Clinical Evidence of the Association Between Serum Perioperative Changes in Xanthine Metabolizing Enzymes Activity and Early Post-transplant Kidney Allograft Function

Barbara Dołęgowska, PhD, Wojciech Błogowski, MD, Leszek Domański, MD, PhD

BACKGROUND: Xanthine oxidoreductase (XOR) and its active forms, dehydrogenase (XD) and oxidase (XO), act as double-edged swords during ischemia-reperfusion injury. On the one hand, their action generates antioxidants, such as uric acid (UA); however, they may strongly enhance production of free radicals. In this study, we examined the association between post-transplant graft function and perioperative xanthine metabolizing enzymes (XME) activity in kidney transplant recipients divided into early (EGF), slow (SGF), and delayed graft function (DGF) groups.

STUDY DESIGN: XME activity and UA levels were measured in blood samples collected directly before and during the first and fifth minutes of reperfusion.

RESULTS: Results demonstrated an increase in XO and XOR activity in all groups; however, these parameters were lower in the EGF than in the DGF group ($p < 0.005$; $p < 0.05$). XD activity increased in SGF and DGF patients ($p = 0.01$); nevertheless, the XD/total XOR coefficient decreased only in DGF individuals ($p = 0.0007$). XME sensitivity, specificity, and positive and negative predictive values in discriminating SGF/DGF from EGF were 73.3% to 78%, 54% to 62.5%, 76% to 78.6%, and 56.5%, respectively. Moreover, mixed model analysis revealed that recipients classified according to results of XOR(5) and XO(5) significantly differ in 1-year post-transplant allograft function ($p = 0.04$ and $p = 0.02$, respectively), but not in the frequency of acute rejection episodes ($p = 0.66$ and $p = 0.90$, respectively).

CONCLUSIONS: During renal transplantation, significant changes in XME occur that are associated with early post-transplant graft function and have potential value to discern between EGF and SGF/DGF.
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Renal ischemia/reperfusion injury (I/R) is a clinically significant problem and an invariable consequence of kidney transplantation; it plays a major role in delayed graft function (DGF) development, and may affect long-term allograft function. This problem begins at the onset of the ischemic phase, which leads to lower adenosine triphosphate (ATP) generation and to intensified adenosine catabolism (through hypoxanthine to xanthine), as well as to increased permeability and expression of adhesion molecules on activated endothelium, which attracts leukocytes and platelets to transmigrate into the vascular wall. In addition, adherent leukocytes intensively generate reactive oxygen species (ROS), which interact with cellular DNA and membrane components leading to DNA damage, lipid peroxidation, and finally, to cellular destruction.[1-5] Although researchers have already demonstrated increased ROS levels in the renal vein after ischemia/reperfusion, and the efficacy of antioxidants in minimizing the consequences of this process,[2,6] the precise origin of ROS in ischemic and reperfused kidney still remains to be elucidated.

One of the most important endogenous sources of ROS, during I/R injury, may be the activity of xanthine oxidoreductase (XOR), an enzyme that catalyses 2 final steps in the degradation of purines such as adenosine. XOR ex-

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From the Departments of Biochemistry and Medical Chemistry (Dołęgowska), Laboratory Diagnostics and Molecular Medicine (Błogowski), and Nephrology, Transplantation and Internal Medicine (Domański), Pomeranian Medical University, Szczecin, Poland.

Correspondence address: Wojciech Błogowski, Department of Laboratory Diagnostics and Molecular Medicine, Pomeranian Medical University, Al. Powstancow Wlkp 72, 70-111 Szczecin, Poland. email: drannab@wp.pl

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Abbreviations and Acronyms

AUC = area under the curve
DGF = delayed graft function
EGF = early graft function
I/R = ischemia/reperfusion injury
ROS = reactive oxygen species
SGF = slow graft function
UA = uric acid
XD = xanthine dehydrogenase
XME = xanthine metabolizing enzymes
XO = xanthine oxidase
XOR = xanthine oxidoreductase

ists in 2 main active irreversible forms: as xanthine dehydrogenase (XD), which predominates in healthy tissues, and as xanthine oxidase (XO), which appears to have an important role in cell and tissue injuries[7] (Fig.1). Interestingly, both XOR active forms act antagonistically, namely, XD favors NAD^+ as its primary electron acceptor, and catalyses the reaction in which uric acid (UA) is synthesized. UA is not only an anti-inflammatory effector with numerous protective roles in the body (including in the kidney), but it also acts as a cellular and systemic antioxidant and free radical scavenger.[8] On the contrary, XO is unable to bind NAD^+, and uses O_2 in a reaction associated with intensified synthesis of ROS and reactive nitrogen species, which are important second messengers that accelerate the inflammatory response via activation of the complement system or modulation of endothelial cell surface P-selectin expression.[9,10]

XOR itself may act as a double-edged sword; in the presence of NAD^+ as a dehydrogenase, and with O_2 as an oxidase. XOR's ability to rapidly convert from an antioxidative to a pro-oxidative form, under various kinds of tissue injury and damage, makes it an ideal component for a fast innate immunity response, which, in some situations, eg, bacterial or fungal infections, is beneficial.[7,11] However, in kidney I/R injury, especially during the reperfusion phase, when high XO's expression with its substrate – oxygen coincidence, this property of XOR should rather be considered disadvantageous.

Several authors have already highlighted the significant role of active XOR forms in liver I/R injury.[12] Unfortunately, the influence of XOR activity has never been determined in human renal transplantations. Therefore, in this study, we examined the dynamics of XD, XO, XOR, and UA changes, which occur during the first 5 minutes of kidney allograft reperfusion in humans, and we correlated our findings with patients' early clinical outcomes. Our hypothesis was that early graft function may be determined by changes in xanthine metabolizing enzymes (XME) activity, which occurs during the first 5 minutes of reperfusion.

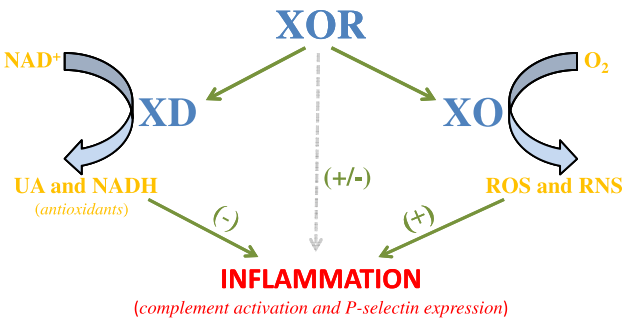

Figure 1. The influence of xanthine oxidoreductase's isoforms, dehydrogenase and oxidase, on modulation of the inflammatory process. NAD, nicotinamide adenine dinucleotide; RNS, reactive nitrogen species; ROS, reactive oxygen species; UA, uric acid; XD, xanthine dehydrogenase; XO, xanthine oxidase; XOR, xanthine oxidoreductase.

METHODS

This study included 69 consecutive recipients who received transplants in our center. They were retrospectively divided into 3 groups depending on graft reactivation: early (immediate), slow, and delayed graft function (EGF, SGF, and DGF, respectively), according to previously described criteria.[13-15] General characteristics of donors and recipients are summarized in Table 1. Patients with immediate allograft activation, defined by serum creatinine levels below 3 mg/dL on the fifth postoperative day, were assigned to the EGF group. Individuals with graft reactivation problems were divided into SGF (creatinine level higher than 3 mg/dL on the fifth postoperative day, but no dialysis treatment required) and DGF (needing dialysis in the first week after transplantation) groups. All patients received standard immunosuppressive protocols with triple drug therapy including cyclosporine A, azathioprine, and steroids. A 5 mg/kg dose of cyclosporine was administered orally on the day of transplantation. From the second day on, patients received 10 mg/kg of cyclosporine per day. In case of allograft reactivation problems, this dose was reduced by 50%. During follow-up, cyclosporine doses were adjusted to achieve troughs of 200 to 250 ng/mL for first 3 to 6 months, with maintenance levels of 120 to 180 ng/mL afterwards. The dose of prednisolone was gradually reduced to 5 mg/day over 3 to 6 months. Acute rejection episodes were defined using Banff criteria and were biopsy proven. Renal allografts were received from patients who died from craniocerebral trauma (n = 25), subarachnoid hemorrhage (n = 11), intracerebral hemorrhage (n = 19), stroke (n = 5), brain tumor (n = 4), neuroinfection (n = 2), or suicide (n = 3). Allocation of donor kidneys to