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Effects of Long-term Exposure to Hydrogen Sulfide on Human Red Blood Cells

A Saeedi, A Najibi, A Mohammadi-Bardbori

Abstract

Background: Hydrogen sulfide (H_2S) exhibits both physiological and toxicological roles in the biological systems. Acute exposure to high levels of H_2S is life threatening while long-term exposure to ambient levels of H_2S elicits human health effects.

Objective: To study the harmful effects of long-term exposure to low levels of H_2S on human blood cells.

Methods: 110 adult workers from Iran who were occupationally exposed to 0–90 ppb H_2S for 1–30 years were studied. The participants aged between 18 and 60 years and were exposed directly or indirectly to sulfur compounds (exposed group). The origin of H_2S was natural gas processing plants. A control group consisting of 110 males who were not in contact with H_2S was also studied. For all participants, hematological profile including total hemoglobin and red blood cell count and sulfhemoglobin, methemoglobin levels were measured.

Results: Among all parameters evaluated in this study the mean methemoglobin and sulfhemoglobin levels were significantly higher among workers who were exposed to sulfur compounds than the control group. Major differences throughout the study period for sulfhemoglobinemia among exposed groups were observed.

Conclusion: Long-term exposure to even low levels of H_2S in workplaces may have potential harmful effects on human health.

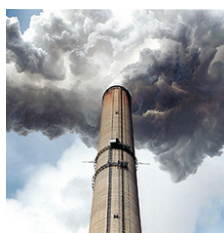
Keywords: Hydrogen sulfide; Sulfhemoglobin; Methemoglobin; Hemoglobins; Erythrocyte count

Introduction

Sweetening of sour gas is a procedure that takes place in natural gas processing plants where hydrogen sulfide (H_2S) is removed from the natural gas.¹ The level of H_2S in the sour gas varies from low concentrations to 85% and even more. Harmful effects of H_2S on the human health have so far been described in

the literature. Acute exposure to high levels of H_2S is life threatening.²⁻⁵ Accumulating data indicated that acute exposure to high levels of H_2S elicit several organ toxicity leading to death including neurotoxicity,⁶ acute respiratory failure,⁷ and even death.^{8,9} Acute exposure to concentrations below 50 ppm can cause eye and respiratory irritation; exposure to concentrations higher than 500 ppm is lethal.¹⁰ Despite

Department of
Pharmacology and
Toxicology, School
of Pharmacy, Shiraz
University of Medical
Sciences, Shiraz, Iran



Correspondence to
Afshin Mohammadi-
Bardbori, PhD
School of Pharmacy,
Shiraz University of
Medical Sciences, Shi-
raz 71345-1583, Iran
E-mail: toxicology@
sums.ac.ir

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the fact that endogenous H₂S plays an important role in the central nervous system, cardiovascular and immune systems,¹¹⁻¹³ long-term exposure to the ambient levels of H₂S can cause ophthalmic lesions,¹⁴ and malignant disorders,¹⁵ among other health effects. Like hydrogen cyanide (HCN) poisoning, H₂S toxicity is due to the inhibition of mitochondrial cytochrome C oxidase.¹⁶⁻¹⁸ H₂S also interferes with the normal function of some enzymes and proteins. It seems that H₂S-induced methemoglobinemia is the potential mechanisms that rescue human and animals from the toxic effects of sulfides.¹⁹⁻²¹

There are scarce information on chronic effect of long-term exposure to ambient levels of H₂S on hematologic profile. We therefore conducted this study to assess changes in hematological parameters including total hemoglobin level and red blood cell count, as well as sulfhemoglobin and methemoglobin levels among a group of workers who were exposed to ambient levels of H₂S in natural gas processing plants.

Materials and Methods

Participants

Using a cross-sectional design, we studied 110 Iranian male workers aged between 18 and 60 years who were occupationally exposed to 0–90 ppb H₂S for 1–30 years. They were exposed directly or indirectly to sulfur compounds (exposed group). The origin of H₂S was natural gas processing plants. The exposed group workers were selected from sulfur recovery unit (n=20), steam generation and distribution unit (n=10), fuel gas system unit (n=10), caustic soda unit (desulfurization) (n=20), amine treating unit (n=20), gas plant unit (n=20), and other departments (n=10). An age-matched control group consisting of 110 males who were not in contact with H₂S

was also studied. They were selected from driving department (n=20), telephone department (n=10), sentry department (n=10), operating department (n=10), administrative department (n=25), and a group of medical students (n=35).

This study was approved by the Ethics Committee of Shiraz University of Medical Sciences. Before the experiment, participants were informed of the objectives of the study and gave consents to participate in the study.

Blood Parameters Analysis

Two mL of venous blood was taken from each participant and collected in an EDTA glass tube. The concentrations of methemoglobin, sulfhemoglobin, total hemoglobin and red blood cell count were measured according to previously described methods.^{22,23} Briefly, blood samples was divided into three aliquots and kept on ice during the experiment. One aliquot was analyzed by an automated hematology analyzer (KX-21N™) to measure red cell count and total hemoglobin. The second aliquot was used to measure methemoglobin. Meth-

TAKE-HOME MESSAGE

- Sweetening of sour gas is a procedure that takes place in natural gas processing plants where hydrogen sulfide (H₂S) is removed from the natural gas.
- Acute exposure to high levels of H₂S is life-threatening.
- Acute exposure to high levels of H₂S elicit several organ toxicity leading to death including neurotoxicity, acute respiratory failure, and eye and respiratory irritation.
- Chronic exposure to ambient levels of H₂S in natural gas processing plants induces methemoglobinemia and sulfhemoglobinemia in workers.
- The length of chronic exposure to ambient level of H₂S did not have any significant effect on the methemoglobin level in the exposed group.

Table 1: Parameters measured in the study. Values are mean (SD).

Parameter	Exposed group (n=110)	Control group (n=110)	p value
Methemoglobinemia (%)	3.07 (1.16)	0.92 (0.26)	<0.001
Sulfhemoglobinemia (%)	0.04 (0.01)	0.05 (0.01)	<0.001
Total hemoglobin (g/dL)	15.4 (1.1)	15.6 (0.8)	0.125
Red cell count (10 ⁶ /μL)	5.23 (0.36)	5.21 (0.35)	0.677

moglobin concentration was determined in hemolysates in 37 mM phosphate buffer at pH 7.3 at 560, 576 and 630 nm wavelength according to previously described method.²² The last aliquot was used to measure sulfhemoglobin level according to the Bagarinao and Vetter method.²³

Statistical Analysis

All results are expressed as mean (SD). The two studied groups were compared with two-tailed *Student's t* tests. A p value <0.05 was considered statistically significant.

Results

Exposed workers had a significantly (p<0.001) higher mean methemoglobin concentration than the control group (Table 1). The length of exposure did not have any significant effect on the methemoglobin level in the exposed group (Fig 1). The mean sulfhemoglobin concentration was significantly (p<0.001) lower in the exposed group compared to the control (Table 1). Those who were exposed between 11 and 15 months had a significantly (p<0.001) lower sulfhemoglobin level than others (Fig 1). The studied groups had no significant differences in terms of hemoglobin level and red cell counts (Table 1).

Discussion

We found that chronic exposure to ambient levels of H₂S in natural gas processing plants induces methemoglobinemia and sulfhemoglobinemia in workers. The exposure, however, does not have any significant effects on total hemoglobin and red cell counts.

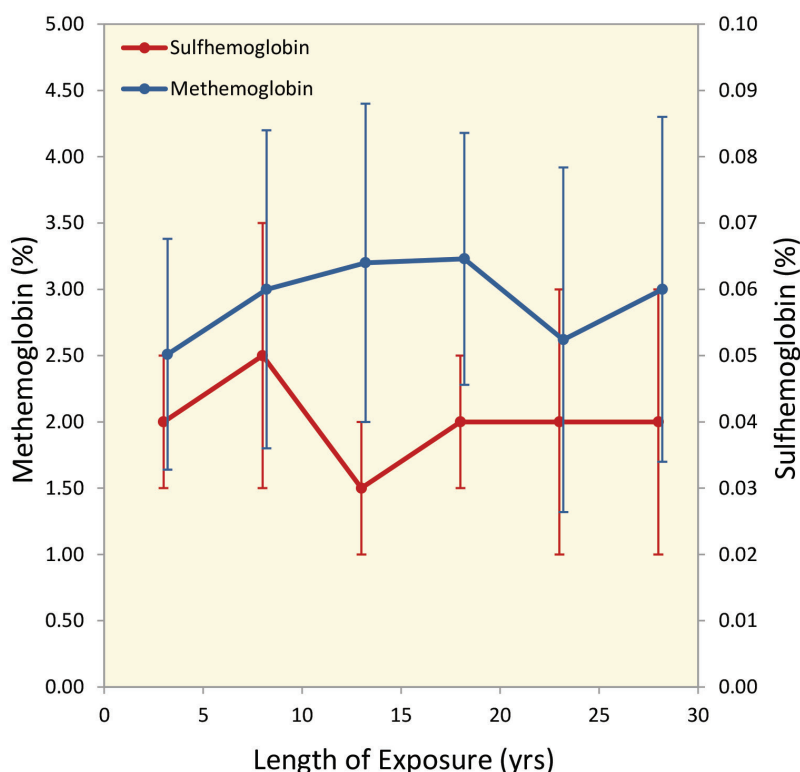


Figure 1: Mean methemoglobin and sulfhemoglobin concentrations in the study group with various lengths of exposure. Error bars represent SD. Note the graph has two vertical axes.

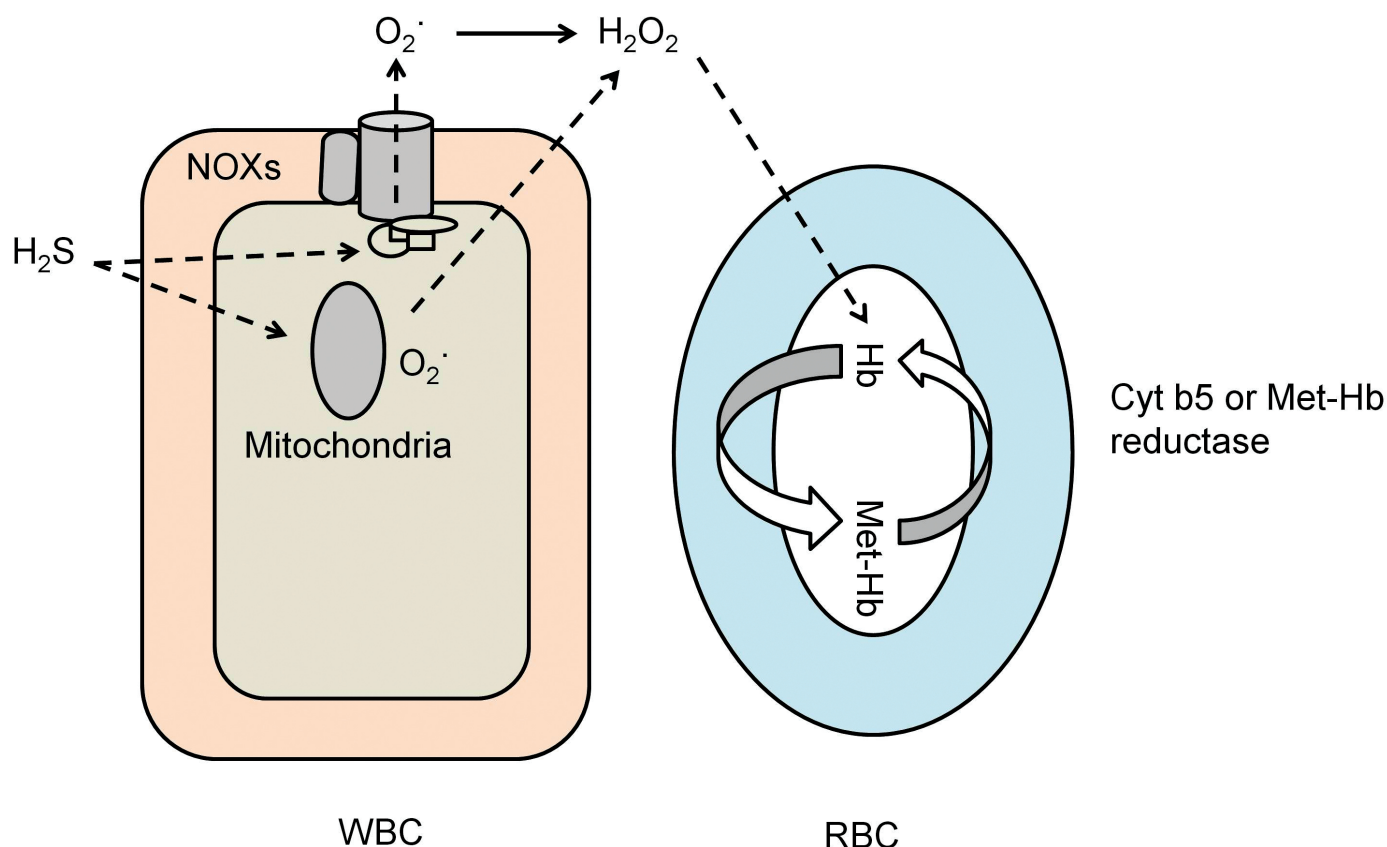


Figure 2: Direct and indirect methemoglobin (Met-Hb) formation after H_2S exposure. Activation of the NADPH oxidase (NOXs) systems or inhibition of mitochondrial respiration can result in H_2O_2 formation in white blood cells. The diffusible H_2O_2 can cross the red cell membrane and induce methemoglobinemia.

Human has historically been exposed to the naturally emitted H_2S mostly from the geothermal and volcanic eruptions, from the industrial processes and concentrated animal farming operations. In addition, H_2S is endogenously produced in the body at very low concentrations and suggested to be an important signaling molecule in the physiological pathways.¹² H_2S is absorbed rapidly by inhalation and distributed throughout the body and metabolized via oxidation, methylation and reaction with metalloproteins.²⁴

We think that the observed increase in methemoglobin concentration might be a developmental adaptation mechanism that would protect human beings and animals from the lethal toxicity of sulfur and

similar compounds.¹⁹⁻²¹ H_2S interacts with a number of enzymes and macromolecules such as hemoglobin, myoglobin and cytochrome C oxidase.^{18,25} It seems that interaction of H_2S with hemoglobin is a way for its elimination from the circulation and protects mitochondria against H_2S toxicity.²⁶ It is well known that inhibition of cytochrome C oxidase can lead to formation of reactive oxygen species (ROS)²⁷ and exposure to high levels of H_2S can cause oxidative stress.²⁸ We believe that the methemoglobin formation after H_2S exposure is either through oxidative stress or a result of interaction between H_2S and hemoglobin (Fig 2). Activation of the NADPH oxidase systems or inhibition of mitochondrial respiration²⁸ leads to formation of a

lot of H₂O₂. The diffusible H₂O₂ produced by white blood cells can then cross the red cell membrane and induce methemoglobinemia (Fig 2).

Use of methemoglobin as an antidote to hydrogen-cyanide poisoning has been suggested since long ago;^{26,29} methemoglobinemia protects cytochrome C oxidase from H₂S, hydrogen-cyanide and azide toxicity.^{30,31}

Despite a high level of methemoglobin and sulfhemoglobin in those chronically exposed to H₂S at workplace, there were no significant differences between the two studied groups in terms of other parameters measured. This observation was similar to that reported earlier.³² Formation of methemoglobin and sulfhemoglobin is irreversible. Sulfhemoglobinemia and methemoglobinemia due to the air pollution have been documented.³³ It has also been reported that ingestion of inorganic sulfate in drinking water can induce sulfhemoglobinemia and methemoglobinemia without alteration in the total hemoglobin concentration.³⁴

In conclusion, it seems that long-term exposure to even acceptable concentrations of H₂S at workplace is a potential risk for human health. Therefore, re-consideration in workplace exposure limits for H₂S is warranted.

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Conflicts of Interest: None declared

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