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Cyclooxygenase-2 expression is associated with the renal macula densa of patients with Bartter-like syndrome

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Background. Bartter-like syndrome (BLS) is a heterogeneous set of congenital tubular disorders that is associated with significant renal salt and water loss. The syndrome is also marked by increased urinary prostaglandin E_2 (PGE_2) excretion. In rodents, salt and volume depletion are associated with increased renal macula densa cyclooxygenase-2 (COX-2) expression. The expression of COX-2 in human macula densa has not been demonstrated. The present studies examined whether COX-2 can be detected in macula densa from children with salt-wasting BLS versus control tissues.

Methods. The intrarenal distribution of COX-2 protein and mRNA was analyzed by immunohistochemistry and in situ hybridization in 12 patients with clinically and/or genetically confirmed BLS. Renal tissue rejected for transplantation, from six adult patients not affected by BLS, was also examined.

Results. The expression of COX-2 immunoreactive protein was observed in cells of the macula densa in 8 out 11 patients with BLS. In situ hybridization confirmed the expression of COX-2 mRNA in the macula densa in 6 out of 10 cases. COX-2 protein was also detected in the macula densa in a patient with congestive heart failure. The expression of COX-2 immunoreactive protein was not observed in cells associated with the macula densa in kidneys from patients without disorders associated with hyper-reninemia.

Conclusion. These studies demonstrate that COX-2 may be detected in the macula densa of humans. Since macula densa COX-2 was detected in cases of BLS, renal COX-2 expression may be linked to volume and renin status in humans, as well as in animals.

Bartter-like syndrome (BLS) comprises a set of autosomal recessively inherited renal tubular disorders characterized by hyperprostaglandinuria, hyper-reninemia, and hyperaldosteronism with hypokalemia and metabolic alkalosis but normal blood pressure [1, 2]. Mutations in at least three genes essential for chloride reabsorption in the thick ascending limb of Henle [3–5] and distal convoluted tubule [6] are associated with phenotypically distinct tubulopathies [1, 7]. Patients with classic Bartter's syndrome are characterized by mutations encoding the basolateral chloride channel. In contrast, hyperprostaglandin E_2 syndrome or antenatal Bartter's syndrome (HPS/aBS) is caused by mutations in genes encoding the apical furosemide-sensitive Na-K-2Cl cotransporter and the inwardly rectifying renal potassium channel (RomK). The metabolic disturbances in these patients may be ameliorated by administration of cyclooxygenase (COX)–inhibiting nonsteroidal anti-inflammatory drugs (NSAIDs) [1].

Cyclooxygenase-mediated prostaglandin formation yields a family of labile, bioactive lipids that modulate a variety of physiological and pathophysiological processes, including renal glomerular filtration, renin release, and epithelial salt and water transport [8]. NSAIDs such as indomethacin block the formation of prostaglandins by inhibiting the enzymatic activity of COX-1 and COX-2 [9]. COX-1 has been proposed to be a housekeeping enzyme, and its inhibition is associated with the well-known side effects of NSAIDs, particularly gastric ulcers and bleeding [9]. COX-2 is usually not expressed in most tissues but can be induced by growth factors and inflammatory cytokines [10]. In contrast to most other tissues, the kidney constitutively expresses both COX isozymes. COX-1 is expressed predominantly within cells of the collecting duct [11], while in rodents, COX-2 has been shown to be expressed in medullary interstitial cells and in cells in the macula densa [12–14].

The macula densa is a specialized cluster of the cortical thick ascending limb cells that are thought to sense luminal salt concentration and thereby control glomerular perfusion [15, 16]. In rodents, salt depletion [14] and renal artery stenosis [17] induce expression of COX-2 in

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Table 1. Expression of COX-2 mRNA and protein in patients with Bartter-like syndrome

Genetic defect	COX-2 ISH	COX-2 Histo	Combined ISH and Histo
ClCnKB	neg	pos	pos
ClCnKB	pos	neg	pos
? (HPS/aBS with SND)	pos	pos	pos
$NaKCl_2$	neg	pos	pos
$NaKCl_2$	pos	pos	pos
$NaKCl_2$	pos	neg	pos
RomK	pos	pos	pos
RomK	ND	pos	pos
RomK	neg	pos	pos
RomK	neg	pos	pos
?	pos	neg	pos
Total	6/10	8/11	11/11

Abbreviations are: COX, cyclooxygenase-2; ISH, in situ hybridization; Histo, immunohistochemistry; pos, positive; neg, negative; ND, not done.

the macula densa. A stimulatory effect of COX-2–derived products on renin release has been suggested by studies showing that selective COX-2 inhibitors reduce renin release in animals with renal artery stenosis or salt and volume depletion [17, 18]. In the present studies, we examined whether COX-2 is also expressed in the renal macula densa of patients with clinically and/or genetically defined BLS, a disease associated with activation of the renin-angiotensin system. In addition, COX-2 expression was analyzed in several patients without underlying BLS.

METHODS

Patients

Renal biopsies were performed in children with genetically or clinically defined BLS to evaluate the effects of long-term indomethacin treatment. Informed written consent from the parents was obtained. In addition, we analyzed renal tissue ($N = 6$) from kidneys deemed unsuitable for kidney transplantation. Medical history did not indicate conditions associated with salt or volume depletion in five cases. One patient had congestive heart failure and required an aortic balloon pump for pressure support prior to death. Approval by the local ethics committee was obtained.

Immunostaining

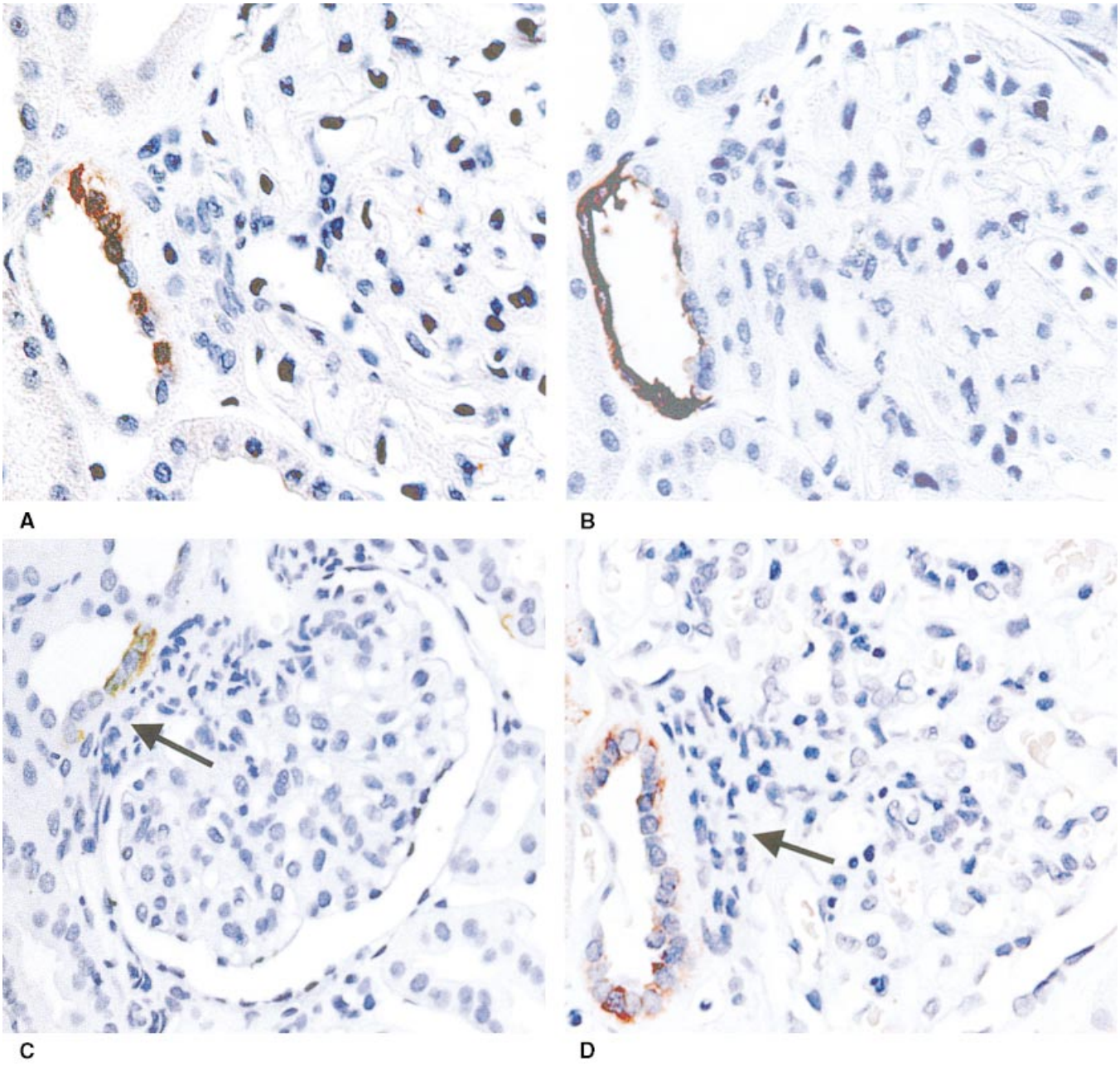
Sections were cut at 3 μm thickness, deparaffinized in xylene, and incubated for 30 minutes in methanol containing 0.3% H_2O_2 to block endogenous peroxidase activity. Primary antibodies were obtained from Santa Cruz (goat polyclonal anti-human COX-2: C-20, sc#1745; Santa Cruz, CA, USA). For detection of COX-2, immunoreactive protein sections were microwaved for three minutes in phosphate-buffered saline (PBS) containing 0.1 mol/L sodium citrate, pH 6.0. Polyclonal anti-Tamm-Horsfall and anti-COX-2 antibodies were diluted 1:200 in TBST (50 mmol/L Tris, pH 7.5, 300 mmol/L NaCl, and 0.05% Tween 20) containing 1% bovine serum albumin (BSA), 5% normal horse serum, and 1% nonfat dry milk. Immunolabeling was detected using a biotinylated rabbit anti-goat antibody followed by visualization with an avidin-biotin horseradish peroxidase labeling kit (Vectastain ABC kit) and diaminobenzidine staining.

In situ hybridization

In situ hybridization was performed as previously described [19]. Briefly, prior to hybridization, tissue sections were deparaffinized, refixed in paraformaldehyde, treated with proteinase K (20 μg/mL), washed with PBS, refixed in 4% paraformaldehyde, and treated with triethanolamine plus acetic anhydride (0.25% vol/vol). ^{35}S-labeled antisense and sense riboprobes synthesized from the COX-2 specific 3′ untranslated region of the human COX-2 cDNA (471 bp) were hybridized to the section at 55°C for 18 hours. After hybridization and stringency washes, sections were treated with RNase A (10 μg/mL) at 37°C for 30 minutes. Slides were dehydrated, air-dried, dipped in photoemulsion (Ilford K5, Knutsford, Cheshire, UK), and exposed for four to five days at 4°C. Photomicrographs were viewed with a Zeiss Axioskop microscope using either bright-field or dark-field optics. Pictures were captured with a digital camera (Spot-Cam; Diagnostic Instruments, Sterling Heights, MI, USA), and color composites were generated by using Adobe Photoshop version 4.0 on a Power Macintosh.

RESULTS

In renal tissue deemed unsuitable for transplantation, COX-2 immunoreactive-protein (ir) was observed in cells of the macula densa in one of the six cases, that being a kidney from a patient with congestive heart failure (Fig. 1A). Only some maculae densae showed COX-2 labeling. Typically, COX-2 ir-protein was restricted to 5 to 10 cells. Serial sections processed with anti-Tamm-Horsfall antibodies (Fig. 1B) showed that COX-2 ir-protein expression was restricted to the cells of the macula densa and did not overlap with Tamm-Horsfall immunoreactive protein. In five cases with no underlying renal or cardiovascular pathology, COX-2 immunoreactive protein was not observed in the macula densa but was present in interstitial cells in the medulla (data not shown). In patients with BLS COX-2 immunoreactivity was also detected in the macula densa in 8 out of 11 biopsies. The underlying mutations in these patients are provided in Table 1. Figure 1C shows expression of COX-2 in a patient with a genetic defect in the gene encoding RomK; Figure 2D demonstrates COX-2 in a patient with a genetic defect in the gene encoding the furosemide sensitive NaK2Cl transporter. Typically, COX-2 protein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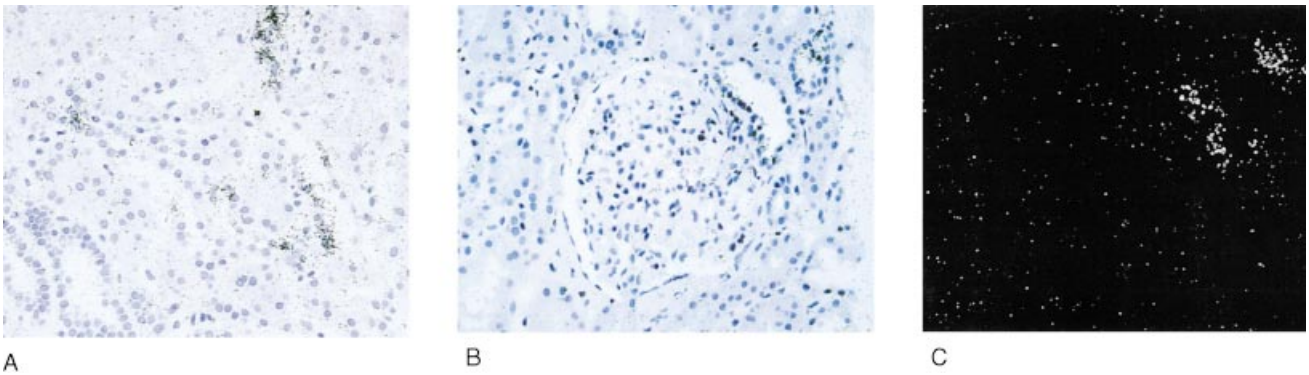
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B
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Fig. 1. Expression of cyclooxygenase-2 (COX-2) immunoreactive protein. Serial sections of renal cortex from a patient with congestive heart failure were processed with anti–COX-2 (*A*) and anti-Tamm-Horsfall antibodies (*B*). COX-2 immunoreactive protein is expressed in the cells of the macula densa but not in the surrounding cells of the thick ascending limb of Henle, which are identified by expression of Tamm-Horsfall protein (B). Expression of COX-2 immunoreactive protein was also observed in a patient with a genetic defect in the gene encoding RomK (*C*) and in a patient with a genetic defect in the furosemide-sensitive NaK2Cl transporter (*D*). Publication of this figure in color was made possible by an unrestricted educational grant from Merck Pharmaceuticals, Inc.

expression was not abundant. Hyperplasia of the juxtaglomerular apparatus was also seen (Fig. 1 C, D, arrows).

In 6 out of 10 biopsies, COX-2 mRNA expression was also detected by in situ hybridization (Table 1). In one patient with a mutation in the gene encoding a basolateral chloride channel, COX-2 mRNA was expressed in the renal medulla (Fig. 2A) in a nephron segment, possibly the thick ascending limb of Henle (TALH). In a patient with clinically confirmed BLS, COX-2 mRNA is expressed in the macula densa (Fig. 2B, brightfield; Fig. 2C, darkfield). Expression of COX-2 protein and mRNA was also observed in a HPS/aBS patient with chronic renal failure and sensorineural deafness (HPS/aBS with SND).

DISCUSSION

Expression of COX-2 in the macula densa has been reported in rats, rabbits, and mice [12–14], and salt depletion has been shown to induce COX-2 in the macula densa [14, 18]. In contrast, previous reports did not detect COX-2 in macula densa of primates or humans [20, 21]. We now report that COX-2 is also expressed in the renal macula densa of humans with documented BLS or congestive heart failure, diseases that are characterized by activation of the renin-angiotensin system. The paucity of glomeruli within biopsy tissue and the initial observation [14] that only a minority of rat maculae densae display expression of COX-2, even when induced by a low-salt diet, may explain the finding that not all of the renal biopsies analyzed were positive. COX-2 labeling was not detected in kidneys from control subjects, consistent with earlier studies that also failed to detect COX-2 in human macula densa [21]. The observation that COX-2 is present in human macula densa reinforces the basis for extrapolating experimental data regarding the role of COX-2 in the kidney, from animals to humans.

In BLS, the administration of indomethacin suppresses plasma renin activity [1]. The present studies suggest COX-2 activity in the macula densa could contribute to the increased renin levels in BLS. A recent report showed that treatment of female volunteers with nimesulide, a clinically approved COX-2 inhibitor, suppressed furosemide-induced renin levels [22]. This supports a relationship between COX-2 and plasma renin activity in humans. COX-2 was also abundantly expressed in the macula densa of a patient with congestive heart failure, which is in keeping with the concept that reduced luminal salt delivery or entry of salt into the macula densa cells may be the stimulus that drives COX-2 expression.

Increased renal production of prostaglandin E_2 is both a characteristic feature and an important indicator of therapeutic efficacy in BLS [1]. Our finding of COX-2 in human macula densa and possibly thick ascending limb of Henle salt suggests that the excessive prostaglandin E_2 formation in BLS may be at least partly attributed to COX-2. This finding provides a rationale for testing COX-2 inhibitors as a therapeutic alternative to nonselective COX inhibitors such as indomethacin in patients with BLS, to avoid gastrointestinal toxicity.

In summary, we have shown that COX-2 is expressed in the macula densa of patients with BLS and in a patient with congestive heart failure. This may reflect enhanced renal COX-2 expression in these syndromes. By analogy with studies in rodents, we speculate that macula densa-associated COX-2 expression may be involved in the regulation of renin release in humans. Given the common gastrointestinal side-effects observed with the high doses of indomethacin required for treatment of BLS, the findings of this study support investigational therapeutic trials with COX-2 inhibitors in patients with BLS.

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Fig. 2. Expression of cyclooxygenase-2 (COX-2) mRNA in patients with Bartter-like syndrome (BLS). COX-2 mRNA is expressed in a nephron segment in the renal medulla in patient with a defect in the basolateral chloride channel. COX-2 mRNA expression in the macula densa is shown in a patient with clinically confirmed BLS (*B*, brightfield; *C*, darkfield). Publication of this figure in color was made possible by an unrestricted educational grant from Merck Pharmaceuticals, Inc.

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