Hydrogen Sulphide-Induced Hypometabolism Prevents Renal Ischemia/Reperfusion Injury

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ABSTRACT

Hydrogen sulphide (H2S) can induce a hypometabolic, hibernation-like state in mammals when given in subtoxic concentrations. Pharmacologically reducing the demand for oxygen is a promising strategy to minimize unavoidable hypoxia-induced injury such as ischemia/reperfusion injury during renal transplantation. Here we show that H2S reduces metabolism in vivo, ex vivo, and in vitro. Furthermore, we demonstrate the beneficial effects of H2S-induced hypometabolism in a model of bilateral renal ischemia/reperfusion injury using three different treatment strategies. The results demonstrate striking protective effects on survival, renal function, apoptosis, and inflammation. A hypometabolic state induced by H2S might have therapeutic potential to protect kidneys that suffer from hypoxia.

nal damage in the control group, leading to an impaired 3-d survival caused by renal failure (Figure 2A). Both groups in which mice were pretreated with H2S had 100% survival after 3 d (P < 0.001), whereas mice that only received H2S during reperfusion showed similar survival to the control group (P = NS). Serum creatinine and urea measurements were performed to quantify the renal function loss associated with bilateral renal ischemia/reperfusion. Control and post-treatment animals showed highly elevated levels of creatinine and urea (Figure 2B, Supplementary Figure 1), whereas animals pretreated with H2S had only slightly higher levels than sham-operated animals (P = NS). These measurements indicate massive renal failure in the control and post-treatment groups, which is the most likely cause of the diminished survival in these groups.

We assessed structural renal damage in periodic acid–Schiff-stained sections and found a similar pattern to the renal function measurements, as expected. Massive acute tubular necrosis was detected in control animals at day 1, whereas mice in both pretreated groups had no or minimal renal damage (Figure 2, D, J through N, Supplementary Figure 3). Post-treatment with H2S showed a significant reduction in tubular damage compared with controls, although it was not as extensive as in pretreated animals. After 3 d, a similar pattern was seen (Supplementary Figure 2). Post-treatment did not have significant protective effects at this time point, although these results are confounded to some extent, because animals with large amounts of renal damage had already deceased at this point.

Active Caspase3 staining using immunohistochemistry indicated that ischemia/reperfusion injury (IRI)-induced apoptosis is also prevented by H2S pre-treatment. (Figure 2C, Supplementary Figure 4). A less pronounced but statistically significant effect was seen in the post-treatment group. Real-time PCR measurements showed that mRNA expression of proapoptotic Bax was 2.5 times higher in control kidneys compared with sham-operated animals (Supplementary Figure 5A). Expression was not significantly increased in animals pretreated with H2S. The expression of anti-apoptotic BCL-2 did not differ between groups (Supplementary Figure 5B), indicating that the anti-apoptotic effects of H2S are not mediated through induction of BCL-2 mRNA expression. Whether H2S directly or indirectly inhibits increased expression of Bax is not clear. Transmission electron microscopy
of a few samples implies that H₂S treatment protected against loss of mitochondrial integrity and mitochondrial swelling (Supplementary Figure 6). In literature, proapoptotic as well as antiapoptotic effects of H₂S are described, and it is not known whether H₂S can directly modulate apoptotic pathways, or that increased mitochondrial integrity and reduced mitochondrial stress caused by reduced mitochondrial activity caused the reduction in Caspase 3 activity in the post-treatment group.

We studied the inflammatory component of IRI by immunohistochemical staining for Mac-1 (CD11b, which is present on macrophages, monocytes, granulocytes, and natural killer cells) and Ly-6G (which is expressed on mature granulocytes). (Figure 2, E through I, Supplementary Figure 7). The influx of Mac-1 and Ly-6G-positive cells was greatly reduced by H₂S pretreatment (P < 0.05) but was not significantly affected by post-treatment.

These results indicate that the reduction in metabolism before ischemia is highly protective in reducing ischemia-induced injury with predictable onset, such as during transplantation or surgical intervention. The mechanism of H₂S-induced hypometabolism is unknown as of yet but is most likely mediated through reversible inhibition of complex IV (cytochrome oxidase), the terminal enzyme of the mitochondrial electron transport chain. Inhibition of this complex might be the mechanism of the reduction in mitochondrial membrane potential caused by H₂S treatment. It seems unlikely that H₂S directly and effectively...
inhibits necrotic, apoptotic, and inflammatory pathways after an ischemic insult. The observation that protection is greatest when H$_2$S is given before and during, but much less when given directly after the hypoxic period, supports the notion that the reduction in O$_2$ demand during hypoxia prevents the activation of these detrimental pathways. The moderate effects of H$_2$S in the post-treatment group could be caused by the inhibition of reactive O$_2$ species production by decreasing mitochondrial activity. Protection could also be mediated through direct antioxidant action, or increased glutathione levels caused by H$_2$S.$^3$

Recent literature shows beneficial effects of gaseous H$_2$S on survival in models of hypoxia$^4$ and hemorrhagic shock.$^{12}$ Other groups have studied the protective effects of soluble forms of H$_2$S (such as sodium hydrosulfide or sodium sulfide) in models of ischemia. These studies show beneficial effects of H$_2$S on renal,$^{13}$ cardiac,$^5$ hepatic,$^{14}$ and pulmonary ischemia.$^{15}$ One paper suggests an association between H$_2$S treatment and reduced activation of multiple signal transduction molecules, such as p38, ERK, and JNK; however, a direct relationship between H$_2$S and kinase activation was not proven. We found that phosphorylation of ERK1/2 was stimulated by ischemia in our model, but no modulation was seen in H$_2$S-treated animals (Supplementary Figure 8). Our study shows a novel relation between H$_2$S treatment and hypometabolism, which has not been previously investigated. The protective effects of H$_2$S treatment posthypoxia are less pronounced in our experiments. However, a recent paper indicated that injection of sodium sulfide just before reperfusion in a model of myocardial infarction caused a great reduction in infarct size and protected mitochondrial integrity and function.$^5$ This indicates that post-treatment with H$_2$S might still be a promising intervention in cutting back on the detrimental effects of hypoxia after the event. We conclude that hypometabolism induced by gaseous H$_2$S is a novel treatment regimen with high therapeutic potential in reducing renal damage associated with ischemic insults.

**CONCISE METHODS**

**Animals**

Male, 6- to 8-wk-old C57BL/6 mice and 250- to 300 g Fischer F344 rats (Harlan, The Netherlands) were housed under standard conditions. Experimental procedures were in agreement with institutional and legislator regulations and approved by the local committee for animal experiments.

**H$_2$S Treatment and Respirometry**

H$_2$S treatment and measurement of O$_2$ consumption were performed using an advanced, modular respirometry system (TR-3 system, Sable Systems, Las Vegas, NV). Compressed air and 500 ppm H$_2$S/nitrogen (Air Products, Amsterdam, The Netherlands) were mixed in a 4:1 ratio, producing a 100 ppm H$_2$S/17% O$_2$ mixture.

**IRI Protocol**

Renal ischemia/reperfusion in mice was performed as described previously.$^{16}$ In short, both renal pedicles were clamped for 30 min using nontraumatic vascular clamps through both renal pedicles were clamped for 30 min before the kidney and from the renal vein, respectively. In these samples, pO$_2$ was measured using an ABL700 blood gas analyzer (Radiometer Medical, Denmark).

**REFERENCES**


**DISCLOSURES**

None.

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