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Hydrogen sulfide may attenuate methylmercury-induced neurotoxicity via mitochondrial preservation

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ABSTRACT

Hydrogen sulfide (H_2S) is a protective molecule and a novel gaseous mediator. Here we explored whether H_2S donor (NaHS) could attenuate methylmercury (MeHg)-induced neurotoxicity in rats. The adult rats were randomly divided into four groups, i.e., control, NaHS, MeHg, and NaHS + MeHg groups. Rats of the NaHS + MeHg group were intraperitoneally (i.p) injected with 5.6 mg/kg/d of NaHS together with 5 μg/kg/d of MeHg. Rats of the MeHg group and NaHS group were injected with 5 μg/kg/d of MeHg and 5.6 mg/kg/d of NaHS, respectively. All treatments were continued for 20 d, and the cerebral cortex of the rats was evaluated. The results showed that NaHS significantly reduced MeHg-induced oxidative stress, as indicated by reduced lipid peroxide content, and increased glutathione levels and glutathione peroxidase and thioredoxin reductase activities. NaHS attenuated MeHg-induced mitochondrial damage, as indicated by increased mitochondrial activity, reduced mitochondrial swelling, and the release of cytochrome C and apoptosis-inducing factors. NaHS also decreased the number of apoptotic cells compared to that observed in MeHg only-treated rats, as indicated in a TUNEL assay. Finally, NaHS increased DNA and RNA content, and the activities of acetylcholinesterase and Na^+/K^+-ATPase. These indices were all lower in the MeHg group than in the control group, and NaHS alone did not observably influence any of these indices compared to the control. Our results demonstrate that H_2S may protect against MeHg-induced neurotoxicity, and the mechanisms appear to involve the inhibition of oxidative stress and the protection of mitochondria.

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1. Introduction

Due to either natural events or anthropogenic sources, mercury (Hg) is present in food. Hg is among the most harmful heavy metals to which humans can be exposed, and has no known physiological role in humans. Inorganic Hg salts and organic Hg chemicals are the dominant forms of Hg in the environment. The organic Hg chemicals have a higher solubility in lipids than the inorganic species, and diffuse more easily through the lipid bilayer of biological membranes, which increases their potential toxicity [1]. As an organic Hg, methylmercury (MeHg) is formed as a result of the biomethylation of inorganic mercury compounds that are released in the aquatic environment, and MeHg-containing fish represent a major source of human Hg exposure. The human body lacks effective mechanisms to excrete MeHg, thus MeHg accumulates in the body, especially in nerve tissues and primarily in the spinal dorsal root ganglia followed by the cerebral cortex and cerebellum [2]. MeHg causes neurological deficits to a variable extent according to the exposure dose and duration [1]. Serious human symptoms of MeHg poisoning include paresthesias, constriction of the visual field, an impairment of hearing and speech, cerebellar ataxia and psychiatric symptomatology [1]. Although the mechanism of MeHg toxicity is still unclear, most studies agree that MeHg has a high affinity for thiol groups (-SH) and seleno groups (-SeH) that are

Abbreviations: H_2S, Hydrogen sulfide; MeHg, methylmercury; AChE, acetylcholinesterase; GPx, Glutathione peroxidases; TrxR, thioredoxin reductases; GSH, glutathione; TUNEL, Terminal-deoxynucleoitidyl Transferase Mediated Nick End Labeling; Cyt-C, cytochrome C; AIF, apoptosis-inducing factor; MTT, methyl thiazolyl tetrazolium; ROS, reactive oxygen species; i.p., intraperitoneally; T-TBS, tris-buffered saline.

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present in amino acids, proteins and enzymes, such as glutathione (GSH, a -SH containing molecule) and glutathione peroxidase (GPX, a -SeH containing protein). MeHg disrupts the biological function of these molecules [1]. The MeHg binding unbalances the redox state, generates free radicals, leads to mitochondrial damage, apoptotic cell death, and neurodegenerative diseases [3]. Despite a massive search for strategies to counteract MeHg toxicity, no ideal approach to completely abolish MeHg toxicity has been identified. Some chelating agents can assist the body in the elimination of MeHg; however, these drugs are of limited use because of their adverse side effects [2].

In recent decades, hydrogen sulfide (H_2S) has been recognized as a protective molecule under many circumstances and as novel gaseous mediator, along with nitric oxide and carbon monoxide. Endogenous H_2S in the mammalian body is produced by cystathionine γ-lyase, cystathionine β-synthase, and 3-mercaptopyruvate sulfurtransferase. In peripheral systems, H_2S regulates vascular tone, protects the heart against ischemic injury, and controls the release of insulin, among other functions [4,5]. In the brain, a higher level of endogenous H_2S has been detected than in other organs. Emerging evidences suggest that H_2S might function as a neuromodulator in the brain, and H_2S deficiency plays a crucial role in the pathogenesis of some neurodegenerative diseases, such as Alzheimer's disease and Parkinson's disease [6,7]. Exogenous H_2S acts as a neuroprotectant [8]. Kimura et al. [9] demonstrated that H_2S protected primary rat cortical neurons from glutamate-induced oxidative stress. Since then, several studies have shown that H_2S protects neuronal cells that have been exposed to different stressors, such as peroxynitite [10], rotenone [11], and β-amyloid [12]. The mechanisms underlying the neuroprotective roles of H_2S as a reducing gas include reducing oxidative stress [13]and inflammation [14], modulating cell proliferation and apoptosis, and protecting mitochondria [11].

It is reported that H_2S reacts directly with MeHg to form a less toxic metabolite [15]. In addition, a H_2S-producing enzyme attenuates MeHg-induced toxification in mammalian cells [16]. However, it is unknown whether exogenous H_2S is an effective treatment for MeHg-induced neurotoxicity. In the present study, we explored whether H_2S donor (NaHS) can attenuate MeHg-induced neurotoxicity in rats. We focused on the roles of NaHS in MeHg-induced oxidative stress and mitochondrial damage in the cerebral cortex, where MeHg easily accumulates.

2. Materials and methods

The experiment protocol was approved by the Animal Ethics Committee of Shenyang University, and was compliant with the World Medical Association Declaration of Helsinki.

2.1. Major materials

Methyl mercuric chloride was purchased from Sigma Chemicals (St. Louis, MO, USA). Primary and secondary antibodies were provided by Santa Cruz Biotechnology Inc. (Dallas, TX, USA). A Terminal deoxynucleotidyl transferase (TdT)-mediated biotinylated deoxyuridine triphosphate nick end-labeling (TUNEL) apoptosis test kit was purchased from the Wuhan Boster Biotech. Co. Ltd. (Wuhan, China). Other reagents and kits were mostly provided by the Beijing Dingguo Changsheng Biotechnology Co. Ltd. (Beijing, China).

Specific pathogens-free grade male Sprague-Dawley rats (220 ± 20 g) were obtained from the Liaoning Provincial Laboratory Animal Public Service Center (China, Benxi City).

2.2. Animal experimental design

The rats were housed at 22 °C ± 2 °C under a 12-h light–dark cycle, with access to standard laboratory animal feed and water *ad libitum*. After 1 w of acclimation, the rats were divided randomly into control, NaHS, MeHg, and NaHS + MeHg treatment groups; each group contained 10 rats. Rats of the NaHS group were intraperitoneally (i.p.) injected with 5.6 mg/kg/d of NaHS (normal saline as the vehicle). This NaHS dosage was previously reported to have neuroprotective effects [17]. Rats of the MeHg group were i.p. injected with 5 μg/kg/d of MeHg (normal saline as the vehicle), the exposure method was previously described [18]. Rats of the NaHS + MeHg group were i.p. injected with 5.6 mg/kg/d of NaHS, and one half hour later, 5 μg/kg/d of MeHg was i.p. injected. Control animals were i.p. injected with only an equal volume of normal saline. All treatments were maintained for 20 d. At 21 d, all animals were euthanized with phenobarbital sodium (50 mg/kg, i.p.), and brain samples were taken. Right brain sagittal sections were used for cerebral cortex mitochondrial analysis and other assays. The left brain hemispheres were used in a TUNEL assay, as described below. The mitochondrial analysis needed to be performed immediately.

2.3. Cerebral cortex oxidative stress analysis and other enzyme assays

The cerebral cortex of rats was minced and homogenized (10%, w/v) separately in ice-cold 1.15% KCl–0.01 M sodium potassium phosphate buffer (pH 7.4) in a glass homogenizer. The homogenate was centrifuged at 10,000 g for 20 min at 4 °C, and the resultant supernatant was used for several different assays. Lipid peroxide was assayed using a protocol developed by Draper and Hadley [19]. Reduced glutathione (GSH) was measured using an enzymatic recycling assay proposed by Akerboom & Sies [20]. The activity of thioredoxin reductases (TrxR, EC 1.8.1.9) was determined using the insulin reduction endpoint assay proposed by Arnér and Holmgren [21]. Glutathione peroxidase (GPX, EC 1.11.1.9) activity was assayed by measuring nicotinamideadenine dinucleotide phosphate (NADPH) oxidationusing t-butyl-hydroperoxide as a substrate, as described by Maral et al. [22]. Acetylcholinesterase (AChE, EC 3.1.1.7) activity was estimated using the method of Ellman et al. (1961) [23]. Na^+/K^+-ATPase (EC 3.6.3.9) activity was determined as described by Kreutzet al. [24].

2.4. Assay of the cerebral cortex nucleic acid content

The cerebral cortex was homogenized in 5 ml of 0.5-M perchloric acid, and water bath heated at 90 °C for 10 min. After cooling, the homogenate was centrifuged at 3000 rpm for 10 min to obtain the supernatant. The DNA content was estimated using the diphenylamine reaction method, and the RNA content was analyzed using the color reaction method [2].

2.5. Cerebral cortex mitochondrial assays

In living mitochondria, succinate dehydrogenase can reduce MTT to water insoluble violet crystal formazan, which has absorbance at 570 nm in solvent. To determine the mitochondrial activity, mitochondria were prepared as described previously [17]. Briefly, a freshly prepared mitochondria suspension (100 μL) was rapidly placed into the microtiter plate. Methyl thiazolyl tetrazolium (MTT, 5 g L^{-1}, 40 μL) was added and the suspension was incubated at 30 °C for 30 min. Next, isopropanol (100 μL) was added for 20 min, and the mitochondrial activity was measured based on the change in the absorbance at 570 nm [25].

To assay the mitochondrial permeability transition, freshly

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