. Flow cytometric (FCM, Beckman-Coulter, Miami, FL, USA) analysis was applied to determine the apoptosis rate.

1.5 Measurement of intracellular reactive oxygen species (ROS) generation

Intracellular ROS were determined by oxidative conversion of cell permeable DCFH-DA to fluorescent 2′, 7′-dichlorfluorescein (DCF)[50–51]. The cells were collected by pipetting and washed once with PBS. DCFH-DA (2.5 μmol/L) was added to cell cultures for 20 min at 37℃, following twice washes with PBS. DCF fluorescence was measured by FCM. The mean fluorescent intensity (MFI) of the positive cells in ten thousand cells per sample represents the amount of ROS.

1.6 Measurement of PON-1 arylesterase activity

Arylesterase activity was measured using phenyl acetate (Santa Cruz Biotechnology, USA) as the substrate. The initial rates of hydrolysis were determined spectrophotometrically at 270 nm. Fifty microliter homogenate of PC12 cells was added to the reaction mixtures containing 100 mmol/L Tris/HCl (pH 8.0), 2 mmol/L $CaCl_2$, and the substrate 0.5 mmol/L phenyl acetate in a total volume of 1.5 ml. The reaction lasted for 5 min at 37℃ and was stopped by adding 200 μl EDTA (0.5 mol/L). The absorbance at 270 nm was recorded at 0 and 5 min after substrate addition. One unit of arylesterase activity is equal to 1 μmol of phenyl acetate hydrolyzed/ml/min.

1.7 Western blot analysis for PON-1, Bcl-2, and cleaved caspase-3 expression

After heated at 100℃ for 5 min, equal amounts of protein from the indicated groups were loaded. The total proteins were separated in 12% SDS-PAGE by electrophoresis, and then transferred to a PVDF membrane. After blocked with 5% BSA in TBST, the membranes were incubated with the primary antibodies at 4℃ over night. After 3 washes with TBST, the membranes were incubated with horseradish peroxidase-conjugated secondary antibodies at room temperature for 2 h. The membranes were washed again and developed with an enhanced chemiluminescence system (ECL, Zsbio, China), and then the signals were exposed to X-ray films. The integrated optical density for the protein band was calculated by ImageJ 1.46i software.

1.8 Statistical analysis

All the data are expressed as $\bar{x} \pm s$, and analyzed with SPSS13.0 software. The significance of inter-group differences was evaluated by one-way analyses of variance (ANOVA: Tukey's test for post hoc comparisons). Differences were considered significant at two-sided $P < 0.05$.

2 Results

2.1 H_2S inhibits formaldehyde-induced downregulation of PON-1 protein expression

To examine the contribution of PON-1 to H_2S-induced neuroprotection, the expression of PON-1 in PC12 cells was detected. As shown in Figure 1, exposure of PC12 cells to FA (120 μmol/L, 24 h) significantly reduced the expression of PON-1 protein. However, this inhibitory effect of FA on PON-1 protein expression was markedly abolished by cotreatment with H_2S (NaHS, 200 μmol/L), indicating that the neuroprotective effect of H_2S is associated with its upregulation of PON-1 expression.

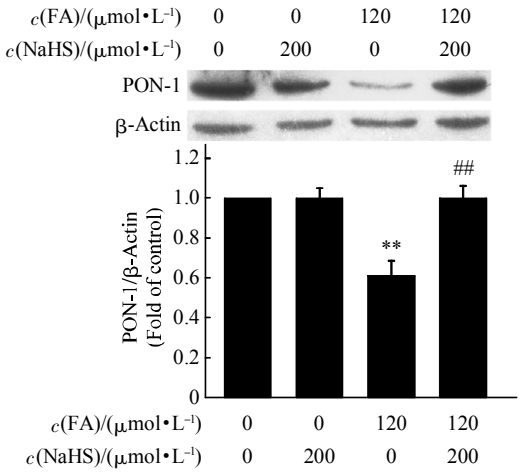

Fig. 1 Effects of H_2S on the expression of PON-1 in PC12 cells

PC12 cells were exposed to formaldehyde (FA, 120 μmol/L) in the presence or absence of NaHS (200 μmol/L) for 24 h. The levels of PON-1 expression in PC12 cells were determined by Western blot using an anti-PON-1 antibody. Western blot images show representative results from three independent experiments. In all blots, staining for β-actin was used as a loading control. The level of PON-1 expression obtained in each experimental condition was calculated as a fold of the control. Data are expressed as $(\bar{x} \pm s)$ of three independent experiments. $^{**}P < 0.01$, versus control; $^{\#\#}P < 0.01$, versus FA-treated alone group.

2.2 H_2S enhances the activity of PON-1 and prevents formaldehyde-induced decrease in PON-1 activity

The effects of H_2S on the activity of PON-1 in

PC12 cells treated with or without FA were also examined. As shown in Figure 2, treatment with H_2S (NaHS, 200 μmol/L, 24 h) significantly increased the activity of PON-1 in PC12 cells. Furthermore, cotreatment with H_2S (NaHS, 200 μmol/L) markedly reduced FA-induced decrease in PON-1 activity. These results indicated that the inhibitory effect of H_2S on FA-induced suppression in PON-1 activity involves its neuroprotective effect.

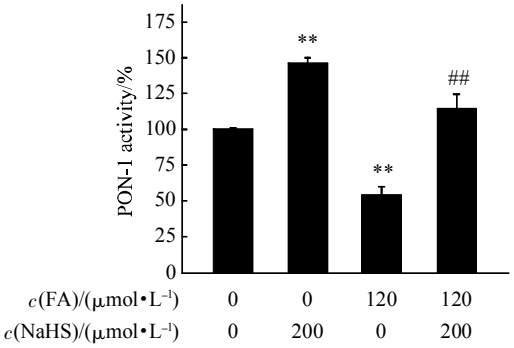

Fig. 2　Effects of H_2S on the activity of PON-1 in PC12 cells

PC12 cells were exposed to formaldehyde (FA, 120 μmol/L) in the presence or absence of NaHS (200 μmol/L) for 24 h. The activity of PON-1 was measured by spectrophotometry. Data are expressed as ($\bar{x} \pm s$) of three independent experiments. $^{**}P < 0.01$, versus control; $^{\#\#}P < 0.01$, versus FA-treated alone group.

2.3　Inhibited PON-1 by 2-HQ reverses H_2S-induced protective effect on formaldehyde-caused cytotoxicity in PC12 cells

To determine whether PON-1 mediates H_2S-exerted protection against FA neurotoxicity, the effect of 2-hydroxyquinoline (2-HQ), a specific inhibitor of PON-1, on the inhibitory effect of H_2S on FA-induced cytotoxicity was investigated by CCK-8 assay. As shown in Figure 3, the cytotoxic effect of FA (120 μmol/L, 24 h) on PC12 cells was significantly reduced by H_2S (NaHS, 200 μmol/L); however, this protective effect of H_2S was markedly attenuated by pretreatment with 2-HQ (200 μmol/L, 30 min), indicating that inhibition of PON-1 prevents the neuroprotective action of H_2S.

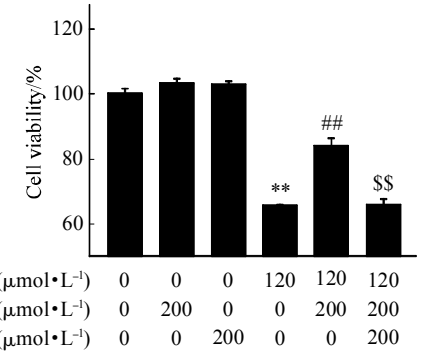

Fig. 3　Effects of 2-hydroxyquinoline on the protection of H_2S against formaldehyde cytotoxicity

PC12 cells were pretreated with 200 μmol/L of 2-hydroxyquinoline (2-HQ), a specific inhibitor of PON-1, for 30 min, and then co-treated with formaldehyde (FA, 120 μmol/L) and NaHS (200 μmol/L) for further 24 h. Cell viability was determined by trypan blue exclusion analysis. Data are expressed as ($\bar{x} \pm s$) of three independent experiments. $^{**}P < 0.01$, versus no-treated control; $^{\#\#}P < 0.01$, versus FA-treated alone group; $^{\$\$}P < 0.01$, versus FA and NaHS co-treated group.

2.4　Inhibited PON-1 by 2-HQ attenuates H_2S-exerted antiapoptotic effect on formaldehyde-induced apoptosis in PC12 cells

The effect of 2-HQ on the inhibitory effect of H_2S on FA-induced apoptosis was evaluated using FCM analysis after PI staining. As illustrated in Figure 4, cotreatment of PC12 cells with H_2S(NaHS, 200 μmol/L) significantly reduced the rate of apoptosis induced by

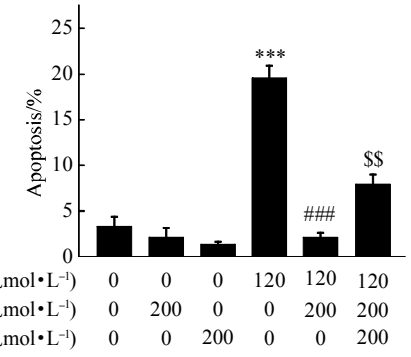

Fig. 4　Effects of 2-hydroxyquinoline on the antiapoptotic role of H_2S in formaldehyde-induced apoptosis in PC12 cells

PC12 cells were pretreated with 200 μmol/L of 2-hydroxyquinoline (2-HQ), a specific inhibitor of PON-1, for 30 min, and then co-treated with formaldehyde (FA, 120 μmol/L) and NaHS (200 μmol/L) for further 24 h. The percentage of apoptotic cells was quantitatively examined by FCM analysis after PI staining. Data are expressed as ($\bar{x} \pm s$) of three independent experiments. $^{***}P < 0.001$, versus no-treated control; $^{\#\#\#}P < 0.001$, versus FA-treated alone group; $^{\$\$}P < 0.01$, versus FA and NaHS co-treated group.

24 h exposure to FA (120 μmol/L); however, this antiapoptotic effect of H_2S was significantly prevented by pretreatment with 2-HQ (200 μmol/L, 30 min). The cells treated with H_2S (NaHS, 200 μmol/L) or 2-HQ (200 μmol/L) showed weak apoptosis similar to that in the untreated cells. These data indicated that inhibition of PON-1 reverses the antiapoptotic effect of H_2S.

2.5 Inhibited PON-1 by 2-HQ prevents H_2S-caused inhibitory effect on formaldehyde-induced accumulation of intracellular ROS in PC12 cells

As antioxidant effect of H_2S contributes to its neuroprotective action [40, 52], we also investigated whether 2-HQ diminishes the inhibitory effect of H_2S on FA-caused intracellular ROS accumulation. As shown in Figure 5, pretreatment of PC12 cells with 200 μmol/L of 2-HQ for 30 min significantly attenuated the inhibitory action of H_2S (NaHS, 200 μmol/L) in FA-induced accumulation of intracellular ROS, indicating that inhibition of PON-1 reverses the inhibitory effect of H_2S on FA-induced accumulation of intracellular ROS.

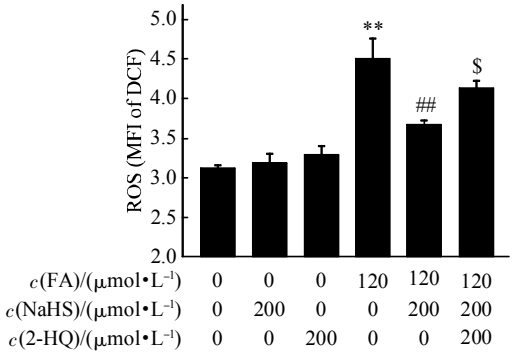

Fig. 5 Effects of 2-hydroxyquinoline on the inhibitory effect of H_2S on formaldehyde-induced accumulation of intracellular ROS

PC12 cells were pretreated with 200 μmol/L of 2-hydroxyquinoline (2-HQ), a specific inhibitor of PON-1, for 30 min, and then co-treated with formaldehyde (FA, 120 μmol/L) and NaHS (200 μmol/L) for further 24 h. The changes of ROS in different treatment groups were quantitatively detected by FCM analysis after DCFH-DA staining. Data are expressed as ($\bar{x} \pm s$) of three independent experiments. $^{**}P < 0.01$, versus no-treated control; $^{\#\#}P < 0.01$, versus FA-treated alone group; $^{\$}P < 0.05$, versus FA and NaHS co-treated group.

2.6 Inhibited PON-1 by 2-HQ abolishes the suppressive effect of H_2S on formaldehyde-induced caspase-3 activation

We have previously reported that H_2S suppresses caspase-3 activation induced by FA[40]. To confirm the important role of PON-1 in H_2S-exerted protection against FA neurotoxicity, the effect of 2-HQ on H_2S-suppressed caspase-3 activation was also investigated. As shown in Figure 6, pretreatment of PC12 cells with 200 μmol/L of 2-HQ for 30 min significantly reversed the inhibitory effect of H_2S (NaHS, 200 μmol/L) on FA-induced activation of caspase-3, which indicated that inhibition of PON-1 abolishes H_2S-suppressed activation of caspase-3.

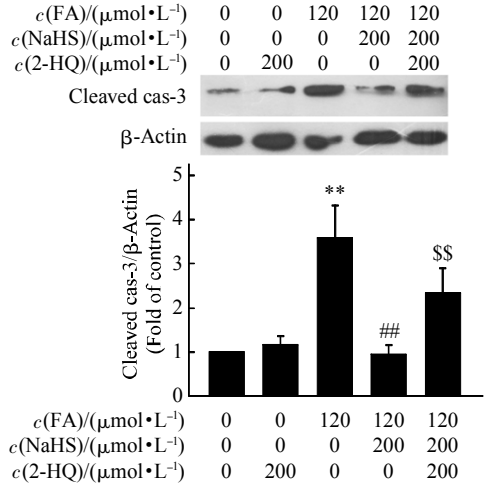

Fig. 6 Effects of 2-hydroxyquinoline on the suppressive effect of H_2S on formaldehyde-induced caspase-3 activation

PC12 cells were pretreated with 200 μmol/L of 2-hydroxyquinoline (2-HQ), a specific inhibitor of PON-1, for 30 min, and then co-treated with formaldehyde (FA, 120 μmol/L) and NaHS (200 μmol/L) for further 24 h. The activation of caspase-3 was determined by Western blot using the cleaved caspase-3 antibody. Western blot images show representative results from three independent experiments. In all blots, staining for β-actin was used as a loading control. The level of cleaved caspase-3 expression obtained in each experimental condition was calculated as a fold of the control. Data are expressed as ($\bar{x} \pm s$) of three independent experiments. $^{**}P < 0.01$, versus no-treated control; $^{\#\#}P < 0.01$, versus FA-treated alone group; $^{\$\$}P < 0.01$, versus FA and NaHS co-treated group.

2.7 Inhibited PON-1 by 2-HQ prevents the reverse effect of H_2S on formaldehyde-induced downregulation of Bcl-2 expression

We have previously reported that H_2S reverses FA-induced downregulation of Bcl-2 [40]. To confirm the important role of PON-1 in H_2S-exerted protection against FA neurotoxicity, the effect of 2-HQ in H_2S-exerted modulation of Bcl-2 protein expression

was also investigated. As shown in Figure 7, pretreatment of PC12 cells with 200 μmol/L of 2-HQ for 30 min significantly reversed the inhibitory effect of H_2S (NaHS, 200 μmol/L) on FA-induced downregulation of Bcl-2 protein expression, which indicated that inhibition of PON-1 abolishes H_2S-exerted upregulation of Bcl-2 expression.

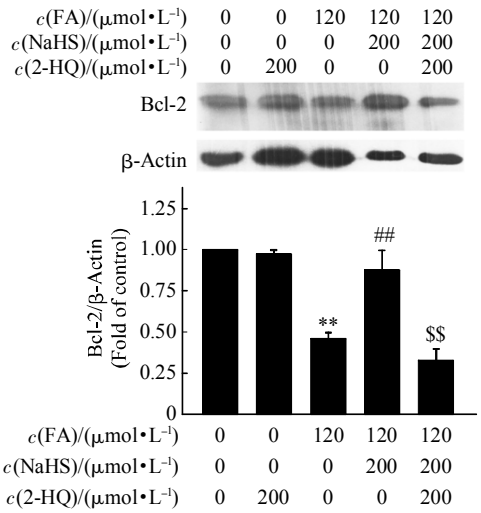

Fig. 7 Effects of 2-hydroxyquinoline on the reserve role of H_2S in formaldehyde-downregulated Bcl-2 expression

PC12 cells were pretreated with 200 μmol/L of 2-hydroxyquinoline (2-HQ), a specific inhibitor of PON-1, for 30 min, and then co-treated with formaldehyde (FA, 120 μmol/L) and NaHS (200 μmol/L) for further 24 h. The expression of Bcl-2 protein was determined by Western blot using the Bcl-2 antibody. Western blot images show representative results from three independent experiments. In all blots, staining for β-actin was used as a loading control. The level of Bcl-2 protein expression obtained in each experimental condition was calculated as a fold of the control. Data are expressed as ($\bar{x} \pm s$) of three independent experiments. $^{**}P < 0.01$, versus no-treated control; $^{\#\#}P < 0.01$, versus FA-treated alone group; $^{\$\$}P < 0.01$, versus FA and NaHS co-treated group.

3 Discussion

We have previously determined the protective role of H_2S in FA-induced neurotoxicity. In this study, to address the mechanisms underlying H_2S-triggered protection against the neurotoxicity of FA, we investigated whether PON-1 plays an important role in this protective effect of H_2S. We demonstrated that H_2S not only upregulates the activity of PON-1，but also significantly restores FA-induced downregulation of PON-1 activity and expression in PC12 cells. We also showed that inhibited PON-1 by 2-HQ (a specific inhibitor of PON-1) significantly blocks H_2S-caused protection against FA-induced cytotoxicity, apoptosis, and accumulation of intracellular ROS in PC12 cells. Furthermore, 2-HQ blocks H_2S-caused downregulation of caspase-3 activation and upregulation of bcl-2 expression in PC12 cells. These findings revealed that PON-1 mediates the protection of H_2S against FA-induced neurotoxicity.

Studies have shown that FA is toxic to neuronal cells [5, 8, 37-38]. Elevated brain FA is a pathogenic factor involved in AD and age-dependent memory decline [21-24]. Therefore, it is of utmost importance to develop new therapeutic approaches to halt the neurotoxicity of FA. Our previous findings demonstrated that H_2S inhibits FA-neurotoxicity [40]. Understanding the molecular mechanisms underlying the neuroprotection of H_2S is helpful for further investigating H_2S as a novel protectant for FA neurotoxicity.

Increasing evidence demonstrated that oxidative damage is one of the most critical effects of FA exposure [10, 35-38]. Oxidative stress is associated with increased production of oxidizing species or a significant decrease in the effectiveness of antioxidant defenses. When ROS formation is unbalanced in proportion to protective antioxidants, the excessive ROS lead to cell death. PON-1 is a pivotal endogenous antioxidant[53]. It has been shown that the PON-1 gene is involved in AD etiopathology [54]. It is therefore logical to determine whether FA-induced oxidative stress in neuron is involved in the reduction of PON-1 expression and activity. In this work, we found that PON-1 protein expresses in PC12 cells and that the expression and activity of PON-1 in PC12 cells were attenuated by FA. These data lead to the conclusion that the downregulation of PON-1 is responsible for the FA-induced accumulation of intracellular ROS, which lead to the neurotoxicity[12].

The antioxidant effects of H_2S have been extensively reported. Kimura *et al.*[34] has for the first time revealed that H_2S protects primary rat cortical neurons from glutamate-induced oxidative stress. It has been subsequently reported that H_2S produces protective effects against oxidative damage induced by peroxynitite[42], hypochlorous acid[43], H_2O_2[55], $A\beta_{25-35}$[56], MPP^+[57], rotenone[58] and homocysteine[59] in neuronal cells. Recently, we showed that H_2S prevents the accumulation of intracellular ROS and neurotoxicity

induced by FA[40]. These findings indicate that H_2S is an endogenous antioxidant gas[60] and suggest that H_2S acts as a neuroprotectant counteracting an oxidative stress to neurons induced by FA. In the present work, we showed that downregulation of PON-1 is responsible for FA-induced oxidative stress and neurotoxicity. Thus, we raised the question whether the antioxidant mechanism of H_2S against FA-induced neurotoxicity involves modulation of PON-1.

It has been shown that upregulation of PON-1 contributes to the neuroprotections both of hypoxic preconditioning against toxic insult in visual cells[61] and of ASC-6 against homocysteine neurotoxicity[62]. Therefore, we investigate whether H_2S protects PC12 cells against FA-induced neurotoxicity by modulating the expression and activity of PON-1. Notably, our data showed that H_2S not only increases the activity of PON-1 but also prevented FA-induced down-regulation of PON-1 expression and activity, which suggested that the neuroprotection of H_2S is associated with its role in upregulation of PON-1. Furthermore, we found that pretreatment with 2-HQ, the inhibitor of PON-1, triggered a drastic reduction of the protective effect of H_2S against FA-induced cytotoxicity and apoptosis. From these results we proposed that modulation of PON-1 contributes to the protection of H_2S against FA-induced neurotoxicity.

Overexpression of PON-1 can scavenge free radical[63]. PON-1 knockout mice are characterized by increased macrophage ROS levels, while decreased macrophage oxidative stress have been shown in PON-1 transgenic mice[64]. In the present work, we showed that pretreatment with 2-HQ, the inhibitor of PON-1, triggered a drastic reduction of the protective effect of H_2S against FA-induced accumulation of intracellular ROS. We thus demonstrate that H_2S-induced upregulation of PON-1 is of major importance for its protective function against FA-triggered oxidative stress. Apoptosis is a cellular self-destruction mechanism involved in a variety of biological events. We have previously demonstrated that antiapoptotic effect of H_2S contributes to its role in restraining FA-induced toxicity to neuronal cells by upregulation of Bcl-2 expression and downregulation of caspase-3 activation[40]. Our present work provided evidence that inhibited PON-1 by 2-HQ reversed the role of H_2S in upregulation of Bcl-2 expression and downregulation of caspase-3 activation. Our data thus indicated that H_2S-induced upregulation of PON-1 plays an important role in its protective function against FA-induced activation of caspase-3 and downregulation of Bcl-2.

In conclusion, the present study showed that H_2S upregulates PON-1 and inhibition of PON-1 blocks the protective function of H_2S against FA-induced cytotoxicity, apoptosis, accumulation of intracellular ROS, activation of csapase-3, and downregulation of Bcl-2. The present findings clearly identify that H_2S provides significant protection against FA-induced neurotoxicity by upregulating PON-1. Our findings expand our understanding of the mechanism involved in H_2S-offered protection against FA neurotoxicity.

References

[1] Franklin P, Dingle P, Stick S. Raised exhaled nitric oxide in healthy children is associated with domestic formaldehyde levels. Am J Respir Crit Care Med, 2000, **161**(5): 1757−1759

[2] Binetti R, Costamagna F M, Marcello I. Development of carcinogenicity classifications and evaluations: the case of formaldehyde. Ann Ist Super Sanita, 2006, **42**(2): 132−143

[3] Thrasher J D, Kilburn K H. Embryo toxicity and teratogenicity of formaldehyde. Arch Environ Health, 2001, **56**(4): 300−311

[4] Andersen M E, Clewell H J, 3rd, Bermudez E, *et al*. Formaldehyde: integrating dosimetry, cytotoxicity, and genomics to understand dose-dependent transitions for an endogenous compound. Toxicol Sci, 2010, **118**(2): 716−731

[5] Songur A, Ozen O A, Sarsilmaz M. The toxic effects of formaldehyde on the nervous system. Rev Environ Contam Toxicol, 2010, **203**: 105−118

[6] Kilburn K H. Neurobehavioral impairment and seizures from formaldehyde. Arch Environ Health, 1994, **49**(1): 37−44

[7] Kilburn K H, Warshaw R, Thornton J C. Formaldehyde impairs memory, equilibrium, and dexterity in histology technicians: effects which persist for days after exposure. Arch Environ Health, 1987, **42**(2): 117−120

[8] Aslan H, Songur A, Tunc A T, *et al*. Effects of formaldehyde exposure on granule cell number and volume of dentate gyrus: a histopathological and stereological study. Brain Res, 2006, **1122**(1): 191−200

[9] Liu Y, Ye Z, Yang H, *et al*. Disturbances of soluble N-ethylmaleimide-sensitive factor attachment proteins in hippocampal synaptosomes contribute to cognitive impairment after repetitive formaldehyde inhalation in male rats. Neuroscience, 2010, **169**(3): 1248−1254

[10] Lu Z, Li C M, Qiao Y, *et al*. Effect of inhaled formaldehyde on learning and memory of mice. Indoor Air, 2008, **18**(2): 77−83

[11] Pitten F A, Kramer A, Herrmann K, *et al*. Formaldehyde neurotoxicity in animal experiments. Pathol Res Pract, 2000, **196**(3): 193−198

[12] Tang X Q, Ren Y K, Chen R Q, *et al*. Formaldehyde induces

neurotoxicity to PC12 cells involving inhibition of paraoxonase-1 expression and activity. Clin Exp Pharmacol Physiol, 2011, **38**(4): 208−214

[13] Tang X Q, Zhuang Y Y, Zhang P, *et al*. Formaldehyde impairs learning and memory involving the disturbance of hydrogen sulfide generation in the hippocampus of rats. J Mol Neurosci, 2013, **49**(1): 140−149

[14] Luo W, Li H, Zhang Y, *et al*. Determination of formaldehyde in blood plasma by high-performance liquid chromatography with fluorescence detection. J Chromatogr B Biomed Sci Appl, 2001, **753**(2): 253−257

[15] Lyles G A. Mammalian plasma and tissue-bound semicarbazide-sensitive amine oxidases: biochemical, pharmacological and toxicological aspects. Int J Biochem Cell Biol, 1996, **28**(3): 259−274

[16] Yu P H, Cauglin C, Wempe K L, *et al*. A novel sensitive high-performance liquid chromatography/electrochemical procedure for measuring formaldehyde produced from oxidative deamination of methylamine and in biological samples. Anal Biochem, 2003, **318**(2): 285−290

[17] Lu J, Miao J, Su T, *et al*. Formaldehyde induces hyperphosphorylation and polymerization of Tau protein both *in vitro* and *in vivo*. Biochim Biophys Acta, 2013, **1830**(8): 4102−4116

[18] Yu P H. Involvement of cerebrovascular semicarbazide-sensitive amine oxidase in the pathogenesis of Alzheimer's disease and vascular dementia. Med Hypotheses, 2001, **57**(2): 175−179

[19] Unzeta M, Sole M, Boada M, *et al*. Semicarbazide-sensitive amine oxidase (SSAO) and its possible contribution to vascular damage in Alzheimer's disease. J Neural Transm, 2007, **114**(6): 857−862

[20] Jiang Z J, Richardson J S, Yu P H. The contribution of cerebral vascular semicarbazide-sensitive amine oxidase to cerebral amyloid angiopathy in Alzheimer's disease. Neuropathol Appl Neurobiol, 2008, **34**(2): 194−204

[21] Tong Z, Han C, Luo W, *et al*. Accumulated hippocampal formaldehyde induces age-dependent memory decline. Age (Dordr), 2013, **35**(3): 583−596

[22] Tong Z, Zhang J, Luo W, *et al*. Urine formaldehyde level is inversely correlated to mini mental state examination scores in senile dementia. Neurobiol Aging, 2011, **32**(1): 31−41

[23] Tong Z, Han C, Luo W, *et al*. Aging-associated excess formaldehyde leads to spatial memory deficits. Sci Rep, 2013, **3**: 1807

[24] Tong Z, Han C, Luo W, *et al*. Accumulated hippocampal formaldehyde induces age-dependent memory decline. Age (Dordr), 2013, **35**(3): 583−596

[25] Wang R. Physiological implications of hydrogen sulfide: a whiff exploration that blossomed. Physiol Rev, 2012, **92**(2): 791−896

[26] Predmore B L, Lefer D J, Gojon G. Hydrogen sulfide in biochemistry and medicine. Antioxid Redox Signal, 2012, **17**(1): 119−140

[27] Chen X, Jhee K H, Kruger W D. Production of the neuromodulator H_2S by cystathionine beta-synthase *via* the condensation of cysteine and homocysteine. J Biol Chem, 2004, **279**(50): 52082−52086

[28] Chiku T, Padovani D, Zhu W, *et al*. H_2S biogenesis by human cystathionine gamma-lyase leads to the novel sulfur metabolites lanthionine and homolanthionine and is responsive to the grade of hyperhomocysteinemia. J Biol Chem, 2009, **284**(17): 11601−11612

[29] Renga B. Hydrogen sulfide generation in mammals: the molecular biology of cystathionine-beta- synthase (CBS) and cystathionine-gamma-lyase (CSE). Inflamm Allergy Drug Targets, 2011, **10**(2): 85−91

[30] Kimura H, Shibuya N, Kimura Y. Hydrogen sulfide is a signaling molecule and a cytoprotectant. Antioxid Redox Signal, 2012, **17**(1): 45−57

[31] Calvert J W, Coetzee W A, Lefer D J. Novel insights into hydrogen sulfide--mediated cytoprotection. Antioxid Redox Signal, 2010, **12**(10): 1203−1217

[32] Kimura H, Nagai Y, Umemura K, *et al*. Physiological roles of hydrogen sulfide: synaptic modulation, neuroprotection, and smooth muscle relaxation. Antioxid Redox Signal, 2005, **7**(5−6): 795−803

[33] Jha S, Calvert J W, Duranski M R, *et al*. Hydrogen sulfide attenuates hepatic ischemia-reperfusion injury: role of antioxidant and antiapoptotic signaling. Am J Physiol Heart Circ Physiol, 2008, **295**(2): H801−806

[34] Kimura Y, Kimura H. Hydrogen sulfide protects neurons from oxidative stress. FASEB J, 2004, **18**(10): 1165−1167

[35] Gurel A, Coskun O, Armutcu F, *et al*. Vitamin E against oxidative damage caused by formaldehyde in frontal cortex and hippocampus: biochemical and histological studies. J Chem Neuroanat, 2005, **29**(3): 173−178

[36] Zhou D X, Qiu S D, Zhang J, *et al*. The protective effect of vitamin E against oxidative damage caused by formaldehyde in the testes of adult rats. Asian J Androl, 2006, **8**(5): 584−588

[37] Zararsiz I, Kus I, Akpolat N, *et al*. Protective effects of omega-3 essential fatty acids against formaldehyde-induced neuronal damage in prefrontal cortex of rats. Cell Biochem Funct, 2006, **24**(3): 237−244

[38] Zararsiz I, Kus I, Ogeturk M, *et al*. Melatonin prevents formaldehyde-induced neurotoxicity in prefrontal cortex of rats: an immunohistochemical and biochemical study. Cell Biochem Funct, 2007, **25**(4): 413−418

[39] Tang X Q, Fang H R, Zhou C F, *et al*. A novel mechanism of formaldehyde neurotoxicity: inhibition of hydrogen sulfide generation by promoting overproduction of nitric oxide. PLoS One, 2013, **8**(1): e54829

[40] Tang X Q, Ren Y K, Zhou C F, *et al*. Hydrogen sulfide prevents formaldehyde-induced neurotoxicity to PC12 cells by attenuation of mitochondrial dysfunction and pro-apoptotic potential. Neurochem Int, 2012, **61**(1): 16−24

[41] Laggner H, Muellner M K, Schreier S, *et al*. Hydrogen sulphide: a novel physiological inhibitor of LDL atherogenic modification by HOCl. Free Radic Res, 2007, **41**(7): 741−747

[42] Whiteman M, Armstrong J S, Chu S H, *et al*. The novel

neuromodulator hydrogen sulfide: an endogenous peroxynitrite 'scavenger'?. J Neurochem, 2004, **90**(3): 765−768

[43] Whiteman M, Cheung N S, Zhu Y Z, *et al*. Hydrogen sulphide: a novel inhibitor of hypochlorous acid-mediated oxidative damage in the brain?. Biochem Biophys Res Commun, 2005, **326**(4): 794−798

[44] Carballal S, Trujillo M, Cuevasanta E, *et al*. Reactivity of hydrogen sulfide with peroxynitrite and other oxidants of biological interest. Free Radic Biol Med, 2011, **50**(1): 196−205

[45] Aviram M, Rosenblat M. Paraoxonases and cardiovascular diseases: pharmacological and nutritional influences. Curr Opin Lipidol, 2005, **16**(4): 393−399

[46] Uzun H, Yanardag H, Gelisgen R, *et al*. Levels of paraoxonase, an index of antioxidant defense, in patients with active sarcoidosis. Curr Med Res Opin, 2008, **24**(6): 1651−1657

[47] Borowczyk K, Shih D M, Jakubowski H. Metabolism and neurotoxicity of homocysteine thiolactone in mice: evidence for a protective role of paraoxonase 1. J Alzheimers Dis, 2012, **30**(2): 225−231

[48] Jakubowski H. The role of paraoxonase 1 in the detoxification of homocysteine thiolactone. Adv Exp Med Biol, 2010, **660**(1): 113−127

[49] Zhang C, Peng W, Jiang X, *et al*. Transgene expression of human PON1 Q in mice protected the liver against CCl_4-induced injury. J Gene Med, 2008, **10**(1): 94−100

[50] Cathcart R, Schwiers E, Ames B N. Detection of picomole levels of hydroperoxides using a fluorescent dichlorofluorescein assay. Anal Biochem, 1983, **134**(1): 111−116

[51] Grieve A, Butcher S P, Griffiths R. Synaptosomal plasma membrane transport of excitatory sulphur amino acid transmitter candidates: kinetic characterisation and analysis of carrier specificity. J Neurosci Res, 1992, **32**(1): 60−68

[52] Fujita K, Yamafuji M, Nakabeppu Y, *et al*. Therapeutic approach to neurodegenerative diseases by medical gases: focusing on redox signaling and related antioxidant enzymes. Oxid Med Cell Longev, 2012, **2012**: 324256

[53] Ng C J, Shih D M, Hama SY, *et al*. The paraoxonase gene family and atherosclerosis. Free Radic Biol Med, 2005, **38**(2): 153−163

[54] Leduc V, Theroux L, Dea D, *et al*. Involvement of paraoxonase 1 genetic variants in Alzheimer's disease neuropathology. Eur J Neurosci, 2009, **30**(9): 1823−1830

[55] Lu M, Hu L F, Hu G, *et al*. Hydrogen sulfide protects astrocytes against H_2O_2-induced neural injury *via* enhancing glutamate uptake. Free Radic Biol Med, 2008, **45**(12): 1705−1713

[56] Tang X Q, Yang C T, Chen J, *et al*. Effect of hydrogen sulphide on beta-amyloid-induced damage in PC12 cells. Clin Exp Pharmacol Physiol, 2008, **35**(2): 180−186

[57] Yin W L, He J Q, Hu B, *et al*. Hydrogen sulfide inhibits MPP(+)-induced apoptosis in PC12 cells. Life Sci, 2009, **85**(7−8): 269−275

[58] Hu L F, Lu M, Wu Z Y, *et al*. Hydrogen sulfide inhibits rotenone-induced apoptosis *via* preservation of mitochondrial function. Mol Pharmacol, 2009, **75**(1): 27−34

[59] Tang X Q, Shen X T, Huang Y E, *et al*. Hydrogen sulfide antagonizes homocysteine-induced neurotoxicity in PC12 cells. Neurosci Res, 2010, **68**(3): 241−249

[60] Nakao A, Sugimoto R, Billiar T R, *et al*. Therapeutic antioxidant medical gas. J Clin Biochem Nutr, 2009, **44**(1): 1−13

[61] Thiersch M, Raffelsberger W, Frigg R, *et al*. Analysis of the retinal gene expression profile after hypoxic preconditioning identifies candidate genes for neuroprotection. BMC Genomics, 2008, **9**: 73

[62] Tang X Q, Chen R Q, Dong L, *et al*. Role of paraoxonase-1 in the protection of hydrogen sulfide-donating sildenafil (ACS6) against homocysteine-induced neurotoxicity. J Mol Neurosci, 2013, **50**(1): 70−77

[63] Aviram M, Rosenblat M, Billecke S, *et al*. Human serum paraoxonase (PON 1) is inactivated by oxidized low density lipoprotein and preserved by antioxidants. Free Radic Biol Med, 1999, **26**(7−8): 892−904

[64] Rozenberg O, Shiner M, Aviram M, *et al*. Paraoxonase 1 (PON1) attenuates diabetes development in mice through its antioxidative properties. Free Radic Biol Med, 2008, **44**(11): 1951−1959

Paraoxonase-1 介导硫化氢的抗甲醛神经毒性作用 *

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摘要　我们以往的研究工作证实了硫化氢 (hydrogen sulfide，H_2S) 对甲醛神经毒性和氧化应激具有拮抗作用．Paraoxonase-1 (PON-1) 是机体重要的内源性抗氧化剂．本研究的目的是探讨 PON-1 是否可介导 H_2S 的抗甲醛神经毒性作用．采用甲醛损伤 PC12 细胞为甲醛神经毒性的细胞模型．硫氢化钠 (NaHS，一种 H_2S 的供体) 不仅可以上调 PC12 细胞 PON-1 的活力，还可恢复甲醛对 PC12 细胞 PON-1 表达与活力的抑制作用．2-hydroxyquinoline (2-HQ) 是一种选择性 PON-1 抑制剂，它可显著降低 H_2S 对甲醛细胞毒性、凋亡和活性氧(reactive oxygen species, ROS)累积的抑制作用．而且，2-HQ 可阻止 H_2S 逆转甲醛激活 PC12 细胞 caspase-3 和下调 PC12 细胞 bcl-2 表达．结果提示 H_2S 依赖 PON-1 去保护 PC12 细胞对抗甲醛的神经毒性．我们的这一发现表明 PON-1 有希望成为防治甲醛神经损伤的新靶点．

关键词　甲醛，硫化氢，paraoxonase-1，凋亡，活性氧
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