Cryobiology 70 (2015) 71–75

Contents lists available at ScienceDirect

Cryobiology

journal homepage: www.elsevier.com/locate/ycryo

Ex vivo use of a Rho-kinase inhibitor during renal preservation improves graft function upon reperfusion ☆,☆☆

Carmen Kirchner [a], Bastian Lüer [b], Patrik Efferz [b], Jeremias Wohlschlaeger [c], Andreas Paul [a], Thomas Minor [b,*]

[a] Clinic of General, Visceral and Transplantation Surgery, University Hospital of Essen, Germany
[b] Surgical Research Division, University Clinic of Surgery, Bonn, Germany
[c] Dpt. for Pathology, University Hospital of Essen, Germany

ARTICLE INFO

Article history:
Received 23 October 2014
Accepted 22 December 2014
Available online 31 December 2014

Keywords:
Renovasculature
Rho-kinase
Preservation
Kidney perfusion

ABSTRACT

Background: Activation of the Rho–Rho-kinase pathway has been shown to cause vasoconstriction in renal afferent arterioles. Vascular dysfunction plays a pivotal role in triggering reperfusion injury after kidney transplantation. Therefore, the effect of a Rho-kinase inhibitor, added to the preservation solution, on renal function after 18 h of storage at 4 °C was evaluated.
Methods: Porcine kidneys were preserved with cold HTK-solution. During preservation, in the study group, HTK was supplemented with the Rho-kinase inhibitor HA1077, whereas the control group received no further treatment ($n = 6$, respectively). Kidney function after 18 h of storage at 4 °C was evaluated by 90 min of isolated reperfusion *in vitro*.
Results: Rho-kinase inhibition (RKI) was associated with significantly higher renal perfusate flow compared to the control group. Endothelial function, as measured by perfusate levels of nitric oxide and gene expression of eNOS, was significantly increased in the study group. In our model, RKI also significantly improved glomerular function (clearance of creatinine) as well as tubular cell integrity as reflected by reduced fractional sodium excretion and release of fatty acid binding protein, a specific tubular cell marker.
Conclusion: Our results indicate that blocking the Rho-kinase pathway during cold preservation may lead to a better graft function upon reperfusion.

© 2014 Elsevier Inc. All rights reserved.

Introduction

Optimal organ preservation plays a crucial role in performing a successful organ transplantation. In this context ischemic reperfusion injury (IRI) represents one of the main risk factors for acute kidney failure after kidney transplantation as well as long-term graft function. One of the patho-mechanisms responsible for postischemic renal dysfunction has been recognized in an inadequate vasoconstrictive state of afferent vessels supplying glomeruli and tubular system [11].

Besides its negative effect on renal filtration rate, reduced renal perfusion may also favor secondary tissue hypoxia and mitochondrial failure [1,2,11]. Ongoing postischemic vasoconstriction thus increases the risk for acute tubular necrosis (ATN) and functional failure of the graft [21]. The balance between renal vasoconstriction and dilation is, amongst others, regulated by G-protein mediated pathways [17]. The activation of the GTPase Rho, targeting the Rho-kinase (ROCK) is known to be involved in inducing vasoconstriction via regulating the contractility of smooth muscle cells [6]. Interestingly, Rho GTPases are upregulated during ischemia reperfusion injury, suggesting an important impact on vascular dysfunction on the level of actin cytoskeletal reorganization involving cell motility and apoptosis [5].

Activation of Rho-kinase results in an inactivation of the myosin light chain phosphatase. This enzyme together with its counterpart myosin light chain kinase is responsible for a balance

Abbreviations: Ccl K18, caspase cleaved keratin 18; eNOS, endothelial nitric oxide synthase; FE Na, fractional excretion of sodium; KLF-2, Krüppel-like factor 2; LFABP, liver type fatty acid binding protein; MLC, myosin light chain; RPL19, ribosomal protein L19; TNF, tumour necrosis factor.

☆ *Statement of funding:* This work was supported by Institutional Funds.
☆☆ Parts of this paper have been presented at the 22nd congress of the German Transplantation Society, October 2013 in Frankfurt.

* Corresponding author at: Surgical Research Division, University Clinic of Surgery, 53127 Bonn, Germany. Fax: +49 228 287 15199.

E-mail addresses: Carmen.Kirchner@uk-essen.de (C. Kirchner), b.l.1982@gmx.de (B. Lüer), Patrik.Efferz@ukb.uni-bonn.de (P. Efferz), Jeremias.wohlschlaeger@uk-essen.de (J. Wohlschlaeger), Andreas.Paul@uk-essen.de (A. Paul), minor@uni-bonn.de (T. Minor).

http://dx.doi.org/10.1016/j.cryobiol.2014.12.008
0011-2240/© 2014 Elsevier Inc. All rights reserved.

of phosphorylation and dephosphorylation of the regulatory light chain of myosin II (MLC20) [20]. While increased phosphorylation of MLC20 represents a crucial step in creating vasospasm [6], other effects of the Rho/Rho-kinase pathway include a central role in the pathogenesis of endothelial dysfunction and inflammatory processes [12].

Therefore, the aim of our study was to investigate the putatively beneficial effects of an *ex vivo* blocking of the Rho-kinase pathway in the pretransplant setting during organ preservation in the porcine kidney.

Materials/methods

All experiments were performed with approval of the respective authorities (Landesamt für Natur, Umwelt und Verbraucherschutz Nordrhein Westfalen) and controlled by the animal welfare officers of the University of Bonn.

Porcine kidneys were procured from German Landrace pigs weighing between 25 and 30 kg. Premedication was performed with ketamine (90 mg/kg), xylazine (10 mg/kg) and atropine (10 μg/kg) 10 min before inducing anesthesia. After administration of midazolam (0.5 mg/kg), pancuronium (0.2 mg/kg) and fentanyl (12.5 μg/kg) intravenously via the ear vein, an intravenous jugular vein catheter was inserted for continuous infusion of fentanyl (0.004 mg/kg/h) and propofol (3 mg/kg/h) to maintain general anaesthesia. After intubation, mechanical ventilation was implemented with an air/oxygen mixture (70/30%) and adjusted to achieve an end-expiratory CO_2 concentration of 38–42 mm Hg.

After nephrectomy the procured organ was flushed on the back table with approx. 100 ml HTK solution at 4 °C using gravity perfusion (100 cm H_2O). In the control group ($n = 6$) HTK solution was used without additives.

In the study group ($n = 6$) the Rho-kinase inhibitor HA 1077 (Fasudil, Sigma–Aldrich Chemicals, St. Louis, MO) was added to the flushout solution at a concentration of 20 μM). The drug is water soluble and could easily be dissolved in the preservation solution.

All kidneys were subsequently cold stored for 18 h at 4 °C.

Reperfusion model

Isolated kidney perfusion *in vitro* was performed using an established model according to earlier studies with some modifications [13].

Thus, the perfusion medium consisted of 1000 ml freshly prepared Krebs–Henseleit buffer containing 2.2% bovine serum albumin and 20 ml of concentrated amino acid solution (RPMI 1640–50×). Creatinine (0.01 g/l) was added to the perfusate to allow for calculation of respective renal clearances. The ureter had been cannulated with PE-tubing and urine was collected throughout the reperfusion period.

Kidneys were placed in a moist temperature chamber and perfused at 37 °C. Perfusate was oxygenated in a temperature controlled hollow fiber oxygenator (Hilite LT 1000, Medos, Stolberg, Germany) with a mixture of 95% oxygen and 5% carbon dioxide. Temperature was regulated by a circulating thermostat, connected to perfusion chamber and oxygenator.

Kidney perfusion pressure was set at 90 mmHg and automatically maintained by servocontrolled roller-pump, connected to a pressure sensor placed in the inflow line immediately prior to the renal artery.

Immediately prior to reperfusion, all organs were exposed to no flow conditions at room temperature for 20 min in order to imitate warm ischemia time in the clinical setting.

Perfusate concentrations of caspase cleaved keratin 18 were assessed photometrically as indicator of renal apoptosis upon reperfusion [7] using a commercialized ELISA-kit (M30, Previa, Stockholm, Sweden).

Concentrations of creatinine were determined in perfusate and corresponding urine samples in a routine fashion at the laboratory centre of the University Hospital Bonn.

Clearances for creatinine were calculated for the respective intervals as urinary creatinine × urine flow (ml/min)/perfusate creatinine.

Total RNA was isolated from snap frozen samples using TRIreagent (Applied Biosystems, Darmstadt). Equal amounts of RNA were quantified by Nano Drop (Thermo Fisher) complementary DNA by incubation with High Capacity cDNA RT Kit (Applied Biosystems, Darmstadt). The PCR reaction mix was prepared by using TaqMan GenEx Master Mix (Applied Biosystems). The amount of specific mRNA in the tissue was expressed in arbitrary units after normalization for the respective individual quantities of transcripts of ribosomal protein L19 (RPL19), which was analyzed as house-keeping gene.

Primers for RPL19 (n°Ss03375624_g1) and endothelial nitric oxide synthase – eNOS (n°Ss03383940_u1) were purchased from Applied Biosystems.

Sequences of the PCR primers for Kruppel-like Factor 2 (KLF-2), customized by Applied Biosystems (custom TaqMan Gene Expression Assay, Part Number 4331348), were as follows: sense GCGCTGGGCTTGGC and antisense GCGGCGTGAGGAGACC.

Analytic kits from the following companies were used according to the instructions of the manufacturers to analyse perfusate levels of liver type fatty acid binding protein – LFABP (USCN life science, Wuhan, China), Interleukin 6, TNF alpha and total nitric oxide (NO) (R&D Systems, Wiesbaden, Germany).

Measurements were done on a fluorescence micro plate reader (Tecan, Grailsheim, Germany).

Renal injury was scored by an experienced pathologist blinded for the groups. Assessment was carried out following Torras et al. [18]: In each slide, 10 visual fields were investigated assessing 6 morphological parameters indicating renal parenchyma injury (tubular dilatation, epithelial vacuolization, epithelial shedding, epithelial necrosis, interstitial edema and inflammation). A 5-point scale was applied for each parameter: 0 = no damage; 1 = lesions affecting < 10% of the field; 2 = 10–25%; 3 = 25–50%; 4 = 50–75% and 5 > 75%.

Statistics

Data are presented as means (±SEM). Differences between groups were tested by unpaired, 2-sided *t*-tests, unless otherwise indicated.

In all tests, an a priori α-error $p < 0.05$ was considered statistically significant.

Results

Vascular perfusion

Renal perfusate flow upon pressure constant reperfusion was significantly different in both groups. While cold preservation resulted in an early decline of renal perfusion to about 120 ml/min, normal postischemic flow pattern seen after Rho-kinase inhibition by HA1077, showing a progressive rise upon early reperfusion and ending up at stable flow values of approximately 250 ml/min after 60 min (Fig. 1).

The molecular expressions of selected genes pertinent to vascular function are depicted in table 1.

It was found that inhibition of Rho-kinase during preservation resulted in a significantly better preservation of renal eNOS

Download English Version:

https://daneshyari.com/en/article/2168396

Download Persian Version:

https://daneshyari.com/article/2168396

Daneshyari.com