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The role of cycloxygenase-2 in the rodent kidney following ischaemia/reperfusion injury *in vivo*

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Abstract

The role of cyclooxygenase-2 (COX-2) in the pathophysiology of renal ischaemia/reperfusion injury is still not fully understood. In order to elucidate the role of COX-2 in ischaemia/reperfusion injury of the kidney, we have evaluated the effects of ischaemia/reperfusion on renal dysfunction and injury in (i) rats treated with either vehicle or the selective COX-2 inhibitor parecoxib, and (ii) wild-type mice or mice in which the gene for COX-2 has been deleted (COX-2 knock-out mice or COX-$2^{-/-}$). Rats were subjected to bilateral renal ischaemia (45 min) and reperfusion (6 h), and received parecoxib (20 mg/kg, i.v.) 30 min prior to ischaemia and 3 h after the commencement of reperfusion. Serum urea, serum creatinine, serum aspartate aminotransferase, creatinine clearance and fractional excretion of sodium were all used as indicators of renal dysfunction and injury. Mice (wild-type and COX-$2^{-/-}$) were subjected to bilateral renal ischaemia (30 min) and reperfusion (24 h) after which renal dysfunction (serum urea and creatinine) and renal injury was assessed by histological analysis. Parecoxib significantly augmented the degree of renal dysfunction and injury caused by ischaemia/reperfusion in the rat. In addition, the degree of renal injury and dysfunction caused by ischaemia/reperfusion was also significantly augmented in COX-$2^{-/-}$ mice when compared to their wild-type littermates. These findings support the view that metabolites of COX-2 protect the kidney against ischaemia/reperfusion injury, and (ii) that selective inhibitors of COX-2 may worsen renal dysfunction and injury in conditions associated with renal ischaemia.

Keywords: Kidney; Ischaemia/reperfusion; Parecoxib; Cyclooxygenase

1. Introduction

Prostaglandins are essential regulators of tissue homeostasis, reproduction and inflammation. The biosynthesis of prostaglandins, as well as leukotrienes, is preceded by the release of arachidonic acid from membrane phospholipids and is mediated by phospholipase A_2 (Gijon and Leslie, 1999). The cyclooxygenase (COX) or lipoxygenase pathways metabolize arachidonic acid to form prostaglandins or leukotrienes, respectively (Goetzl et al., 1995). COX exists as several isozymes: COX-1, COX-2 and the recently discovered COX-3 (a splice variant of COX-1) (Willoughby et al., 2000). The current dogma regarding COX isozyme expression is that COX-1 is constitutively expressed, whereas COX-2 is induced in response to many pathological stimuli. However, even under physiological conditions, the kidney expresses both COX-1 and COX-2 with abundance in the collecting ducts, renal vasculature, glomeruli and papillary interstitial cells (Harris et al., 1994; Komhoff et al., 1997; Smith and Bell, 1978).

The physiology of the kidney is somewhat dependent on prostaglandins because they modulate glomerular haemodynamics

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and regulate distal nephron function by way of having an active role in vascular tone and salt and water homeostasis (Currie and Needleman, 1984; Dunn, 1987). Therefore, any inhibition of COX in the kidney may affect its function. In addition to gastrointestinal side effects (Hawk et al., 1951), non-selective COX inhibitors (i.e. non-steroidial anti-inflammatory drugs (NSAIDs)) may cause acute ischaemic renal failure, fluid and electrolyte disturbances, and, possibly renal papillary necrosis (Nies, 1988; Whelton and Hamilton, 1991). Therefore, there has been great interest in developing specific and selective inhibitors of COX-2. It has become evident recently, however, that the long-term use of novel selective COX-2 inhibitors increases cardiovascular side effects (Furberg et al., 2005), as they inhibit the COX-2-derived release of prostacyclin (a vasodilator), but not the COX-1-derived release of thromboxane A_2 (a vasoconstrictor).

The specific COX-2 inhibitor celecoxib has been to shown to protect the lung (Cuzzocrea et al., 2002), gut (Cuzzocrea et al., 2001), pancreas (Alhan et al., 2004) and the brain (Chu et al., 2004) from conditions associated with inflammation. However, celecoxib is dissolved in dimethyl sulfoxide, which is not only toxic itself, but is also a known oxygen radical scavenger (Aita et al., 2005; Yu and Quinn, 1994). Thus, we have used parecoxib, which is the first highly water-soluble prodrug of the second-generation selective COX-2 inhibitor valdecoxib, to investigate the role of COX-2 in the kidney following ischaemia/reperfusion. We found that paracoxib enhanced the renal injury and dysfunction caused by ischaemia/reperfusion. In order to confirm that the inhibition of COX-2 activity is indeed responsible for these detrimental effects of parecoxib, we have subsequently compared the effects of ischaemia/reperfusion on renal injury and dysfunction in wild-type mice and in COX-2 knock-out (COX-$2^{-/-}$) mice.

2. Methods

2.1. Experimental protocol (rat)

Forty-five male Wistar rats (Charles River Ltd, Margate, UK) weighing 250 to 320 g were used in this part of the study. Rats received a standard diet and water *ad libitum*, and were cared for in accordance with both the *UK Home Office Guidance in the Operation of the Animals (Scientific Procedures) Act 1986*, published by Her Majesty's Stationery Office, London, UK and the *Guide for the Care and Use of Laboratory Animals*, published by the American Physiological Society. All rats were anaesthetised with sodium thiopentone (Intraval® Sodium, 120 mg/kg i.p.; Merial Animal Health Ltd., Harlow, Essex, UK) and anaesthesia was maintained by supplementary injections (~10 mg/kg i.v.) of sodium thiopentone. Animals were randomly allocated into four groups as described below:

- Ischaemia/reperfusion group: control, rats which underwent bilateral renal ischaemia for 45 min followed by reperfusion for 6 h and were administered saline (vehicle for parecoxib, 2 ml/kg, i.v.) 30 min prior to ischaemia and 3 h after the commencement of reperfusion ($N=9$).
- Ischaemia/reperfusion parecoxib group: rats which underwent bilateral renal ischaemia for 45 min followed by reperfusion for 6 h and were administered parecoxib (20 mg/kg, 2 ml/kg, i.v.) 30 min prior to ischaemia and 3 h after the commencement of reperfusion ($N=9$).
- Ischaemia/reperfusion pre-ischaemia parecoxib group: rats which underwent bilateral renal ischaemia for 45 min followed by reperfusion for 6 h and were administered parecoxib (20 mg/kg, 2 ml/kg, i.v.) 30 min prior to ischaemia ($N=6$).

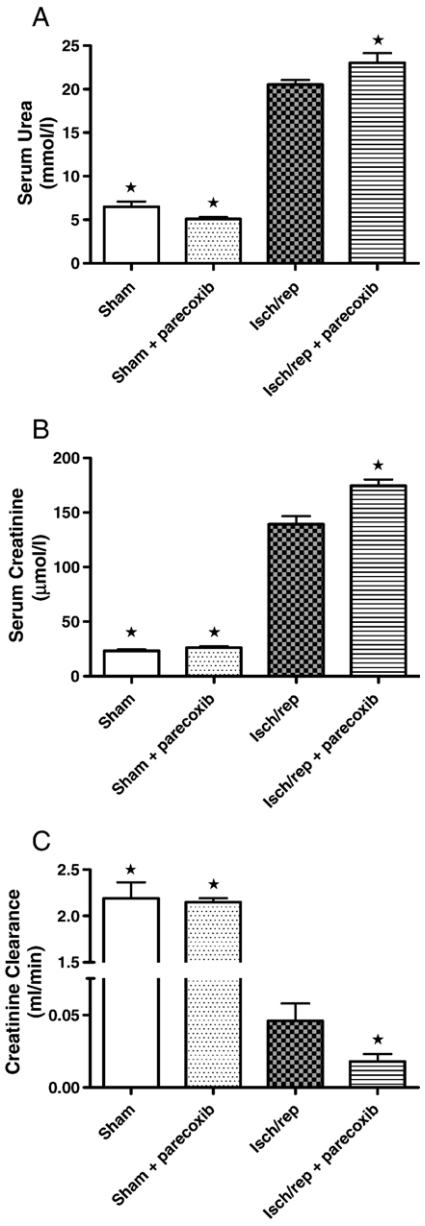

Fig. 1. Effect of parecoxib on glomerular dysfunction mediated by ischaemia/reperfusion in rats. (A) serum urea levels, (B) creatinine levels and (C) creatinine clearance were measured subsequent to sham-operation (Sham, $N=9$) or renal ischaemia/reperfusion (Isch/rep, $N=9$). Rats were administered parecoxib (20 mg/kg, i.v.) 30 min prior to ischaemia and 3 h after the commencement of reperfusion (Isch/rep+parecoxib, $N=9$). A further group of rats received parecoxib (20 mg/kg, i.v.) 30 min prior to sham ischaemia and 3 h after the commencement of sham reperfusion (Sham+parecoxib, $N=6$). Data are expressed as means±S.E.M. for N number of observations. ★$P<0.05$ vs. ischaemia/reperfusion.

- Ischaemia/reperfusion post-ischaemia parecoxib group: rats which underwent bilateral renal ischaemia for 45 min followed by reperfusion for 6 h and were administered parecoxib (20 mg/kg, 2 ml/kg, i.v.) 3 h after the commencement of reperfusion ($N=6$).
- Sham group: rats were subjected to the same surgical procedures as above, except for renal ischaemia/reperfusion. Rats were administered saline (vehicle for parecoxib, 2 ml/kg, i.v.) at times equivalent to those described in the ischaemia/reperfusion parecoxib group ($N=9$).
- Sham parecoxib group: rats were subjected to the same surgical procedures as above, except for renal ischaemia/reperfusion. Rats were administered parecoxib (20 mg/kg, 2 ml/kg, i.v.) at times equivalent to those described in the ischaemia/reperfusion parecoxib group ($N=6$).

The dose of parecoxib used was based on that previously shown to provide protection against ischaemia/reperfusion injury in the rodent (Sivarajah et al., 2005). Anaesthetised rats were placed onto a thermostatically controlled heating mat (Harvard Apparatus Ltd., Kent, UK) and body temperature was maintained at 37±1 °C by means of a rectal probe attached to a homeothermic blanket. A tracheostomy was performed and a small section of polyethylene tube was inserted into the airway (Internal Diameter 1.67 mm, Portex, Kent, UK) to maintain airway patency and

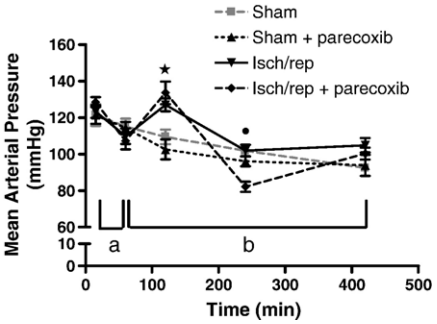

Fig. 3. Effect of parecoxib on mean arterial pressure. Mean arterial pressure was monitored throughout renal ischaemia/reperfusion in rats subjected to sham-operation (Sham, $N=9$) or renal ischaemia/reperfusion (Isch/rep, $N=9$). Rats were administered parecoxib (20 mg/kg, i.v.) 30 min prior to ischaemia and 3 h after the commencement of reperfusion (Isch/rep+parecoxib, $N=9$). A further group of rats received parecoxib (20 mg/kg, i.v.) 30 min prior to sham ischaemia and 3 h after the commencement of sham reperfusion (Sham+parecoxib, $N=6$). a: ischaemic period; b: reperfusion period. Data are expressed as means±S.E.M. for N number of observations. ★$P<0.05$ sham vs. ischaemia/reperfusion and •$P<0.05$ ischaemia/reperfusion vs. ischaemia/reperfusion+parecoxib.

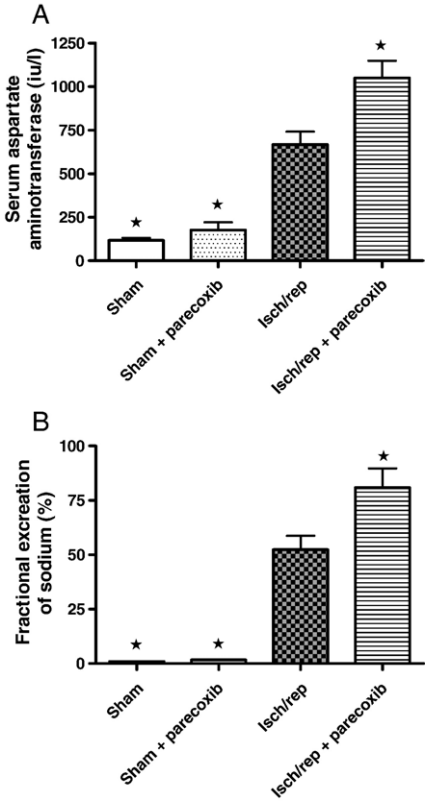

Fig. 2. Effect of parecoxib on renal injury and tubular dysfunction subsequent to ischaemia/reperfusion in rats. (A) serum aspartate aminotransferase levels and (B) fractional excreation of sodium subsequent to sham-operation (Sham, $N=9$) or renal ischaemia/reperfusion (Isch/rep, $N=9$). Rats were administered parecoxib (20 mg/kg, i.v.) 30 min prior to ischaemia and 3 h after the commencement of reperfusion (Isch/rep+parecoxib, $N=9$). A further group of rats received parecoxib (20 mg/kg, i.v.) 30 min prior to sham ischaemia and 3 h after the commencement of sham reperfusion (Sham+parecoxib, $N=6$). Data are expressed as means±S.E.M. for N number of observations. ★$P<0.05$ vs. ischaemia/reperfusion.

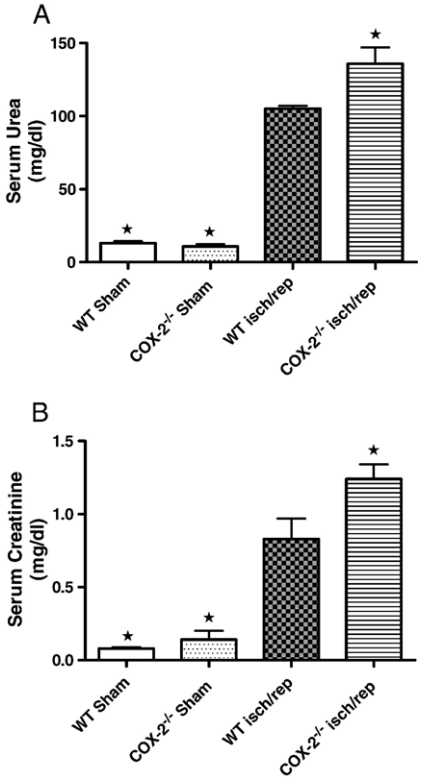

Fig. 4. Renal dysfunction in COX-$2^{-/-}$ mice following ischaemia/reperfusion injury. (A) serum urea and (B) creatinine levels were measured, from COX$2^{-/-}$ mice, as biochemical markers of renal dysfunction subsequent to sham-operation (WT [wild-type] Sham, $N=8$; COX-$2^{-/-}$ Sham, $N=5$) or renal ischaemia/reperfusion (WT isch/rep, $N=7$; COX-$2^{-/-}$ isch/rep, $N=10$). Data represent mean±SEM for N observations; ★$P<0.05$ vs. WT ischaemia/reperfusion group.

facilitate spontaneous respiration. The right carotid artery was cannulated (Internal Diameter 0.58 mm, Portex) and connected to a pressure transducer (Capto SP 844 Physiological Pressure Transducer, AD Instruments, Hastings, UK) for the measurement of mean arterial blood pressure and derivation of the heart rate from the pulse waveform, which were displayed on a data acquisition system (PowerLab 8e, Chart v4.24, AD Instruments, Hastings, UK) installed on an IBM compatible personal computer. MAP and HR were monitored for the duration of each experiment. The right jugular vein was cannulated (Internal Diameter 0.40 mm, Portex) for the administration of solutions and continuous infusion of saline. A midline laparotomy was performed and the bladder was cannulated (Internal Diameter 0.58 mm, Portex) for the collection of urine. The kidneys were located inside the peritoneum and the renal pedicles, containing the renal artery, vein, and nerve supplying each kidney, were carefully isolated. Rats were subjected to bilateral renal occlusion for 45 min using non-traumatic arterial clamps (Dieffenbach Bulldog Clamps, Harvard Apparatus Ltd., Kent, UK) to clamp the renal pedicles, followed by reperfusion for 6 h. After the renal clamps were removed, the kidneys were observed for a further 5 min to ensure reflow after which 2 ml saline at 37 °C was injected into the abdomen to ensure gut motility. Sham-operated rats underwent identical surgical procedures to rats undergoing ischaemia/reperfusion except that arterial clamps were not applied. All groups described above received a continuous infusion of 0.9% (w/v) saline (2.4 ml/kg/h, i.v.) throughout the reperfusion period.

2.2. Experimental protocol (mouse)

Fifteen male C57BL/6J wild-type mice and 15 male COX-2$^{-/-}$ mice (25–30 g, purchased from Jackson Laboratories, Harlan Nossan, Italy) were used in this part of the study. Mice received a standard diet and water *ad libitum*, and were cared for in accordance with Italian regulations on protection of animals used for experimental and other scientific purposes (D.M. 116192), as well as with the European Economic Community regulations (O.J. of E.C. L358/1 12/18/1986). Mice were anaesthetised using chloral hydrate (125 mg/kg, i.p.) and core body temperature maintained at 37 °C using a homoeothermic blanket. After performing a midline laparotomy, mice were then divided into the following four groups:

- Ischaemia/reperfusion wild-type group; wild-type mice, which underwent renal ischaemia for 30 min followed by reperfusion for 24 h ($N=7$);
- Ischaemia/reperfusion COX-2$^{-/-}$ group; COX-2 knock-out mice, which underwent renal ischaemia for 30 min followed by reperfusion for 24 h ($N=10$);
- Sham wild-type group; wild-type mice, which were subjected to the surgical procedures described above, but were not subjected to renal ischaemia/reperfusion ($N=8$);
- Sham COX-2$^{-/-}$ group; COX-2 knock-out mice, which were subjected to the surgical procedures described above, but were not subjected to renal ischaemia/reperfusion ($N=5$).

Mice were maintained under anaesthesia for the duration of ischaemia (i.e. 30 min). After performing a midline laparotomy, mice from the ischaemia/reperfusion groups were subjected to bilateral renal ischaemia for 30 min, during which the renal arteries and veins were occluded using microaneurysm clamps (Chatterjee et al., 2003). The time of ischaemia chosen was based on that found to maximize reproducibility of renal functional impairment, while minimizing mortality in these animals (Chatterjee et al., 2003). After the renal clamps were removed,

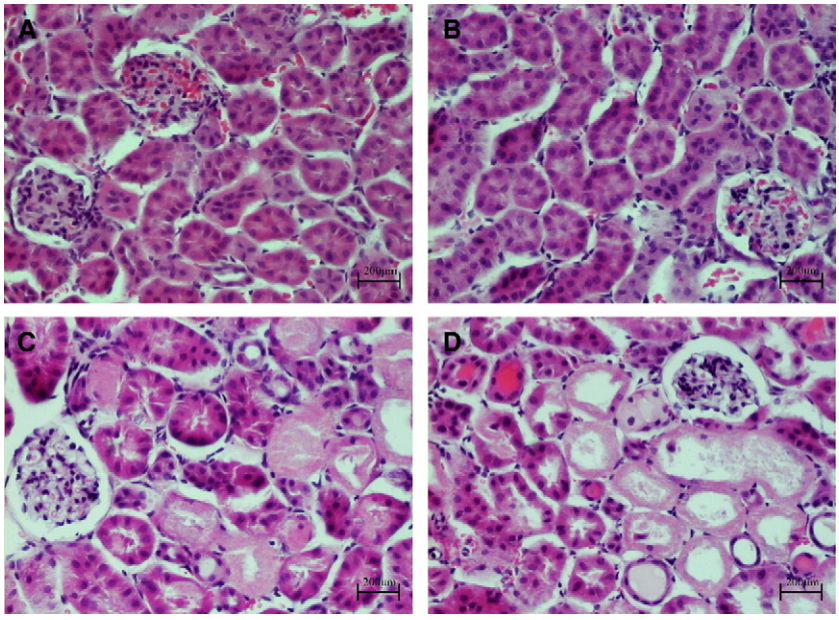

Fig. 5. Renal injury (histological examination) in COX-2$^{-/-}$ mice following ischaemia/reperfusion injury. Renal sections taken from (A) a wild-type sham-operated mouse, (B) a COX-2$^{-/-}$ sham-operated mouse, (C) a wild-type mouse subjected to renal ischaemia/reperfusion and (D) a COX-2$^{-/-}$ mouse subjected to renal ischaemia/reperfusion. Hematoxylin and eosin, figures are representative of at least 3 experiments performed on different days ($N=5$–10 for all groups).

the kidneys were observed for 5 min further to ensure reflow after which 1 ml saline at 37 °C was injected into the abdomen and the incision was sutured in two layers. Mice were then returned to their cages where they were allowed to recover from anaesthesia and observed for 24 h. Sham-operated mice underwent identical surgical procedures to ischaemia/reperfusion mice except that microaneurysm clamps were not applied.

2.3. Determination of renal injury and dysfunction

At the end of the reperfusion period, 1 ml blood samples were collected via the carotid artery for the rat and via cardiac puncture for the mouse into S/1.3 tubes containing serum gel (Sarstedt, Germany), after which the heart was removed to terminate the experiment. The samples were centrifuged (6000 ×*g* for 3 min) to separate serum from which biochemical parameters were measured within 24 h (Vetlab Services, Sussex, UK). Serum urea and creatinine concentrations were used as indicators of impaired renal (glomerular) function (Chatterjee and Thiemermann, 2003). The rise in the serum level of aspartate aminotransferase was used as an indicator of reperfusion-injury (Thiemermann et al., 2003). Urine samples were collected during the reperfusion period and the volume of urine produced was recorded. Urinary creatinine was measured and was used in conjunction with serum creatinine concentration and urine flow to calculate creatinine clearance using standard formulae, which was used as an indicator of glomerular function (Chatterjee and Thiemermann, 2003). Urinary sodium was measured at the end of the reperfusion period and used in conjunction with serum sodium to estimate fractional excretion of sodium using standard formulae, and which was used as an indicator of tubular function (Chatterjee et al., 2004).

2.4. Histological evaluation of renal injury (murine model)

Kidneys were removed from rats at the end of the experimental period after tying the renal pedicle and cut in a sagital section into two halves. These tissue samples were fixed by immersion in 10% (w/v) formaldehyde in phosphate-buffered saline (PBS; 0.01 M; pH 7.4) at room temperature for 1 week. After dehydration using graded ethanol, the tissue was embedded in Paraplast (Sherwood Medical, Mahwah, NJ) and cut in fine (8 μm) sections and mounted on glass slides. Sections were then deparaffinized with xylene, counterstained with hematoxylin and eosin, and viewed under a light microscope (Dialux 22, Leitz, Milan, Italy).

2.5. Materials

Unless otherwise stated, all compounds used in this study were purchased from Sigma-Aldrich Company Ltd. (Poole, Dorset, UK). Parecoxib was purchased from Sequoia Research Products (Oxford, UK) All solutions used were prepared using non-pyrogenic saline (0.9% [w/v] NaCl; Baxter Healthcare Ltd., Thetford, Norfolk, UK). Thiopentone sodium (Intraval® Sodium) was purchased from Rhone Merieux (Harlow, Essex, UK).

2.6. Statistical analysis

All values described in the text and figures are expressed as mean±standard error of the mean (S.E.M.) for *N* observations. Each data point represents biochemical measurements 9 separate animals. One-way analysis of variance with Dunnett's post hoc-test was performed on all biochemical data using GraphPad Prism version 4.03 for Windows (GraphPad Software, San Diego, California, USA, www.graphpad.com) and a *P* value of less than 0.05 was considered to be significant.

3. Results

3.1. Effect of parecoxib on glomerular dysfunction caused by ischaemia/reperfusion in rats

In a set of preliminary experiments rats that underwent renal ischaemia/reperfusion that were administered parecoxib either 30 min prior to ischaemia or 3 h after the commencement of reperfusion exhibited no significant differences in serum creatinine levels [154.75±9.18 (Ischaemia/reperfusion pre-ischaemia parecoxib group) vs. 139.22±7.30 (Ischaemia/reperfusion group), $P>0.05$; 132.50±6.54 (Ischaemia/reperfusion post-ischaemia parecoxib group) vs. 139.22±7.30 (Ischaemia/reperfusion group), $P>0.05$] or creatinine clearance [0.029±0.008 (Ischaemia/reperfusion pre-ischaemia parecoxib group) vs. 0.046±0.012 (Ischaemia/reperfusion group), $P>0.05$; 0.076±0.008 (Ischaemia/reperfusion post-ischaemia parecoxib group) vs. 0.046±0.012 (Ischaemia/reperfusion group), $P>0.05$] when compared rats subjected to renal ischaemia/reperfusion only.

Rats that underwent renal ischaemia/reperfusion exhibited a significant increase in the serum levels of urea and creatinine compared with sham-operated rats (Fig. 1A and B). Compared to rats subjected to ischaemia/reperfusion only (control), administration of parecoxib 30 min prior to ischaemia (20 mg/kg, i.v.) and 3 h after the commencement of reperfusion (20 mg/kg, i.v.) significantly increased the serum levels of urea and creatinine (Fig. 1A and B).

In order to discount the possibility of a rapid increase in serum creatinine levels due to increased release of creatinine from muscle during ischaemia/reperfusion, creatinine clearance was also measured (Fig. 1C). Rats subjected to renal ischaemia/reperfusion demonstrated a significant attenuation in creatinine clearance compared to sham-operated rats (Fig. 1C). Compared to rats subjected to ischaemia/reperfusion only, administration of parecoxib produced a significant fall in creatinine clearance (Fig. 1C).

3.2. Effect of parecoxib on renal injury and tubular dysfunction caused by ischaemia/reperfusion in rats

Renal ischaemia/reperfusion produced a significant increase in the serum concentrations of aspartate aminotransferase (a marker of renal tissue injury) on comparison with levels obtained from sham-operated rats (Fig. 2A). The administration of parecoxib to rats subjected to renal ischaemia/reperfusion significantly increased aspartate aminotransferase (Fig. 2A).

Compared with sham-operated rats, renal ischaemia/reperfusion produced a significant increase in fractional excretion of sodium indicating (Fig. 2B). Treatment of rats with parecoxib significantly raised the ischaemia/reperfusion-mediated increase in fractional excretion of sodium (Fig. 2B).

3.3. Effect of parecoxib on mean arterial pressure caused by ischaemia/reperfusion

When compared to sham-operated rats, renal ischaemia/reperfusion caused a transient and significant rise in mean arterial pressure 1 h after the commencement of reperfusion (Fig. 3), which was not inhibited in rats subjected to ischaemia/reperfusion and administered parecoxib. However, rats subjected to renal ischaemia/reperfusion that were administered parecoxib produced a transient, but significant fall in mean arterial pressure 4 h after the commencement of the reperfusion period compared to rats subjected to renal ischaemia/reperfusion alone (Fig. 3). However, sham-operated rats administered parecoxib did not demonstrate this transient fall in mean arterial pressure (Fig. 3).

3.4. Renal dysfunction in COX-2$^{-/-}$ mice caused by ischaemia/reperfusion

Wild-type mice subjected to bi-lateral renal ischaemia for 30 min followed by reperfusion for 24 h exhibited a significant increase in the serum levels of urea and creatinine compared with sham-operated mice (Fig. 4A and B). When compared to wild-type mice subjected to ischaemia/reperfusion, serum levels of urea and creatinine and, therefore, renal dysfunction, were significantly higher in COX-2$^{-/-}$ mice subjected to ischaemia/reperfusion (Fig. 4A and B).

3.5. Renal injury in COX-2$^{-/-}$ mice caused by ischaemia/reperfusion

When compared to sham-operated mice (Fig. 5A and B), histological examination of kidneys obtained from wild-type mice subjected to ischaemia/reperfusion demonstrated a significant degree of renal injury (Fig. 5C). Specifically, kidneys obtained from these animals exhibited degeneration of tubular structure, tubular dilatation, swelling and necrosis and luminal congestion. Renal sections obtained from COX-2$^{-/-}$ mice that underwent ischaemia/reperfusion (Fig. 5D) demonstrated a marked enhancement in the severity of these histological features of renal injury, when compared with kidneys obtained from wild-type mice subjected to ischaemia/reperfusion only (Fig. 5C).

4. Discussion

The most common drugs taken for pain, inflammation and fever are NSAIDs. We demonstrate here that paracoxib enhances the renal dysfunction and injury associated with bi-lateral renal ischaemia (45 min) and reperfusion (6 h). Specifically, in rats subjected to renal ischaemia/reperfusion, parecoxib (20 mg/kg given 30 min prior to reperfusion and 3 h after the commencement of reperfusion) significantly enhanced the degree of (i) glomerular dysfunction (increases in serum creatinine and urea, and decrease in creatinine clearance); (ii) reperfusion-injury (increase in serum aspartate aminotransferase) and (iii) tubular dysfunction (increase in fractional excretion of sodium). Additionally, COX-2$^{-/-}$ mice subjected to renal ischaemia/reperfusion also significantly enhanced the degree of renal dysfunction and injury compared to their wild-type littermates. We show here that COX-2 plays an essential role in the function of the kidney during ischaemia/reperfusion of both the rat and mouse kidney.

Parecoxib was also unable to reverse the significant rise in mean arterial pressure 1 h after the commencement of reperfusion as seen in rats subjected to ischaemia/reperfusion alone and significantly reduced mean arterial pressure 4 h after the commencement of reperfusion when compared to rats subjected to ischaemia/reperfusion alone. The rise in mean arterial pressure at 1 h of reperfusion might be the result of the activation of the renin–angiotensin system, a major endocrine regulatory system of cardiovascular homeostasis, which is involved in reperfusion-injury of the kidney (Pazoki-Toroudi et al., 2003). However, it has been shown that renin release is inhibited by specific COX-2 inhibitors and even genetic deletion of COX-2 (see review (Harris et al., 2004)). This suggests that other mediators may be involved in influencing mean arterial pressure following ischaemia/reperfusion injury of the kidney, such as catecholamines. Further investigations would need to be carried out to determine this. The significant reduction in mean arterial pressure 4 h after the commencement of reperfusion may be due to the continual inhibition of renin release by parecoxib allowing mediators such as nitric oxide from inducible nitric oxide synthase (Chatterjee et al., 2003) to exert significant vasodilatation.

The effects (blood pressure and renal function) of inhibiting COX-2 with standard doses of celecoxib, rofecoxib, diclofenac or naproxen in individuals with normal renal function seems to be silent (Dilger et al., 2002; Schwartz et al., 1999). However, several studies have already focused on the role of COX-1 and -2 in the compromised kidney with conflicting results. It has been demonstrated recently that the pre-treatment of rats (i.e. before the kidney is subjected to an insult) with either indomethacin (non-selective COX inhibitor) or rofecoxib (selective COX-2 inhibitor) is able to ameliorate renal tissue damage induced by ischaemia/reperfusion injury, with a concomitant decline in the COX-2/COX-1 expression ratio (Feitoza et al., 2005). Another recent study has shown continuous intrarenal infusion of parecoxib (40 mg per pig) beneficially influenced kidney function during suprarenal aortic cross-clamping (Hauser et al., 2005). However, deficiency of the COX-1 gene is associated with sodium loss (Athirakul et al., 2001), whereas sodium restriction increases COX-2 expression (Lucas et al., 2005) and inhibition of COX-2 leads to sodium-sensitive hypertension (Zewde and Mattson, 2004). In patients, especially the elderly with renal complications, the administration of selective COX-2 inhibitors causes reduced glomerular filtration rate and reduced urinary sodium excretion (Swan et al., 2000). These data suggest that COX-2 inhibitors do have the potential to cause acute renal failure but most likely depends on the actual function of the kidney at the time of COX-2 inhibitor administration and the dosing regime used.

In our study, we demonstrate that the water-soluble selective COX-2 inhibitor, parecoxib, enhances the renal dysfunction and injury observed in the rat *in vivo* following ischaemia/reperfusion injury. This data is supported for the first time by COX-2$^{-/-}$ mice subjected to renal ischaemia/reperfusion injury. This suggests that COX-2 plays a pivotal role in the pathogenesis of acute renal faliure.

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