CRITICAL CARE

COMMENTARY

Endogenous H_2S in hemorrhagic shock: innocent bystander or central player?

Enrico Calzia*[1], Peter Radermacher[1] and Kenneth R Olson[2]

See related research by Van de Louw and Haouzi, http://ccforum.com/content/16/5/R178

Abstract

The role of the gaseous mediator hydrogen sulfide (H_2S) in hemorrhagic shock is still a matter of debate. This debate is emphasized by the fact that available literature data on blood and tissue H_2S concentrations vary by three orders of magnitude, both under physiological conditions as well as during stress states. Therefore, in a rat model of unresuscitated, lethal hemorrhagic shock, Van de Louw and Haouzi tested the two hypotheses of whether blood and tissue H_2S levels would increase due to the shock-related tissue hypoxia, and whether vitamin B12 would attenuate organ injury and improve survival as a result of enhanced H_2S oxidation. Hemorrhage did not affect the blood and tissue H_2S content, and, despite the increased capacity to oxidize H_2S, vitamin B12 did not affect any parameter of shock severity. The authors concluded that H_2S concentrations cannot be used as a marker of shock, most probably as a result of tissue's capacity to oxidize H_2S even under conditions of severe oxygen debt. This research paper elegantly re-adjusts the currently available data on blood and tissue H_2S levels, and thereby adds an important piece to the puzzle of whether H_2S release should be enhanced or lowered during stress conditions associated with tissue hypoxia.

In a recent issue of *Critical Care*, Van de Louw and Haouzi report on the effects of lethal hemorrhage on blood and tissue levels of hydrogen sulfide (H_2S) [1]. The role of H_2S during hemorrhage is a matter of debate: while both inhaled H_2S and intravenous sodium sulfide and sodium hydrosulfide improved survival [2-4], other authors reported that sodium sulfide did not exert any beneficial effects [5]. Moreover, blocking H_2S biosynthesis by inhibiting cystathione-γ-lyase attenuated circulatory failure and organ injury [6,7]. Since hypoxic conditions decrease [8,9] and supplemental vitamin B12 (hydroxocobalamin) increases (due to the rise in oxidative capacity) the rate of H_2S metabolism, the authors hypothesized that hemorrhage would increase plasma and tissue H_2S levels, and that vitamin B12 would improve survival. Rats were hemorrhaged by five times withdrawal of 5 ml/kg blood (that is, approximately 30% of the calculated blood volume). The total H_2S content was measured in the first and last blood samples, using the methylene blue assay [10]. Indirect calorimetry for oxygen uptake and carbon dioxide production before and at the end of the hemorrhage period allowed determination of the shock-induced oxygen deficit. The major finding was that, despite a severe cumulative oxygen debt (100 to 140 ml/kg), H_2S blood and tissue concentrations did not change, rendering them useless as markers of shock severity. In line with this finding, vitamin B12 failed to exert any therapeutic effects despite an increased capacity to oxidize H_2S.

What do we learn from this study? According to the authors' standard curve for the methylene blue assay, the plasma light absorbance peak at 670 nm would correspond to ~8 μM H_2S [1]. This absorbance, however, was due to turbidity rather than the presence of the blue dye. The true H_2S concentrations were most probably therefore much lower, possibly even below the detection limit of 1.5 μM.

There is considerable discrepancy in the literature on blood H_2S concentrations. In rats, baseline values of 25 to 50 μM have been reported, which increased up to 80 μM after hemorrhage, endotoxin exposure and injection of sulfide donors [6,11,12]. However, bolus (4 mg/kg) or continuous intravenous (20 mg/kg/hour) sodium sulfide only increased blood H_2S levels from 0.4 to 0.9 μM to 4.0 to 4.5 μM when the monobromobamine assay [10] was used to determine H_2S concentrations [13]. In mice, 10 mg/kg endotoxin either decreased (from ~2.3 to

*Correspondence: enrico.calzia@uni-ulm.de
[1]Sektion Anästhesiologische Pathophysiologie und Verfahrensentwicklung, Klinik für Anästhesiologie, Universitätsklinikum Ulm, Helmholtzstrasse 8-1, D-89081 Ulm, Germany
Full list of author information is available at the end of the article

~1.8 μM [14]) or increased (from ~34 to ~65 μM [15]) the blood sulfide content. Finally, inhaling up to 200 ppm gaseous H_2S in mice increased the sulfide content by <1.5 μM [13,16,17].

According to the available literature, the blood H_2S content may vary by three orders of magnitude – so which H_2S concentrations are real? At physiological pH, dissolved H_2S gas represents 20 to 50% of the total sulfide [9,10,17], which can of course escape into the headspace [9,10]. Van de Louw and Haouzi carefully avoided any H_2S loss related to volatilization. Furthermore, blood-borne H_2S is rapidly bound and/or metabolized: using a polarographic sensor with a detection limit for H_2S gas corresponding to 100 nM total sulfide in blood at pH 7.4, a 10 μM sodium sulfide spike only transiently increased sulfide from undetectable levels to about 0.5 μM [18]. Finally, the odor threshold of H_2S is 0.01 to 0.3 ppm [9,10], and simply smelling the blood allows one to verify that plasma H_2S concentrations are at, or below, 1 μM: in a phosphate buffer, the human nose can detect as little as 1 μM H_2S [9,19]. The gas/water coefficient of distribution for H_2S is 0.39 [9,10]. Assuming that only 20% of the dissolved gas (that is, 4 to 10% of the total free sulfide) disappears from the blood sample due to volatilization during the 2 or 3 seconds of sniffing [10], a 10 ml blood sample would have a total free sulfide concentration of 20 to 50 μM. This is within the range reported for rat blood, but clearly rat blood does not smell like rotten eggs!

Based on the results of their study, the authors conclude that 'H_2S in the blood cannot be used as a marker of hemorrhagic shock', because any H_2S accumulation resulting from 'tissue hypoxia must be reconciled with the ability of tissues to oxidize H_2S' [1]. Clearly, H_2S consumption via the sulfide quinone reductase system [20] is reduced under hypoxic conditions, which in turn would cause H_2S accumulation: in lung tissue homogenates and pulmonary artery smooth muscle cells [8,9], H_2S consumption dropped at oxygen concentrations of 10 μM (that is, ~8 mmHg) – a 50% drop occurring at oxygen partial pressure values of ~4 mmHg. In the present study, at the end of the hemorrhage the arterial oxygen partial pressure was still normal and, despite a 50% reduction of minute ventilation, the arterial carbon dioxide partial pressure was reduced to 31 mmHg. The hypoxia was probably therefore not sufficiently low to significantly impair H_2S oxidization. Unfortunately, the authors only reported lactatemia, and not pH, so the severity of lactic acidosis cannot be definitively estimated. Finally, core temperature was maintained at 35 to 36°C. Mild hypothermia of 34°C is well established to attenuate lactic acidosis and better preserve tissue oxygenation. Consequently, albeit ultimately lethal, the authors' model might have been unable to detect the hypoxia threshold necessary to cause H_2S accumulation. In this context, it is tempting to speculate whether any local H_2S accumulation might decrease energy expenditure and thereby oxygen consumption due to inhibition of cytochrome c oxidase, which in turn would restore the oxygen partial pressure and thereby at least partially resume H_2S oxidation. Furthermore, it is noteworthy that H_2S can even sustain ATP synthesis during mild hypoxia [21], and thus presumably attenuate tissue lactic acidosis.

Van de Louw and Haouzi meticulously conducted a study in a "hot" field. The authors have the merit of re-adjusting the data available on blood H_2S concentrations. Moreover, lacking any observable increase in H_2S levels during hemorrhage coupled with the currently available knowledge on the role of cystathione-γ-lyase for oxygen sensing and vascular homeostasis [8,9,22], blocking endogenous H_2S production most probably has little therapeutic benefit and may actually prove to be contra-indicated. Additional studies are mandatory on the exogenous H_2S supplementation during hemorrhagic shock, in order to answer the question of whether H_2S is an innocent bystander or a central player under these conditions.

Abbreviations

H_2S, hydrogen sulfide.

Competing interests

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Author details

[1]Sektion Anästhesiologische Pathophysiologie und Verfahrensentwicklung, Klinik für Anästhesiologie, Universitätsklinikum Ulm, Helmholtzstrasse 8-1, D-89081 Ulm, Germany. [2]Department of Physiology, Indiana University School of Medicine, 1234 Notre Dame Avenue, South Bend, IN 46617, USA.

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