Hibernating mitochondria, the cool key to cellular protection and transplant optimization

Hendriks, Koen

DOI:
10.33612/diss.160451743

Hydrogen sulphide-induced hypometabolism in human-sized porcine kidneys

Hanno Maassen
Koen D.W. Hendriks
Rob H. Henning
Sijbrand H. Hofker
Harry van Goor
Henri G.D. Leuvenink
Annemieke M. Coester

Published: PLoS One. (2019)
DOI: 10.1371/journal.pone.0225152

7

ABSTRACT

Since the start of organ transplantation, hypothermia-forced hypometabolism has been the cornerstone in organ preservation. Cold preservation showed to be protective compared to warm ischemia, however, damage still occurs and improvement in preservation techniques is needed. We showed, for the first time, that hydrogen sulphide (H_2S) can be used as such new preservation technique, by inducing a reversible hypometabolic state in human sized kidneys during normothermic machine perfusion.

Porcine kidneys were connected to an ex-vivo isolated, oxygen supplemented, normothermic blood perfusion set-up. Experimental kidneys (n=5) received 85mg NaHS infusion of 100 ppm and were compared to controls (n=5). As reflection of the metabolism, oxygen consumption, mitochondrial activity and tissue ATP levels were measured. Kidney function was assessed by creatinine clearance and fractional excretion of sodium. To rule out potential damage kidneys were studied for biochemical markers and histology.

NaHS strongly decreased oxygen consumption ($p<0.001$), which was associated with a marked decrease in mitochondrial function, without affecting ATP levels. Renal biological markers and histology did not change by H_2S treatment, or showed a trend of improvement.

In conclusion, we showed that hydrogen sulphide can induce a controllable hypometabolic state in a human sized organ, without damaging the organ itself. Highlighting this treatment as promising therapeutic alternative for cold preservation in renal transplantation.

INTRODUCTION

Renal transplantation is the preferred treatment for end-stage renal disease[1]. The on-going increase in the needed number of renal transplantations and lack of suitable donors results in the increased use of suboptimal donors, donation after circulatory death (DCD)[2] and extended criteria donors (ECD). Organs from these donors start with a lower spare capacity and are more prone to injury caused by warm and cold ischemia, resulting in increased ischemia-reperfusion injury (IRI) and graft failure following transplantation[3]. Especially the warm ischemic time, together with extraction- and cooling time, are crucial and relates to survival in, for instance, liver transplantation[4]. IRI leads, via mitochondrial failure, to cell death, inflammation[5] and fibrosis[6]. In addition, mitochondrial dysfunction might be a surrogate for tissue health after transplantation[7]. Therefore, targeting mitochondria in order to reduce IRI improves preservation of these organs[8]. Gasotransmitters could play a vital role during the process of transplantation[9], especially hydrogen sulphide (H_2S) is a potent therapeutic intervention[10].

Preservation could be improved by inducing a fast hypometabolic state by directly inactivating mitochondria, instead of the slower cold-forced inactivity, and thereby decreasing the damage obtained by warm ischemia during extraction and bypassing the negative effects of a cold environment. Interestingly, exploiting the gasotransmitter H_2S to a higher concentration, induces a hypometabolic state. H_2S induces this hypometabolism through reversible inhibition of complex IV (cytochrome c oxidase) of the mitochondrial electron transport chain (ETC)[11,12]. Next to ETC inhibition, H_2S protects the ETC by different mechanisms[13]. Indeed, gaseous administration of H_2S in mice induces a hypometabolic state of suspended animation[12], prevents renal injury in mice during IRI[14] and is promising in decreasing ROS damage[15-16]. Besides the direct mitochondrial effects, H_2S acts anti-inflammatory[17] and inhibits apoptosis[18]. Although H_2S showed protective effects during room-temperature static storage[19], until now, neither systemically administered[20] or gaseous administered[21] H_2S induced successful hypometabolism in larger mammals.

H_2S is traditionally known for its toxicity with numerous cases of intoxication and death. Though, in these cases of intoxication signs of protection against hypoxic injury are seen[22], promoting its capacity of reducing ischemic injury in a human body by means of hypometabolism. In the current study, we show that H_2S can induce a fast hypometabolic state in isolated perfused porcine kidneys during normothermic machine perfusion as measured by oxygen consumption, mitochondrial function and ATP production, without damaging the organ. A promising new way of organ preservation in donation after cardiac death donors.

MATERIALS AND METHODS

Animals

Porcine (Dutch landrace pigs, 5 months, 130 kilograms on average) kidneys (296 grams on average) were obtained from a local slaughterhouse. Pigs died from circulatory arrest.

Perfusion

After circulatory arrest, kidneys were exposed to a standardized 30 min of warm ischemia after which they were flushed with 180 ml cold 0.9% saline and connected to a hypothermic perfusion machine (HMP) for 4h, to bridge the time between circulatory arrest and start of the experiment. HMP: perfused with 500 ml University of Wisconsin solution (UW-MPS, Belzer), 4°C, mean pressure of 45 mmHg, 100 ml/min oxygen supplied. Before normothermic perfusion, kidneys were flushed with 50 ml cold 0.9% saline to remove the UW-MPS. Afterwards, the kidneys were connected to our normothermic perfusion set-up: mean pressure of 80 mmHg, 500 ml of leukocyte depleted blood diluted with 300 ml of Ringerslactate and enriched with 7,5 mg/L Mannitol, 7,5 mg/L Dexamethasone, 10 ml 8,4% Sodium bicarbonate, 10 ml glucose 5%, 112,5 mg/L Creatinine, 100mg/200mg Augmentin, 125 µl/L (20mg/ml) sodium nitroprusside, two constant infusion solutions: 82 ml Aminosol, 2.5 ml 8,4% Sodium bicarbonate and 17 IU insulin (infusion at 20 ml/h) and 5% glucose (infusion at 5 ml/h). Oxygenated with carbogen (95% O_2 and 5% CO_2 at 500 ml/min). Kidneys were first gradually rewarmed to 21°C during 1h, then warmed to 37°C during 1h, in which the experimental group received 85 mg of the NaHS dissolved in 10 ml 0.9% saline. NaHS was infused at 100 ppm, corrected for the current flow (approximately 5 min). Next, the kidneys were perfused at 21°C for 1h.

Perfusion equipment

Perfusion was performed using a Kidney Assist (Organ Assist, Netherlands) with heart assist software and a centrifugal pumphead (eltastream DP3, MEDOS Medizintechnik AG, Germany). Temperature was regulated using a Jubalo water heating system. An integrated heat exchanger (HILITE 1000, MEDOS Medizintechnik AG, Stolberg, Germany) was built in the oxygenator. The flow sensor is a Clamp-on flow sensor (ME7PXL clamp, Transonic Systems Inc., Ithaca, NY). The pressure sensor is a Truewave disposable pressure transducer (Edwards lifesciences, Irvine California, USA).

Live registration

Oxygen, temperature, flow and diuresis were constantly monitored during the experiment. Oxygen measurements were performed continuously using the PreSens Fibox 4 oxygen-measurement system. Oxygen consumption was shown as (pO_2 [hPa] arterial – pO_2 venous[hPa]) · (flow [ml/min] / weight [gr]). Temperature was measured by the integrated sensor of the kidney assist. Flow was constantly measured and noted every 10 min. Urine was constantly collected in a beaker, which was replaced every 15 min.

Biological markers

Serially taken urine and plasma samples were analysed for creatinine, sodium, lactate, pH and potassium at the Clinical Chemical Laboratory of the UMCG. Cortical biopsies were taken for ATP levels (sonification buffer) and histology (formalin). ATP levels were measured using the ATP Bioluminescence Assay Kit CLS II according to manufacturer's protocol and expressed relative to the protein concentration (Pierce™ BCA Protein Assay Kit). As a marker for reactive oxygen species (ROS) induced damage, lipid oxidation was quantified in tissue samples (taken 90 min after H_2S infusion) by measurement of malondialdehyde (MDA) using the OxiSelect TBARS assay kit (Cell Biolobs) according to manufacturer's protocol, including a butanol extraction. Fluorescence was measured using the Synergy 2 Multi-Mode plate reader (BioTek). Lipid peroxidation levels were expressed as µM corrected for protein levels (Bradford assay, Biorad).

Tissue examination

Periodic acid-Schiff (PAS) staining was performed on the paraffin embedded biopsies taken 75 min after H_2S infusion and analysed by an experienced pathologist.

Mitochondrial function

Mitochondria were freshly isolated using a standard differential centrifugation protocol and protein concentration was determined (Bradford, Biorad). 5 µg of mitochondria were resuspended in a total volume of 100 µl mitochondrial buffer containing JC-1 (Sigma Aldrich) with NaHS (0-5 mM) or Carbonyl cyanide-p-trifluoromethoxyphenylhydrazone (FCCP, 2µM). After 30 min incubation (37°C), mitochondrial membrane potential was fluorescently measured by quantifying the fluorescence emission shift from green (529 nm) monomers to red (590 nm) aggregates. Data expressed as ratio red / green, relative to control.

Statistics

Data were analysed using SPSS 25.0 (SPSS inc., Chicago, IL, USA). A linear mixed model was used to analyze the repeated measurements. The model was designed to handle missing data. A student t-test was used to analyze the lipid peroxidation. GraphPad PRISM 5.04 (GraphPad, San Diego, CA, USA) was used to create the graphs.

RESULTS

H_2S infusion induces a rapid and reversible decrease in oxygen consumption

Immediately upon H_2S infusion, oxygen consumption decreased strongly from 409 to 160 ΔhPa·ml/min/gr (figure 1A, p<0.001) which restored rapidly after ending the H_2S administration. Interestingly, a temporary increased oxygen consumption was observed for 20 min after the H_2S infusion. To compare the hypometabolic effects of H_2S to hypothermia, we cooled the organ at the end of the experiment. Gradually cooling to 21°C decreased oxygen consumption to 220 ΔhPa·ml/min/gr (figure 1A).

To examine whether the drop in oxygen consumption is a result of mitochondrial depression, mitochondrial membrane potential was measured in H_2S treated mitochondria. Increasing NaHS concentrations resulted in decreased mitochondrial activity, where 100 ppm NaHS resulted in a strong decrease in mitochondrial membrane potential compared to non-treated mitochondria (figure 1C). Despite the decrease in mitochondrial activity during H_2S infusion, ATP levels did not alter directly after H_2S infusion, but increased over time compared to control levels after H_2S infusion (figure 1B).

Besides the inhibited metabolism, H_2S decreased the flow shortly during H_2S infusion, after which an increase can be seen (figure 1D). In addition, during cooling the flow decreases, most potently in H_2S treated kidneys.

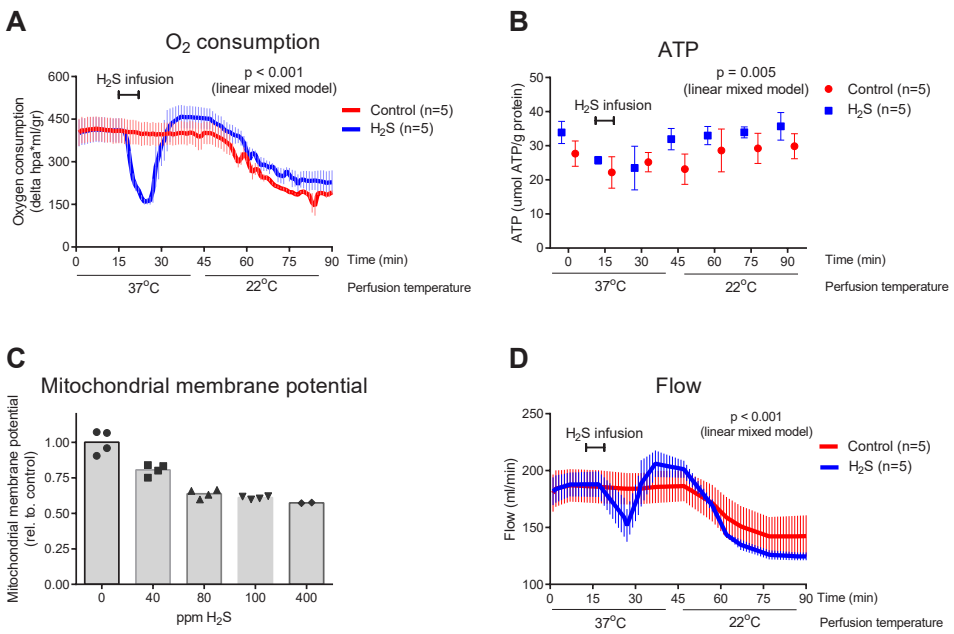

Figure 1, H_2S effects on kidney perfusion and oxygen consumption. A: *after H_2S infusion at 37°C, a significant (p<0.001) decrease from 409 to 160 ΔhPa·ml/min/gr is seen which restores to normal oxygen consumption levels with a temporary increase within 20 minutes after NaHS infusion.* ***B:*** *ATP levels in renal tissue, data expressed as µmol ATP/g protein, showing no clear alteration after H_2S infusion but remain higher after infusion of H_2S.* ***C:*** *Mitochondrial membrane potential in H_2S treated pig kidney mitochondria, data expressed as ratio red / green relative to control, showing a 39% decrease in mitochondrial membrane potential in 100 ppm NaHS treated mitochondria compared to non-treated mitochondria.* ***D:*** *As a result of H_2S administration, flow reduced from 188 ml/min till 152 ml/min. After 20 minutes, the reduced flow restored to slightly above normal levels at 206 ml/min but restores to control levels within 40 minutes after NaHS administration. Figure A, B, D, presented as mean + SEM.*

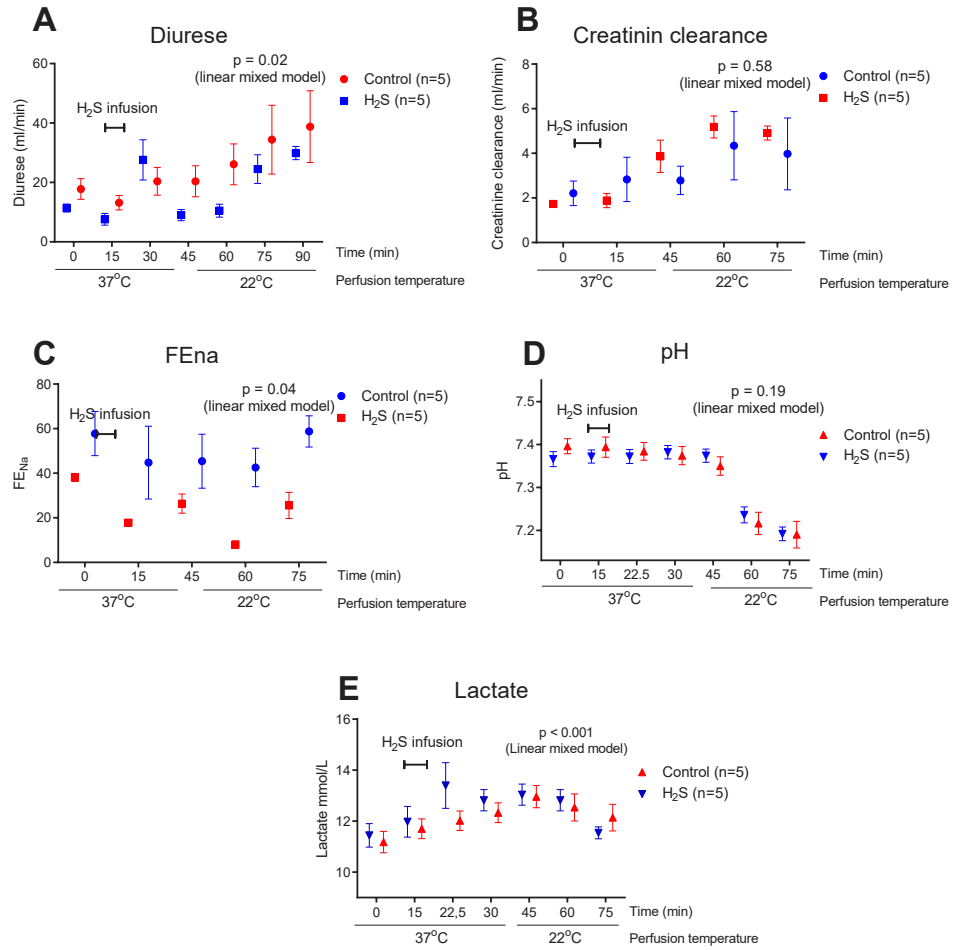

Figure 2, Kidney function during H_2S treatment. A: *Diuresis (mL) showing an 363% increase after H_2S infusion, restored to control levels within 30 min.* ***B:*** *Creatinine clearance (mL/min) showing no difference between the H_2S and control group.* ***C:*** *Fractional excretion of sodium (FEna) showing difference between the H_2S and control group.* ***D:*** *pH level of perfusion fluid* ***E:*** *lactate level (mmol/L) of perfusion fluid showing a higher venous lactate level of the H_2S treated group after infusion of H_2S.*

Preserving effects of H_2S on renal function

H_2S increased diuresis during infusion by 3-fold, which restored to control levels within 30 min (figure 2A, p=0.027). Renal function, expressed as fractional excretion of sodium (FEna) shows significant improved function (p=0.033) in the H_2S treated group, whereat creatinine clearance, shows a positive trend (p=0.50) upon H_2S treatment. Higher venous lactate levels were seen, probably matching increased glycolysis, without alterations in pH (figure 2E).

No damage response was observed after the H_2S treatment.

Kidneys were histologically examined for tubular necrosis and ischemic damage, which showed no changes between H_2S and control kidneys (figure 3A). ASAT and LDH showed a small increase over time, but no differences were observed between the H_2S treated and non-treated groups (p=0.70 and p=0.76) (figure 3B,C). As a marker for reactive oxygen species (ROS), lipid oxidation was measured in samples before and after perfusion with H_2S. The H_2S-induced hypometabolic state did not lead to increased oxidative damage. On top of that, we found a trend of protection, a trend towards decreased MDA levels in the H_2S treated kidneys (figure 3D).

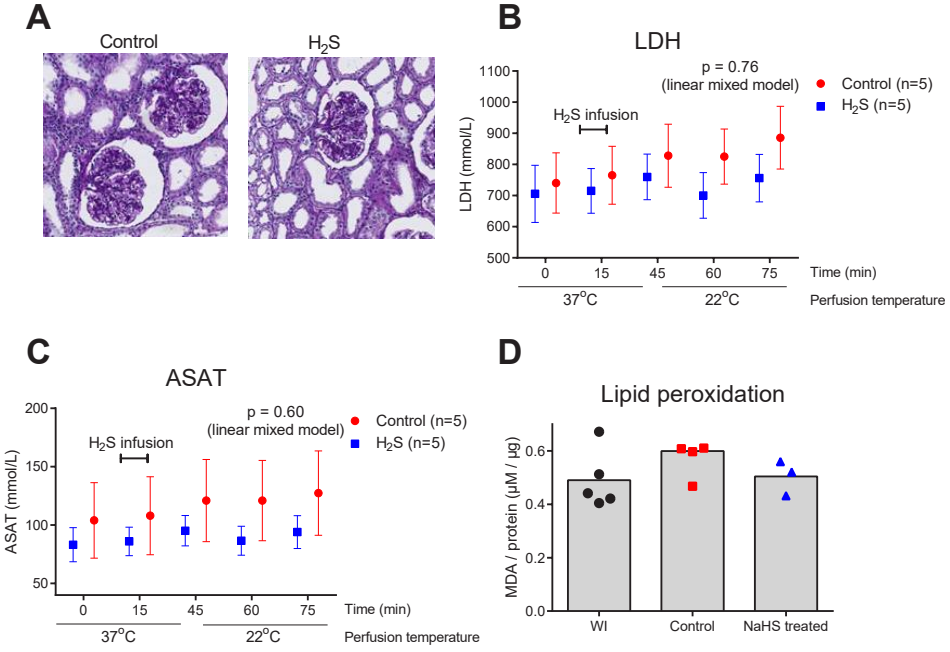

Figure 3, renal damage response A: *PAS stained tissue with no difference between H_2S treated and control.* ***B:*** *ASAT level in perfusion fluid (mmol/L) showing no difference between the H_2S treated and control group.* ***C:*** *LDH level in perfusion fluid (mmol/L) showing no difference between the H_2S treated and control group.* ***D:*** *Lipid peroxidation, expressed as µM corrected for protein level, showing a trend towards decreased MDA levels in the H_2S treated kidneys. Data expressed as mean with SEM.*

DISCUSSION

Traditionally, kidneys are preserved by cold storage or, more recently, by hypothermic machine perfusion. Both techniques are bas on cold temperatures, lowering metabolism and prolonging safe conservation of the organ compared to warm ischemia[23]. However, hypothermia is known to be detrimental to cellular processes[24]. Indeed, the length of cold ischemic times is related to an increased risk of graft failure and/or mortality following renal transplantation[25]. Both indicating that improved preservation techniques are needed.

We showed that H_2S can induce a safe and reversible hypometabolic state in human sized porcine kidneys during isolated normothermic perfusion, as advocated by decreased oxygen consumption and mitochondrial activity without any short-term damage and signs of renal function improvement. Therefore, H_2S showed to be a very potent alternative preservation technique. By inducing a hypometabolic state, H_2S has shown to reduce ischemic injury[14], scavenge ROS[15-16] and inhibit apoptosis[18,26]. Although H_2S treatment can mitigate renal graft IRI during cold storage following renal transplantation in rats[27] and shows potentially cytoprotective and anti-inflammatory effects following renal IRI in CLAWN miniature swine[28], the hypometabolic effect of H_2S combined with (sub) normothermic preservation and human sized organs is still unknown.

H_2S is a gasotransmitter, produced by the conversion of L-cysteine by cystathionine β-synthase (CBS), cystathionine γ-lyase (CSE) and cysteine aminotransferase (CAT), all three mainly located in the cytosol. Additionally, H_2S is produced directly within mitochondria by 3-mercaptopyruvate sulfur-transferase (3MST)[29]. CBS and CSE translocate to mitochondria during cellular stress such as hypoxia[30]. Displaying the considerable role of mitochondria in H_2S production and regulation. H_2S suppresses metabolism via reversible inhibition of mitochondrial complex IV (also known as cytochrome c oxidase). This mechanism has been proposed as the driven force behind the hypometabolic state induced by H_2S when used in high dosages, as in our experiment. A shift towards more glycolysis could be expected due to loss of the mitochondrial energy production by decreased oxidative phosphorylation. Indeed, a slight increase in venous lactate levels were found. Interestingly, ATP levels did not directly alter after H_2S infusion, but are increased compared to controls after the infusion, suggesting a lower ATP consumption or alternative production.

The fast but limited effects of H_2S on different parameters can be explained by the H_2S concentration and time of infusion. NaHS, as very rapid acting H_2S donor, is known to increase the H_2S concentration fast, after which H_2S is rapidly lost from the solution by volatilization in laboratory conditions or transferred across

respiratory membranes[31], in this experiment, the oxygenator. Explaining the short and limited effects of H_2S on injury markers. In addition, the moment of infusion, halfway normothermic perfusion, limited the potential protective properties[14].

We showed a complete restoration to normal kidney function after H_2S treatment. Biochemical parameters (ASAT and LDH) were not altered by H_2S treatment and histology showed no difference, indicating that short-term damage is absent. In addition, as mitochondrial ROS production is one of the major damaging routes during IRI[32], and H_2S is a known for ROS inhibition[33], we evaluated lipid peroxidation levels as marker for ROS before and after perfusion. Although a trend of decreased MDA was seen in the experimental group, no significant differences were found.

The effect of H_2S on the increase of diuresis and flow can be explained by vasorelaxation, as seen in earlier experiments in rats[34]. Vasorelaxation and decreased blood pressure, caused by opening of K_{atp} channels[34], can both influence the flow and diuresis. Moreover, similar effects of decreased blood pressure have been seen in a porcine reperfusion model[16]. In addition, CSE knockout mice develop hypertension, indicating that endogenously produced H_2S modulated blood pressure[35]. The absence of changes in creatinine clearance advocates that H_2S does not affect renal function. However, fractional excretion of sodium was lower in the H_2S group. When H_2S substrate l-cysteine is infused into renal arteries of rats it causes an increase in GFR and urinary excretion of Na^+ and K^{+}[36], possibly explaining the partly better renal function.

Summarizing, our model shows that a reversible hypometabolism can be induced using H_2S. H_2S could be used clinically at different moments during renal donation and transplantation procedure. Instead of waiting till the organ has cooled during extraction of the organ, inducing a fast hypometabolic state by infusion of H_2S could reduce ischemic injury. In addition, H_2S can be used during transportation of the organ, thereby inducing a hypometabolic state in normothermic circumstances, potentially avoiding the deleterious effects of low temperatures. In addition, its antioxidant capacity could reduce IRI[15].

This study shows that H_2S is applicable for clinical purposes by means of its capacity to induce a rapid reversible state of hypometabolism in the absence of functional or structural deterioration and signs of renal function improvement. More research is needed to determine long term effects of H_2S and its use in the transplantation setting.

REFERENCES

1. Cavallo MC, Sepe V, Conte F, Abelli M, Ticozzelli E, Bottazzi A, et al. Cost-effectiveness of kidney transplantation from DCD in Italy. Transplant Proc. 2014 46(10):3289-96.
2. Ojo AO, Hanson JA, Meier-Kriesche H, Okechukwu CN, Wolfe RA, Leichtman AB, Agodoa LY, Kaplan B, Port FK. Survival in recipients of marginal cadaveric donor kidneys compared with other recipients and wait-listed transplant candidates. J Am Soc Nephrol. 2001 12(3):589-97.
3. Kokkinos C, Antcliffe D, Nanidis T, Darzi AW, Tekkis P, Papalois V. Outcome of Kidney Transplantation From Nonheart-Beating Versus Heart-Beating Cadaveric Donors. Transplantation. 2007 15;83(9):1193-9.
4. Jochmans I, Fieuws S, Tieken I, Samuel U, Pirenne J. The Impact of Hepatectomy Time of the Liver Graft on Post-transplant Outcome: A Eurotransplant Cohort Study. Ann Surg. 2017 21.
5. Slegtenhorst BR, Dor FJMF, Elkhal A, Rodriquez H, Yang X, Edtinger K, Quant M, Chong AS, Tullius SG. Mechanisms and consequences of injury and repair in older organ transplants. Transplantation. 2014 15;97(11):1091-9.
6. Kalogeris T, Baines CP, Krenz M, Korthuis RJ. Cell biology of ischemia/reperfusion injury. Int Rev Cell Mol Biol. 2012;298:229–317.
7. Garonzik-Wang, JM, Lonze, BE, Ruck, JM, et al. Mitochondrial membrane potential and delayed graft function following kidney transplantation. Am J Transplant. 2019; 19: 585– 590.
8. Lobb, I, Jiang, J, Lian, D, Liu, W, Haig, A, Saha, MN, Torregrossa, R, Wood, ME, Whiteman, M & Sener, A. Hydrogen Sulfide Protects Renal Grafts Against Prolonged Cold Ischemia–Reperfusion Injury via Specific Mitochondrial Actions. Am J Transplant 2017; 17: 341– 352
9. Snijder, P. M., van den Berg, E. , Whiteman, M. , Bakker, S. J., Leuvenink, H. G. and van Goor, H. Emerging Role of Gasotransmitters in Renal Transplantation. American Journal of Transplantation. 2013 13: 3067-3075.
10. Dugbartey GJ, Bouma HR, Saha MN, Lobb I, Henning RH, Sener A: A Hibernation-Like State for Transplantable Organs: Is Hydrogen Sulfide Therapy the Future of Organ Preservation? Antioxid. Redox Signal. 2018, 28, 1503–1515.
11. Blackstone E, Morrison M, Roth MB. H2S induces a suspended animation-like state in mice. Science. 2005 22;308(5721):518.
12. Baumgart K, Radermacher P, and Wagner F. Applying gases for microcirculatory and cellular oxygenation in sepsis: Effects of nitric oxide, carbon monoxide, and hydrogen sulfide. Curr Opin Anesthesiol 2009 22: 168–176
13. Wetzel MD, Wenke JC: Mechanisms by which hydrogen sulfide attenuates muscle function following ischemia-reperfusion injury: effects on Akt signaling, mitochondrial function, and apoptosis. J Transl Med. 2019, 17(1):33.
14. Bos EM, Leuvenink HG, Snijder PM, Kloosterhuis NJ, Hillebrands JL, Leemans JC, Florquin S, van Goor H. Hydorgen sulfide-induced hypometabolism prevents renal ischemia/reperfusion injury. J Am Soc Nephrol. 2009 20(9):1901-5.
15. Geng B, Chang L, Pan C, Qi Y, Zhao J, Pang Y, Du J, Tang C: Endogenous hydrogen sulfide regulation of myocardial injury induced by isoproterenol. Biochem Biophys Res Commun. 2004, 318(3):756-63.
16. S. A. Hosgood, M. L. Nicholson. Hydrogen sulphide ameliorates ischaemia-reperfusion injury in an experimental model of non-heart-beating donor kidney transplantation. Br J Surg. 2010. 97(2):202-9.
17. Jensen AR, Drucker NA, Khaneki s, Ferkowicz MJ, Markel TA: Hydrogen sulfide improves intestinal recovery following ischemia by endothelial nitric oxide-dependent mechanisms. Am J Physiol Gastrointest Liver Physiol. 2017, 312(5): G450–G456.

18. Li H, Zhang C, Sun W, Li L, Wu B, Bai S, Li H, Zhong X, Wang R, Wu L et al: Exogenous hydrogen sulfide restores cardioprotection of ischemic post-conditioning via inhibition of mPTP opening in the aging cardiomyocytes. Cell Biosci. 2015, 30;5:43.
19. Smriti Juriasingani, Masoud Akbari, Justin YH. Chan, Matthew Whiteman, Alp Sener. H2S supplementation: A novel method for successful organ preservation at subnormothermic temperatures. Nitric Oxide 2018. 1;81:57-66.
20. Dirkes MC, Milstein DMJ, Heger M, van Gulik TM. Absence of Hydrogen Sulfide-Induced Hypometabolism in Pigs: A Mechanistic Explanation in Relation to Small Nonhibernating Mammals. Eur Surg Res. 2015;54(3-4):178-91.
21. Philippe Haouzi, Véronique Notet, Bruno Chenuel, Bernard Chalon, Isabelle Sponne, Virginie Ogier, Bernard Bihain. H2S induced hypometabolism in mice is missing in sedated sheep. Respiratory Physiology & Neurobiology 2008. 160(1):109-15
22. Eline A.Q. Mooyaart, Egbert L.G. Gelderman, Maarten W. Nijsten, Ronald de Vos, J. Manfred Hirner, Dylan W. de Lange, Henri D.G. Leuvenink, Walter M. van den Bergh. Outcome after hydrogen sulphide intoxication. Resuscitation 2016. 103:1-6
23. Bon, Delphine ; Chatauret, Nicolas; Giraud, Sébastien ; Thuillier, Raphael; Favreau, Frédéric ; Hauet, Thierry. New strategies to optimize kidney recovery and preservation in transplantation. Nature Reviews Nephrology. 2012. 8(6):339-47
24. Hendriks, Koen D. W.; Lupi, Eleonora; Hardenberg, Maarten C.; Hoogstra-Berends, Femke; Deelman, Leo E.; Henning, Robert H. Differences in mitochondrial function and morphology during cooling and rewarming between hibernator and non-hibernator derived kidney epithelial cells. Scientific Reports, 2017. 7(1):1548
25. Debout A, Foucher Y, Trebern-Launay K, Legendre C, Kreis H, Mourad G, Garrigue V, Morelon E, Buron F, Rostaing L, Kamar N, Kessler M, Ladrière M, Poignas A, Blidi A, Soulillou JP, Giral M, and Dantan E. Each additional hour of cold ischemic time significantly increases the risk of graft failure and mortality following renal transplantation. Kidney Int 2015. 87: 343–349.
26. Meng G, Wang J, Xiao Y, Bai W, Xie L, Shan L, Moore PK, and Ji Y. GYY4137 protects against myocardial ischemia and reperfusion injury by attenuating oxidative stress and apoptosis in rats. J Biomed Res 2015. 29: 203–213.
27. I. Lobb, A. Mok, Z. Lan, W. Liu, B. Garcia, A. Sener. Supplemental hydrogen sulphide protects transplant kidney function and prolongs recipient survival after prolonged cold ischaemia-reperfusion injury by mitigating renal graft apoptosis and inflammation BJU Int, 2012. 110 E1187-E1195
28. Mitsuhiro Sekijima, Hisashi Sahara, Katsuyuki Miki, Vincenzo Villani, Yuichi Ariyoshi, Takehiro Iwanaga, Yusuke Tomita, Kazuhiko Yamada. Hydrogen sulfide prevents renal ischemia-reperfusion injury in CLAWN miniature swine. Journal of Surgical Research. 2017. 219:165-172
29. Shibuya N, Tanaka M, Yoshida M, Ogasawara Y, Togawa T, Ishii K, Kimura H: 3-Mercaptopyruvate Sulfurtransferase Produces Hydrogen Sulfide and Bound Sulfane Sulfur in the Brain. Antioxid. Redox Signal 2009. 11(4):703-14.
30. Teng H, Wu B, Zhao K, Yang G, Wu L, Wang R: Oxygen-sensitive mitochondrial accumulation of cystathionine β-synthase mediated by Lon protease. Proc Natl Acad Sci U S A. 2013, 31:12679-84.
31. Kashfi K and Olson KR. Biology and therapeutic potential of hydrogen sulfide and hydrogen sulfide-releasing chimeras. Biochem Pharmacol 2013. 85: 689–703.
32. Anzell, A. R., Maizy, R., Przyklenk, K. & Sanderson, T. H. Mitochondrial Quality Control and Disease: Insights into Ischemia-Reperfusion Injury. Mol. Neurobiol. 2017. 10.1007/s12035-017-0503-9
33. Snijder PM, de Boer RA, Bos EM, van den Born JC, Ruifrok WPT, Vreeswijk-Baudoin I, van Dijk MCRF, Hillebrands J, Leuvenink HGD, van Goor H. Gaseous Hydrogen Sulfide Protects against Myocardial Ischemia-Reperfusion Injury in Mice Partially Independent from Hypometabolism. PLoS One. 2013 8(5):e63291.

34. Zhao W, Zhang J, Lu Y, Wang R. The vasorelaxant effect of H(2)S as a novel endogenous gaseous K(ATP) channel opener. EMBO J. 2001 20(21):6008-16.
35. G. Yang, L. Wu, B. Jiang, W. Yang, J. Qi, K. Cao, et al.H2S as a physiologic vasorelaxant: hypertension in mice with deletion of cystathionine gamma-lyase. Science. 2008. 322 (5901) 587-590
36. Koning AM, Frenay AS, Leuvenink HGD, van Goor H. Hydrogen sulfide in renal physiology, disease and transplantation – The smell of renal protection. 2015. 46:37-49

ABBREVIATIONS

ASAT	aspartate aminotransferase
ATP	adenosine triphosphate
DCD	donation after cardiac death
ECD	extended criteria donors
ETC	electron transport chain
LDH	lactate dehydrogenase
MDA	malondialdehyde
PAS	periodic acid–Schiff
ROS	reactive oxygen species