Clinical Toxicology (2011) **49**, 161–166

ISSN 1556-3650 print/ISSN 1556-9519 online
DOI: 10.3109/15563650.2011.565419

informa
healthcare

RESEARCH

Myocardial and lung injuries induced by hydrogen sulfide and the effectiveness of oxygen therapy in rats

NA WU, XUQIN DU, DIXIN WANG, and FENGTONG HAO

Department of Occupational Diseases and Clinical Toxicology, Beijing Chaoyang Hospital, Capital Medical University, Beijing, China

Objective. To study myocardial and lung injuries initiated by hydrogen sulfide, and evaluate the role and effectiveness of normobaric and hyperbaric oxygen (HBO) treatment in rats. *Methods*. One hundred healthy male Wistar rats were randomly divided into five groups: A: Normal control group (no H_2S); B: H_2S-exposed group; C: H_2S+33% oxygen treatment group; D: H_2S+50% oxygen treatment group; E: H_2S+HBO group. The rats in groups C, D and E were exposed to H_2S in an exposure chamber (1 m^3) and were made to inhale 300 ppm hydrogen sulfide for 60 min, and then they were subjected to normobaric or HBO therapy. Normobaric oxygen was at concentrations of 33% or 50%, HBO was for 100 min including compression and decompression; the rats in group A inhaled air under the same conditions. Blood was sampled immediately after the experiment for analysis of arterial blood gases, myocardial enzymes and cardiac troponin I. Lung was rapidly removed to be made into tissue homogenates and then cytochrome c oxidase activity was measured; myocardial and lung ultrastructural changes were observed by electron microscopy. *Results*. Arterial blood gases: partial pressure of O_2 (mmHg) (Group A, 97.6 $\pm$ 8.38; B, 76.5 $\pm$ 6.95*; C, 83.2 $\pm$ 2.66*; D, 86.20 $\pm$ 10.75*; E, 93.50 $\pm$ 4.97: *$p < 0.01$ compared to group A) was significantly lower than that in group in all but HBO rats. For myocardial enzymes and cardiac troponin I every parameter in groups B and C was significantly higher than that in group A ($p < 0.01$),with no difference in D and E. Cytochrome c oxidase activity (u/mg) of lung tissue was reduced compared to group A after all treatments (A, 1.76 $\pm$ 0.02; B, 0.36 $\pm$ 0.04; C, 0.50 $\pm$ 0.12; D, 0.56 $\pm$ 0.07; E, 0.68 $\pm$ 0.05 (A vs. B $p < 0.01$; B vs. C,D,E $p < 0.05$ or $p < 0.01$), with a graded effect of oxygen dose in C, D and E. Pathological changes: (1) Myocardium – Mitochondrial swelling and autolysis with blurred or broken cristae was observed in the myocardium of H_2S-exposed group; in group E, mitochondrial structure was basically normal, and clear cristae were found. (2) Lung tissue – In H_2S-exposed group, alveolar epithelial cells disappeared, vacuolization of the organelle occurred, nuclear membrane was irregular and marginal condensation of heterochromatin was present; nucleus showed relatively normal morphology in group E, although some vacuoles still persisted within them. *Conclusions*. HBO therapy can effectively improve arterial oxygen partial pressure, and significantly reduce myocardial damage, as well as potentially relieve lung injury in this model. Further work in humans appears warranted.

Keywords Hydrogen sulfide poisoning; Hyperbaric oxygen; Cardiac troponin I; Cytochrome c oxidase; Tissue ultrastructure

Introduction

Hydrogen sulfide (H_2S) is a potentially toxic environmental and lethal industrial hazard.[1] In China, 130 H_2S poisoning events were reported between 1990 and 2003, and 636 people poisoned resulting in 266 deaths (41.8% mortality).[2]

The primary mechanism for the toxic action of H_2S is direct inhibition of cytochrome c oxidase (CCO). This action interrupts oxidative phosphorylation and cellular aerobic metabolism, which causes tissue hypoxia.[3–7]

Providing oxygen therapy in a timely manner is extremely important in the emergency treatment of acute H_2S poisoning. Normobaric and hyperbaric oxygen (HBO) treatment have been widely used in clinics. Few studies on the effectiveness of varying oxygen therapy have been reported except for case reports.[8–13] Theoretically, HBO therapy should have a beneficial effect in reversing the cellular anoxia caused by H_2S poisoning. However, the documented benefit of HBO treatment remains largely anecdotal, and has not become the standard of care.[8,13] We report cardiac and lung injuries induced by inhaled H_2S in rats, and the impact and effectiveness of different concentrations of normobaric and HBO were also evaluated.

Received 16 December 2010; accepted 19 February 2011.

Address correspondence to Dixin Wang, Department of Occupational Diseases and Clinical Toxicology, Beijing Chaoyang Hospital, Capital Medical University, Beijing, China. E-mail: wdxlsq42@yahoo.com.cn

Materials and methods

H_2S exposure and oxygen treatment

One hundred healthy male Wistar rats weighing 200–250 g were kept at 20–25°C with free access to water and food.

They were randomly divided into five groups, 20 rats/group: A: normal control group, B: H_2S-exposed group, C: H_2S+33% oxygen treatment group, D: H_2S+50% oxygen treatment group, and E: H_2S +HBO group. The rats in groups B–E were placed into poisoning tanks (1 m^3). H_2S was then added to the tanks, at a concentration of 300 ppm. All rats were exposed for 60 min, and the effects were observed and recorded. Within 20 min of the end of exposure to the poisonous gas, the rats in groups C–E were subjected to normobaric or HBO therapy. Rats in groups C and D were transferred to transparent tanks (1 m^3) to inhale oxygen at a concentration of 33% and 50%, respectively for 100 min (normobaric oxygen therapy); the rats of group E were placed in HBO chamber with pure oxygen ventilation for 10 min; after the pressure was boosted up to 0.2 MPa (2 ATA) after 10 min of first exposure, a constant pressure was administered for 60 min, then 20 min of decompression was conducted. Thus, the whole inhalation time was 100 min; the rats in group A were subjected to the same environmental conditions and were made to inhale air.

Detection of blood parameters and histopathological examination

After therapy, all rats were anesthetized by intraperitoneal injection of 0.3% pentobarbital l (1 ml/100 g), and a blood sample of 1 ml was collected immediately from the abdominal aorta. Blood gas analyzer Gem PREMIER 3000 was utilized for blood gas analysis; 3 ml blood was drawn from the heart without anticoagulation, Beckman DXC800 fully automatic biochemistry analyzer was applied to detect serum levels of cardiac enzymes and cardiac troponin I (cTnI); right lung was removed and made into lung tissue homogenate, and CCO activity was determined by UV spectrophotometry; two animals of each groups were dissected, a small amount of lung tissue (left lung) and heart muscle tissue (the anterior left ventricular wall) was harvested, underwent initial-fixation in 3% glutaraldehyde, and post-fixation in 1% osmium tetroxide, and then dehydrated in a series of ascending concentrations of acetone, embedded with plastic resin vcd4206, the thin sections of 40 nm thickness were cut with an LKB ultra microtome, the sections were stained with uranyl acetate and lead citrate, ultrastructure changes of lung and cardiac tissues were observed using a Hitachi H2600 TEM.

Statistics

SPSS 11.5 software was used for statistical analysis. Unless otherwise noted, the results were expressed as mean $\pm$ standard deviation ($\bar{x} \pm s$). Parametric data were analyzed using an analysis of variance (ANOVA). A probability value of <0.05 was used as the critical level of significance for all statistical tests.

Results

Detection of blood parameters

Partial pressure of O_2 in arterial blood (PO_2) (Table 1)

The PO_2 in rats from group B was much reduced compared with those in group A ($p < 0.01$). After oxygen therapy, the PO_2 in group-E rats returned to normal.

Determination of serum cardiac enzymes and cTnI (Table 2)

Cardiac enzymes include creatine kinase (CK), lactate dehydrogenase (LDH), aspartate aminotransferase (AST). Each parameter in group B was significantly higher than that in group A ($p < 0.01$), the indicators in group D and E obviously returned to normal, except AST in group D which was still higher than that in group A ($p < 0.01$).

Cytochrome c oxidase activity in lung tissue (Table 3)

CCO activity in group B was remarkably reduced than that in group A ($p < 0.01$); after oxygen therapy, CCO activities in group C and D were higher than that in group B

Table 1. Determination of the PO_2 in rats of different experimental groups ($\bar{x} \pm s$).

	A	B	C	D	E
PO_2 (mmHg)	97.6 $\pm$ 8.38	76.5 $\pm$ 6.95*	83.2 $\pm$ 2.66*	86.2 $\pm$ 10.75*	93.5 $\pm$ 4.97

Groups B–E are compared with group A. *$p < 0.01$. $n = 8$.

Table 2. Determination of serum levels of cardiac enzymes and cTnI in rats of different experimental groups ($\bar{x} \pm s$).

Group	AST (U/L)	CK (U/L)	LDH (U/L)	cTnI (U/L)
A	127 $\pm$ 8.57	1071 $\pm$ 182	1043 $\pm$ 170	8.21 $\pm$ 2.62
B	246 $\pm$ 27.9*	1501 $\pm$ 83.2*	1691 $\pm$ 191*	38.1 $\pm$ 4.70*
C	174 $\pm$ 27.9*	1340 $\pm$ 221*	1382 $\pm$ 293*	36.2 $\pm$ 9.66*
D	163 $\pm$ 17.6*	1067 $\pm$ 149	1117 $\pm$ 179	7.22 $\pm$ 0.81
E	130 $\pm$ 7.75	1049 $\pm$ 125	1104 $\pm$ 170	8.64 $\pm$ 2.18

AST, aspartate aminotransferase; CK, creatine kinase; LDH, lactate dehydrogenase; cTnI, cardiac troponin I. Groups B–E are compared with group A. *$p < 0.01$. $n = 8$.

Table 3. Measurement of CCO activities in the lungs of rats in different experimental groups ($\bar{x} \pm s$).

	A	B	C	D	E
CCO (U/mg)	1.76 ± 0.02	0.36 ± 0.04**	0.50 ± 0.12*	0.56 ± 0.07*	0.68 ± 0.05**

Group B is compared with group A, and groups C–E are compared with group B. *$p < 0.05$, **$p < 0.01$. $n = 8$.

($p < 0.05$), and group E exhibited more significant enhancement compared with group B ($p < 0.01$).

Histopathological changes

Myocardium

Mitochondrial swelling and autolysis with blurred or broken cristae were present in myocardial cells of group B; Mitochondrial swelling, decrease or disappearance of mitochondria crista displaying focal cavitation were observed in groups C and D; the cells in group E were rich in mitochondria with clear structure and complete morphology of mitochondria, which demonstrated an obvious repair (Figs. 1–3).

Lung

In group B, alveolar epithelial cells disappeared, vacuolization of the organelle occurred, nuclear membrane was irregular and marginal condensation of heterochromatin was present showing "crescent" apoptosis; ins group C and D, organelles were vacuolized with irregular nucleus and nuclear membrane, and no significant repair was found; in group E, the nucleus appeared relatively normal with slight vacuolization, presenting mild repair (Figs. 4–6).

Discussion

H_2S dissociates into HS^- in the body, which can combine with Fe^{3+} contained in CCO thereby blocking the respiratory electron transport chain and cellular oxygen utilization, which causes tissue hypoxia.[3–7] In this experiment, acute animal models of H_2S poisoning were successfully established by inhalation in Wistar rats. The rats were immediately given HBO treatment or normobaric oxygen treatment with different concentrations; the PO_2, cardiac enzymes (AST, CK, LDH), cTnI and CCO activity in lung tissue, ultrastructural changes in the lung and cardiac tissues were detected and compared, in an attempt to identify sensitive indicators for the effect of oxygen therapy, thus providing a theoretical basis for establishing reasonable on-site oxygen therapy programs.

Detection of blood parameters

PO_2 is the most efficient and direct indicator reflecting the degree of hypoxia. This experiment indicated that HBO therapy can significantly improve the PO_2, and actively reverse the hypoxic state of tissues and organs in H_2S-exposed rats.

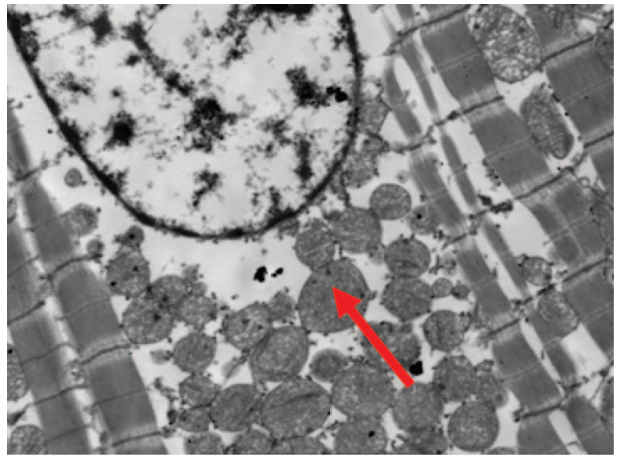

Fig. 1. Cardiac mitochondria in group B ×6000.

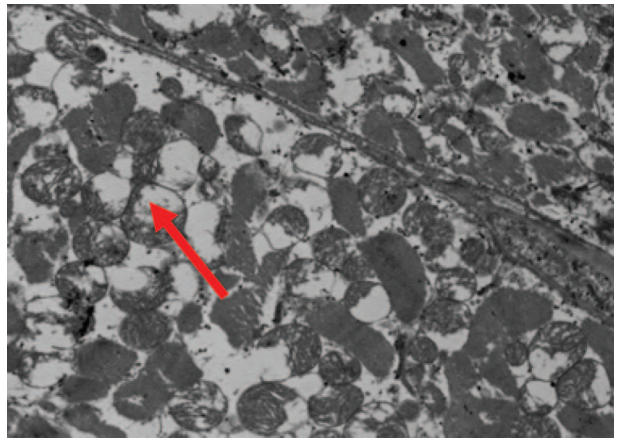

Fig. 2. Cardiac mitochondria in group D ×6000.

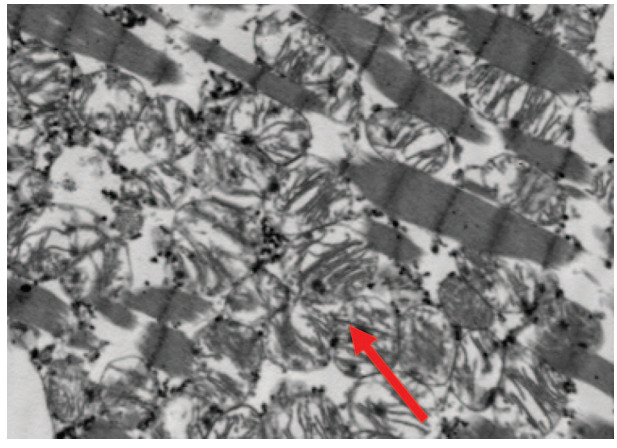

Fig. 3. Cardiac mitochondria in group E ×6000.

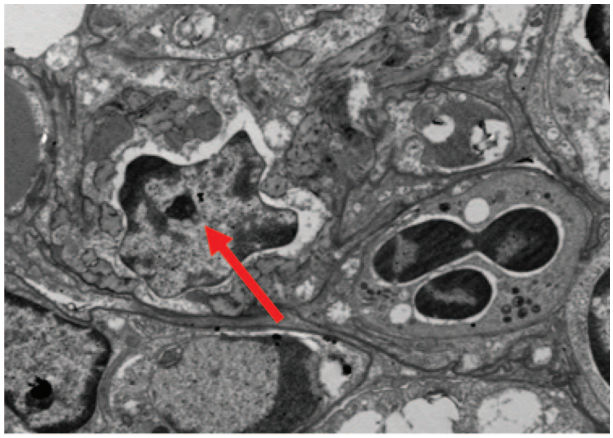

Fig. 4. Plural alveoli in group B ×6000.

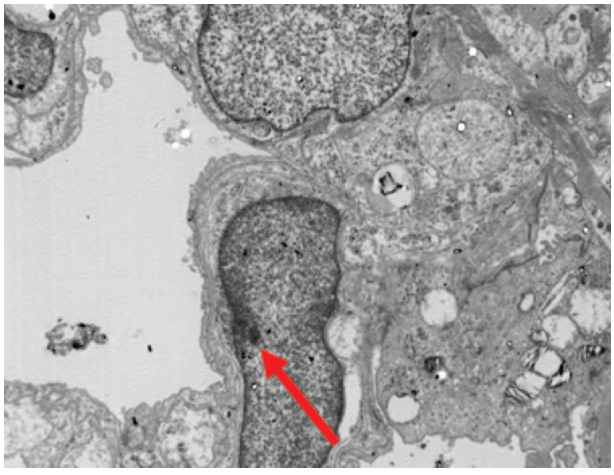

Fig. 5. Plural alveoli in group D ×6000.

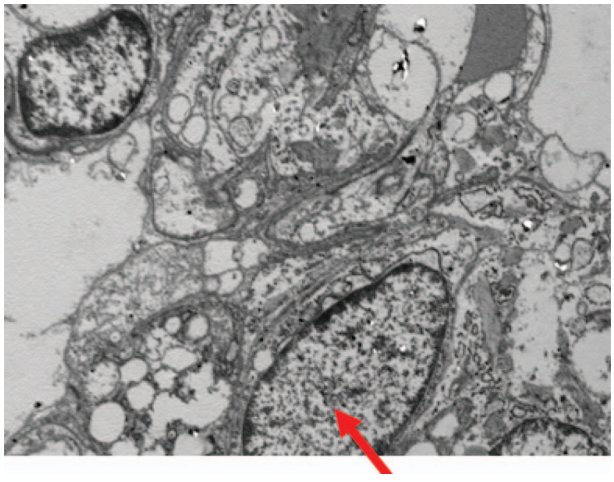

Fig. 6. Plural alveoli in group E ×6000.

cTnI is a cardiac specific antigen, which has high sensitivity as well as specificity for myocardial injury especially for minor myocardial injury, and it has become the optimum marker for condition monitoring myocardial disease.[14,15] Thus, cTnI can be used as a sensitive indicator for evaluating therapeutic effect of oxygen therapy due to its high specificity and sensitivity. This study demonstrated that the levels of myocardial enzymes and cTnI in H_2S-exposed group were significantly higher than that in the normal control group, indicating significant myocardial damage; the cardiac enzymes and cTnI decreased significantly both in the 50% oxygen treatment group and the HBO group as compared to control. Based on the changes in myocardial enzymes and cTnI levels, the degree of myocardial injury was reduced both in 50% oxygen treatment group and HBO group. When we compared the two groups, the HBO group had significantly better efficacy than that of 50% oxygen treatment group. The relationship between serum levels of cTnI and prognosis posterior to HBO treatment at different times was observed in acute, moderate and severely H_2S-poisoned patients by Ji-Qin Wang et al.,[16] which suggested that the earlier the patients accessed HBO treatment, the faster the cTnI values recovered, the less the myocardial damage was, and the better the prognosis.

CCO, located at the end of the respiratory chain, catalyzing the redox reaction with cytochrome C and oxygen as substrates, is a rate-limiting enzyme of cellular aerobic respiration.[1,17,18] This study demonstrates that lung CCO activity of H_2S-exposed rats is significantly inhibited, and this decrease to some extent prevented by oxygen therapy, and the decrease in the HBO treatment group was significantly less than that in the other groups. Nevertheless, only 50% of the lung CCO activity loss was prevented by those various oxygen therapies.

Histopathological changes

H_2S is a strong inhibitor of cytochrome c oxidase, which can make the latter lose its ability to transfer electrons, thereby resulting in cell asphyxia.[3–4] The heart is highly ATP-dependent. The mitochondria are well known as the "power plant" of cellular respiration and energy supply, and mitochondrial damage is one of the earliest signs during cellular hypoxia. Myocardial cells are characterized by abundant and large mitochondria, accounting for 30% of their cytoplasm.[19] Mitochondrial swelling and autolysis with blurred or broken cristae were present in myocardial cells of H_2S-exposed rats in this research, which indicated significant damage in myocardial mitochondria. For those rats subjected to oxygen therapy at concentrations of 33% and 50%, obvious mitochondrial vacuolization was observed, whereas, mitochondria with clear structure and complete morphology was found in those rats undergoing HBO treatment. This provides evidence that HBO treatment delivered in the early stages of H_2S poisoning can significantly reduce the myocardial injury in rats. The histopathological changes on electron microscopy were basically consistent with the alterations in serum cardiac enzymes and cTnI, which further provides supporting evidence for discernible effects of oxygen therapy on H_2S

poisoning. HBO therapy appeared more effective than other methods of oxygen therapy.

In a case of large H_2S inhalation reported by Christia-Lotter et al.,[20] creatine phosphokinase (CPK) and LDH levels were elevated to 6641 IU/L and 715 IU/L, respectively 7 h after exposure. Echocardiogram and cardiac ultrasound revealed hypokinesia in the postero-lateral wall of the left ventricle, and the patient died within 24 h. Changes were seen in the myocardium at autopsy, and large areas of myocardial necrosis were detected on pathological examination.

H_2S is cytotoxic to the lung, and leads to severe injuries to a range of lung cells and tissues and significant pulmonary edema. Nevertheless, few studies on pathological changes of lung have been reported.[21–23] According to the pulmonary ultrastructure changes of different groups in the present study, obvious lung damage resulting from H_2S were present in those rats subjected to normobaric oxygen therapy with oxygen concentrations of 33% and 50%. In contrast in the HBO group, alveolar epithelial cells were relatively normal, even though nuclear vacuolization was still remained.

Fu-Ming Ye et al.[24] found that extensive lung injuries involving pulmonary vessels, alveolar epithelium, bronchial epithelium and pulmonary interstitium occurred 15 days after exposure to 100 ppm H_2S for 3 h in rats. The basic pathologic changes were: (1) pulmonary hemorrhage and edema induced by extensive pulmonary vascular injury; (2) varying degrees of degeneration of alveolar and bronchial epithelium; (3) interstitial inflammation and hyperplasia of connective tissue. The extent of these lesions varied over time after exposure. Initially, the pulmonary edema developed in pulmonary interstitium, and edema fluid was located around the vasculature. Then it progressively extended to the other parts of the interstitium, and the scope of the pulmonary edema expanded. Mitochondrial swelling and expansion of the endoplasmic reticulum was seen in the alveolar epithelium, endothelium, macrophages, and fibroblasts. In addition, patchy shedding of whole epithelial cells were observed 3, 6 and 24 h after exposure. More obvious damage was noted 2, 3 and 7 days after poisoning. Fifteen days after exposure, type II alveolar epithelial cells and fibroblast cells increased, collagen proliferated and alveolar wall thickening occurred. An animal experiment conducted by Brenneman et al.[21] proved that acute exposure to >80 ppm H_2S can result in reversible injury of respiratory and olfactory mucosa. Another case reported by Duong et al.[25] described a patient who suffered from pulmonary interstitial fibrosis 4 years after H_2S poisoning.

Conclusions

In summary, this comparative study of varied oxygen therapy in rats reveals that HBO treatment is superior to normobaric oxygen treatment in the early stage of H_2S poisoning. Studies at the subcellular level have confirmed that HBO therapy can significantly alleviate myocardial injury, and slightly reduce the degree of lung injury. Nevertheless, the lung CCO activity will remain at low level when only a single oxygen therapy is performed. Among all the indicators, cTnI is a more sensitive parameter of treatment efficacy and has specificity and high sensitivity. Normobaric oxygen treatment administered at 33% and 50% used in this study simulates the oxygen treatment modes of nasal oxygen catheters and high-flow oxygen masks, respectively. Based on these data, if HBO treatment is not available, then high-flow oxygen therapy should be given for H_2S poisoning. Further studies are needed to confirm the efficacy of HBO in humans.

Acknowledgments

The authors thank Qing-Sheng Lu and Jian-Zhong Liu from Beijing Centers for Disease Control and Prevention and Ye Han from SEM Room of Peking Union Medical University; they also thank Yu-Jing Xia and Zhou Shuo from Department of Occupational Diseases and Clinical Toxicology of Beijing Chao-Yang Hospital for expert assistance with these studies.

Declaration of interest

The study was funded in part by social welfare projects (No.2008020) of the Ministry of Science and Technology of P.R. China. Wu N and Du XQ play an equally important role in this experiment.

References

1. Dorman DC, Moulin FJ, McManus BE, Mahle KC, James RA, Struve MF. Cytochrome oxidase inhibition induced by acute hydrogen sulfide inhalation: correlation with tissue sulfide concentrations in the rat brain, liver, lung, and nasal epithelium. Toxicol Sci 2002; 65:18–25.
2. Yue MX, Xu BX, Li T, Dong ZJ, Cao J. The characteristics and hydrogen sulfide inhalation injury and emergency treatment. Chinese J Emerg Med 2005; 14:175.
3. Beauchamp RO, Bus JS, Popp JA, Borieko CJ, Andjelkovich DA. A critical review of the literature on hydrogen sulfide toxicity. CRC Crit Rev Toxicol 1984; 13:25–97.
4. Nicholson RA, Roth SH, Zhang AG, Zheng J. Inhibition of respiratory and bioenergetic mechanisms by hydrogen sulfide in mammalian brain. J Toxicol Environ Health: Part A 1998; 54:491–507.
5. Truong D, Eghbal M, Hindmarsh W, Roth SH, O'Brien P. Molecular mechanisms of hydrogen sulfide toxicity. Drug Metab Rev 2006; 38:733–744.
6. Eghbal MA, Pennefather PS, O'Brien PJ. H_2S cytotoxicity mechanism involves reactive oxygen species formation and mitochondrial depolarization. Toxicology 2004; 203:69–76.
7. Thompson RW, Valentine HL, Valentine WM. Cytotoxic mechanisms of hydrosulfide anion and cyanide anion in primary rat hepatocyte cultures. Toxicology 2003; 188:149–159.
8. Gerasimon G, Bennett S, Musser J, Rinard J. Acute hydrogen sulfide poisoning in a dairy farmer. Clin Toxicol 2007; 45: 420–423.
9. Whitcraft DD, Bailey TD, Hart GB. Hydrogen sulfide poisoning treated with hyperbaric oxygen. J Emerg Med 1985; 3:23–25.
10. Belley R, Bernard N, Côté M, Paquet F, Poitras J. Hyperbaric oxygen therapy in the management of two cases of hydrogen sulfide toxicity from liquid manure. CJEM 2005; 7:257–261.

11. Snyder J, Safir E, Summerville G. Occupational fatality and persistent neurologic sequelae after mass exposure to hydrogen sulfide. Am J Emerg Med 1995; 13:199–203.
12. Gunn B, Wong R. Noxious gas exposure in the outback: two cases of hydrogen sulfide toxicity. Emerg Med 2001; 13:240–246.
13. Lindenmann J, Matzi V, Anegg U, Neuboeck N, Porubsky C, Fell B, Raber T, Ratzenhofer-Komenda B, Renner H, Klemen H, Greilberger J, Haas J, Maier A, Smolle-Juettner F. Hyperbaric oxygen in the treatment of hydrogen sulphide intoxication. Acta Anaesthesiol Scand 2010;54:784–785.
14. Moal EL, Giuliani I, Bertinchant JP, Polge A, Larue C, Saussine SV. Earlier detection of myocardial infarction by an improved cardiac TnI assay. Clin Biochem 2007; 40:1065–1073.
15. Bar-Or D, Thomas GW, Bar-Or R, Rael L, Winkler JV. Diagnostic potential of phosphorylated cardiac troponin I as a sensitive, cardiac-specific marker for early acute coronary syndrome: preliminary report. Clin Chim Acta 2005; 362:65–70.
16. Wang JQ, Sun KY, Cheng DS, Han B, Cai ZL. Effect of early HBO therapy on serum cTnI level and prognosis in acute hydrogen sulfide poisoning. Chinese J Crit Care Med 2007; 27:1115–1116.
17. Horvat S, Beyer C, Arnold S. Effect of hypoxia on the transcription pattern of subunit isoforms and the kinetics of cytochrome c oxidase in cortical astrocytes and cerebellar neurons. J Neurochem 2006; 99:937–951.
18. Khan AA, Coppock RW, Schuler MM, Prior MG. Biochemical effects of subchronic repeated exposures to low and moderate concentrations of hydrogen sulfide in Fischer 344 rats. Inhal Toxicol 1998; 11:1037–1044.
19. Zhao LM, Gao CJ, Wang GZ, Ge H, Chen ZB, Wang M. The impact of hyperbaric oxygen on myocardial ultrastructural changes in acute carbon monoxide poisoning in rats. Chin J Naut Med Hyperbar Med 2004; 3:1–4.
20. Christia-Lotter A, Bartoli C, Piercecchi-Marti MD, Demory D, Pelissier-Alicot AL, Sanvoisin A, Leonetti G. Fatal occupational inhalation of hydrogen sulfide. Forensic Sci Int 2007; 169:206–209.
21. Brenneman KA, James RA, Gross EA, Dorman DC. Olfactory neuronal loss following subchronic inhalation exposure to low levels of hydrogen sulfide in adult male CD rats. Toxicol Pathol 2000; 28:326–333.
22. Brenneman KA, Meleason DF, Marshall MW, James RA, Gross EA, Martin JT, Dorman DC. Olfactory mucosal necrosis in male CD rats following acute inhalation exposure to hydrogen sulfide: reversibility and the possible role of regional metabolic capacity in lesion distribution. Toxicol Pathol 2002; 30:200–208.
23. Tanaka S, Fujimoto S, Tamagaki Y. Bronchial injury and pulmonary edema caused by hydrogen sulfide poisoning. Am J Emerg Med 1999; 17:427–429.
24. Ye FM, Chen YS, Ou-Yang ZQ, Wang GC, Yu ZP. Morphologic study of lung in hydrogen sulfide-exposed rats. Acta Academiae Medicinae Militaris Tertiae 1992; 14:343–346.
25. Duong TX, Suruda AJ, Maier LA. Interstitial fibrosis following hydrogen sulfide exposure. Am J Ind Med 2001; 40:221–224.