

Editorial

Role of Nitric Oxide Synthase (NOS) During *Ex Vivo* Warm Kidney Perfusion Prior to Renal Transplantation

Kaye M. Reid and David A. Geller

Department of Surgery, Starzl Transplantation Institute,
University of Pittsburgh, Pittsburgh, PA 15213, USA
*Corresponding author: Kaye M. Reid, reidkm@
msx.upmc.edu

Received 10 February 2003, revised and accepted for
publication 18 February 2003

Introduction

Due to the shortage of available organ donors and the growing renal transplantation waiting list, most transplant centers are utilizing kidneys from an expanded donor pool that consists of older kidneys and those with longer cold ischemia times. In addition, some centers are re-addressing the issue of pumping kidneys (*ex vivo* hypothermic perfusion) to optimize marginal grafts as a technique to expand the donor pool. In the accompanying report by Brasile et al. (1), the investigators describe excellent function following long-term (48 h) *ex vivo* warm perfusion of canine autotransplanted kidneys.

The kidney perfusion method utilizes exsanguineous metabolic support (EMS) with a near-normothermic acellular solution. This system moves away from the more traditional hypothermic storage of a donated organ until the time of transplantation. The solution mimics a cell-culture-like system and contains essential nutrients necessary for metabolic function. In the current study, canine kidneys perfused via EMS (for 24 or 48 h) at near normothermic conditions (32 °C) had lower peak serum creatinine levels following autotransplantation compared to control grafts stored on ice with Viaspan (UW solution) or grafts receiving hypothermic perfusion with standard Belzer's Machine Perfusate. Even more interesting is the finding that discarded human kidneys, ViaSpan-preserved on ice for average 38 h and then warm-perfused for up to 48 h, maintained stable perfusion pressures, flow rates, and oxygen consumption. Further, urine flow was re-established in these kidneys and post-EMS histology showed no significant abnormalities.

To investigate the mechanism of preserved vascular integrity, the role of nitric oxide synthase (NOS) was examined

in the canine kidneys. In control long-term *ex vivo* warm-perfused kidneys, a noted rise in NO_3^- accumulation [Nitric Oxide (NO) end-product] was observed in the perfusate. The addition of the nonselective NOS inhibitor N^6 -nitro-L-arginine methyl ester (L-NAME) increased perfusion pressure and graft edema. When iNOS-specific inhibitors were used, a similar increase in graft edema was noted without changes in flow. Predictably, all the NOS inhibitors decreased NO_3^- levels. These results are noteworthy and suggest that NOS plays an important role in maintaining vascular integrity during *ex vivo* warm perfusion.

Since its presentation as molecule of the year in 1992, nitric oxide (NO) has held the attention of the research community. NO is synthesized from L-arginine by a family of three NOS isoforms. Two of the NOS enzymes are constitutively expressed in endothelial cells (eNOS) or neurons (nNOS), while the third is inducible (iNOS) following cytokine or inflammatory mediator activation. Numerous reports have shown a protective role for the L-arginine/NOS pathway during the ischemia/reperfusion (I/R) injury associated with renal, liver, and other organ transplants (2–5).

During I/R injury, there is decreased production of NO by the endothelium. Diminished NO production following graft reperfusion leads to microvascular constriction and localized reduction in blood flow. In addition, oxidative stress associated with I/R injury leads to free radical and chemoattractant production, causing neutrophil-endothelial cell damage and further vascular injury. The cytoprotective effects of NO in the early post-transplant setting involve tissue protection through regulation of vascular tone, inhibition of platelet aggregation, and neutralization of free radical injury. This has to be balanced with the potential cytotoxic effects of excessive NO leading to peroxynitrite and hydroxyl radical production that can contribute to the organ damage.

In the current work, Brasile et al. examined the role of NOS in *ex vivo* warm-perfused canine kidneys. The finding of significant NO_3^- accumulation in the perfusate implies maintenance of eNOS activity. An additional protective role for iNOS is suggested by the exacerbation of graft edema when iNOS-selective inhibitors were used. Direct detection of tissue eNOS/iNOS expression was not performed and would have strengthened the interpretation of the inhibitor studies. Moreover, a benefit from NO donors or L-arginine supplementation would also have corroborated the

findings. Nonetheless, all NOS inhibitors tested did worsen graft edema, consistent with a protective role for NOS in the vascular bed. Others have shown a biphasic role for NO in renal warm ischemia models (3). In another study, oral treatment with the NOS substrate L-arginine significantly hastened the return of serum creatinine to baseline (2).

Taken together, a cytoprotective role for NO in renal ischemia/reperfusion injury is supported. Overall, the results suggest that protective strategies optimizing the L-arginine/NOS pathway may prove to be useful to combat tissue damage following organ preservation. The potential benefit of warm *ex vivo* renal perfusion (with or without pharmacologic NOS manipulation) must be balanced with the significant expense and practical limitations inherent in warm *ex vivo* organ perfusion.

References

1. Brasile L, Stubenitsky BL, Booster MH, Haisch C, Kootstra G. NOS: The Underlying Mechanism Preserving Vascular Integrity and During *Ex Vivo* Warm Kidney Perfusion. *Am J Transplant* 2003; 3: 674–679.
2. Shoskes DA, Xie Y, Gonzalez-Cadavid NF. Nitric oxide synthase activity in renal ischemia–reperfusion injury in the rat: implications for renal transplantation. *Transplantation* 1997; 27: 495–500.
3. Weight SC, Furness PN, Nicholson ML. Biphasic role for nitric oxide in experimental renal warm ischaemia-reperfusion injury. *Br J Surg* 1999; 86: 1039–1046.
4. Yagnik GP, Takahashi Y, Tsoufas G, Reid K, Murase N, Geller DA. Blockade of the L-arginine/NO synthase pathway worsens hepatic apoptosis and liver transplant preservation injury. *Hepatology* 2002; 36: 573–581.
5. Shah V, Kamath PS. Nitric oxide in liver transplantation. *Pathobiology and clinical implications. Liver Transpl* 2003; 9: 1–11.