Protein kinase C-β inhibition attenuates the progression of nephropathy in non-diabetic kidney disease

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Abstract

Background. Activation of protein kinase C (PKC) has been implicated in the pathogenesis of diabetic nephropathy where therapy targeting the β isoform of this enzyme is in advanced clinical development. However, PKC-β is also increased in various forms of human glomerulonephritis with several potentially nephrotic factors, other than high glucose, resulting in PKC-β activation. Accordingly, we sought to examine the effects of PKC-β inhibition in a non-diabetic model of progressive kidney disease.

Methods. Subtotally nephrectomized (STNx) rats were randomly assigned to receive either the selective PKC-β inhibitor, ruboxistaurin or vehicle. In addition to functional and structural parameters, gene expression of the podocyte slit-pore diaphragm protein, nephrin, was also assessed.

Results. STNx animals developed hypertension, proteinuria and reduced glomerular filtration rate (GFR) in association with marked glomerulosclerosis and tubulointerstitial fibrosis. Glomerular nephrin expression was also attenuated the impairment in GFR and reduced the extent of both glomerulosclerosis and tubulointerstitial fibrosis in STNx rats. In contrast, neither proteinuria nor the reduction in nephrin expression was improved by ruboxistaurin.
Conclusions. These findings indicate firstly that PKC-β inhibition may provide a new therapeutic strategy in non-diabetic kidney disease and secondly that improvement in GFR is not inextricably linked to reduction in proteinuria.

Keywords: glomerulosclerosis; nephritis; protein kinase C; proteinuria; tubulointerstitial fibrosis

Introduction

The epidemic of diabetic nephropathy [1] has placed it at the forefront of attempts to discover new therapies for progressive kidney disease. However, non-diabetic kidney disease, principally due to various forms of glomerulopathy, remains a major contributor to the burden of patients entering dialysis and transplantation programmes. While the aetiologies of these two major categories of kidney disease are clearly different, they share common clinical manifestations such as hypertension, proteinuria and declining glomerular filtration rate (GFR) as well as major histopathological characteristics, including glomerulosclerosis, tubulointerstitial fibrosis and macrophage infiltration. Indeed, numerous findings also suggest common pathogenetic mechanisms that link diabetic and non-diabetic kidney disease, such as the renin–angiotensin system (RAS) and the elaboration of profibrotic growth factors. Together, these findings raise the possibility that treatments designed to target the diabetic kidney might also be effective in the non-diabetic setting.

As a consequence of increased glycolytic flux, hyperglycaemia leads to the excessive production of di-acyl glycerol, a cofactor for the activation of classical and novel isoforms of protein kinase C (PKC) [2]. Studies examining the effects of PKC inhibition had until recently been confined to cell culture or short-term animal studies because of toxicity and/or lack of specificity of inhibitors such as staurosporine and calphostin. However, over the past decade less toxic approaches have been developed and are currently undergoing extensive preclinical and clinical evaluation [3]. Of these, ruboxistaurin, a specific PKC-β inhibitor, is the most advanced in its clinical development, having completed phase II and III trials in diabetic nephropathy and retinopathy, respectively [4].

While PKC-β is highly expressed during glomerulonephritis [5], little if any is present in the adult human kidney [6]. In contrast, biopsies from patients with various forms of glomerulonephritis reveal abundant PKC-β [6]. Moreover, several potentially nephrotic factors (apart from high glucose), such as inflammatory mediators, growth factors, hypoxia and vasoactive hormones have all been shown to activate PKC-β [7–9]. Consistent with these findings, PKC-β activation has been shown to mediate injury in the non-diabetic setting in extra-renal sites, such as in the lung following ischaemia–reperfusion injury [9] and in the heart after myocardial infarction [10]. Accordingly, the present study sought to determine whether PKC-β inhibition might also be effective in non-diabetic kidney disease, using the remnant kidney (subtotal nephrectomy, STNx) model of progressive disease that not only shares the major hallmarks of kidney injury seen in humans [11], but also its responsiveness to blood pressure reduction [12] and blockade of the RAS [13].

Methods

Animals

Thirty male Sprague-Dawley rats weighing 200–250 g were randomized to three groups of 10 animals each. Anaesthesia was achieved by the intraperitoneal administration of pentobarbitial (7 mg/100 g body weight; Boehringer Ingelheim, Artarmon, NSW, Australia). The control group underwent sham surgery consisting of laparotomy and manipulation of both kidneys before wound closure. The other 20 rats all underwent STNx performed by right subcapsular nephrectomy and infarction of approximately two-thirds of the left kidney by selective ligation of two of three to four extrarenal branches of the left renal artery [11]. Animals were then randomly assigned to two groups: STNx alone or STNx with the PKC-β inhibitor ruboxistaurin (10 mg/kg/day; Eli Lilly and Co., Indianapolis, IN, USA) in rat chow, or non-drug control chow (Certified Rodent Diet #5002, LabDiet, St. Louis, Missouri, USA) for 12 weeks. Rats were housed in a temperature (22°C)-controlled room with ad libitum access to water and food during the entire study. Rats from each group were sacrificed at 12 weeks post surgery. At sacrifice the remnant (left) kidney was then sliced sagitally and one half immersion fixed in 10% neutral buffered formalin for in situ hybridization and histopathology and the other half fixed in 4% paraformaldehyde (0.1 M phosphate buffer, pH 7.4) for immunohistochemistry. All tissues were subsequently embedded in paraffin. All experiments adhered to the guidelines of the local Animal Welfare and Ethics Committees.

Renal function

Body weight was measured weekly. GFR was measured prior to sacrifice by a single-shot Tc99m-DTPA clearance at the end of the study [11]. Systolic blood pressure was measured in conscious rats using an occlusive tail-cuff plethysmograph attached to a pneumatic pulse transducer (Narco Bio-system Inc., Houston, TX, USA) [14]. Before sacrifice, rats were housed in metabolic cages for 24 h for subsequent measurement of urinary protein excretion using the benzthonium chloride method.

Histopathology

Changes in kidney structure were assessed in a masked protocol in at least 25 randomly selected tissue sections from each group studied, as previously reported [15]. Sections were stained with either Mayer’s haematoxylin and eosin (H&E), periodic acid Schiff’s stain (PAS) or Masson’s modified trichrome to demonstrate collagen matrix. The glomerulus was considered as the area internal to and including Bowman’s capsule. In 3-μm kidney sections stained with PAS, 50–80 glomeruli from rats were examined in a masked protocol. The degree of sclerosis in each glomerulus was subjectively graded on a scale of 0–4 as previously described [11]: Grade 0, normal; Grade 1, sclerotic area up to 25% (minimal); Grade 2, sclerotic area 25–50% (moderate); Grade 3, sclerotic area 50–75% (moderate to severe) and Grade 4, sclerotic area 75–100% (severe). Glomerulosclerosis was defined as GBM thickening, mesangial hypertrophy and capillary occlusion. A glomerulosclerotic index (GSI) was then calculated using the formula

\[ GSI = \frac{4}{\sum_{i=1}^{n} Fi (i)} \]

where Fi is the percentage of glomeruli in the rat with a given score (i) [11].

Quantification of interstitial fibrosis

The accumulation of interstitial matrix was quantified on Masson’s trichrome-stained sections, using computer-assisted image analysis, as previously reported [15]. Briefly, five random nonoverlapping fields from six rats per group were captured and digitized using a BX50 microscope attached to a Fujix HC5000 digital camera. Digital images were then loaded onto a Pentium III IBM computer. An area of blue (matrix on Masson’s trichrome stain) was selected for its colour range. The proportional area stained blue was then determined using image analysis (AIS; Analytical Imaging Station version 6.0, St Catharines, ON, Canada).
Immunohistochemistry

For PKC-β, 6-µm frozen sections were post-fixed in 4% paraformaldehyde for 20 min and incubated for 20 min with normal goat serum (NGS) diluted 1:10 with 0.1 M phosphate-buffered saline (PBS) at pH 7.4. Sections were then incubated for 18 h at 4°C with specific mouse anti-rat monoclonal PKC-β antibody (1:750, Zymed, CA, USA). Sections incubated with 1:10 NGS instead of the primary antiserum served as the negative control. After thorough washing with PBS (×5 min changes), the sections were incubated with FITC-labelled goat anti-rabbit IgG (Dakopatts, Glostrup, Denmark) diluted 1:200 with PBS for 1 h at room temperature. Sections were rinsed with PBS (×2 min), rinsed in tap water for 5 min and mounted for microscope viewing.

For PKC-α, 5-µm sections were placed into histosol to remove the paraflin wax, hydrated in graded ethanol and immersed into tap water before being incubated for 20 min with NGS diluted 1:10 with 0.1 M PBS at pH 7.4. Sections were then incubated for 18 h at 4°C with specific primary polyclonal antiserum to PKC-α (Santa Cruz Biotechnology, CA, USA). Sections incubated with 1:10 NGS instead of the primary antiserum served as the negative control. After thorough washing with PBS (×5 min changes), the sections were flooded with a solution of 5% hydrogen peroxide, rinsed with PBS (×2 min) and incubated with biotinylated goat anti-rabbit IgG (Dakopatts) diluted 1:200 and goat anti-mouse IgG diluted 1:200 (Dakopatts) with PBS. Sections were rinsed with PBS (×2 min) and incubated with an avidin-biotin peroxidase complex (Vector, Burlingame, CA, USA) diluted 1:200 with PBS. Following rinsing with PBS (×5 min), sections were incubated with 0.05% diaminobenzidine and 0.05% hydrogen peroxide (Pierce, Rockford, IL, USA) in PBS at pH 7.6 for 1–3 min, rinsed in tap water for 5 min, counterstained in Mayer’s haematoxylin, differentiated in Scott’s tap water, dehydrated, cleared and mounted in DPX.

The proportional area of the glomerulus that was immunolabelled was determined by image analysis in 50–80 glomeruli from each animal by image analysis, as described above.

Collagen synthesis

In light of the reduction in glomerulosclerosis and tubulointerstitial fibrosis with ruboxistaurin observed in five of six nephrectomized animals, we considered whether PKC-β inhibition might attenuate TGF-β-induced collagen formation. A well-characterized, cloned mesangial cell line (1097) isolated from Sprague-Dawley rats [16] was used between passages 20 and 40. Cells were cultured in Dulbecco’s Modified Eagle’s Medium (DME, In-vitrogen, Grand Island, NY, USA) containing 10% fetal calf serum (FCS), 100 µM penicillin and 100 µg/ml streptomycin in humidified 5% CO2 atmosphere at 37°C. Mesangial cells were plated out in 24-well plates in DME/5% FCS and allowed to adhere overnight. The subconfluent cells were then starved overnight in DME/0.5% FCS supplemented with 150 µM L-ascorbic acid.

L-proline incorporation was used as an index of collagen production. The cells were pretreated with 0-20 µM ruboxistaurin for 4 h prior to the addition of TGF-β (50 ng/ml) and 1 µCi/well [2,3,4,5-3H] proline (Perkin-Elmer, Boston, MA, USA). Cells were cultured for a further 44 h, washed twice in ice-cold PBS, incubated in ice-cold 10% TCA for 30 min, followed by a final wash in ice-cold 10% TCA. Cells were dissolved in 1 M sodium hydroxide before being neutralized with hydrochloric acid, prior to scintillation counting. Replicates of three wells were used.

In addition, total collagen was also assessed by determining the quantity of hydroxyproline produced by cultured mesangial cells, according to the method of Villarreal et al. [17]. In brief, cells were seeded in equal numbers into 100-mm culture dishes in DME/10% PBS and allowed to adhere overnight. Cells were then starved overnight in DME/0.5% FBS/150 µM ascorbic acid prior to pre-treatment with or without ruboxistaurin for 4 h, followed by stimulation with TGF-β (5 ng/ml) for 48 h. During treatment, ascorbic acid was replenished after 24 h by direct addition into the culture medium.

Cells and media were collected, boiled for 20 min and precipitated at −20°C overnight by the addition of 2 volumes of absolute ethanol. After precipitation, samples were centrifuged at 30 000 g for 30 min. The pellet was air dried and then hydrolyzed in 2 ml of 6 M HCl overnight at 110°C. After evaporation to dryness, samples were reconstituted in 0.25 ml of 50% isopropanol, microfuged to remove insoluble debris and 100 µl duplicate samples assayed for hydroxyproline content according to the method of Bergmann and Loxley [18].

In situ hybridization

Since the proteinuria induced in this model was not attenuated by ruboxistaurin, we explored the effects of the injury and its treatment on the expression of nephrin, an integral component of the glomerular slit-pore membrane that modulates the transglomerular passage of protein. In situ hybridization was performed as previously described [19]. In brief, a 293-base-pair cDNA coding for rat nephrin was cloned into pBEM-T (Promega), linearized with Not I and an antisense riboprobe was produced using T7 RNA polymerase. Purified riboprobe length was adjusted to ~150 bases by alkaline hydrolysis. Four micrometre-thick sections were cut onto slides precoated with 3-aminopropyltriethoxysilane and baked overnight at 37°C. Tissue sections were dewaxed and rehydrated and in situ hybridization was performed using radiolabelled riboprobe as previously described [11]. Following hybridization, slides were washed in 2× SSC for 45 min at 55°C, dehydrated in graded ethanol, air dried and exposed to Kodak BioMax MR autoradiographic film for 3 days. Slides were then dipped in Amersham nuclear emulsion (GE Healthcare, Little Chalfont, Buckinghamshire, UK), stored in a light-free box with desiccant at 4°C for 21 days, developed in Ilford Phenosol, fixed in Ilford Hypam and stained with H&E.

Quantitative autoradiography

Quantitative in situ hybridization by autoradiographic film densimetry that permits the assessment of gene expression equivalent to northern blot analysis was used to determine the magnitude of gene expression, as previously reported by our group [20]. In brief, film densitometry of autoradiographic images was performed by computer-assisted image analysis as previously described [21] using a Micro Computer Imaging Device (MCID; Imaging Research, St Catherine’s, Ontario, Canada). In this method, quantitation of transcript is based on the changes in X-ray film density that follows exposure to the radioactive emissions of radio-labelled nephrin mRNA. In situ autoradiographic images were placed on a uniformly illuminating fluorescent light box (Northern Light Precision Luminator Model C60; Image Research) and captured using a video camera (CCD72 Video Camera Module CCD; Dage MTI, Japan) connected to an IBM AT computer with a 512 × 512 pixel array imaging board with 256 grey levels. In view of the focal nature of rat glomerular nephrin mRNA, the outline of 20 glomeruli/section was defined by interactive tracing for each kidney section, as previously described [22]. Following appropriate calibration, by constructing a curve of optical density versus radioactivity, quantitation of digitalized autoradiographic images was performed using MCID software. Data were expressed as nCi/g relative to control kidneys.

All sections were cut in a uniform manner in the mid-saggital plane, hybridized in the same experiment and analysed in duplicate under identical conditions. All analyses were performed with the observer masked to the animal study group.

Statistics

Data are expressed as means ± SEMs unless otherwise stated. Statistical significance was determined by ANOVA with a Tukey’s post hoc comparison. Analyses were performed using Prism 5.0 (GraphPad Software Inc., La Jolla, CA, USA) on an Apple MacBook Pro computer (Apple Computer Inc., Cupertino, CA, USA). A P-value < 0.05 was regarded as statistically significant.

Results

Renal functional and biochemical studies

Compared with sham-operated rats, rats that underwent STNx had hypertension that was unaffected by ruboxistaurin. GFR was reduced in STNx rats (P < 0.01, Table 1), while treatment with ruboxistaurin for 12 weeks significantly attenuated this reduction. In contrast, STNx developed severe proteinuria that was not altered by ruboxistaurin.

Renal histopathology

Subtotal nephrectomy was associated with a significant increase in sclerotic glomeruli that was attenuated by the
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Table 1. Animal data

<table>
<thead>
<tr>
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<th>Sham</th>
<th>STNx</th>
<th>STNx + Rbx</th>
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<tr>
<td>Body weight (g)</td>
<td>543 ± 22</td>
<td>488 ± 27</td>
<td>478 ± 25</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>115 ± 5</td>
<td>160 ± 10*</td>
<td>166 ± 12*</td>
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<tr>
<td>GFR (ml/min)</td>
<td>5.8 ± 0.2</td>
<td>0.65 ± 0.2†</td>
<td>1.99 ± 0.3†</td>
</tr>
<tr>
<td>Proteinuria (mg/day)</td>
<td>0.99 ± 0.14</td>
<td>5.4 ± 1.1†</td>
<td>5.7 ± 1.1†</td>
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Systolic blood pressure, SBP
Data are expressed as mean ± SEM.
*P < 0.05, †P < 0.01 versus sham. ‡P < 0.05 versus STNx.

administration of ruboxistaurin (Figure 1). Similarly, the kidneys of nephrectomized rats also showed prominent interstitial fibrosis that was significantly attenuated, though not normalized by ruboxistaurin treatment (Figure 2).

In vitro studies

TGF-β induced a robust increase in ³H-proline incorporation by mesangial cells that was attenuated by ruboxistaurin at 10 and 20 nM (Figure 3). A similar pattern was noted when total collagen was assessed by the measurement of hydroxyproline concentration in mesangial cell culture medium [control, 108 ± 8 µg/µl (mean ± SEM); TGF-β, 150 ± 1 µg/µl; TGF-β + ruboxistaurin, 76 ± 1 µg/µl; P < 0.05 control versus TGF-β and TGF-β versus TGF-β + ruboxistaurin]. Mesangial cells remained viable, as evidenced by trypan blue exclusion and the maintenance of normal mesangial cell appearance.

Immunohistochemistry

Immunostainable PKC-β and PKC-α were both increased in the glomeruli of subtotal nephrectomized rats (Figures 4 and 5). However, while ruboxistaurin treatment was associated with a decline in PKC-β, PKC-α was unaffected.

Nephrin expression

In situ hybridization autoradiography revealed punctate cortical expression of nephrin mRNA consistent with localization of transcript to glomeruli (Figure 6). The intensity of labelling was greatest in sham-operated animals. When compared with sham animals, STNx rats showed reduced nephrin mRNA that was not attenuated by ruboxistaurin. Sections labelled with sense probe (negative control) showed no hybridization (not shown).

Emulsion-dipped, H&E-counter-stained sections localized nephrin mRNA exclusively to the glomerulus. Nephrin mRNA was detected in a predominantly peripheral distribution consistent with expression in visceral epithelial cells in sham-operated rat kidneys (Figure 7). Fewer autoradiographic grains were detected in glomeruli of STNx rats evidenced by trypan blue exclusion and the maintenance of normal mesangial cell appearance.

Fig. 1. Representative PAS-stained sections from rats that underwent sham surgery (A), subtotal nephrectomy (B) or STNx with ruboxistaurin treatment (C). In sham-operated rats (A), only minimal glomerular thickening was noted, while subtotal nephrectomy surgery (B) was associated with a dramatic glomerulosclerosis. Treatment of rats that were subjected to STNx with ruboxistaurin (C) was associated with a reduction in the glomerulosclerosis. Original magnification ×350. Quantification of glomerulosclerotic index (GSI) is represented in (D). Data are shown as mean ± SEM. *P < 0.05 versus sham-operated rats, †P < 0.05 versus untreated STNx rats.
Fig. 2. Representative trichrome-stained sections from rats that underwent sham surgery (A), subtotal nephrectomy (SB), or rats that underwent STNx followed by ruboxistaurin treatment (C). In sham operated rats, there is sparse collagen within the interstitium. Subtotal nephrectomy surgery was associated with substantial fibrosis that was attenuated with ruboxistaurin treatment. A dramatic increase in the extent of fibrosis was noted in rats subjected to subtotal nephrectomy that was significantly attenuated by ruboxistaurin treatment (D). Original magnification ×250.

Fig. 3. In vitro effects of 5–20 nM ruboxistaurin on transforming growth factor-β (50 ng/ml) induced 3H-proline incorporation in cultured rat mesangial cells. Values are expresses as mean ± SEM. *P < 0.05 versus control, †P < 0.01 versus TGF-β-stimulated cells without ruboxistaurin.

treated with or without ruboxistaurin. No hybridization was noted in sections incubated with nephrin sense riboprobe.

Discussion

The principal findings of the present study are that following subtotal nephrectomy, ruboxistaurin, without affecting blood pressure, attenuated the decline in GFR in association with a reduction in glomerulosclerosis and tubulointerstitial fibrosis. However, despite these changes in structure and function, proteinuria, a key manifestation of progressive kidney disease was unaffected by PKC-β inhibition.

PKC is a widely expressed family of serine–threonine kinases that transduce a myriad of cellular processes by substrate-specific phosphorylation [23]. Of the 12 different PKC isoforms, most studies in kidney diseases have been confined to the diabetic context and have included examination of classical (alpha, beta), novel (delta, epsilon) and atypical (zeta) isoforms [24–27]. In the in vivo setting in diabetes, gene deletion studies suggest that individual PKC isoforms may account for some, but not all of the pathophysiological changes of diabetic nephropathy. For instance, while the diabetic PKC-β−/− mouse shows reduced glomerular hypertrophy and TGF-β expression, the magnitude of albuminuria is unaffected [28]. In the current study, we found that while both PKC-α and -β were increased in the glomeruli of subtotal nephrectomized rats, only PKC-β was reduced by the administration of ruboxistaurin. Together these studies suggest that PKC-β may be more prominent in the pathogenesis of glomerulosclerosis while other isoforms, such as alpha, may be more relevant to the development of proteinuria, explaining, at least in part why in the present study ruboxistaurin effectively reduced glomerulosclerosis but not albuminuria.

In the present study, we noted that inhibiting the activity of PKC-β with ruboxistaurin also led to a reduction in PKC-β, but not PKC-α protein. These findings are
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Fig. 4. Immunofluorescence and quantitative analysis for PKC-β in glomeruli from sham (A), subtotally nephrectomized (STNx) (B) and STNx rats treated with ruboxistaurin (C). Magnification ×1020. Abundant PKC-β immunolabelling was noted in the glomeruli of STNx rats compared with sham rats that was reduced by ruboxistaurin. *P < 0.01 versus sham, †P < 0.05 versus subtotal nephrectomy.

Fig. 5. Immunohistochemistry and quantitative analysis for PKC-α in glomeruli from sham (A), subtotally nephrectomized (STNx) (B) and STNx rats treated with ruboxistaurin (C). Magnification ×1020. Abundant PKC-α immunolabelling was noted in the glomeruli of STNx rats compared with sham rats but was unaffected by ruboxistaurin. *P < 0.01 versus sham.
consistent with the recently described auto-regulatory feedback loop whereby increased PKC-β activity leads to enhanced PKC-β transcription in an isoform-specific manner [29,30]. Indeed, this mechanism is also thought to underlie the findings in human diabetic nephropathy biopsies where high glucose increases not only the PKC-β activity but also its mRNA [31].

Proteinuria and declining GFR are cardinal features of many forms of chronic glomerulopathy that often progress concomitantly with advancing disease. However, in several settings, such as minimal change and anti-GBM disease, the relationship between proteinuria and GFR is less constant. Treatment directed against the RAS attenuates the decline in GFR as well as reducing proteinuria. However, there is uncertainty as to whether the improvement in GFR and its major structural correlates, glomerulosclerosis and tubulointerstitial fibrosis, are simply a consequence of lowering urinary protein or whether other actions of RAS blockade might be responsible. In the present study, we found that ruboxistaurin attenuated glomerulosclerosis, tubulointerstitial fibrosis and the decline in GFR to a similar extent to that reported with angiotensin-converting enzyme inhibition and angiotensin receptor blockade, in the same model [11]. However, unlike these two latter agents, ruboxistaurin did not reduce urinary protein, suggesting that while impairment of GFR and proteinuria are common manifestations of kidney disease, their response to therapy are not inextricably linked.

One of the key components of the barrier to the transglomerular passage of protein is the slit-diaphragm protein nephrin, a mutation which leads to congenital nephrotic syndrome (NPHS1), manifested by massive proteinuria soon after birth [32]. However, in addition to such inborn errors, nephrin expression is also reduced in a range of acquired proteinuric diseases such as diabetic nephropathy [33], focal glomerulosclerosis and minimal change disease [34]. In the present study, we found that nephrin expression was reduced in nephrectomized rats and was unaffected by ruboxistaurin, contrasting the reduction in glomerulosclerosis achieved with this agent. These findings are reminiscent of those of the diabetic PKC-β−/− mouse in which neither the increase in albuminuria nor the reduction in nephrin expression were attenuated in comparison with wild-type mice [28].

While the pathogenesis of kidney fibrosis is undoubtedly complex, one growth factor, TGF-β, has emerged as preeminent [35]. Among a range of intracellular signalling pathways, TGF-β binding to its cognate receptor leads to activation of PKC [36]. Previous studies have shown that the TGF-β-induced collagen production by cultured mesangial
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Fig. 7. In situ hybridization emulsion-dipped photomicrograph for nephrin mRNA in sham (A), subtotally nephrectomized (STNx) (B) and STNx rats treated with ruboxistaurin (C). Abundant nephrin mRNA was noted in the glomeruli of sham rats in comparison with STNx rats treated with or without ruboxistaurin. Hybridization with sense riboprobe showed no hybridization (D). Magnification ×1020.

cells can be abrogated in cells infected with a vector expressing dominant negative PKC-δ [37]. The present study demonstrates that inhibition of the β-isoform of PKC can also attenuate TGF-β-induced collagen production.

In the setting of its apparent safety in clinical trials in diabetic subjects, the findings of the present study suggest that PKC-β inhibition might also be considered as a potential therapeutic strategy in non-diabetic kidney disease.

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Adequate phosphate binding with lanthanum carbonate attenuates arterial calcification in chronic renal failure rats

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Abstract

Background. Hyperphosphataemia is a risk factor for arterial calcification contributing to the high cardiovascular mortality in patients with chronic kidney disease. Calcium-based phosphate binders can induce hypercalcaemia and are associated with progression of vascular calcification. Therefore, the effect of lanthanum carbonate, a non-calcium phosphate binder, on the development of vascular calcification was investigated in uraemic rats.

Methods. Chronic renal failure (CRF) was induced by feeding rats an adenine-enriched diet for 4 weeks. After 2 weeks, 1% or 2% lanthanum carbonate was added to the diet for 6 weeks. Calcification in the aorta, carotid and femoral arteries was evaluated histomorphometrically, biochemically