SHORT REPORT

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

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J Clin Pathol 2002;**55**:631–633

This report investigated the origin of H_2S in a newborn boy with sulfhaemoglobin 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_2S 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 a sulfhaemoglobin induced cyanosis, with emphasis on the clinical aspects of the case.[1] Sulfhaemoglobin is a green pigmented haemoglobin with a H_2S derived sulfur on the haem iron.[2] In this paper we focus on the production and on the origin of H_2S. Therefore, we collected and studied frozen material from this newborn boy after death.

PATIENT'S HISTORY

Because of a progressive sulfhaemoglobin 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.[1] 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 SULFHAEMOGLOBINAEMIA

The cyanosis resulted from the presence of sulfhaemoglobin. Just before death, sulfhaemoglobin (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°C or −70° C. Quantitative analysis of protein persulfide in the body fluids and tissues was performed by head space gas chromatography, essentially as described for methanethiol.[3] 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_2S was sampled and assayed by gas chromatography, as described previously.[3] To check for a continuous slow release of H_2S 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_2S

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_2S from protein bound sulfane sulfur or persulfides (in both cases 3.1µM; reference values, 0.0–0.7µM).[4] The addition

Table 1 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_2S from protein bound persulfide) and 60mM DTT (H_2S from protein bound persulfide and from DTT itself). For comparison, a normal control serum was also included

	Hydrogen sulfide released in the presence of			
	5mM DTT		60mM DTT	
Material	After 4 minutes	Increase after 2 hours	After 4 minutes	Increase after 2 hours
Serum	3.1	0	14	193
Abdominal fluid	3.1	0	3	35
Lung (left)	3.4	0	<15*	123
Lung (right)	14.4	0	<15*	138
Liver	59	0	124	123
Kidneys	38	0	72	132
Control serum	0.6	0	0.7	0

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_2S (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_2S

Because DTT is a specific reagent for the quantitative reduction of disulfide bonds,[5] the initial reaction between serum or abdominal fluid and DTT, resulting in the release of H_2S, suggested an increased amount of protein bound sulfane sulfur or persulfide.[4 6] However, in contrast to the finite fast release of methanethiol from methanethiol mixed disulfides normally seen on reaction with 60mM DTT,[3] we saw a finite fast release of H_2S from protein bound sulfane sulfur or persulfides, in addition to a continuous slow release of large amounts of H_2S 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_2S 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_2S 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_2S 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_2S releasing activity was seen because in healthy persons the organs had not deteriorated. In our patient enzymes from deteriorating organ cells released into the bloodstream were probably responsible for the continuous slow H_2S releasing activity. The abdominal fluid showed the same continuous slow release of activity. The same enzymes might also have released H_2S from sulfur containing amino acids inside our patient.

These results also suggest that the sulfane sulfur or persulfide pools in serum and abdominal fluid were raised in our patient, and that sulfur volatiles, especially H_2S, methanethiol, and dimethylsulfide (the last two being methylated products of H_2S)[7] were mainly responsible for the patient's foul smelling breath and urine.[8]

> "The raised concentrations of H_2S probably contributed in several ways to organ malfunctioning and the fatal outcome"

In our patient, *E coli* septic shock was complicated by extreme sulfhaemoglobinaemia. The sulfhaemoglobin possibly aggravated the Gram negative shock by limiting the oxygen transport capacity. The raised concentrations of H_2S probably also contributed in other ways to organ malfunctioning and the fatal outcome. H_2S is an extremely toxic metabolite,[9] transforming three dimensional protein structures by reacting with cystine disulfide bonds, and reacting with nearly all free and accessible protein bound heavy metals. Consequently, various heavy metal containing proteins (such as haemoglobin, myoglobin, cytochromes, and enzymes) may irreversibly lose their physiological function. Thus, H_2S mediated protein inactivation may prevent adequate electron transport and consequently reduce the respiratory oxygen consumption.

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_2S 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

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.[10 11] Haemolysins are cytolytic toxins that damage the bilayer structure of cellular and mitochondrial membranes,[12] and exhibit little target specificity.[12] 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.[12] 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.[12] This family includes enterohaemorrhagic O157 *E coli* haemolysin, the haemolysins of *Proteus vulgaris* and *Morganella morganii*, and the haemolysins and leukotoxins of *Actinobacillus* spp.[12]

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_2S from sulfur containing amino acids. The extremely toxic H_2S may have contributed to the fatal outcome in the Gram negative septic shock by aggravating tissue acidification,[1] as a result of reduced oxygen transport. The role of the (invasive) *E coli* and its haemolysin needs further study.

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Accepted for publication 14 February 2002

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FOOD FOR THOUGHT

We pathologists, wise men say, are obsessed with food,
I, for once, dunno 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,
Give you a biliary and renal colic;
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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