Hydrogen Sulfide Treatment Mitigates Renal Allograft Ischemia-Reperfusion Injury during Cold Storage and Improves Early Transplant Kidney Function and Survival Following Allogeneic Renal Transplantation

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Abbreviations and Acronyms

Adamts1 = ADAM metallopeptidase with thrombospondin type 1

Atf3 = activating transcription factor 3

ATN = acute tubular necrosis

BN = Brown Norway

Cxcl = chemokine (C-X-C motif) ligand

Fg = fibrinogen

Ifn-γ = interferon-γ

IRI = ischemia-reperfusion injury

Kim-1 = kidney injury molecule 1

Myc = Myelocytomatosis oncogene

Ngal = neutrophil gelatinase-associated lipocalin

Olr1 = oxidized LDL receptor 1

POD = postoperative day

RTx = renal transplantation

SerpinE1 = serpin peptidase inhibitor clade

Timp1 = TIMP metalloproteinase inhibitor

UW = University of Wisconsin preservation solution

Purpose: Ischemia-reperfusion injury is unavoidable during organ transplantation. Prolonged ischemia-reperfusion injury is detrimental to short-term and long-term graft function and survival. H_2S is a recently characterized, endogenously produced gaseous molecule with important physiological roles that has been shown to be cytoprotective during tissue ischemia-reperfusion injury. The current study aimed to determine whether H_2S could mitigate cold renal ischemia-reperfusion injury in the clinically relevant context of allogeneic renal transplantation.

Materials and Methods: Following bilateral native nephrectomy Lewis rats underwent renal transplantation with kidneys from Brown Norway donor rats that were flushed with cold (4C) standard University of Wisconsin preservation solution (University of Wisconsin preservation solution group) or cold University of Wisconsin preservation solution plus 150 μM NaHS (H_2S group) solution. Kidneys were stored for 6 hours at 4C in the same solution. Recipient animals were monitored for 14 days or until sacrifice using metabolic cages to assess various parameters of renal graft function.

Results: H_2S treatment improved early allograft survival and function, and decreased early levels of necrosis, apoptosis and Kim-1 compared to University of Wisconsin preservation solution alone. H_2S treatment did not affect allograft rejection. Rather, it modulated the early allograft transcriptome to decrease the expression of renal injury, coagulation and cellular stress response genes, and increase the expression of cellular proliferation and Ifn-γ induced genes compared to University of Wisconsin preservation solution alone.

Conclusions: To our knowledge our findings are the first to show that H_2S protects donor kidneys against cold ischemia-reperfusion injury in the context of allogeneic renal transplantation. This potentially represents a novel cost-effective therapeutic solution to mitigate ischemia-reperfusion injury and improve the clinical outcomes of renal transplantation.

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ISCHEMIA-REPERFUSION injury is an unavoidable consequence of organ transplantation that is severely detrimental to renal graft function and survival.[1] Prolonged periods of cold ischemia during RTx have been widely associated with increased rates of delayed graft function and decreased long-term graft survival.[2,3] In addition, to keep up with growing transplant wait lists many transplant programs accept grafts with extended cold ischemic periods, thus, further contributing to overall tissue injury after reperfusion. Therefore, it is not surprising that IRI continues to be a major determining factor of the overall success of transplanted organs.

Gasotransmitters are a family of small, endogenously produced gaseous molecules with anti-inflammatory, antioxidant and anti-apoptotic effects that have led to recent investigation as novel therapy to mitigate tissue injury during IRI.[4] These molecules include nitric oxide, CO and most recently H_2S.[5] Hydrogen sulfide, which is endogenously produced by 3 major enzymes, including CSE (cystathionine-γ-lyase), CBS (cystathionine-β-synthase) and 3-MST (3-mercaptopyruvate sulfurtransferase), modulates many important physiological functions such as vasodilation and cellular signaling.[6] H_2S treatment has been shown to abrogate ischemia-reperfusion associated renal injury following warm and cold ischemia, and it has been identified as a potential therapy in improving RTx outcomes.[7,8]

We have previously reported that H_2S treatment drastically improved survival and function, and decreased graft injury and the expression of apoptotic and inflammatory markers during syngeneic RTx following prolonged cold organ storage.[9] While this study proved the protective capacity of H_2S against cold IRI associated with RTx, these findings have limited clinical value since the majority of human kidney transplants are allogeneic. Allogeneic transplantation is complicated by enhancement of innate and adaptive recipient immune response against allografts due to mismatched antigens present on donor tissue, resulting in increased graft injury compared to syngeneic transplantation.

Therefore, in the current study we investigated the potential of H_2S to mitigate renal IRI in a murine model of allogeneic RTx after cold organ storage. To our knowledge we report for the first time that H_2S treatment during cold storage protects allografts from IRI during allogeneic RTx, improving early allograft function and survival, limiting allograft injury and modulating the allograft transcriptome to promote allograft recovery and potential cellular regeneration.

MATERIALS AND METHODS

Experimental Animals

A total of 26 and 28 male BN and Lewis rats, respectively (Charles River Canada, Saint-Constant, Quebec, Canada), weighing 300 to 350 gm were maintained at University of Western Ontario according to standard conditions. We used the fewest number of animals in our study to firmly establish biological and statistical relevance in accordance with institutional animal use subcommittee regulations, which stress and enforce a reduce-reuse-recycle philosophy when performing animal studies.

Surgical Procedure and Postoperative Monitoring

Allogeneic renal transplantation was performed using the left kidney from BN rat donors and Lewis rat recipients, a model which elicits a robust recipient immune response against donor tissue. Rats were randomized to treatment groups, anesthetized with ketamine (30 mg/kg) and maintained under anesthesia with 1% isoflurane during surgery. Using aseptic techniques donor kidneys were procured and flushed with a 28 gauge Angiocath™ with cold (4C) UW as the UW group of 9 rats or cold UW plus H_2S donor molecule (150 μM NaHS, Sigma-Aldrich®) as the H_2S group of 7 until venous effluent was clear. Grafts were placed in 50 ml of the same perfusion solution and stored at 4C for 6 hours, representing a moderate level of clinical cold storage time.

Following bilateral nephrectomy recipient rats underwent RTx with donor kidneys, which were removed from cold storage and transplanted orthotopically in the left renal fossa using 10-zero Prolene® suture as previously described.[10] Five Lewis sham operated rats with a midline incision only were also followed to establish a baseline for survival, serum creatinine and allo-antibody binding analysis. Three BN sham operated rats were sacrificed at PODs 1 to 2 and used as a baseline for allograft histology and RNA microarray analysis.

After RTx rats were monitored in metabolic cages to assess urine output, water intake, urine and serum creatinine, and urinary physiological parameters for 14 days or until sacrifice. In an additional subset of animals the allograft was removed preemptively at PODs 1 to 2, including 2 UW and 4 H_2S group rats. These allografts were combined with those from survival analysis animals sacrificed at PODs 1 to 2 (3 UW rats) and PODs 6 to 9 (6 UW and H_2S rats each) for further analysis.

Half of the sagittally bivalved kidney was placed in formalin for histological analysis and the other half was stored at −80C for subsequent RNA microarray analysis. All surgeries were performed by the same microsurgeon. Operative time in the recipient was approximately 2 to 3 hours in both treatment groups. There was no difference in operative time between the treatment groups. Renal failure was assumed in animals sacrificed prematurely while showing elevated serum creatinine. At sacrifice surgical complications were ruled out as a cause of death.