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Thromboxane receptor signalling in renal ischemia reperfusion injury

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Abstract

F_2-isoprostanes are formed by oxidative modification of arachidonic acid and are the gold standard for detection of oxidative stress *in vivo*. F_2-isoprostanes are biologically active compounds that signal through the thromboxane A_2 (TP) receptor; infusion of F_2-isoprostanes reduces glomerular filtration in the kidney by constricting afferent arterioles. This study investigated whether endogenous F_2-isoprostanes contribute to the pathogenesis of ischemic acute kidney injury, which is associated with oxidative stress and reduced glomerular filtration. TP receptor knockout mice—that lack F_2-isoprostanes and thromboxane A_2 signalling—and wild-type control mice underwent 30 min of renal ischemia and 24 h of reperfusion. Kidney dysfunction, histological injury and the number of infiltrated neutrophils were similar between the two mouse strains, whereas TP receptor knockout mice had significantly more apoptotic cells and tissue lipid peroxidation than their wild-type counterparts. F_2-isoprostanes and thromboxane B_2 were readily detectable in urine collections after surgery. The findings indicate that F_2-isoprostanes and thromboxane A_2 signalling do not contribute critically to the pathogenesis of ischemic acute kidney injury and more generally provide evidence against a prominent role for F_2-isoprostanes signalling in exacerbating acute disease states associated with oxidative stress.

Keywords: *Acute kidney injury, F_2-isoprostanes, ischemia reperfusion injury, thromboxane A_2, TP receptor*

Introduction

Acute kidney injury is a frequent complication after ischemia and reperfusion due to cardiovascular surgery and haemorrhagic shock and is independently associated with increased in-hospital mortality [1]. In renal transplantation, acute kidney injury results in delayed graft function which is associated with an increased incidence of acute rejection and graft loss [2]. At reperfusion, re-introduction of oxygen to the ischemic kidney rapidly leads to the formation of reactive oxygen species that may damage cellular lipids, proteins and DNA when anti-oxidant defences have been exhausted. The resulting oxidative stress is an important cause of acute kidney injury, since administration of various anti-oxidants has been shown to attenuate renal dysfunction and histological injury in animal models of renal ischemia and reperfusion [3–5].

F_2-isoprostanes are formed by oxidative modification of arachidonic acid in cell membranes. They are cleaved and released into the plasma by platelet-activating factor acetylhydrolase [6,7]. Measurement of plasma and urinary F_2-isoprostanes has become accepted as the gold standard for detection of oxidative stress *in vivo* [8] and their production has been demonstrated in a rat model of renal ischemia and reperfusion [9]. Interestingly, next to being accurate biomarkers of oxidative stress, F_2-isoprostanes are biologically active compounds that signal through the thromboxane A_2 (TP) receptor [10]. Intra-arterial administration of F_2-isoprostanes to rats and pigs results in dose-dependent reductions in renal blood

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flow and glomerular filtration rate due to vasoconstriction of afferent glomerular arterioles [9,11]. This renal vasoconstriction has also been observed in human acute kidney injury [12].

Taken together, the vasoconstrictive properties of locally produced F_2-isoprostanes may contribute to the reduction of glomerular filtration rate in ischemic acute kidney injury and may explain the protective effects of anti-oxidants in animal models of renal ischemia and reperfusion. To address this hypothesis, we studied the effects of genetic disruption of the TP receptor—which eliminates F_2-isoprostanes signalling [13]—in a mouse model of renal ischemia and reperfusion.

Materials and methods

Renal ischemia and reperfusion in mice

Homozygous TP receptor knockout breeding pairs (backcrossed on C57BL/6J mice for 13 generations) were used [14]. Wild-type C57BL/6J mice were obtained from Charles River (L'arbresle Cedex, France). Animals were housed in standard laboratory cages with free access to food and water. In random order, male wild-type and TP receptor knockout mice aged 9–11 weeks were anaesthetized with ketamine and xylazine (100 and 10 mg/kg s.c., respectively). Body temperature was maintained at 37°C by a heating lamp until the animals had recovered from anaesthesia. Ischemia was induced by clamping the left renal pedicle for 30 min using a non-traumatic vascular clamp through a midline abdominal incision. The surgeon was blinded for mouse genotype. Upon clamp removal, the kidney was inspected for restoration of blood flow and the contralateral kidney was excised. The abdomen was closed in two layers, 0.25% bupivacaine was applied topically for post-operative pain management and 1 mL pre-warmed PBS was given subcutaneously to prevent dehydration. Animals were euthanized at 24 h after reperfusion, when blood was collected by puncture of the vena cava. The left kidney was recovered for further analysis. Blood was stored on ice in heparanized tubes until centrifugation at 900 g at 4°C for 10 min. Plasma creatinine and blood urea nitrogen (BUN) concentrations were measured. In a separate set of experiments, the contralateral kidney was left *in situ* and the animals were housed in metabolic cages to collect urine for 24 h before and after surgery.

Histological assessment of renal tissue

Renal tissue was formalin-fixed and stained with periodic acid-Schiff for blinded assessment of renal injury by an experienced nephropathologist. Tubular dilation, casts and debris, brush border loss and necrosis at the corticomedullary junction were scored in 10 fields at 400 × magnification on a scale of 0–5 (0 is normal; 1 involves < 10% of cortex; 2 involves 10–25% of cortex; 3 involves 25–50% of cortex; 4 involves 50–75% of cortex; and 5 involves > 75% of cortex) [15].

For detection of neutrophils, tissue sections were incubated with polyclonal rabbit anti-human myeloperoxidase (MPO) antibodies (1:50 dilution; Hycult Biotech, Uden, The Netherlands) for 2 h and with biotinylated polyclonal swine anti-rabbit IgG antibodies (1:500 dilution; Dako, Glostrup, Denmark) for 30 min. Endogenous peroxidase activity and non-specific binding were blocked with 0.6% H_2O_2 and 10% normal pig serum. After incubation with streptavidin and horseradish peroxidase conjugated biotin (Dako), slides were developed with H_2O_2 and AEC and were counterstained with haematoxylin. The number of positive cells at the corticomedullary junction were counted in 10 fields at 400 × magnification by a blinded investigator.

Apoptotic cells were detected by terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick-end labelling (TUNEL; *in situ* cell death detection kit; Roche Diagnostics, Indianapolis, IN) according to the manufacturer's instructions [16]. Before labelling, nuclei were permeabilized by incubating the tissue sections with 300 μg/mL proteinase K (Sigma-Aldrich, St. Louis, MO) for 15 min. Endogenous peroxidase activity and non-specific binding were blocked with 0.6% H_2O_2 and 3% bovine serum albumin. The slides were developed with H_2O_2 and AEC and the number of positive nuclei at the corticomedullary junction were counted in 10 fields at 400 × magnification by a blinded investigator.

TP receptor genotyping

Genomic DNA was isolated from renal tissue according to the manufacturer's instructions (Wizard genomic DNA purification kit; Promega, Madison, WI). DNA was amplified using the primers 5'-GGGGGTAGCTATGGTGTTC-3' (for the wild-type allele), 5'-CTTCCTCGTGCTTTACGGTA-3' (for the mutant allele with PGK-neomycin insert) and 5'-GTGAGAAGGGCCGTGTGAT-3' (for both alleles) in 30 cycles at 94°C, 60°C and 72°C (60 s each). The PCR product was run on a 1.2% agarose gel with a DNA basepair ladder. The predicted amplicon sizes from the wild-type and mutant alleles were 150 and 700 base pairs, respectively.

Measurement of urine thromboxane B_2 and F_2-isoprostanes

Urine was collected on ice for 24 h before and after surgery, centrifuged at 900 g at 4°C for 10 min and stored at −80°C. Urinary thromboxane B_2 and F_2-isoprostanes concentrations were measured using commercially available enzyme immunoassays (Cayman

Chemical, Ann Arbor, MI) according to the manufacturer's instructions and were adjusted for urinary creatinine concentrations.

Measurement of tissue malondialdehyde

Malondialdehyde (MDA) was measured in homogenized renal tissue that was sampled and snap-frozen at 24 h after reperfusion and stored at −80°C until analysis. A 100 µL sample was added to 900 µL reagent (0.12 M 2-thiobarbituric acid, 0.32 M *O*-phosphoric acid, 0.68 mM butylated hydroxytoluene, 0.01% EDTA) and incubated for 1 h at 100°C. MDA products were extracted with 500 µL butanol and their concentrations were determined by HPLC using a Nucleosil C18 column (150 × 3.2 mm, 5 µm particle size; Supelco) eluted with 35% methanol in 25 mM phosphate buffer (pH 4.8). Detection was done by fluorescence using excitation at 532 nm and emission at 553 nm. Malonaldehyde bis(diethylacetal) was used as internal standard.

Laboratory animal use and ethical considerations

Assuming a standard deviation of plasma creatinine of 15% of the mean, 10 mice per experimental group are necessary to detect a 20% difference between groups with statistical significance; we consider this difference to be biologically relevant. *In vivo* experiments were approved by local laboratory animal and national genetically modified organism review boards (DEC 2007-139 and GGO IG 07-110).

Statistical methods

Continuous variables are presented as means and standard deviations. Independent samples *t*-tests were used to compare continuous variables between experimental groups. For comparisons of continuous variables within experimental groups, paired samples *t*-test were used. $p < 0.05$ was considered statistically significant.

Results

Effect of TP receptor genotype on renal ischemia and reperfusion injury

Homozygous TP receptor knockout mice and wild-type controls were randomly subjected to 30 min of renal ischemia ($n = 10$ per group) or sham surgery ($n = 5$ per group). No complications occurred during surgery and all animals survived until euthanasia at 24 h after reperfusion. Mean body weight of TP receptor knockout and wild-type mice was 24 ± 0.8 and 24 ± 1.2 g, respectively, before surgery and 22 ± 1.5 and 23 ± 1.0 g at euthanasia. TP receptor genotype of each animal was confirmed by PCR of genomic DNA (data not shown).

Temporary renal ischemia induced severe kidney dysfunction, as indicated by increased plasma concentrations of creatinine and BUN at 24 h after reperfusion (Figure 1). TP receptor knockout and wild-type control mice experienced a similar extent of renal dysfunction after ischemia and reperfusion ($p = 0.73$ for creatinine and $p = 0.19$ for BUN). Furthermore, substantial renal injury was observed on histological assessment of tissue sections after ischemia and reperfusion, indicated by tubular dilation, cast deposition, brush border loss and necrosis at the corticomedullary junction (Figure 2). In line with its effect on renal function, disruption of the TP receptor gene did not significantly reduce the extent of tissue injury after ischemia and reperfusion ($p = 0.58$ for tubular dilation, $p = 0.06$ for cast deposition, $p = 0.32$ for brush border loss and $p = 0.06$ for necrosis).

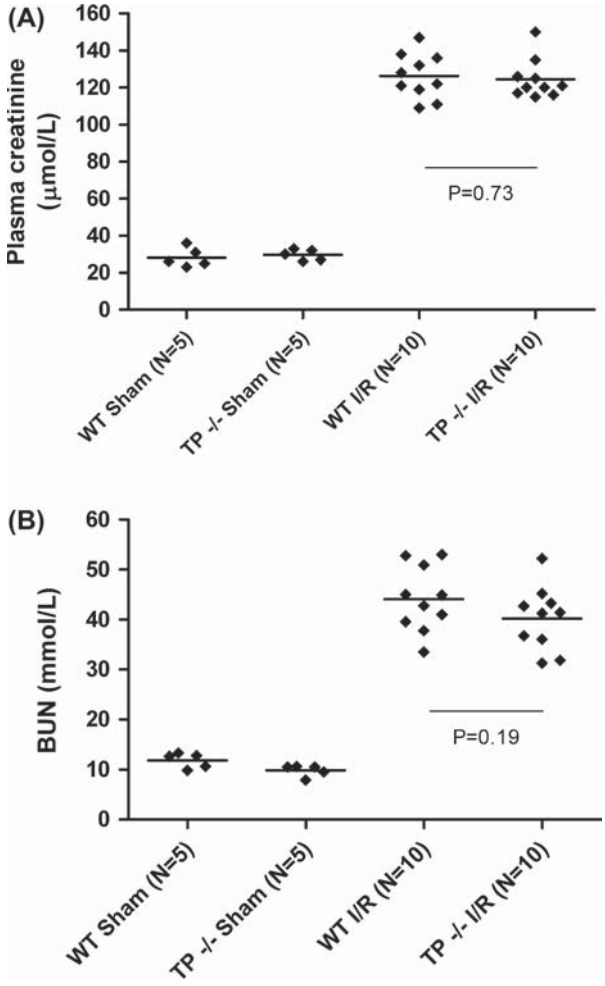

Figure 1. Kidney function is impaired to a similar extent in TP receptor knockout (TP -/-) and wild-type control mice (WT) after 30 min of renal ischemia and 24 h of reperfusion (I/R). (A) Plasma creatinine concentration. (B) Blood urea nitrogen (BUN) concentration. Diamonds and lines represent individual mice and group means, respectively.

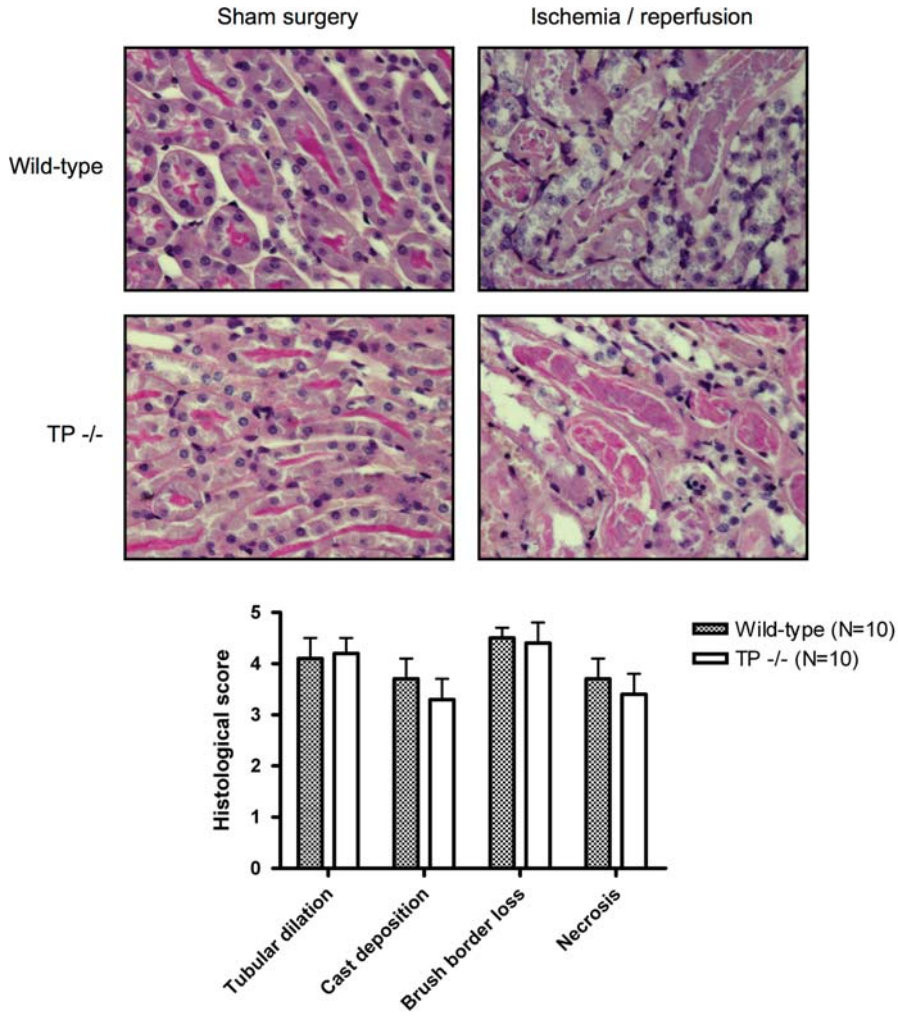

Figure 2. TP receptor knockout (TP -/-) and wild-type control mice experience a similar extent of renal injury after 30 min of renal ischemia and 24 h of reperfusion. Representative pictures of periodic acid Schiff stained tissue sections at 400 × magnification are shown. Tubular dilation, casts deposition, brush border loss and necrosis were blindly scored by an experienced nephropathologist on a scale of 0–5 [15]. Data are presented as means and standard deviations.

Effect of TP receptor genotype on neutrophil infiltration, apoptosis and lipid peroxidation

Tissue injury activates innate immunity and leads to infiltration of inflammatory cells into the damaged tissue [17]. Influx of neutrophils into the kidney occurs relatively early after reperfusion and has been shown to exacerbate ischemic acute renal injury [18]. In our experiment, renal ischemia and reperfusion resulted in massive accumulation of neutrophils around the injured tubules at the corticomedullary junction (Figure 3A). The extent of neutrophil infiltration was similar for TP receptor knockout and wild-type control mice ($p = 0.96$). Furthermore, after renal ischemia and reperfusion, tubular epithelial cells undergo apoptotic cell death which may aggravate acute kidney injury [19]. In line with our other findings, disruption of the TP receptor gene did not attenuate the induction of apoptosis in tubular epithelial cells at the corticomedullary junction after renal ischemia and reperfusion. On the contrary, TP receptor knockout mice had significantly more apoptotic cells than their wild-type counterparts ($p = 0.01$, Figure 3B). Finally, we measured MDA concentrations in renal tissue to study lipid peroxidation after ischemic acute kidney injury. Unexpectedly, renal MDA concentrations were significantly higher in TP receptor knockout mice than in wild-type animals, both at baseline (76 ± 23 vs 43 ± 11 nmol/g, $p = 0.02$) and at 24 h after reperfusion (66 ± 25 vs 45 ± 7 nmol/g, $p = 0.02$).

Production of F_2-isoprostanes and thromboxane A_2

Production of the TP receptor ligands F_2-isoprostanes and thromboxane A_2 increases after renal ischemia and reperfusion in rats [9,20]. Since genetic disruption of the TP receptor did not alter the susceptibility to ischemic acute kidney injury in mice, we investigated whether TP receptor ligands were present in our experimental model. In urine collections before and

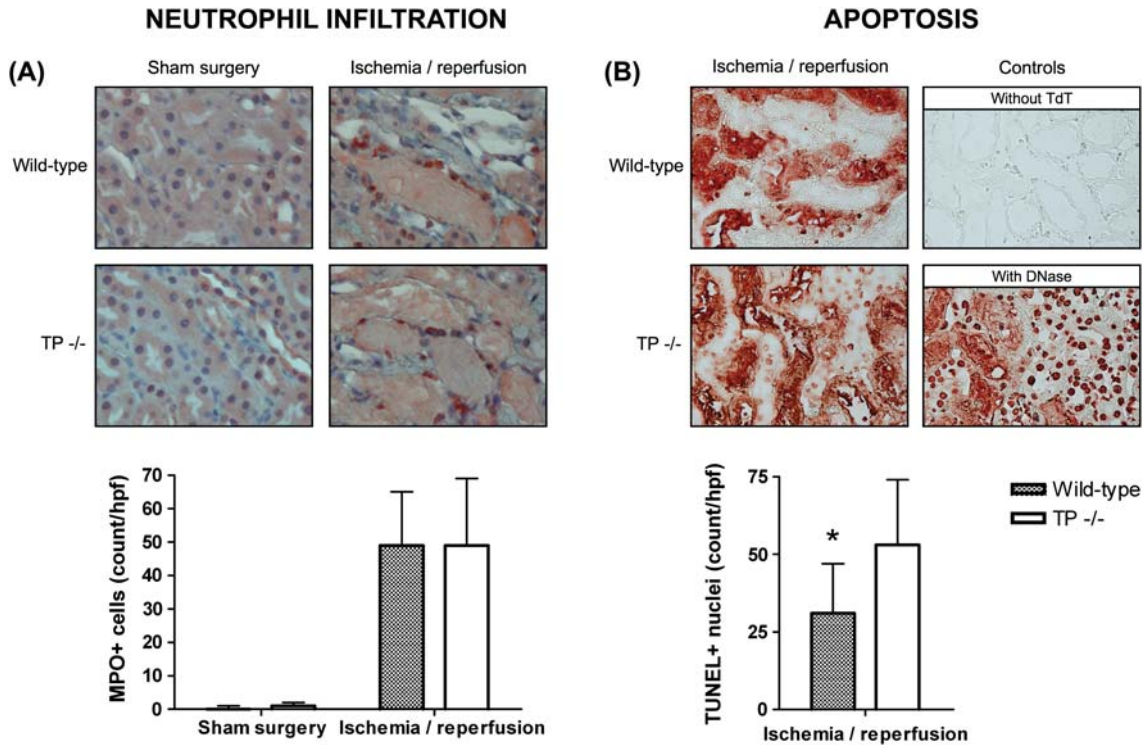

Figure 3. TP receptor knockout (TP -/-) have a similar extent of (A) neutrophil infiltration into the kidney and (B) significantly more tubular epithelial cell apoptosis after 30 min of renal ischemia and 24 h of reperfusion as compared to wild-type control mice. Representative pictures of myeloperoxidase (MPO) and terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick-end labelling (TUNEL) stained tissue sections at 400 × magnification are shown. Tubular debris was diffusely positive for TUNEL staining and was not taken into account when quantifying apoptotic nuclei. Data are presented as means and standard deviations; the asterisk indicates statistical significance ($p = 0.01$).

after temporary renal ischemia, F_2-isoprostanes and thromboxane B_2 (the stable metabolite of thromboxane A_2) concentrations from TP receptor knockout and wild-type control mice were similar (Table I). Urine thromboxane B_2 concentrations increased by 44 ± 35% following renal ischemia and reperfusion ($p = 0.01$), whereas urine F_2-isoprostanes concentrations were constant ($p = 0.27$). These findings confirm the presence of the two major TP receptor ligands after renal ischemia and reperfusion.

Table I. F_2-isoprostanes and thromboxane B_2 concentrations (ng/mg creatinine) in 24-h urine collections before and after renal ischemia and reperfusion.[a]

	Before ischemia and reperfusion	After ischemia and reperfusion	*p*
F_2-Isoprostanes			
Wild-type controls	4.1 ± 2.1	3.3 ± 0.8	0.27
TP receptor knockouts	4.2 ± 1.3	3.4 ± 1.1	
Thromboxane B_2			
Wild-type controls	8.8 ± 2.3	12.2 ± 3.1	0.01
TP receptor knockouts	8.8 ± 2.3	11.5 ± 1.7	

[a]Data are presented as mean (standard deviation). *p*-values represent comparisons of urine concentrations before and after renal ischemia and reperfusion after pooling of both mouse strains.

Discussion

The current study was designed to establish the biological effects of locally produced F_2-isoprostanes in a mouse model of renal ischemia and reperfusion. We found that mice with genetic deletion of the TP receptor—which eliminates F_2-isoprostanes and thromboxane A_2 signalling [13]—suffered renal dysfunction of similar severity as wild-type mice with the same genetic background at 24 h after reperfusion. Furthermore, the degree of histological injury and the number of neutrophils in the outer renal medulla were comparable between the two groups of mice. TP receptor knockout mice had significantly more apoptosis and a trend towards less necrosis than their wild-type counterparts, suggesting a change in the mode of cell death after renal ischemia and reperfusion when TP signalling is lost. Taken together, however, F_2-isoprostanes and thromboxane A_2 signalling do not seem to contribute critically to the development of ischemic acute kidney injury.

The biological effects of F_2-isoprostanes have been studied in various experimental models. Administration of synthetic F_2-isoprostanes increases systemic blood pressure in rodents and causes vasoconstriction of human arteries and veins *in vitro* [13,21,22]. The vasoconstrictive actions of F_2-isoprostanes are

particularly evident in the renal microcirculation: intra-renal arterial administration of 1 μg/kg/min 15-F_2-isoprostane acutely reduces glomerular filtration rate by 35% in pigs and 49% in rats, whereas systemic haemodynamic effects are not observed until administration of 10 μg/kg/min 15-F_2-isoprostane [9,11,13]. Other biological effects of F_2-isoprostanes include stimulation of leukocyte adhesion to endothelium and proliferation of endothelial and vascular smooth muscle cells, promoting angiogenesis and atherosclerosis *in vivo* [23,24]. Conflicting reports have been published on the effects of F_2-isoprostanes on platelets, some groups finding increased activation of platelets whereas others report that F_2-isoprostanes prevent aggregation of platelets *in vitro* [13,25]. These paradoxical effects may be explained by partial agonistic activity of F_2-isoprostanes on TP receptors or by the presence of a second, inhibitory F_2-isoprostanes receptor on platelets [10].

It has been established that administration of *synthetic* F_2-isoprostanes causes a variety of biological effects that are highly comparable to the actions of other TP receptor agonists. It has repeatedly been proposed that *endogenous* formation of F_2-isoprostanes as a result of oxidative stress may elicit similar effects, thereby contributing to the pathogenesis of conditions as diverse as ischemia-reperfusion injury, atherosclerosis, tumour angiogenesis and asthmatic bronchoconstriction. However, biological actions of synthetic F_2-isoprostanes have typically been observed after adding these compounds at concentrations two to three orders of magnitude greater than the physiological plasma concentrations of F_2-isoprostanes in healthy volunteers. At sites of oxidative stress, F_2-isoprostanes may nevertheless reach concentrations at which adverse biological effects have been observed [26].

The current study is the first to investigate the pathogenic actions of locally produced F_2-isoprostanes in renal ischemia-reperfusion injury. This experimental model is well suited for assessment of the biological effects of F_2-isoprostanes, since oxidative stress plays an important role in the pathophysiology of ischemic acute kidney injury and because the actions of F_2-isoprostanes are particularly evident in the renal microcirculation [9,11]. The haemodynamic effects of F_2-isoprostanes are entirely mediated by TP receptors that are expressed in the glomeruli and intra-renal arteries of human and rodent kidneys [27–29]. Mice with genetic deletion of the TP receptor are viable and are characterized by normal renal haemodynamics with attenuated renal vasoconstriction after administration of endotoxin or angiotensin-II [14,30,31]. In the current study, we found that ischemia and reperfusion induces acute kidney injury in TP receptor knockout mice to a similar extent as in wild-type mice, indicating that neither F_2-isoprostanes nor thromboxane A_2 signalling is critically involved in the pathophysiology of ischemic acute kidney injury. Our study does not account for potential TP receptor-independent actions of F_2-isoprostanes. It has been proposed that esterified F_2-isoprostanes may cause cellular injury in the absence of TP receptors by disruption of the fluidity and integrity of cell membranes, similar to other products of lipid peroxidation [32].

Our findings seem to contradict previous experiments that have suggested thromboxane A_2 signalling to contribute to the pathogenesis of ischemic acute kidney injury [33]. Specific inhibitors of thromboxane A_2 synthase have been shown to attenuate acute kidney injury in rodent models of renal ischemia and reperfusion [33]. However, inhibition of thromboxane A_2 synthesis increases the production of the vasodilator prostacyclin as a result of the increased availability of their common precursor prostaglandin H_2—a phenomenon termed endoperoxide shunting [34]. Since the beneficial effects of thromboxane A_2 synthase inhibitors on ischemic acute kidney injury were eliminated when prostacyclin synthesis was blocked [35], these effects may have been caused by an increase in prostacyclin signalling as a result of endoperoxide shunting rather than by a decrease in thromboxane A_2 signalling. A separate study found that pharmacological inhibition of TP receptor signalling attenuated renal dysfunction in a rat model of 60 min of renal ischemia followed by 6 h of reperfusion [20]. In contrast, we observed no effect of genetic deletion of TP receptors on acute kidney injury after 30 min of renal ischemia and 24 h of reperfusion in mice. These conflicting findings may be caused by differences in the applied experimental models or by cross-reactivity of the drug used to inhibit TP receptor signalling [36].

Finally, we observed increased lipid peroxidation in kidneys of TP receptor knockout mice as compared to those of wild-type animals, both at baseline and after renal ischemia and reperfusion. This novel and unexpected finding suggests that TP receptor signalling by F_2-isoprostanes may induce endogenous anti-oxidant defence mechanisms that limit lipid peroxidation in the kidney. It would be interesting to study this hypothesis on negative feedback on anti-oxidant regulation by F_2-isoprostanes in future studies.

Conclusions

F_2-isoprostanes and thromboxane A_2 signalling do not contribute critically to the pathogenesis of ischemic acute kidney injury. Tissue injury due to ischemia and reperfusion is characterized by extensive oxidative stress and the biological actions of F_2-isoprostanes are particularly evident in the renal microcirculation. Therefore, our findings more generally argue against a prominent role for F_2-isoprostanes signalling in exacerbating acute disease states associated with oxidative stress.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

[1] Chertow GM, Burdick E, Honour M, Bonventre JV, Bates DW. Acute kidney injury, mortality, length of stay, and costs in hospitalized patients. J Am Soc Nephrol 2005;16: 3365–3370.

[2] Yarlagadda SG, Coca SG, Formica RN, Jr, Poggio ED, Parikh CR. Association between delayed graft function and allograft and patient survival: a systematic review and meta-analysis. Nephrol Dial Transplant 2009;24:1039–1047.

[3] Paller MS, Hoidal JR, Ferris TF. Oxygen free radicals in ischemic acute renal failure in the rat. J Clin Invest 1984; 74:1156–1164.

[4] Koyama I, Bulkley GB, Williams GM, Im MJ. The role of oxygen free radicals in mediating the reperfusion injury of cold-preserved ischemic kidneys. Transplantation 1985;40: 590–595.

[5] Baker GL, Corry RJ, Autor AP. Oxygen free radical induced damage in kidneys subjected to warm ischemia and reperfusion. Protective effect of superoxide dismutase. Ann Surg 1985;202:628–641.

[6] Stafforini DM, Sheller JR, Blackwell TS, Sapirstein A, Yull FE, McIntyre TM, Bonventre JV, Prescott SM, Roberts LJ, 2nd. Release of free F2-isoprostanes from esterified phospholipids is catalyzed by intracellular and plasma platelet-activating factor acetylhydrolases. J Biol Chem 2006;281:4616–4623.

[7] Morrow JD, Hill KE, Burk RF, Nammour TM, Badr KF, Roberts LJ, 2nd. A series of prostaglandin F2-like compounds are produced *in vivo* in humans by a non-cyclooxygenase, free radical-catalyzed mechanism. Proc Natl Acad Sci USA 1990;87:9383–9387.

[8] Kadiiska MB, Gladen BC, Baird DD, Germolec D, Graham LB, Parker CE, Nyska A, Wachsman JT, Ames BN, Basu S, Brot N, Fitzgerald GA, Floyd RA, George M, Heinecke JW, Hatch GE, Hensley K, Lawson JA, Marnett LJ, Morrow JD, Murray DM, Plastaras J, Roberts LJ, 2nd, Rokach J, Shigenaga MK, Sohal RS, Sun J, Tice RR, Van Thiel DH, Wellner D, Walter PB, Tomer KB, Mason RP, Barrett JC. Biomarkers of oxidative stress study II: are oxidation products of lipids, proteins, and DNA markers of CCl4 poisoning? Free Radic Biol Med 2005;38:698–710.

[9] Takahashi K, Nammour TM, Fukunaga M, Ebert J, Morrow JD, Roberts LJ, 2nd, Hoover RL, Badr KF. Glomerular actions of a free radical-generated novel prostaglandin, 8-epi-prostaglandin F2 alpha, in the rat. Evidence for interaction with thromboxane A2 receptors. J Clin Invest 1992;90: 136–141.

[10] Khasawneh FT, Huang JS, Mir F, Srinivasan S, Tiruppathi C, Le Breton GC. Characterization of isoprostane signaling: evidence for a unique coordination profile of 8-iso-PGF(2alpha) with the thromboxane A(2) receptor, and activation of a separate cAMP-dependent inhibitory pathway in human platelets. Biochem Pharmacol 2008;75:2301–2315.

[11] Krier JD, Rodriguez-Porcel M, Best PJ, Romero JC, Lerman A, Lerman LO. Vascular responses *in vivo* to 8-epi PGF(2alpha) in normal and hypercholesterolemic pigs. Am J Physiol Regul Integr Comp Physiol 2002;283:303–308.

[12] Alejandro V, Scandling JD, Jr, Sibley RK, Dafoe D, Alfrey E, Deen W, Myers BD. Mechanisms of filtration failure during postischemic injury of the human kidney. A study of the reperfused renal allograft. J Clin Invest 1995;95:820–831.

[13] Audoly LP, Rocca B, Fabre JE, Koller BH, Thomas D, Loeb AL, Coffman TM, FitzGerald GA. Cardiovascular responses to the isoprostanes iPF(2alpha)-III and iPE(2)-III are mediated via the thromboxane A(2) receptor *in vivo*. Circulation 2000;101:2833–2840.

[14] Thomas DW, Mannon RB, Mannon PJ, Latour A, Oliver JA, Hoffman M, Smithies O, Koller BH, Coffman TM. Coagulation defects and altered hemodynamic responses in mice lacking receptors for thromboxane A2. J Clin Invest 1998;102: 1994–2001.

[15] Leemans JC, Stokman G, Claessen N, Rouschop KM, Teske GJ, Kirschning CJ, Akira S, van der Poll T, Weening JJ, Florquin S. Renal-associated TLR2 mediates ischemia/reperfusion injury in the kidney. J Clin Invest 2005;115: 2894–2903.

[16] Kelly KJ, Sandoval RM, Dunn KW, Molitoris BA, Dagher PC. A novel method to determine specificity and sensitivity of the TUNEL reaction in the quantitation of apoptosis. Am J Physiol Cell Physiol 2003;284:1309–1318.

[17] Thurman JM. Triggers of inflammation after renal ischemia/reperfusion. Clin Immunol 2007;123:7–13.

[18] Kelly KJ, Williams WW, Jr, Colvin RB, Meehan SM, Springer TA, Gutierrez-Ramos JC, Bonventre JV. Intercellular adhesion molecule-1-deficient mice are protected against ischemic renal injury. J Clin Invest 1996;97:1056–1063.

[19] Daemen MA, van 't Veer C, Denecker G, Heemskerk VH, Wolfs TG, Clauss M, Vandenabeele P, Buurman WA. Inhibition of apoptosis induced by ischemia-reperfusion prevents inflammation. J Clin Invest 1999;104:541–549.

[20] Kramer HJ, Mohaupt MG, Pinoli F, Backer A, Meyer-Lehnert H, Schlebusch H. Effects of thromboxane A2 receptor blockade on oliguric ischemic acute renal failure in conscious rats. J Am Soc Nephrol 1993;4:50–57.

[21] Cracowski JL, Stanke-Labesque F, Devillier P, Chavanon O, Hunt M, Souvignet C, Bessard G. Human internal mammary artery contraction by isoprostaglandin f(2alpha) type-III [8-iso-prostaglandin F(2alpha)]. Eur J Pharmacol 2000 ;397: 161–168.

[22] Gardan B, Cracowski JL, Sessa C, Hunt M, Stanke-Labesque F, Devillier P, Bessard G. Vasoconstrictor effects of iso-prostaglandin F2alpha type-III (8-iso-prostaglandin F2alpha) on human saphenous veins. J Cardiovasc Pharmacol 2000;35:729–734.

[23] Benndorf RA, Schwedhelm E, Gnann A, Taheri R, Kom G, Didie M, Steenpass A, Ergun S, Boger RH. Isoprostanes inhibit vascular endothelial growth factor-induced endothelial cell migration, tube formation, and cardiac vessel sprouting *in vitro*, as well as angiogenesis *in vivo* via activation of the thromboxane A(2) receptor: a potential link between oxidative stress and impaired angiogenesis. Circ Res 2008 ;103:1037–1046.

[24] Tang M, Cyrus T, Yao Y, Vocun L, Pratico D. Involvement of thromboxane receptor in the proatherogenic effect of isoprostane F2alpha-III: evidence from apolipoprotein E- and LDL receptor-deficient mice. Circulation 2005;112:2867–2874.

[25] Minuz P, Andrioli G, Degan M, Gaino S, Ortolani R, Tommasoli R, Zuliani V, Lechi A, Lechi C. The F2-isoprostane 8-epiprostaglandin F2alpha increases platelet adhesion and reduces the antiadhesive and antiaggregatory effects of NO. Arterioscler Thromb Vasc Biol 1998;18:1248–1256.

[26] Iuliano L, Pratico D, Greco C, Mangieri E, Scibilia G, FitzGerald GA, Violi F. Angioplasty increases coronary sinus

F2-isoprostane formation: evidence for *in vivo* oxidative stress during PTCA. J Am Coll Cardiol 2001;37:76–80.
[27] Abe T, Takeuchi K, Takahashi N, Tsutsumi E, Taniyama Y, Abe K. Rat kidney thromboxane receptor: molecular cloning, signal transduction, and intrarenal expression localization. J Clin Invest 1995;96:657–664.
[28] Mannon RB, Coffman TM, Mannon PJ. Distribution of binding sites for thromboxane A2 in the mouse kidney. Am J Physiol 1996;271:1131–1138.
[29] Brown GP, Venuto RC. Thromboxane receptors in human kidney tissues. Prostaglandins Other Lipid Mediat 1999;57: 179–188.
[30] Boffa JJ, Just A, Coffman TM, Arendshorst WJ. Thromboxane receptor mediates renal vasoconstriction and contributes to acute renal failure in endotoxemic mice. J Am Soc Nephrol 2004;15:2358–2365.
[31] Kawada N, Dennehy K, Solis G, Modlinger P, Hamel R, Kawada JT, Aslam S, Moriyama T, Imai E, Welch WJ, Wilcox CS. TP receptors regulate renal hemodynamics during angiotensin II slow pressor response. Am J Physiol Renal Physiol 2004;287:753–759.
[32] Niki E. Lipid peroxidation: physiological levels and dual biological effects. Free Radic Biol Med 2009;47:469–484.
[33] Lelcuk S, Alexander F, Kobzik L, Valeri CR, Shepro D, Hechtman HB. Prostacyclin and thromboxane A2 moderate postischemic renal failure. Surgery 1985;98:207–212.
[34] Nowak J, FitzGerald GA. Redirection of prostaglandin endoperoxide metabolism at the platelet-vascular interface in man. J Clin Invest 1989;83:380–385.
[35] Klausner JM, Paterson IS, Kobzik L, Rodzen C, Valeri CR, Shepro D, Hechtman HB. Vasodilating prostaglandins attenuate ischemic renal injury only if thromboxane is inhibited. Ann Surg 1989;209:219–224.
[36] Narumiya S, FitzGerald GA. Genetic and pharmacological analysis of prostanoid receptor function. J Clin Invest 2001;108:25–30.

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