Immunolocalization of cyclooxygenase-2 in the macula densa of human elderly

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Abstract To gain insight into the role of prostanoids in human kidney function, we examined the distribution of cyclooxygenase (COX) 1 and COX-2 by immunofluorescence and immunohistochemistry in human kidneys from adults of various age groups. COX-1 was detected in the collecting ducts, thin loops of Henle and portions of the renal vasculature. COX-2 was detected in the renal vasculature, medullary interstitial cells, and the macula densa. In addition, COX-2 immunoreactivity was noted in afferent arteries and the macula densa of the renal cortex and was more evident in the kidneys of older adults.

Key words: Kidney; Human; Cyclooxygenase; Macula densa; Immunohistology

1. Introduction

The cyclooxygenase enzymes (COX) catalyze the formation of prostanoids and are the products of two distinct genes, COX-1 and COX-2. The COX-1 gene is ubiquitous and has been previously linked to the cytoprotective effects of prostanoids in the gut as well as to the gastrointestinal (GI) toxicity associated with sustained non-steroidal anti-inflammatory drug (NSAID) therapy [1,2]. The COX-2 gene is induced by a variety of stimuli and has been linked to inflammatory responses [3–5]. It has been demonstrated that COX-2 selective inhibitors have anti-inflammatory activities with a reduced GI toxicity profile [6–9].

Both COX-1 and COX-2 are expressed in the kidney [10–14], and sustained NSAID therapy has a low incidence of renal toxicity [15,16]. Previous reports have stated that COX-1 is highly expressed in the collecting ducts and the renal vasculature of rats, dogs, rabbits, monkeys, and humans [12–14]. However, the renal distribution of COX-2 differs among these species, in addition to differing from the COX-2 expression pattern found in humans. Although it has been shown that COX-2 is expressed in the macula densa of rats, rabbits and dogs [12–14], this observation has not been reported in humans or non-human primates [10,14]. Moreover, in rats and dogs, the expression of COX-2 in the macula densa is positively regulated by volume depletion caused either by low salt diet or by angiotensin-converting enzyme inhibitors [12,14,17]. In humans, these conditions are more frequent in the elderly who are also more likely to undergo chronic NSAID therapy. Therefore, we examined the distribution of COX-1 and COX-2 from adults within different age groups.

2. Materials and methods

2.1. Sample preparation

Kidney tissues were obtained from 10 patients aged 17–97 years old (average: 52.5, median: 43, see Table 1). Their deaths were unrelated to renal or cardiovascular pathologies (mostly car accidents). The kidneys were fixed in paraformaldehyde, dehydrated, embedded in paraffin and sectioned onto Superfrost Plus slides (Fisher Scientific, Pittsburgh, PA, USA). The samples were encoded for unbiased examination and review.

2.2. Immunohistology

Immunostaining was performed using rabbit polyclonal anti-peptide antisera ovCOX-1 (160108, Cayman Chemical Co., Ann Arbor, MI, USA), hCOX-2 (160107, Cayman), mCOX-2 (160116, Cayman) and rabbit antiserum raised against whole ovCOX-2 protein. All steps were performed at room temperature.

Briefly, sections were deparaffinized, hydrated to dH_2O, and washed in PBS. Following a 30 min treatment with 0.05% blocking buffer (NEN Life Science Products, Boston, MA, USA) sections were incubated for 2 h with COX-specific antisera diluted to working concentrations in the blocking buffer.

Fluorescent detection of bound COX antibodies was accomplished by a subsequent 1 h incubation with Alexa 594-conjugated anti-rabbit IgG (Molecular Probes, Inc., Eugene, OR, USA). Colorimetric demonstration of bound COX antibodies was performed by incubating the sections for 30 min with peroxidase-conjugated anti-rabbit IgG (Boehringer Mannheim) after washing in PBS/0.05% Tween. The anti-rabbit IgG was diluted in 10% Superblock (Pierce) to inhibit non-specific binding. Sections were developed with Enhanced DAB substrate (Pierce). Endogenous peroxidase activity was quenched prior to application of the secondary antibody by a 15 min treatment with Peroxidase Inhibitor (Pierce). The slides were counterstained with Gill's hematoxylin (Fisher) and examined on a Zeiss Axiophot fluorescence microscope equipped with a CCD camera. Non-specific signals were recorded from slides tested with the secondary antibodies.

3. Results

3.1. Cyclooxygenase-1

COX-1 expression in the kidney was primarily confined to the renal medulla. Strong immunoreactivity was detected in the collecting duct epithelium (Fig. 1A,B). Less intense staining was seen in the descending thin loops of Henle (arrows in Fig. 1A,B) and interstitial cells in the papilla (not shown). Many of the arcuate arteries also expressed COX-1 that appeared to be confined to the tunica media (Fig. 1CC). This pattern of COX-1 expression was observed in 10/10 specimens.

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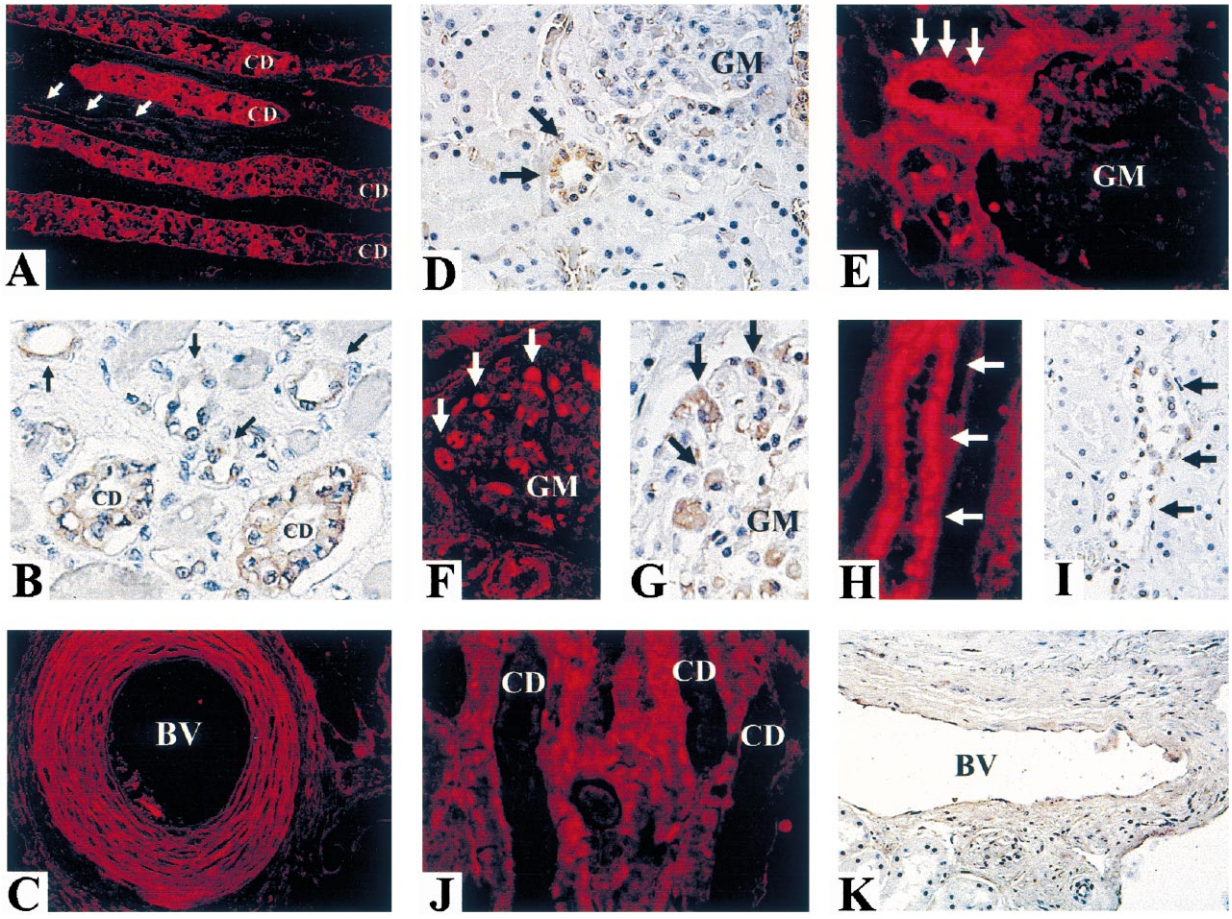

Fig. 1. COX-1 (A–C) and COX-2 (D–K) immunoprotein in human kidney detected by immunofluorescence (A, C, E, F, H, J) and immunohistochemistry (B, D, G, I, K). A positive labeling with COX-1 antibodies is detected in collecting ducts (A, B), thin loop of Henle (arrows in A, B) and arcuate arteries (BV in C). In the cortex, COX-2 immunoreactivity (arrows) was observed in the macula densa (D), afferent arteriole (E), glomerular podocytes (F, G) and a small number of thick ascending loops (H, I). In the medulla, positive COX-2 immunoreactivity was localized to interstitial cells (J) and arcuate arteries (K). GM: glomerulus; CD: collecting ducts; BV: blood vessels.

3.2. *Cyclooxygenase-2*

The expression of COX-2 in human kidneys is shown in Fig. 1D–K. Each of the COX-2 specific antisera gave similar staining patterns where, in contrast to COX-1, COX-2 protein was detected in both the medulla and the cortex. In the cortex, COX-2 immunoreactivity was identified in the macula densa (Fig. 1D), the glomerular afferent arteriole (Fig. 1E), podocytes in a subset of glomeruli (Fig. 1F,G), and in a limited number of thick loops of Henle (Fig. 1H,I). In the medulla, as with COX-1 expression, COX-2 immunoreactivity was limited to interstitial cells (Fig. 1J) and the tunica media of arcuate arteries (Fig. 1K).

Table 1
Distribution of COX-2 protein in human kidney

Subject I.D.	Sex	Age	Pod	MD	TAL	mIC	BV
A97-102-N1	M	17	−	−	±	+	+
A97-107-O1	M	29	±	+	±	+	+
A97-116-O1	M	29	±	−	±	+	+
A97-122-N1	M	38	+	−	±	+	+
A98-86-N1	M	38	±	±	±	+	+
36833	F	48	−	+	±	+	+
15929	M	67	±	+	±	n.d.	+
A98-80-O1	M	79	−	+	±	+	+
A-98-85-O1	F	83	±	+	±	+	+
A98-56-N1	M	97	−	+	±	+	+

Pod: podocytes; MD: macula densa; TAL: thick ascending loop; mIC: medullary interstitial cells; BV: blood vessels.
+: present; ±: present in a subset of cells; −: absent; n.d.: not determined.

The presence of COX-2 in the macula densa was more evident in kidney specimens obtained from older subjects (Table 1). We detected COX-2 immunoreactivity in the macula densa of five of five kidneys (100%) from subjects aged 48 years or greater. Only two of five kidneys (40%) from subjects less than 40 years of age showed COX-2 macula densa immunoreactivity. COX-2 positive immunostaining was also detected within podocytes of the some glomeruli in six of 10 kidneys (60%) (Table 1).

4. Discussion

This is the first report of the presence of COX-2 protein in human macula densa. Previous reports failed to detect COX-2 in this structure [10,14] either because of a lower specific activity of the COX-2 antisera used in this case or because their samples came from donors <50 years old [14]. The macula densa functions as an integral part of the renin-angiotensin system and is thought to monitor Na^+ and K^+ levels in the distal tubule lumen. Renin release, triggered by the macula

densa, results in the eventual secretion of aldosterone that, in turn, promotes reabsorption of sodium ions and water from the glomerular filtrate thereby contributing to the maintenance of plasma volume and blood pressure.

Previous reports have proposed that prostanoids are involved in the regulation of renin release [17–20]. In rats, the COX-2 protein is expressed in the macula densa and other distal tubule cells [12,20]. After administration of a selective COX-2 inhibitor, increases in renal renin mRNA levels are prevented when those animals are maintained on a low sodium diet [19]. Our finding of COX-2 protein in human macula densa suggests the possibility of a similar mechanism for the regulation of renal renin release in humans.

It has been observed in rodents that angiotensin converting enzyme inhibitors and angiotensin antagonists induced the expression of COX-2 in the macula densa. Since renin secretion decreases with aging [21], it is possible that this reduction is involved in the COX-2 up-regulation observed in the elderly subjects.

In conclusion, our results corroborate earlier reports of COX-1 and COX-2 renal expression [10,12–14]. Moreover, we extend the current data to include the novel finding of macula densa COX-2 expression in humans.

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