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Immunohistochemical localisation of renal cyclooxygenase-1 expression in non-steroidal anti-inflammatory drug-treated mice

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ABSTRACT

The utility of cyclooxygenase-inhibiting non-steroidal anti-inflammatory drugs is limited by unwanted side effects that include disturbances in renal function. In order to further understand the mechanisms that underlie these renal side effects, the expression of the prostaglandin-synthesizing enzyme cyclooxygenase (COX) was examined by immunohistochemical methods in murine kidneys after treatment with indomethacin a non-selective inhibitor or nimesulide a inhibitor that preferentially and selectively blocks the COX-2 isoform of the enzyme. In untreated control kidneys, COX-1 protein was expressed in the glomeruli and parietal cells of the glomerular capsule, epithelial cells of the proximal and distal convoluted tubules including the juxtaglomerular region, and the collecting ducts. At therapeutic doses, indomethacin (10mg/kg) did not alter renal COX-1 expression relative to immunoreactivity in untreated control kidney. By contrast, an equipotent therapeutic dose of nimesulide reduced renal COX-1 expression within the first 24 hours of treatment. Taken together with the reports of reduced COX-1 expression prior to renal tissue damage following high dose indomethacin treatment, our findings suggest that effects of NSAIDs on renal COX expression are dependent on dose and may be related to isoform specificity.

Keywords: prostaglandin, kidney, cyclooxygenase, NSAIDs,

INTRODUCTION

The kidney is an important site for the production of prostaglandins, the biosynthesis of which is mediated by the activity of both isoforms of the arachidonic acid converting enzyme

cyclooxygenase (COX-1, COX-2). These bioactive prostanoids are involved in the maintenance of a range of physiological functions in the kidney including regulation of glomerular filtration rate and electrolyte balance, as well as modulation of renal haemodynamics. These functions occur in spatially defined sites in the renal cortex and medulla (such as the juxtaglomerular apparatus, glomerular mesangium, convoluted tubules, collecting duct) (Nasrallah and Herbert, 2005).

Several studies have examined the correlation between PG-mediated renal activity and cyclooxygenase localisation in the kidney. In rats and mice, COX-1 expression has been described in the extraglomerular mesangium, juxtaglomerular apparatus and arteriolar endothelia in the kidney (Campean et al., 2003). Significant expression is also present in cortical collecting ducts and the cortical interstitium but not in the thin limb of the loop of Henle, macula densa and parts of the distal convoluted tubule. Expression of COX-1 has however been reported in glomerular cells and the collecting duct in human kidney (Komhoff et al., 1997). COX-2 is expressed in the glomeruli in the human kidney (Adegboyega and Ololade, 2004) and in the thick ascending loop of Henle and macula densa in the rat kidney (Harris, 2000). The relative importance of both COX isoforms is demonstrated by the finding that kidneys from COX-2 deficient mice fail to develop normally whereas kidneys from COX-1 deficient mice are not associated with any reported abnormalities (Dinchuk et al., 1995; Morham et al., 1995).

COX inhibitors such as nonsteroidal anti-inflammatory drugs (NSAIDs) are widely employed in the treatment and clinical management of a variety of inflammatory, arthritic and neoplastic conditions as well as the provision of analgesia. These inhibitors are classified according to their

ability to preferentially antagonise one or both isoforms of COX; most commonly prescribed inhibitors inactivate both COX-1 and COX-2. The clinical use of these agents is however limited by unwanted gastrointestinal and renal toxicity (Eras and Perazella, 2001; Sigthorsson et al., 2002) with 1-5% of patients developing renal syndromes that necessitate intervention (Whelton, 1999). Decreased renal perfusion and salt excretion as well as renal papillary necrosis are thought to arise from COX-1 inhibition (Brater et al., 1987; Brater et al., 1999; Brater et al., 2001; Whelton, 1999). Is there any correlation between clinical inhibitor therapy and COX-1 enzyme expression in the kidney? To address this question, this study examined the expression of COX-1 in the kidney before and after treatment with two COX inhibitors: indomethacin a non-selective inhibitor and nimesulide a preferentially selective COX-2 inhibitor.

MATERIALS AND METHODS

Animals

Forty-five male CD-1 mice, aged 8 weeks old were used in this study, housed in plastic cages under standard conditions and allowed access to food and water ad libitum. Animals were allocated equally to one of three groups: one group of animals was given indomethacin, a second group received Nimesulide, and the remaining group received vehicle. The vehicle-treated group served as control. There were 15 animals in each group.

Treatments

Each animal received one of the following treatments every 12 hours by intraperitoneal injection: Indomethacin (Sigma-Aldrich, Dublin, Ireland) 10mg/kg body weight, Nimesulide (Helsinn Birex, Dublin, Ireland) 15mg/kg body weight, or vehicle. All treatments were given for 24 hours.

The doses of inhibitors administered were equipotent (Sigthorsson et al., 1998; Sigthorsson et al., 2002) and each treatment solution was made by dissolving the drug in 5ml of vehicle. The vehicle solution was made up of 0.9% saline to which a drop Tween 80 dispersing agent was added. Addition of the dispersing agent was necessary because both indomethacin and nimesulide dissolve very slowly in aqueous media.

Tissue retrieval and processing

Each kidney was delivered by laparotomy, cut transversely into several 1-2mm thick specimen slices and immersion fixed in 10% formal saline for 24 hours. Specimens were processed routinely for embedding in paraffin wax. Sections obtained at a thickness of 5µm were placed on poly-L-lysine coated glass slides.

Immunohistochemistry

Paraffin sections were dewaxed in xylol, rehydrated through graded alcohols, and antigen retrieval performed by heat-treatment in a microwave oven in 10mM sodium citrate buffer solution pH 6.0 for 10 minutes. Immunostaining was carried out according to the protocol of Coons (1958). Sections were treated with 3% hydrogen peroxide in methanol to block endogenous peroxidase activity and pre-incubated with 10% normal goat serum for 1 hour to block extraneous binding sites prior to incubation with primary antibody to COX-1 overnight. The following antibodies were used: rabbit polyclonal raised against murine COX-1 (1:200, Cayman Chemical, Ann Arbor, MI, USA) and biotin-conjugated goat secondary antibody raised against rabbit IgG (Sigma-Aldrich, Dublin, Ireland). Following treatment with secondary biotin-conjugated goat anti-rabbit antibody (1:200) for 1 hour, and subsequently with avidin-biotin

(Vector Laboratories, Burlingame, CA, USA), antibody binding was visualised with diaminobenzidine (DAB) and sections counterstained with haematoxylin. Slide coverslips were mounted with XAM. Specificity of the antibody was confirmed by omitting the primary antibody (negative control) or by preabsorbing the primary antibody for 60 minutes in a 10-fold molar excess of blocking peptide (Cayman Chemical, Ann Arbor, MI, USA). Intensity of COX immunostaining in the sections was evaluated by light microscopy and assessed by blinded review.

Ethical Approval

Ethical approval for this study was sought from and granted under institutional ethics regulatory mechanisms and all procedures carried out, under licence, within the provisions of the Cruelty to Animals Act (Ireland) 1876.

RESULTS

Control kidney:

Immunohistochemical analysis revealed expression of COX-1 in both the renal cortex and medulla with significantly more expression evident within the cortex. Immunoreactivity in the superficial cortex was more pronounced than in the rest of the cortex or the medulla, and was localised predominantly to the cell cytoplasmic compartment. However, immunoreactive protein was also present in the nucleus of a minority of cells within the superficial cortex. COX-1 protein was expressed in the glomeruli and parietal cells of the glomerular capsule, epithelial cells of the proximal and distal convoluted tubules, and the collecting ducts (Fig 1a, b). Cytoplasmic COX-1 protein within the distal tubule was present in the juxtaglomerular region,

adjacent to the glomerulus. Interstitial cells in the renal cortex did not show reactivity for COX-1. Within the medulla, pronounced nuclear and cytoplasmic expression of COX-1 was apparent within the endothelial cells of the renal vasculature but the epithelial cells of the medullary collecting ducts and the interstitial cells in the medulla showed poor reactivity for COX-1.

Indomethacin-treated kidney:

Immunostaining was present in both the cortical and medullary areas of indomethacin-treated specimens. In contrast to the cytoplasmic localisation of COX-1 immunoreactivity in control kidney, COX-1 staining within superficial cortical cells in indomethacin-treated kidneys was diffuse and confined to the nucleus. COX-1 immunoreactive protein was apparent in the outer parietal layer of the Bowman's capsule and in some cells of the glomerulus (Fig 1c). Cytoplasmic staining was observed within cells of the proximal and distal convoluted tubules while nuclear immunoreactivity was evident within the cortical collecting ducts. Localization of cytoplasmic COX-1 within the endothelial cells of the renal blood vessels and the medullary collecting ducts was minimal (Fig 1d). There was no staining for COX-1 immunoreactive protein in the cortical or the medullary interstitium.

Nimesulide-treated kidney:

There was significant expression of COX-1 protein in the superficial cortical region adjacent to the renal capsule and evidence of some COX-1 immunoreactivity in the outer parietal epithelial cell layer of Bowman's capsule (Fig 1e). Very minimal, diffuse cytoplasmic staining was evident in the proximal convoluted tubule. There was COX-1 protein expression within cells of the cortical distal convoluted tubules in both the nucleus and cytoplasm, with significant localisation

within the cortical collecting ducts. Focal cytoplasmic immunostaining was seen in endothelial cells of blood vessels and collecting duct cells in the renal medulla (Fig 1f).

DISCUSSION

Previous studies have documented the expression of COX-1 in human, rat and murine kidney (Campean et al., 2003; Khan et al., 1998; Komhoff et al., 1997; Vitzthum et al., 2002; Zidar et al., 2008). Human COX-1 protein expression was reported in the collecting duct cells, endothelial cells of the renal vasculature (more so in the afferent arteriole at the glomerular entrance), interstitial cells and in the parietal cells of Bowman's capsule (Komhoff et al., 1997). Expression was also localized to the terminal portion of the distal convoluted tubule. In the rat and mouse, COX-1 expression was observed in the juxtaglomerular apparatus of the distal convoluted tubule, the terminal portion of distal convoluted tubule and the cortical collecting duct (Campean et al., 2003). The mesangial cells within the glomerulus also exhibited strong affinity for the COX-1 antibody. In the murine renal tissue significant expression was noted in the cortical interstitium. Findings from our study of COX-1 expression in the kidney of CD-1 mice indicate the presence of COX-1 protein in the juxtaglomerular region of the distal convoluted tubule. Other workers have revealed significant COX-1 expression within the terminal distal convoluted tubule and the connecting tubule (Campean et al., 2003; Stubbe et al., 2003). The distal tubule represents an important location for electrolyte absorption. Of interest is the presence of COX-1 protein in the proximal convoluted tubule, a finding that has not previously been reported. COX-1 expression was also evident within the endothelial cells of the renal vasculature and the cortical and medullary collecting ducts which agree with previous reports (Vitzthum et al., 2002; Zidar et al., 2008). Immunohistochemical analysis revealed COX-

1 staining within the parietal epithelium of the Bowman's capsule. This is in keeping with data from Zidar et al (2008) who localized COX-1 expression in the same area. However this study did not find COX-1 staining within the mesangium of the glomerulus. The cortical and medullary interstitium was negative for COX-1 protein in our study in contrast to Campean et al (2003) who reported significant murine expression of the protein in the cortical interstitium. These differences may be due to interspecies variation in renal localization of cyclooxygenase (Khan et al., 1998).

Cyclooxygenase (COX) exists in two related but unique isoforms in the renal kidney. NSAIDs cause renal toxicity following direct inhibition of COX isoforms. Humans and animals exhibit differences in susceptibility, which may be associated with differences in expression of one or both isoforms in the kidney (Radi, 2009). Rats and dogs exhibit greater susceptibility than humans to the development of renal papillary necrosis due to their sluggish blood supply (Khan and Alden, 2002; Khan et al., 2002). Renal damage as a result of NSAID administration is due to ischemic injury through direct inhibition of the vasodilatory effects of renal prostaglandins (Silva, 2004). Previous studies have looked at COX expression in renal tissue following a single toxic dose (75mg/kg) of indomethacin that caused papillary necrosis (Khan et al., 1998) accompanied by a slight to moderate decrease in papillary COX expression. This decrease was observed 2 hours after administration of the agent and was also seen in tissue that lacked the light microscopic changes of renal papillary necrosis. The possible mechanism that leads to an initial decrease in COX immunoreactivity in indomethacin-treated animals is not known.

Dose and duration of administration of NSAIDs are both important in determining effects in many tissues. Although other studies have employed therapeutic doses of NSAIDs over varying durations, effects on COX expression have not been examined (McGarvey et al., 2007a;

McGarvey et al., 2007b; Sigthorsson et al., 1998) In this study a therapeutic dose (10mg/kg) of indomethacin did not alter COX expression but an equipotent dose of nimesulide (15 mg/kg) caused reduced expression. Taken together with the finding that a toxic dose of indomethacin reduces COX expression prior to renal damage, our results suggest that the effect of NSAIDs on renal COX-1 expression may be dependent on dose and isoform specificity. It would be of interest to determine what effect high/toxic doses or prolonged administration of nimesulide may have on renal tissue. In comparison to the information on the effects of Indomethacin on renal COX-1 expression, there is a lack of information on the effect of selective COX-2 inhibitors such as nimesulide on the expression of COX-1 in renal tissue.

In conclusion this study has documented the expression of COX-1 along the nephron and reported the presence of COX-1 in the proximal convoluted tubule, a finding not previously described. This study has also determined that administration of therapeutic doses of indomethacin and nimesulide have different effects on COX-1 expression in the murine kidney and that these effects may be related to drug dose and isoform specificity.

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FIGURE LEGEND**Figure 1.**

Immunoreactivity to COX-1 protein with haematoxylin counterstaining. Distribution of COX-1 protein in proximal and distal tubules (macula densa) in untreated control kidney (a). There is minimal COX-1 staining on the outer periphery of the glomerulus. COX-1 immunostaining is significant in the vasa recta and collecting duct (b). Indomethacin-treated kidneys (c) show decreased nuclear and cytoplasmic COX-1 immunostaining within the cortical collecting ducts and minimal staining in the glomerular capsule. There is also significant nuclear immunoreactivity within the endothelial cells of the renal vasculature (d) with minimal staining of collecting ducts. In nimesulide-treated kidneys, COX-1 protein was present in the glomerular capsule, proximal and distal tubules (e, f). Untreated kidney with primary antibody omitted from immunostaining protocol (g). Scalebar = 50 micrometer

