ORIGINAL ARTICLE

Inhibition of coagulation proteases Xa and IIa decreases ischemia–reperfusion injuries in a preclinical renal transplantation model

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Coagulation is an important pathway in the pathophysiology of ischemia–reperfusion injuries. In particular, deceased after circulatory death (DCD) donors undergo a no-flow period, a strong activator of coagulation. Hence, therapies influencing the coagulation cascade must be developed. We evaluated the effect of a new highly specific and effective anti-Xa/IIa molecule, with an integrated innovative antidote site (EP217609), in a porcine preclinical model mimicking injuries observed in DCD donor kidney transplantation. Kidneys were clamped for 60 minutes (warm ischemia), then flushed and preserved for 24 hours at 4°C in University of Wisconsin (UW) solution (supplemented or not). EP217609-supplemented UW solution (UW-EP), compared with unfractionated heparin-supplemented UW solution (UW-UFH) or UW alone (UW). A mechanistic investigation was conducted in vitro: addition of EP217609 to endothelial cells during hypoxia at 4°C in the UW solution inhibited thrombin generation during reoxygenation at 37°C in human plasma and reduced tumor necrosis factor alpha, intercellular adhesion molecule 1, and vascular cell adhesion molecule 1 messenger RNA cell expressions. In vivo, function recovery was markedly improved in the UW-EP group. Interestingly, levels of thrombin–antithrombin complexes (reflecting thrombin generation) were reduced 60 minutes after reperfusion in the UW-EP group. In addition, 3 months after transplantation, lower fibrosis, epithelial–mesenchymal transition, inflammation, and leukocyte infiltration were observed. Using this new dual anticoagulant, anti-Xa/IIa activity during kidney flush and preservation is protected by reducing thrombin generation at revascularization, improving early function recovery, and decreasing chronic lesions. Such an easy-to-deploy clinical strategy could improve marginal graft outcome. (Translational Research 2016;■:1–12)

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Abbreviations: α-SMA = α-smooth muscle actin; DCD = donors deceased after cardiac death; EMT = epithelial–mesenchymal transition; FBS = fetal bovine serum; GAPDH = glyceraldehyde 3-phosphate dehydrogenase; HAEC = human aortic endothelial cell; HES = hematoxylin–eosin–saffron; ICAM-1 = intercellular adhesion molecule 1; IL = interleukin; iNOS = inducible nitric oxide synthase; IR = ischemia–reperfusion; IRIs = ischemia–reperfusion injuries; MCP-1 = monocyte chemoattractant protein 1; MMP-2 = matrix metalloproteinase 2; NEP = uninephrectomized; PAI-1 = plasminogen activator inhibitor type-1; PAR = protease-activated receptor; SWC3a = clone anti-swine monocytes/granulocytes; TAT = thrombin–antithrombin; TF = tissue factor; TGF-β = transforming growth factor β; TNF-α = tumor necrosis factor alpha; UFH = unfractionated heparin; UW = University of Wisconsin; VCAM-1 = vascular cell adhesion molecule 1

AT A GLANCE COMMENTARY

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Background

The increasing use of marginal donors highlights the importance of organ quality in transplantation. Renal ischemia–reperfusion injury, which includes a deleterious activation of coagulation, plays a central role in determining graft quality and outcome.

Translational Significance

Using an established porcine renal autotransplantation preclinical model with a high clinical relevance, the benefits of a per-preservation anticoagulation therapy using a new highly specific and effective dual-molecule anti-Xa/IIa were evaluated. This study demonstrates the therapeutic benefits of anticoagulation against the 2 major coagulation proteases at the time of organ washing and preservation.

INTRODUCTION

Transplantation is currently the best therapy for end-stage renal disease. However, this success has led to an organ shortage crisis, with only a quarter of patients on waiting lists having access to organ transplantation. This crisis changed donor demography, toward an increased use of organs from marginal donors and from deceased after circulatory death (DCD) donors, with significant comorbidity factors.[1] These organs are more sensitive to the ischemia–reperfusion injury (IRI), which translates to a negative influence on graft dysfunctions. Indeed, kidneys from marginal or DCD donors are associated with higher levels of delayed graft function and primary nonfunction.[2] This new donor demography imposes the community to investigate methods to improve graft preservation quality. In the present study, we focus on one critical process in IRI: the coagulation cascade.[3] Coagulation takes place in the vascular compartment, the first target of IRI, increasing lesions such as endothelial activation and inflammation.

During organ collection, the coagulation cascade is strongly activated.[3] This coagulation activation acts during organ flush (washing time after collection), somewhat less during cold storage, and mostly during the revascularization time. IRI induces vascular expression of tissue factor (TF)[4] and phosphatidylserine exposure at the outer leaflet of a cell membrane.[5] After damage to blood vessels, factor VII moves into contact with TF forming an activated complex (TF-VIIa), which activates factor X to Xa, and Xa ultimately activates factor II (prothrombin) to IIa (thrombin). The generation and signaling of Xa and IIa result in important thrombosis and inflammation.[6] Indeed, Xa and IIa cleave the protease-activated receptors (PARs), which subsequently induce the production of vascular adhesion, proinflammatory, and profibrotic molecules.[7] It was demonstrated that PAR-1 and PAR-2, activated by coagulation proteases, play a crucial role in IRI[8,9] in association with fibrosis, tissue remodeling, and inflammation.[10,11]

As a consequence, the coagulation cascade is a major target to limit IRI. Currently, no inhibitors of the coagulation proteases are used in clinical settings during graft flush and preservation. However, the results of our laboratory previously demonstrated that the use of anti-IIa (melagatran) or anti-Xa (fondaparinux) molecules during graft preservation could limit early lesions and chronic lesions of transplanted kidneys.[12-14] In addition, another group showed that fondaparinux reduces IRI-induced expression of proinflammatory cytokines and chemokines and neutrophil invasion in a mice kidney ischemia–reperfusion (IR) model.[15] We also demonstrated that the use of unfractionated heparin (UFH), a nonsynthetic anticoagulant that exhibits pharmacokinetic and biological limitations including induction of thrombocytopenia,[16] showed less benefits compared with more specific melagatran and fondaparinux.[12-14]

In view of our previous findings, it appeared judicious to evaluate the impact of EP217609, an innovative powerful dual-action anticoagulant targeting 2 protease factors IIa and Xa. EP217609 is a new anticoagulant combining, in one molecular construct, an analog of fondaparinux (anti-Xa) and an Nα-(2-naphthyl-sulphonyl-glycyl)-DL-p-amidinophenylalanyl-piperidine (α-NAPAP) derivative with direct thrombin