Available online at www.sciencedirect.com
ScienceDirect
European Journal of Pharmacology 562 (2007) 148–154
www.elsevier.com/locate/ejphar

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

Nimesh S.A. Patel [a,*], Salvatore Cuzzocrea [b,c], Massimo Collino [a], Prabal K. Chaterjee [d], Emanuela Mazzon [b,c], Domenico Britti [e], Muhammad M. Yaqoob [a], Christoph Thiemermann [a]

[a] *Centre for Experimental Medicine and Nephrology and Critical Care, William Harvey Research Institute, St. Bartholomew's and The Royal London School of Medicine and Dentistry, Queen Mary — University of London, Charterhouse Square, London, EC1M 6BQ, UK*
[b] *Department of Clinical and Experimental Medicine and Pharmacology, Institute of Pharmacology, University of Messina, Italy*
[c] *IRCCS Centro Neurolesi "Bonino-Pulejo", Messina, Italy*
[d] *School of Pharmacay and Biomolecular Sciences, University of Brighton, UK*
[e] *Department of Veterinary and Agricultural Science, University of Teramo, Italy*

Received 7 November 2006; received in revised form 18 January 2007; accepted 24 January 2007
Available online 8 February 2007

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

* Corresponding author. Tel.: +44 207 882 5810; fax: +44 207 900 2475.
E-mail address: n.s.patel@qmul.ac.uk (N.S.A. Patel).

0014-2999/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2007.01.079

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 ($N=6$).

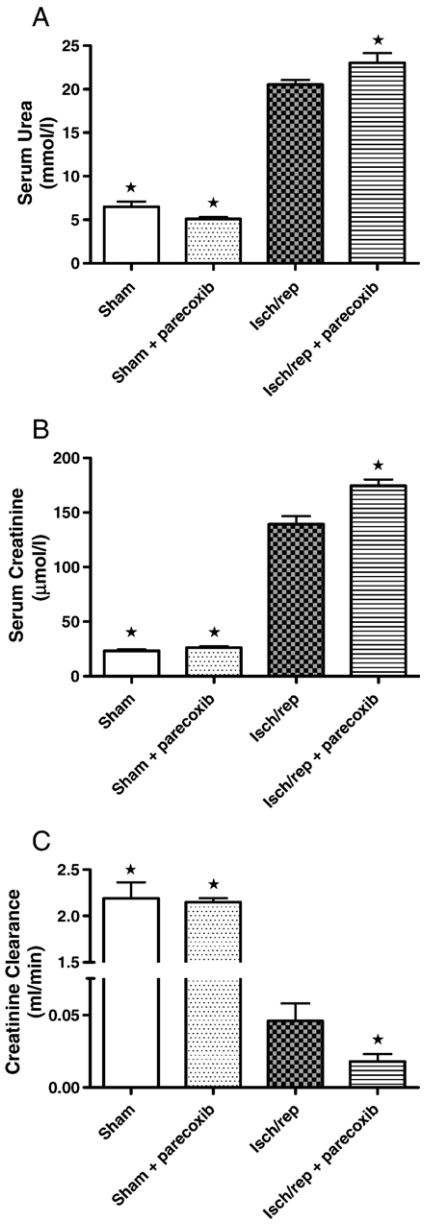

Fig. 1. Effect of parecoxib on glomerular dysfunction mediated by ischaemia/reperfusion in rats. (A) serum urea levels, (B) creatinine levels and (C) creatinine clearance were measured subsequent to sham-operation (Sham, $N=9$) or renal ischaemia/reperfusion (Isch/rep, $N=9$). Rats were administered 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.