The origin of hydrogen sulfide in a newborn with sulfaemoglobin induced cyanosis

A Tangerman, G Bongaerts, R Agbeko, B Semmekrot, R Severijnen

This report investigated the origin of H$_2$S in a newborn boy with sulfaemoglobin induced cyanosis, who died because of multiple organ failure. Frozen material was collected and studied after death. The results suggest that enzymes had been released from deteriorating organs into the blood and abdominal fluid, and that the reaction of one of these enzymes with sulfur containing amino acids might have resulted in increased H$_2$S concentrations. It is hypothesised that this release of enzymes resulted from a haemolysin produced by an invasive haemolytic Escherichia coli that was found in the blood and organs of this patient.

In a previous paper we presented a 3 day old boy with sulfaemoglobin induced cyanosis, with emphasis on the clinical aspects of the case. Sulfaemoglobin is a green pigmented haemoglobin with a H$_2$S derived sulfur on the haem iron. In this paper we focus on the production and on the origin of H$_2$S. Therefore, we collected and studied frozen material from this newborn boy after death.

PATIENT’S HISTORY

Because of a progressive sulfaemoglobin induced cyanosis the boy was admitted to hospital on suspicion of cardiac disease. Important clinical data were: meconium ileus, no passage of stools during the first 3 days of life, deep cyanosis and distended abdomen on admission, foul breath and reeking dark urine, anaemia, and isolation of an invasive haemolytic Escherichia coli from blood and all organs tested. The blood was dark brown coloured until the patient was adequately transfused with packed red blood cells. In addition, the patient’s serum had haemolytic activity. On the second day after admission (the 5th day of life) the boy died from severe septic shock and multiple organ failure. Necropsy showed extensive haemorrhages in nearly all organs and interstitia. Cytogenetic analysis of the patient’s tissue was consistent with the diagnosis of cystic fibrosis (mutation δF508).

PATIENT’S SULFAEMOglobinaemia

The cyanosis resulted from the presence of sulfaemoglobin. Just before death, sulfaemoglobin (normally absent) was 1.5% of the total haemoglobin, but this was measured after numerous transfusions of packed cells. Without transfusions this proportion would probably have been much higher.

MATERIALS AND METHODS

Blood, urine, abdominal fluid, and organs (lungs, liver, and kidney) had been stored at $-20^\circ$C or $-70^\circ$C. Quantitative analysis of protein persulfide in the body fluids and tissues was performed by head space gas chromatography, essentially as described for methanethiol. In a closed evacuated 15 ml glass vial, 200 µl of dithiothreitol (DTT; 5mM) in 0.1M Tris (pH 7.4) was added to 25–100 µl of serum, abdominal fluid, or tissue homogenate. After vortexing for 10 seconds, the mixture was left at room temperature for four minutes and the released H$_2$S was sampled and assayed by gas chromatography, as described previously. To check for a continuous slow release of H$_2$S originating from DTT, the reaction was performed with 60mM instead of 5mM DTT, and the sampling procedure was repeated at several time intervals, extending to two hours after the start of the reaction.

RESULTS

Release of H$_2$S

The addition of 5mM DTT to the patient’s serum or abdominal fluid caused a finite fast release of a slightly increased amount of H$_2$S from protein bound sulfane sulfur or persulfides (in both cases 3.1 µM; reference values, 0.0–0.7 µM). The addition

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Hydrogen sulfide released in the presence of the patient’s serum, abdominal fluid (µM), and several tissues (µmol/kg), determined using 5mM DTT (mainly H$_2$S from protein bound persulfide) and 60mM DTT (H$_2$S from protein bound persulfide and from DTT itself). For comparison, a normal control serum was also included</th>
</tr>
</thead>
<tbody>
<tr>
<td>Material</td>
<td>Hydrogen sulfide released in the presence of 5mM DTT (After 4 minutes, Increase after 2 hours)</td>
</tr>
<tr>
<td>Serum</td>
<td>3.1</td>
</tr>
<tr>
<td>Abdominal fluid</td>
<td>3.1</td>
</tr>
<tr>
<td>Lung (left)</td>
<td>3.4</td>
</tr>
<tr>
<td>Lung (right)</td>
<td>14.4</td>
</tr>
<tr>
<td>Liver</td>
<td>59</td>
</tr>
<tr>
<td>Kidneys</td>
<td>38</td>
</tr>
<tr>
<td>Control serum</td>
<td>0.6</td>
</tr>
</tbody>
</table>

The control serum was also frozen for a long period, as was the case for the patient’s material. *No exact value could be obtained because of a high blank reading.

DTT, dithiothreitol.
of 60mM DTT to the patient’s serum and abdominal fluid caused an additional continuous, although slow, release of extremely large amounts of H$_2$S (table 1), which was not seen in the serum of healthy persons, as shown in table 1 for one control serum. Similar releases were also seen using homogenates of the patient’s liver, lungs, and kidneys (table 1).

**Origin of H$_2$S**

Because DTT is a specific reagent for the quantitative reduction of disulfide bonds, the initial reaction between serum or abdominal fluid and DTT, resulting in the release of H$_2$S, suggested an increased amount of protein bound sulfane sulfur or persulfide. However, in contrast to the finite fast release of methanethiol from methanethiol mixed disulfides normally seen on reaction with 60mM DTT, we saw a finite fast release of H$_2$S from protein bound sulfane sulfur or persulfide in addition to a continuous slow release of large amounts of H$_2$S when the patient’s serum was allowed to react with 60mM DTT. A measurable slow release was not seen during the reaction of either control serum of a healthy person with 60mM DTT, or the patient’s serum with 5mM DTT.

The similar continuous slow release of large amounts of H$_2$S was also seen in the reaction of 60mM DTT with tissue homogenates of this patient (table 1). In animal experiments such a continuous slow release was also seen with tissue homogenates of liver, kidney, and brain of normal rats (A Tangerman, personal communication, 2001). Although we did not study H$_2$S release from tissue homogenates in human controls, these would probably show a similar continuous slow release (as seen in tissue homogenates from control rats and our patient) after the addition of 60mM DTT.

**DISCUSSION**

The results strongly suggest that slow enzymatic H$_2$S releasing activity (originating from 60mM DTT) was present inside the studied organs, which was released upon homogenisation. In human control serum no continuous slow enzymatic H$_2$S releasing activity was seen because in healthy persons the organs had not deteriorated. In our patient enzymes from deteriorating organs were released into the bloodstream, which was shown in table 1 for one control serum. Similar releases were also seen using homogenates of the patient’s liver, lungs, and kidneys (table 1).

In our patient, *E coli* septic shock was complicated by extreme sulfhaemoglobinemia. The sulfhaemoglobin possibly aggravated the Gram negative shock by limiting the oxygen transport capacity. The raised concentrations of H$_2$S probably also contributed in several ways to organ malfunctioning and the fatal outcome:

- In this patient, it appears that enzymes were released from deteriorating organs into the bloodstream and abdominal fluid, and that the reaction of one of these enzymes with sulfur containing amino acids might have resulted in increased H$_2$S concentrations.
- It is thought that these enzymes were released as a result of a haemolysin produced by an invasive haemolytic *Escherichia coli* that was found in the blood and organs of this patient.

Transports and consequently reduce the respiratory oxygen consumption.

Remarkable in this newborn were the presence of (1) an invasive haemolytic *E coli* in organs and blood, (2) haemolysis, and (3) extensive haemorrhages in nearly all organs and interstitia. This strongly suggests an extra-intestinal role of the *E coli* haemolysin, which also plays a role in upper urinary tract infections. Haemolysins are cytolytic toxins that damage the bilayer structure of cellular and mitochondrial membranes, and exhibit little target specificity. Their presence can be recognised by cytolytic activity towards red blood cells. The *E coli* haemolysin has a wide spectrum of cytocidal activity, attacking at least erythrocytes, granulocytes, monocytes, endothelial cells, and renal cells of mice, ruminants, and primates. The *E coli* haemolysin is one of a close family of membrane targeted toxins assumed or known to contribute to haemorrhagic intestinal disease, juvenile periodontitis, pneumonia, and whooping cough. This family includes enterohaemorrhagic *O157 E coli* haemolysin, the haemolysins of *Proteus vulgaris* and *Morganella morgani*, and the haemolysins and leukotoxins of *Actinobacillus* spp.

The haemolysin in our patient was probably produced during the growth of the invasive *E coli*, that is, initially in the obstructed intestinal tract and later in blood and tissues. The cytolytic activity of haemolysin might be responsible for the release of enzymes from tissue or blood cells, which may release H$_2$S from sulfur containing amino acids. The extremely toxic H$_2$S may have contributed to the fatal outcome in the Gram negative septic shock by aggravating tissue acidification, as a result of reduced oxygen transport. The role of the (invasive) *E coli* and its haemolysin needs further study.

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**Take home messages**

- In this patient, it appears that enzymes were released from deteriorating organs into the blood and abdominal fluid, and that the reaction of one of these enzymes with sulfur containing amino acids might have resulted in increased H$_2$S concentrations.
- It is thought that these enzymes were released as a result of a haemolysin produced by an invasive haemolytic *Escherichia coli* that was found in the blood and organs of this patient.

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FOOD FOR THOUGHT

We pathologists, wise men say, are obsessed with food, I, for once, duno if that’s bad or good. When you sit down to a hearty meal, Do you think it’s such a big deal, That a serving of piping hot pea soup Takes you through a typhoidal gut loop? Or, the sight of anchovy sauce makes you shiver, ‘cos it only means—amoebic abscess of the liver!

When soft, creamy pudding studded with sago Reminds one of the spleen you cut not long ago; You switch to plain bread and butter, Not unlike a rheumatic heart that went aflutter. When the aroma of fresh, crunchy popcorn Brings memories of your pal Hodgkin, you know pathology has gotten under your skin. Strawberries and mulberries, worth a lick; The redcurrant jelly gives an ache in the belly, you know the matter ain’t so silly.

Swiss cheese with a dash of nutmeg, buff coloured buns with fried egg, Chicken wire and mutton leg; leave us alone, please, they beg! Let’s be original, rising above the culinary level, Lest we battle indigestion in the bowel, making us moan, groan and cry, and ending up as a “row of tombstones” against a “starry sky”!

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