Hydrogen sulphide-generating pathways in haemodialysis patients: a study on relevant metabolites and transcriptional regulation of genes encoding for key enzymes

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Abstract

Background. Hydrogen sulphide, H2S, is the third endogenous gas with putative cardiovascular properties, after nitric oxide and carbon monoxide. H2S is a vasorelaxant, while H2S deficiency is implicated in the pathogenesis of hypertension and atherosclerosis. Cystathionine β-synthase (CBS), cystathionine γ-lyase (CSE) and 3-mercaptopyruvate sulphurtransferase (MPS) catalyze H2S formation, with different relative efficiencies. Chronic kidney disease (CKD) is characterized by elevation of both plasma homocysteine and cysteine, which are substrates of these enzymes, and by a high prevalence of hypertension and cardiovascular mortality, particularly in the haemodialysis stage. It is possible that the H2S-generating pathways are altered as well in this patient population.

Methods. Plasma H2S levels were measured with a common spectrophotometric method. This method detects various forms of H2S, protein-bound and non-protein-bound. Blood sulphaemoglobin, a marker of chronic exposure to H2S, was also measured, as well as related sulphur amino acids, vitamins and transcriptional levels of relevant genes, in haemodialysis patients and compared to healthy controls.

Results. Applying the above-mentioned methodology, H2S levels were found to be decreased in patients. Sulphaemoglobin levels were significantly lower as well. Plasma homocysteine and cysteine were significantly higher; vitamin B6, a cofactor in H2S biosynthesis, was not different. H2S correlated negatively with cysteine levels. CSE expression was significantly downregulated in haemodialysis patients.

Conclusions. Transcriptional deregulation of genes encoding for H2S-producing enzymes is present in uraemia. Although the specificity of the method employed for H2S detection is low, the finding that H2S is decreased is complemented by the lower sulphaemoglobin levels. Potential implications of this study relate to the pathogenesis of the uraemic syndrome manifestations, such as hypertension and atherosclerosis.

Keywords: cystathionine β-synthase; cystathionine γ-lyase; haemodialysis; homocysteine; hydrogen sulphide

Introduction

The endogenous production of hydrogen sulphide (or sulphidric acid, H2S) has recently gained the interest of the scientific community [1–5]. H2S can be considered as the third gas with modulating actions, along with nitric oxide, NO, and carbon monoxide, CO.

Three enzymes can catalyze H2S formation: cystathionine β-synthase (CBS, EC 4.2.1.22), cystathionine γ-lyase (cystathionase, CSE, EC 4.4.1.1) and 3-mercaptopyruvate sulphurtransferase (MST, EC 2.8.1.2). H2S in the cardiovascular system is mainly produced by CSE, while in the brain CBS plays the most important role [1–6]. MST contributes to a far lesser degree quantitatively, even if it is present in several tissues, including blood [7,8].

CBS catalyzes the formation of cystathionine and water by condensing serine and homocysteine, but it has been also demonstrated that CBS catalyzes the formation of cystathionine and H2S through condensation of cysteine and homocysteine as alternative substrates [9].

CSE catalyzes the conversion of cystathionine to cysteine in the trans-sulphuration pathway. In addition, it catalyzes the formation of H2S in a reaction utilizing cysteine, a spontaneously occurring cysteine oxidation product, producing pyruvate, ammonia and thiocysteine, which in turn decomposes to cysteine and H2S [4]. Pyridoxal 5′-phosphate, a vitamin B6-derived coenzyme, is required for CBS and CSE activities.

MST catalyzes the formation of H2S from 3-mercaptopyruvate, a cysteine metabolite, or it can transfer its sulphur atom to sulphite, which forms thiosulphate. Cysteine formed by CSE can then act as an acceptor of the sulphur transferred from 3-mercaptopyruvate by MST [4].
Aminoguanidine, an inhibitor of NO synthase, considerably decreases CSE activity in the rat brain and leads to a simultaneous increase in MST activity [10].

H$_2$S is also formed non-enzymatically from elemental sulphur, inorganic polysulphides and organic polysulphides, contained for example in garlic, accounting for the latter’s blood pressure-lowering effects [11].

H$_2$S has been shown to reduce blood pressure in rats, and to induce vasodilation in isolated blood vessels [12]. Low H$_2$S generation has been demonstrated in the vasculature of spontaneously hypertensive rats; H$_2$S administration lowers their blood pressure, while chronic administration of a CSE inhibitor induces arterial hypertension [13]. In CSE knock-out mice, H$_2$S was markedly reduced in serum and many tissues; pronounced hypertension and reduced endothelium-dependent vasorelaxation were distinct features [5]. An observational study in coronary heart disease patients, hypertensives and smokers has also shown that plasma H$_2$S is lower compared to normal subjects [14]. Low H$_2$S was found in hypertensive children as well [15].

H$_2$S exerts antiatherosclerotic effects, for example in apolipoprotein E knock-out mice [16]. It has been demonstrated that H$_2$S can protect the endothelium against hydrogen peroxide and oxLDL-mediated endothelial cytotoxicity, and can prevent haemin-mediated oxidative modifications of LDL [17]. H$_2$S is also involved in human corpus cavernosum smooth-muscle relaxation [18], and therefore can be involved in sexual dysfunction.

In the kidney, ischaemia reperfusion reduces CBS-mediated H$_2$S generation, contributing to renal injury, while the administration of an exogenous H$_2$S donor, NaHS, improves renal function [19].

In spite of this evidence, concern has been expressed lately about H$_2$S functions, essentially because H$_2$S detection in blood is fraught with methodological problems [20–23]. Therefore, the normal plasma concentration of H$_2$S is currently debated [20–23]. Wide variations can be found in the literature, due to differences in techniques, animal/human model utilized, etc. It has even been argued that blood H$_2$S concentration is actually in the nanomolar range, instead of the micromolar range, thus ‘tempering the exuberance for this novel signalling system’ [22].

The spectrophotometric method employed in this paper has been found to: ‘measure a form of bound or sequestered H$_2$S rather than “free” H$_2$S’ [21]. That is, this method can measure free H$_2$S, plus its protein-bound and acid-labile moieties [20,21]. However, it has been defined as ‘convenient, relatively robust and at least comparable to other techniques’ [21]. In addition, since H$_2$S adheres to glass, contained for example in plastic tubes. H$_2$S ionization is favoured in solution thus preventing rapid evaporation; however, it is rapidly oxidized in air, and therefore, samples must be prepared at top speed and rapidly complexed with zinc acetate in order to limit loss [24]. In the present paper, the utmost care was given to these precautions.

CKD and uraemia are characterized by a high cardiovascular mortality, and new biomarkers for evaluating cardiovascular risk in CKD patients are being sought [25]. Among these markers, we find thiols, such as homocysteine and cysteine, which are consistently increased in the majority of patients, highest levels being reached in uraemia and in haemodialysis patients [26–30]. These compounds are among the principal substrates of the enzymes involved in H$_2$S formation. Alterations in plasma H$_2$S concentration could be present in these patients.

We measured in a control group, and in a group of haemodialysis patients, plasma H$_2$S concentration and plasma homocysteine and cysteine; the concentration of sulphhaemoglobin in erythrocytes (a marker of chronic exposure to H$_2$S [4,21,31]); plasma vitamin B$_6$; and gene transcription levels of the three enzymes involved in H$_2$S biosynthesis, with real-time PCR, starting from RNA obtained from peripheral blood mononuclear cells. We reasoned in fact that the levels obtained in blood could easily reflect, or be an indicator of a pattern present in tissues, being tissues unavailable from patients. The common C677T polymorphism of the methylene tetrahydrofolate reductase gene (MTHFR) was also assessed, given that the presence of the T allele could theoretically determine an increase in H$_2$S steady-state levels, through altered homocysteine and cysteine formation [32–33].

Subjects and methods

Patients

Controls were volunteer healthy subjects recruited among hospital staff (n = 31, mean age 53 years, age range 29–59 years, F/M 14/17). End-stage renal disease patients were selected (n = 65, mean age 63 years, age range 39–69 years, F/M 29/36), provided that they were not affected by systemic diseases such as lupus erythematosus, and diabetes mellitus, or evidence of other systemic diseases antecedent to renal failure, which would be confounding variables. The patients were also antibody C virus negative. The patients gave their informed consent to the study, which was approved by our institution’s ethics committee (Prot. N. 89 of 02/03/2004). Patients were on regular haemodialysis treatment for at least 3 months and were clinically stable. The patients received regular bicarbonate haemodialysis treatment (Na 140 mM, HCO$_3$ 35 mM, K 2.2 mM, Ca 1.25 mM) thrice weekly, utilizing hollow-fibre no-reuse dialysers. Dialysers consisted in low-flux polysulphone membranes (Helixone, FX-Class, Fresenius Medical Care, Bad Homburg, Germany). Dry weight was achieved in each case as an oedema-free state. Weekly time on dialysis was ~12 h. Kt/V was routinely checked at monthly intervals, and was >1.4 during the study and in the previous 6 months. In addition, patient anaemia was controlled with erythropoietin and iron therapy, as needed. Most patients followed various antihypertensive regimens, which included calcium channel antagonists, beta-blockers and angiotensin-converting enzyme inhibitors, angiotensin II receptor antagonists or a combination of drugs. Most patients were also usually treated for secondary hyperparathyroidism with sevelamer, calcitriol and/or cinacalcet. DOQI guidelines were followed for patient management of anaemia, secondary hyperparathyroidism and dialysis adequacy.

Blood was drawn, immediately before the dialysis session in haemodialysis patients, in the fasting state, by venipuncture, using EDTA (1 mg/ml of blood). The blood samples were drawn in plastic tubes. The samples were processed immediately after withdrawal for the H$_2$S assay, while plasma for Hcy and Cys was kept at −20°C, in the dark.

Reagents

Sigma-Aldrich (St. Louis, MO, USA) provided most of the materials, unless otherwise indicated.

H$_2$S concentration measurement

Once blood was drawn in plastic Vacutainers® using EDTA (1 mg/ml of blood), plasma was immediately obtained through brief (3′) centrifugation at 3000 g and rapidly added to the assay mixture. The H$_2$S concentration was measured spectrophotometrically as follows: in a test tube containing 0.5 ml of 1% zinc acetate, 0.4 ml of FeCl$_3$ in 1.2 M HCl and 0.5 ml
of \(N,N\)-dimethyl-\(\mu\)-phenylene diamine dihydrochloride (C\(3\)H\(12\)N\(2\)) in 20 mM in HCl 7.2 M, 0.4 ml of plasma were added. To deproteinize the sample, 0.7 ml of a 10% solution of trichloroacetic acid were added, and then the mixture was centrifuged at 3000 g for 30 min at room temperature. The absorbance of the supernatant was read at 670-nm wavelength, with a Biorad 3 Thermo Spectronic (Rochester, NY, USA) spectrophotometer. H\(2\)S concentration was referred to a calibration curve obtained with several known H\(2\)S concentrations, in a range between 5 and 160 \(\mu\)M, utilizing the H\(2\)S donor NaHS [34]. Standard curves were repeated daily with triplicate measurement for each point, and freshly made solutions were utilized at all times.

**Sulphaemoglobin determination**

The concentration of sulphaemoglobin in erythrocytes reflects exposure to chronic H\(2\)S blood concentrations [4,21,31].

The sulphaemoglobin concentration was measured as follows [31,35]: venous blood was centrifuged, erythrocytes separated and haemolyzed by freeze-thaw. The absorbance was measured spectrophotometrically at four different wavelengths (622 nm; 636 nm; 535 nm; 560 nm). Values were expressed as the ratio between wavelengths utilizing the following formula: (A\(622\) nm — A\(636\) nm)/(A\(535\) nm + A\(560\) nm) \(\times\) 0.5 \(\times\) 100 [31].

**Plasma homocysteine and cysteine**

Plasma total homocysteine and cysteine were measured in control and uraemic patient on haemodialysis with HPLC separation and fluorescence detection [36]. Data were expressed as microMolar (\(\mu\)M) concentration.

**Gene expression of CBS, CSE and MST utilizing quantitative real-time PCR**

Blood mononuclear cells were first isolated with a Ficoll density gradient. RNA was extracted by a double Trizol–chloroform treatment (Invitrogen, Carsbad, CA, USA), and precipitated with isopropanol. The pellet was washed in 70% ethanol and resuspended in H\(2\)O DEPC. RNA quantity was determined using the Nanodrop ND-1000 spectrophotometer (Cellbio, Milan, Italy) and purity was determined using agarose gel electrophoresis. Complementary DNA was synthesized utilizing the QuantiTect reverse transcriptase (Qiagen, Velencial, CA, USA) kit according to the manufacturer’s instructions, starting with 1 \(\mu\)g of RNA. Primers for genes of interest, designed using the Beacon Designer software package versus 2.0 (Biosoft International, Palo Alto CA, USA), were the following:

- **CBS**: GenBank accession number NM_000071.1
  - Primer forward: 5′ AGG ATA AGG AAG CCA AGG AG 3′
  - Primer reverse: 5′ TGT TGA TTC TGA CCA TAG GG 3′
- **CSE**: GenBank accession number NM_001902.4
  - Primer forward: 5′ GCC TTC ATA ATA GAC TTC GTT TC 3′
  - Primer reverse: 5′ GCA GCC CAG GAT AAA TAA CC 3′
- **MST**: GenBank accession number NM_001013440.1
  - Primer forward: 5′ AGG ACA TCA AGG AGA ACC 3′
  - Primer reverse: 5′ TCA GGA AGT CTG TGA AGG 3′
- **GAPDH**: GenBank accession number NM_002046
  - Primer forward: 5′-TTG GTA TCG TGG AAG GAC TCA TG-3′
  - Primer reverse: 5′-CAG TAG AGG CAG GGA TTA TGC TG-3′

PCRs were done utilizing SYBR green. Differences in gene expression were normalized relatively to the levels of the GAPDH housekeeping gene transcript. All amplifications were performed with an iCycler thermocycler (Bio-Rad, Milan, Italy), with a fluorescence detection system iCycler iQ real-time PCR. The reaction mix contained 1 \(\mu\)l of cDNA (2 \(\mu\)l for CBS), 0.3 \(\mu\)M of each primer and 12.5 \(\mu\)l of master mix QuantiTect SYBR green (Qiagen, Velencial, CA, USA) for CBS and CSE, while for MST iQ Sybr Green Supermix was utilized, plus DEPC-treated H\(2\)O (Invitrogen, Carsbad, CA, USA) with a 25 \(\mu\)l of final volume. cDNA, during quantitative real-time PCR, was amplified under the following conditions: 95°C for 15 min, followed by 35 cycles at 94°C for 15 s (40 s for CBS at 53°C), at 57°C for 30 s and 72°C for 30 s.

Relative expression was calculated using the \(\Delta\)Ct method. For statistical evaluation, each patient was challenged against all controls. The value of \(2^{-\Delta\Delta C T} > 1\) reflects increased expression of the relevant gene, and a value of \(2^{-\Delta\Delta C T} < 1\) points to a decrease in gene expression [37].

**MTHFR polymorphism**

Whole blood necessary for DNA extraction was stored at \(-20^\circ\)C. Genomic DNA was extracted utilizing the salting out technique. The C677T polymorphism of the MTHFR gene was amplified with PCR and digested with the enzyme HinfI, and the 198 bp fragment thus obtained underwent electrophoresis on a 4% agarose gel.

**Vitamin B\(_6\)**

Vitamin B\(_6\) was measured with a commercial kit based on a patented enzymatic method (AntiCancer Inc., San Diego, CA, USA).

**Results**

**H\(2\)S concentration in controls and haemodialysis patients**

The results relative to H\(2\)S concentration measured in serum samples from uraemic patients and controls are shown in Figure 1, panel A. Patient levels were significantly lower with respect to controls.

**Sulphaemoglobin**

Sulphaemoglobin concentration was evaluated in erythrocyte samples from both uraemic patients and controls. Sulphaemoglobin levels were significantly lower in patients (Figure 1, panel B), thus reflecting the findings relative to plasma H\(2\)S concentration.

**Plasma homocysteine and cysteine**

Both plasma homocysteine and cysteine in haemodialysis patients were significantly higher than those in controls (Figure 1, panel C).

**Plasma vitamin B\(_6\) concentration**

Pyridoxal 5′-phosphate is an essential cofactor in H\(2\)S biosynthesis. Its precursor, vitamin B\(_6\), was evaluated in serum samples from both uraemics and controls. The results showed that the vitamin B\(_6\) concentration in controls was 19.41 nmol/L (2.034), \(n = 30\), and in patients was 23.37 (1.892), \(n = 70\), \(P = ns\), respectively.

**Correlations**

All combinations between H\(2\)S and the other compounds were tested with the appropriate statistic tools, and none reached statistical significance. In particular, for example, no significant correlation between H\(2\)S concentrations and vitamin B\(_6\) was found; thus, the low H\(2\)S levels present
in patients cannot be ascribed to a deficit in vitamin B_{6}. One exception was the presence of a negative correlation in patients, not in controls, between H_{2}S and cysteine (linear regression $P < 0.004$; Pearson $P < 0.004$, Figure 2).

**Gene expression of CBS**

CBS expression turned out to be completely absent in circulating mononuclear cells of both patients and controls. To rule out that a poor yield of CBS amplification products was due to an adverse effects of specific experimental conditions, rather than to the absence of mRNA encoding for this messenger, we checked several different conditions (e.g. change of buffers, primers, number of cycles, gradients, temperature, etc.), but repeatedly confirmed the result under all the conditions tested. We also used, as a positive control, the HepG2 cell line (human hepatoma cell line), whose RNA extract is known to express CBS transcript [39].

Cells were grown in D-MEM with 4.5 g/l glucose without glutamine, added with FBS 10% (Gibco, Invitrogen, Grand Island, NY, USA), NEaa 1% (Cambrex Bio Science, Verviers, Belgium), Pen/Strep 1% (Gibco) and glutamine 1% (Gibco). Positive controls always showed that CBS was expressed in substantial amounts. Conversely, an appreciable amount of CBS amplification product could neither be detected in patient nor in control RNA extracts.

**CSE and MST gene expression**

Contrary to CBS, CSE and MST expression was investigated and found to be present both in the control and in the patient population, with, respectively, CSE significantly lower and MST significantly higher in patients with respect to controls (Figure 3).

**MTHFR polymorphism and H_{2}S**

MTHFR is a key enzyme in sulphur amino acid metabolism, in that it catalyzes methyltetrahydrofolate formation, which in turn is the co-substrate for homocysteine remethylation into methionine. The C677T mutation of the MTHFR gene represents the most common polymorphism (related to skin colour) of the gene encoding for enzymes involved in sulphur amino acid metabolism [40].
Fig. 3. Expression of CSE and MST in patients with respect to controls. CSE expression (panel A) and MST expression (panel B) levels were always normalized for GAPDH transcript levels. Results in patients (n = 24) were compared to controls (n = 24) as relative expression: fold (SD).

The patients and controls were characterized with respect to the MTHFR polymorphism. While the H₂S concentration was confirmed to be significantly lower in patients with respect to controls in the three genotypes, the H₂S concentration in patients and controls wild type (CC), heterozygous (CT) and homozygous (TT) for the C677T transition was not significantly different. Therefore, this polymorphism does not seem to exert an effect on H₂S concentration, at least in the patient population studied (data not shown).

Discussion

In this paper, we show for the first time that H₂S is decreased in the plasma of haemodialysis patients. This finding is confirmed by the low sulphhaemoglobin levels present in this patient population, and is accompanied by high plasma homocysteine and cysteine, with a significant negative correlation between cysteine and H₂S. The cofactor of CBS and CSE, vitamin B₆, is not different in patients with respect to controls. The MTHFR polymorphism does not influence H₂S blood levels. Gene expression of CBS in blood is absent, while that of CSE is lower and of MST is over-expressed, realizing a condition were a transcriptional deregulation of genes encoding for key H₂S-producing enzymes is present.

The methods utilized for H₂S detection in blood are cumbersome, and their reliability is hindered by complex analytical problems [20–23]. As a consequence, the normal plasma concentration of H₂S is debated [20–23]. Wide variations can be found in the literature, also due to differences in techniques, animal/human model utilized, etc. It has even been argued that blood H₂S concentration is actually in the nanomolar range, instead of micromolar range, thus casting doubt on the regulatory role of H₂S in circulation [20–23]. However, data by Whitfield et al., prevalently obtained in a trout model, support the “role of sulphidemetabolism as an oxygen “sensor” that may provide the long-sought-after couple in hypoxia-mediated excitation and activation in a variety of cells” [22]. Furne et al. found H₂S concentrations in mouse tissues by far lower than those previously measured by others, but concluded that H₂S roles are compatible with a “rapidly adapting mediator of cellular activity” [23].

The spectrophotometric method employed in this paper has been found to “measure a form of bound or sequestered H₂S rather than “free” H₂S” [21]. In other words, it could measure, apart from free H₂S, also protein-bound and acid-labile H₂S. According to the same authors, however, it is a method: “convenient, relatively robust and at least comparable to other techniques” [21].

Artefactuality due to adherence to glass or to volatility was bypassed by employing maximum care in sample preparation (plastic tubes and rapid sample complexation). The preparation of the standards for the calibration curve was also done daily.

Our results confirm that the normal H₂S concentration is in the low micromolar range in control subjects, which is significantly lower in patients with respect to controls. This in accordance with the idea that H₂S levels are distant from the 100 µM range. In addition, it should be considered that even if the method measures mixed H₂S moieties, levels in patients are nonetheless lower than normal (not higher).

Sulphhaemoglobin can be considered to reflect chronic exposure to H₂S [4,21,31]. Another good marker of exposure is represented by the excretion of thiosulphate in the urine. However, it is usually not possible to measure this compound in haemodialysis patients, who are virtually anuric. We were instead able to measure red cell sulphhaemoglobin, which was, in the uraemic patients’ group, significantly lower than in controls. This finding confirms that plasma H₂S concentration is actually lower in this patient population than in controls.

Both plasma homocysteine and cysteine are known to be consistently higher in haemodialysis patients than in controls. Homocysteine elevation has been linked to an increased cardiovascular risk, in the general population and in haemodialysis patients [25,33]. Homocysteine and cysteine, or their direct derivatives, are utilized as substrates by the enzymes involved in H₂S biosynthesis, namely, CBS, CSE and MST. Plasma homocysteine and cysteine were higher in our patient population than in controls, as already well known. Correlations between the variables
H₂S-generating pathways in haemodialysis patients

Fig. 4. Schematic representation of the metabolic pathways related to H₂S production and relevant alterations in uraemia. The scheme summarizes our major findings, together with data from the literature, fitted in an overall hypothesis relative to the role of H₂S and related metabolite derangement in uraemia. 3MP: 3-mercaptopropionate; αKG: α-ketoglutarate; CAT: cysteine aminotransferase. Thin black arrows indicate the main physiological pathways. A grey background indicates the pyridoxal phosphate-requiring enzymes. Black arrows included in circles and pointing up or down indicate increase or reduction, respectively. Namely, circulating homocysteine and cysteine are increased (see Figure 1, panel C), H₂S is decreased (Figure 1, panel A), as well as its stable derivative sulphhaemoglobin (Figure 1, panel B). Our data showed that, at gene expression level, CSE and MST are oppositely regulated (see also Figure 3). However, CSE is the major enzyme in H₂S production while MST plays a minor role, resulting in a reduced H₂S concentration [4]. This is consistent with the possible role of aminoguanidine in the inverse regulation of CSE and MST according to ref. 10 (grey-shaded arrow connector; see also the ‘Discussion’ section). As evidenced, CSE downregulation can explain both hyperhomocysteinaemia and H₂S decrease in CKD patients. X indicates suppressed renal excretion due to CKD. Open arrows indicate the roles of CBS, CSE and MST in H₂S biosynthesis, as well as their relevant substrates. In particular, H₂S synthesis can be catalyzed directly from cysteine (CBS) or through its oxidation product cysteine (CSE; open arrows in bold line), while MST can produce H₂S from 3MP.

involved were not significant, with one notable exception, that between H₂S and cysteine. The presence of such a negative correlation between these two compounds points to decreased activity of CSE, as confirmed by the gene expression studies.

As recently pointed out by Ruma Banerjee’s research group [41], under conditions where hyperhomocysteinaemia is present, it might be expected that the relative contribution of CSE in H₂S generation might be increased with respect to CBS. In our patients, where CSE activity might be lower due to decreased gene expression, H₂S in fact is decreased. This condition therefore is in accordance with the predicted central role of CSE in sulphur metabolism provided by Banerjee’s model [42]. However, the uraemic milieu may provide a unique example of the hyperhomocysteinaemic condition in which, due to CSE downregulation, high Hcy fails to determine high H₂S overproduction.

Plasma vitamin B₆, a cofactor of both CBS and CSE, was found not to be different from controls. Therefore, a possible reduction in enzyme activity cannot be ascribed to cofactor deficiency.

Gene expression of CBS, CSE and MST, the three enzymes involved in H₂S production, was measured in nucleated blood cells, as a surrogate of the expression present in tissues. Expression of CBS was not found in blood, in any of the several experimental conditions studied. As for CSE expression, this was significantly reduced in patients; possibly this is related to uraemic toxicity, with one or more toxins acting as inhibitors at the gene expression level. This finding is consistent with the decrease in H₂S in plasma. Therefore, we conclude that this decrease is due to a reduced H₂S production caused by less enzymes being produced, in turn due to genetic downregulation.

CSE specificity towards its substrates is unbalanced; in fact, structural and functional studies reveal that CSE is operative in H₂S production from L-cysteine. However, although this γ-lyase reaction is present in humans, it is 20 times slower than the cleavage of the C–S bond in the conversion of L-cystathionine to L-cysteine, in the trans-sulphuration pathway [43]. According to this model, any significant reduction in CSE expression and activity may result in the decrease of H₂S production and, eventually, in a moderate accumulation of the trans-sulphuration precursor...
homocysteine. Our data provide the basis for a role of CSE downregulation in uraemia, in which H2S production is compromised and sulphur amino acid metabolism is deranged, leading to hyperhomocysteinaemia.

MST expression was found to be significantly increased. MST is a multifunctional enzyme, and one of the functions is devoted to the detoxification of cyanide, which is transformed into thiocyanate [7–8]. Interestingly, high cyanide and thiocyanide levels are found in blood of haemodialysis patients [44]. We can therefore speculate that this enzyme’s gene expression is increased because of the necessity to dispose of excess plasma cyanide, typical of these patients. In this respect, it can be also added that aminoguanidine, an inhibitor of NO synthase, considerably decreases CSE activity in the rat brain and leads to a simultaneous increase in MST activity [10]. Since in uraemia many guanidino compounds are elevated in blood [45], and exert many biochemical responses, one can also speculate that a similar phenomenon occurs in uraemia (Figure 4). It can also be mentioned that MST importance in the quantitative H2S formation is limited [4]; therefore, even if upregulated in these patients, the overall effect on H2S formation would be overcome by CSE downregulation.

The reduction in H2S production due to reduced gene expression of CSE can be considered one of the manifold manifestations of the uraemic toxicity syndrome, and can be in turn responsible for some of its features. H2S deficiency is involved in hypertension, atherosclerosis [1–3] and sexual dysfunction [18], which are also distinct characteristics of the uraemic condition [25,45]. Intriguingly, a recent study in a model of uraemic hyperhomocysteinaemic mice found that H2S is reduced in blood, and its supplementation prevented the hyperhomocysteinaemia-associated renal damage [46].

In conclusion, low blood levels of H2S have been found in haemodialysis patients, through transcriptional deregulation of relevant genes. The latter could be also linked to the mechanism of hyperhomocysteinaemia in uraemia. H2S is a gas, endogenously produced, like NO and CO, with many cardiovascular-modulating properties. Such properties are not as yet explored in many pathological conditions and in particular in haemodialysis patients. Hypertension and premature atherosclerosis are important factors influencing the high cardiovascular mortality present in these patients. Exploiting the possibilities that the study of H2S holds in this regard could have unforeseen payoffs [47].

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**Abstract**

**Background.** Almost 30% of chronic haemodialysis (HD) patients are dependent on central venous catheters (CVCs) for their vascular access, and catheter-related bacteraemia (CRB) is the major reason for catheter loss and has been associated with substantial morbidity, including meta-static infections. This systematic review evaluates the benefits and harms of antimicrobial