Early treatment with cGMP phosphodiesterase inhibitor ameliorates progression of renal damage

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Background. Chronic renal disease is associated with oxidative stress and reduced nitric oxide availability which, in turn, promotes hypertension and further progression of renal damage. Most actions of nitric oxide are mediated by cyclic 3',5' guanosine monophosphate (cGMP) which is rapidly degraded by phosphodiesterases (PDE). Therefore, we investigated if inhibition of PDE-5 would retard the progression of chronic renal failure.

Methods. We studied rats with 5/6 nephrectomy treated with sildenafil (2.5 mg/kg−1/day−1) in two experimental protocols. In the first protocol, we started sildenafil therapy immediately after renal ablation and continued treatment for 8 weeks. Control groups consisted of rats with renal ablation treated with drug-free vehicle and sham-operated rats with and without sildenafil treatment.

Results. In these studies, sildenafil treatment prevented hypertension and deterioration of renal function, reduced histologic damage, inflammation and apoptosis, delayed the onset of proteinuria, and preserved renal capillary integrity. In the second protocol we compared sildenafil with losartan (7.5 mg/kg−1/day−1) and the combination of both drugs in established renal disease, starting these drugs 4 weeks after 5/6 nephrectomy. Delayed sildenafil treatment failed to improve proteinuria and glomerulosclerosis but ameliorated hypertension and azotemia.

Conclusion. These observations suggest that currently available PDE-5 inhibitors have potential clinical value in the treatment of chronic renal disease.

The reduction in the number of nephron units by the primary disease process(es) triggers a chain of events that result in glomerular and tubulointerstitial inflammation, progressive losses of the remaining nephrons, and development of chronic renal failure [1, 2]. This phenomenon is associated with diminished nitric oxide availability which is caused by a combination of reduced nitric oxide production (down-regulation of nitric oxide synthase (NOS) and diminished L-arginine uptake) [3–5], accumulation of endogenous NOS inhibitors and depletion/inactivation of nitric oxide by reactive oxygen species [6, 7]. The associated nitric oxide deficiency, in turn, promotes hypertension and accelerates progression of renal disease [7, 8].

Many of the biologic actions of nitric oxide are mediated by cyclic 3',5' guanosine monophosphate (cGMP), which is rapidly degraded by phosphodiesterases (PDE). We, therefore, hypothesized that pharmacologic inhibition of the cGMP-specific PDE-5 may attenuate hypertension and retard progression of renal disease by raising the available pool of cGMP, despite the associated nitric oxide deficiency. To test this hypothesis, we evaluated the effects of sildenafil (Viagra®) in prevention (starting immediately after renal ablation) and in treatment (started 4 weeks after renal ablation) of renal failure in 5/6 nephrectomized rats. In addition, we compared the effects of sildenafil with those of angiotensin receptor 1 (AT1) blockade. We found that early sildenafil treatment prevented hypertension and renal functional deterioration and reduced apoptosis and inflammatory damage in the renal ablation model. When administration of sildenafil was delayed until significant renal damage was present, the severity of proteinuria and glomerulosclerosis were similar to that observed in vehicle-treated rats but blood pressure and serum creatinine changes were ameliorated. These observations suggest that a currently available, relatively safe, class of pharmacologic agents can be of potential value in the management of progressive renal disease.
METHODS
Experimental design

Studies were done in male Sprague-Dawley rats (Instituto Venezolano de Investigaciones Científicas, Los Teques, Venezuela) with a weight of 250 to 350 g housed in institutional facilities and receiving regular rat chow (Protinal) (Purina, Valencia, Venezuela) and with free access to water. Two experimental protocols were used.

Protocol 1 used early administration of sildenafil. After obtaining baseline studies, subtotal renal ablation was done in 20 rats using a single surgical procedure that included right nephrectomy and selective infarction of two thirds of the left kidney, as described previously [9]. Four to five days after surgery, the rats were divided in two groups. One group received sildenafil (2.5 mg/kg/day−1 in 0.5 mL of water) by gastric gavage (N = 10) and one group received vehicle (N = 10). Since the sildenafil powder is not water soluble, the dose was suspended in water by vigorous agitation immediately prior to administration, as previously done by us in other studies with water insoluble drugs [9]. Sham-operated animals (N = 10) had laparotomy and manipulation of the kidneys and renal pedicle without removal of kidney tissue. An additional group of sham-operated rats (N = 5) were treated with sildenafil to evaluate the effects of this drug in control animal.

All animals were followed for 8 weeks after renal ablation at the end of which were sacrificed under nembutal anesthesia. At this time remnant kidneys were harvested, and used for histologic and immunohistologic studies.

Protocol 2 used late administration of sildenafil. In these studies rats were kept under observation with free access to food and water for 4 weeks after 5/6 nephrectomy. At this time, five rats were sacrificed and kidneys were harvested for evaluation of histologic damage at the start of therapy and the rest were randomly assigned to the following treatment groups that were given for the start of therapy and the rest were randomly assigned were harvested for evaluation of histologic damage at the end of which were sacrificed under nembutal anesthesia. At this time remnant kidneys were harvested, and used for histologic and immunohistologic studies.

Histology and immunohistology

Light microscopic studies were done in methyl Carnoy or formalin-fixed, paraffin-embedded biopsies stained with periodic acid-Schiff (PAS), hematoxylin and eosin, and trichrome stains. Glomerulosclerosis was evaluated in a score initially described by Raij, Azar, and Keane [13] used in previous communications form our group [10, 12, 14]. Briefly, glomeruli were graded from 0 to +4: grade 0, normal; grade 1, <25% involvement of the glomerular tuft; grade 2, 25% to 50% involvement of the glomerular tuft; grade 3, 50% to 75%; and grade 4, sclerosis occupying >75% of the glomerular tuft. The glomerulosclerosis score was obtained as follows: [(1 × number of glomeruli with +1) + (2 × number of glomeruli with +2) + (3 × number of glomeruli with +3) + (4 × number of glomeruli with +4)] × 100/total number of glomeruli examined.

Tubulointerstitial damage was scored using a 0 to 5 scale used previously [10–12] depending on the extent of areas with tubular dilatation, interstitial infiltration, and fibrosis (grade 0, no changes; grade 1, <10%; grade 2, 10% to 25%; grade 3, 25% to 50%; grade 4, 50% to 75%; and grade 5, 75% to 100%). determined in successive fields examined in the entire cortical and juxtamedullary areas suited for evaluation of each biopsy, using computer assisted image analysis.

Avidin-biotin-peroxidase methodology was used to study to identify lymphocytes (CD5-positive cells), macrophages [endothelin-1(ED-1)–positive cells], and glomerular and peritubular capillaries and apoptosis as described previously [15]. Immune cell infiltration in glomeruli and tubulointerstitial regions were evaluated separately and results were expressed as positive cells per glomerular cross section (gcs) and positive cells per mm², respectively. Glomerular and peritubular capillary areas identified by the corresponding antibody (see below) were studied by computer image analysis and expressed as the ratio of positive/total areas under examination in successive fields in the cortical and juxtamedullary areas.

Renal function, urinary cGMP, and nitrate/nitrite excretion

Plasma creatinine and proteinuria were determined every 2 weeks and systolic blood pressure was measured by tail-cuff plethysmography every week as described in previous communications [10–12]. Creatinine clearance was determined in five rats of each group at the end of the experiments.

Urinary cGMP excretion was measured in 24-hour urine collections obtained prior to sacrifice in rats of protocol 1, using commercially available assay kits following the instructions of the manufacturers (Direct cGMP Enzyme Immunoassay Kit (Assay Diagnostics, Inc., Ann Arbor, MI, USA). The lower detection limit is 88 fmol/mL. Intra- and interassay % coefficient of variation were 6.5% and 11.2%, respectively. Total urinary nitrate/nitrite excretion [nitric oxide oxidation products (NOx)] was determined with the Griess reaction after conversion of nitrate to nitrite by nitrate reductase, using a commercially available assay kit (Nitrate/Nitrite Colorimetric Assay Kit) (Cayman Chemical Co., Ann Arbor, MI, USA). The lower detection limit using 80 μL sample (200 μL assay volume) is 2.5 μmol/L.
of the biopsies. Apoptosis was determined in frozen kidney sections by in situ peroxidase labeling of free 3’OH DNA termini by a commercially available kit (ApopTag, In Situ Apoptosis Detection Kit) (Serologicals Corporation, Norcross, GA, USA) following the directions of the manufacturer, as described previously [16].

All histologic evaluations were done blinded with respect to the group under evaluation. Computer-assisted image analysis was done with an Olympus BX51 System Microscope (Olympus Corp., Miami, FL, USA) and DP70 microscope digital camera, with software of Sigma Pro (Leesburgh, VA, USA).

Antisera

Anti-CD5 and anti-ED-1 monoclonal antibodies (Biosource, Camarillo, CA, USA) were used to identify lymphocytes and macrophages, respectively. Glomerular and peritubular capillaries were identified with panendothelial monoclonal mouse antirat RECA-1 antibody (clone HIS52) (Serotec Inc., Raleigh, NC, USA). Secondary biotin-conjugated affinity-pure antibodies with minimal reactivity to rat serum proteins were purchased from Accurate Chemical and Scientific Co. (Westbury, NY, USA). Nonrelevant antibodies were used for negative control studies.

Statistical analysis

Differences between groups were evaluated by multigroup analysis of variance (ANOVA) and Tukey post tests. Serial determinations in the same animal were analyzed by repeated measurement ANOVA. Two-tailed \( P < 0.05 \) was considered significant. All the data are presented as mean \( \pm \) SD.

RESULTS

Experimental protocol 1

Clinical parameters, renal function and blood pressure.

The clinical characteristics of the experimental groups after surgery and prior to randomization to the treatments are shown in Table 1. Body weight, blood pressure, serum creatinine, and urinary protein excretion were similar in the group that was to receive vehicle (5/6 nephrectomy group) and the group that was to receive sildenafil (5/6 nephrectomy treated with sildenafil group). Sham-operated rats had essentially similar values (except for serum creatinine) (Table 1).

The plasma creatinine was similarly elevated immediately after renal ablation in both experimental groups but after the second week the group treated with sildenafil had a significant lower serum creatinine levels that stabilized at 0.83 \( \pm \) 0.10 mg/dL and remained significantly below the levels found in the untreated group (Table 2). The sham-operated rats treated with sildenafil (not shown) had normal creatinine throughout the study (0.42 \( \pm \) 0.15 mg/dL at the end of the study).

The systolic blood pressure increased progressively in the rats with renal ablation. In contrast, the similarly nephrectomized rats treated with sildenafil maintained normal blood pressure throughout the experiment (Fig. 2). The sham-operated rats treated with sildenafil (not shown) remained normotensive during the study (systolic blood pressure at the end of the study 123 \( \pm \) 8.1 mm Hg) (see Table 2).

The development of proteinuria was reduced by sildenafil treatment. As shown in Figure 3, proteinuria remained within normal levels until the fourth week when it increased progressively. Nevertheless, at the end of the experiment, the 5/6 nephrectomy treated with sildenafil group had 50% reduction of proteinuria compared to the untreated 5/6 nephrectomy group (\( P < 0.001 \)). Urine protein in the sham-operated rats treated with sildenafil remained within normal limits (values at the end of the experiment are shown in Table 2).

The clinical parameters in the sham-operated groups (untreated and treated with sildenafil), the 5/6 nephrectomy group and the 5/6 nephrectomy treated with sildenafil group at the end of the experimental protocol 1 are shown in Table 2. Body weight, blood pressure, creatinine clearance, and proteinuria were all improved by sildenafil treatment. Urinary NOx and cGMP excretion were increased in the 5/6 nephrectomy treated with sildenafil group (\( P < 0.01 \) vs. 5/6 nephrectomy) (Table 2).

Histologic studies. Histologic studies confirmed the renoprotection resulting from PDE-5 inhibition.

Table 1. Experimental protocol 1: General data prior to the beginning of treatment (5 days after surgery)

<table>
<thead>
<tr>
<th></th>
<th>Sham (N = 15)</th>
<th>5/6 nephrectomy group (N = 10)</th>
<th>5/6 nephrectomy treated with sildenafil group (N = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight g</td>
<td>303 ( \pm ) 35.1</td>
<td>287 ( \pm ) 43.9</td>
<td>291 ( \pm ) 49.3</td>
</tr>
<tr>
<td>Plasma creatinine mg/dL</td>
<td>0.42 ( \pm ) 0.12^a</td>
<td>0.97 ( \pm ) 0.50</td>
<td>0.89 ( \pm ) 0.50</td>
</tr>
<tr>
<td>Systolic blood pressure mm Hg</td>
<td>123 ( \pm ) 6.0</td>
<td>123 ( \pm ) 7.3</td>
<td>125 ( \pm ) 9.1</td>
</tr>
<tr>
<td>Urinary protein excretion g/day</td>
<td>3.5 ( \pm ) 3.5</td>
<td>5.03 ( \pm ) 2.99</td>
<td>5.94 ( \pm ) 1.99</td>
</tr>
</tbody>
</table>

Animals were randomized 5 days after renal ablation to receive vehicle (5/6 nephrectomy group) or sildenafil (5/6 nephrectomy treated with sildenafil) for a total of 8 weeks. There were no significant differences between groups at the beginning of the treatment. Sham-operated rats were subsequently divided in an untreated group (N = 10) and a sildenafil-treated group (N = 5). ^P < 0.05 vs. the rest.
Glomerulosclerosis (Fig. 4) and tubulointerstitial damage scores (Fig. 5) were ameliorated and glomerular and tubulointerstitial capillaries were preserved in the sildenafil-treated rats (Fig. 6A to C).

Glomerular infiltration of lymphocytes (CD5-positive cells) was generally absent but macrophage accumulation (ED-1-positive cells/ges) were increased in the vehicle treated rats with renal ablation (0.46 ± 0.18) compared...
to the sildenafil-treated rats (0.26 ± 0.11) (P < 0.05). The sildenafil-induced reduction in the immune cell infiltration was more significant in tubulointerstitial areas. As shown in Figure 7, sildenafil administration reduced CD5 and ED-1 infiltration to about half of the numbers present in the 5/6 nephrectomized rats treated with vehicle.

As shown in Figure 8, rats with renal ablation had increased numbers of apoptotic cells. Treatment with sildenafil reduced the number of apoptotic cells to numbers essentially similar to those found in sham-operated rats (Fig. 8).

In all the histologic studies the findings in the sham-operated rats treated with sildenafil were essentially similar to those in the untreated sham-operated control group.

**Experimental protocol 2**

*Blood pressure and renal function.* The administration of sildenafil, losartan, and the combination of both treatments ameliorated significantly the hypertension, particularly in the groups that received losartan alone and in combination with sildenafil (Fig. 9). Serum creatinine changes were also improved by sildenafil, losartan, and the combination of both treatments (Fig. 10).

The effects of delayed administration of sildenafil, losartan, and the combination of both on the proteinuria in this model are shown in Figure 11. Proteinuria was reduced by losartan treatment alone or in combination with sildenafil at 6 and 8 weeks after renal ablation (P < 0.05). Sildenafil administration alone did not modify the proteinuria that results from renal mass reduction.

*Histologic studies.* Glomerulosclerosis index and tubulointerstitial damage scores in the delayed treatment protocol are shown in Figure 12. Glomerulosclerosis (Fig. 12A) increased from the fourth to the eighth week after renal ablation in the vehicle-treated rats. Sildenafil treated rats had essentially the same glomerulosclerosis index than the vehicle-treated rats while the losartan treatment was associated with a reduction in the glomerulosclerosis index. Tubulointerstitial damage scores were improved similarly in all treatment groups (Fig. 12B).

**DISCUSSION**

The central finding in this work is the demonstration that early treatment with a cGMP PDE inhibitor retards the progression of renal injury in the renal ablation model. Daily administration of sildenafil started immediately after renal ablation resulted in stabilization of creatinine levels, prevention of hypertension, reduction in proteinuria as well as increments in urinary NOx excretion and cGMP excretion in the 5/6 nephrectomized rats (Table 2).

The changes in serum creatinine levels in the treated group are not due to a reduction in the muscular mass since the body weight of the rats at the time of sacrifice was higher in the sildenafil-treated than in the untreated 5/6 nephrectomy group. Furthermore, creatinine clearance was also significantly higher in the sildenafil-treated 5/6 nephrectomy group than in the untreated 5/6 nephrectomy group (Table 2). Sildenafil administration is known to exert a preconditioning protective effect that results in a reduction in the extension of the experimentally induced myocardial infarction [17]. However, it is unlikely that this effect could have been responsible for a reduction in the size of the renal infarcts in the remnant kidney of the 5/6 nephrectomy treated with sildenafil group.

![Fig. 3. Serial determinations of urinary protein excretion in the rats with renal ablation receiving vehicle (5/6 nephrectomy group (5/6 Nx group)), rats with renal ablation treated with sildenafil (5/6 Nx.SIL), and sham-operated rats. Urine protein data obtained in the sham-operated rats treated with sildenafil (not shown) are given in Table 2. Arrow indicates the time of renal ablation surgery. Data are mean ± SD. *P < 0.05; **P < 0.01; *** P < 0.001 vs. 5/6 nephrectomy group.](image)

![Fig. 4. Glomerulosclerosis damage score (A) in rats with renal ablation (5/6 nephrectomy (5/6 Nx)) is reduced by sildenafil treatment (5/6 Nx.SIL). **P < 0.01; ***P < 0.001. Representative microphotograph of a glomerulus from a rat from the 5/6 nephrectomy group showing an area of sclerosis occupying approximately 25% of the glomerular tuft (B) is contrasted with a glomeruli from (A) and a rat from the 5/6 nephrectomy treated with sildenafil group (C) showing only focal hypercellularity (arrow) and mild mesangial expansion [periodic acid-Schiff (PAS) staining].](image)
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Fig. 4.

Fig. 5.
since we started sildenafil administration 5 days after the surgery.

The sildenafil-treated group remained normotensive throughout the experiments, in contrast with the progressive hypertension induced by renal ablation in the untreated 5/6 nephrectomy group. The severity of hypertension observed in the untreated 5/6 nephrectomy group here is essentially similar to that found in previous studies from our group [9] and others [18] in this model of renal ablation. Inhibition of PDE-5 is known to reduce blood pressure in humans [19] and lower vascular resistance in specific vascular beds [20, 21]. Amelioration of hypertension in the treated group was likely due to a combination of increased nitric oxide bioactivity and preservation of renal function. Increased cGMP could partially compensate for the reduction in nitric oxide bioavailability which is caused by depressed production and enhanced inactivation of nitric oxide by
reactive oxygen species in uremia [7]. In turn, the normalization of blood pressure must have helped to halt progression of renal failure since the severity of glomerulosclerosis is directly correlated with the average systolic blood pressure in this model [22, 23].

Fig. 7. Tubulointerstitial immune cell infiltration is significantly reduced by sildenafil treatment. (A) CD5-positive cells are lymphocytes and endothelin-1 (ED-1)–positive cells are macrophages. **$P < 0.01$; ***$P < 0.001$ vs. the rest. Microphotographs show macrophage infiltration (ED-1–positive cells) in the vehicle-treated (B) and reduced numbers in the sildenafil-treated (C) rats with renal ablation (immunoperoxidase staining).

Fig. 8. Apoptosis is increased in rats with renal ablation and reduced to almost normal (sham-operated) levels by sildenafil treatment. (A) Comparison between sham, 5/6 nephrectomy (5/6 Nx), and 5/6 nephrectomy plus sildenafil (5/Nx.SIL). ***$P < 0.001$ vs. the rest. TUNEL is terminal deoxynucleotidyltransferase (TdT)-mediated deoxyuridine triphosphate (dUTP) nick-end labeling. Microphotographs show increased number of apoptotic cells in vehicle-treated rats with renal ablation (5/6 nephrectomy group) (B) in contrast with rats treated with sildenafil (C) (frozen kidney sections, peroxidase staining).
Attenuation of glomerular structural damage is probably responsible for the delay observed in the appearance of proteinuria (Fig. 3). Glomerular and peritubular capillary integrity (Fig. 6) was preserved by sildenafil treatment. Taken in conjunction with the functional improvement, these findings support the observation of Kang et al [24] who emphasized the contribution of microvascular damage to the progression of renal scarring.

It is of note that in the experiments in protocol 1, proteinuria remained at baseline levels in the sildenafil-treated group until the fourth week after 5/6 nephrectomy and then increased progressively, almost in parallel, with the proteinuria in the untreated 5/6 nephrectomy group.
The observed histologic improvement is in concert with the clinical improvement in the sildenafil-treated group. The glomerular lesions in the 5/6 nephrectomy group were not too severe. A glomerulosclerosis index of 25, found in the untreated 5/6 nephrectomy (Fig. 4), corresponds to sclerotic lesions involving approximately one quarter of the glomerular tuft in one quarter of the remaining glomeruli. Therefore, the relatively large impairment of renal function observed in the 5/6 nephrectomy group (Fig. 1) (Table 2) is more concordant with the more severe tubulointerstitial injury that involved 25% to 50% of the cortical and juxtamedullary regions (Fig. 5); in agreement with studies that demonstrate that the degree of renal functional loss is better correlated with the severity of tubulointerstitial injury than with glomerular histologic damage [25–27].

Apoptosis is increased in the remnant kidney. Increased number of apoptotic cells may reflect the increased number of cells associated with the inflammatory reaction but apoptosis has also been identified as a mechanism of nephron loss in the renal ablation model [28]. Sildenafil has been recently shown to have a strong protective effect against apoptosis through a nitric oxide signaling pathway [29] and the demonstration that that apoptosis is reduced by the administration of sildenafil in our studies (Fig. 8) suggests an additional potential mechanism for the renoprotection.

Our findings in the 5/6 nephrectomy group are in agreement with recent studies that have found a reduced urinary cGMP in the renal ablation model [30]. In the present studies the urinary cGMP excretion in the rats with 5/6 nephrectomy is actually increased in relation to the remnant renal mass since sham-operated rats excrete only two to three times more urinary cGMP than rats with one sixth of the renal mass remaining. This may represent an adaptive response in the rats with renal ablation. The administration of sildenafil almost doubled the cGMP excretion by the remnant kidney and this finding is not only a consequence of PDE-5 inhibition, but also to a large extent the result of preservation of renal function and, hence, nitric oxide production capacity. Similarly, the greater urinary excretion of the nitric oxide metabolites (NOx) is most likely a reflection of improved renal function and structure in the rats with renal ablation treated with sildenafil.

The experiments in which the treatment was delayed were intended to evaluate the effects of PDE-5 inhibition on established renal damage. In the 5/6 nephrectomy model, demonstrable mesangial expansion and glomerulosclerosis is already evident 4 weeks after renal ablation and the histology can be improved with the combined treatment of mycophenolate mofetil and angiotensin II blockade [31, 32]. Our findings indicate that proteinuria and glomerulosclerosis are similar in the sildenafil-treated and the vehicle-treated rats with renal ablation and the addition of sildenafil to losartan does not improve the antiproteinuric effects of the latter, which is known to increase neuronal NOS (nNOS) in the renal cortex in the remnant kidney [3].

The lack of improvement in glomerulosclerosis may account for the lack of improvement in proteinuria. It has been amply demonstrated that proteinuria is associated with progressive renal damage [33]; even though it is a matter of dispute whether proteinuria itself is a risk factor or a marker of disease activity [34]. Therefore, it is reasonable to assume that rats treated with sildenafil alone will go on to develop chronic renal failure earlier than the rats treated with losartan, that have less proteinuria and glomerulosclerosis. Nevertheless, beneficial effects of sildenafil on the blood pressure and serum creatinine levels were observed even when the drug treatment was delayed until renal damage was already established (experimental protocol 2). Similarly, the tubulointerstitial damage was less extensive with sildenafil (Fig. 12B) pointing to a better correlation between tubulointerstitial damage and renal function as discussed earlier.

The explanation for the better preservation of tubulointerstitial structures than glomeruli by sildenafil in established renal damage may not be discerned from the present studies. To our knowledge, there are no studies defining the changes in glomerular hemodynamics associated with PDE-5 inhibition. However, since such effects would likely result from cGMP-mediated vasodilatation, primarily of preglomerular arterioles, it is reasonable to assume that the effects of sildenafil would differ from those of the angiotensin blockers which primarily cause postglomerular vasodilation and thereby attenuate glomerular hyperfiltration.

Several partial explanations may be offered for the beneficial effects of sildenafil on chronic tubulointerstitial injury. First, the increase in the pool of cGMP that partially compensates for reduced nitric oxide availability may, in part, contribute to the observed attenuation of tubulointerstitial inflammation in the sildenafil-treated 5/6 nephrectomy animals. It is of note that nitric oxide mediates the vasodilatory and anti-inflammatory actions of bradykinin which is significantly reduced in various models of renal injury. In fact, administration of kallikrein (by gene transfer) which increases bradykinin production has been shown to increase endothelial NOS (eNOS) activity and attenuate inflammation, fibrosis, apoptosis, and tissue damage [35–37]. The latter observations, which demonstrated the beneficial effects of kallikrein-kinin-mediated increase in nitric oxide availability, parallel the results of the present study which demonstrated beneficial effects of enhancing cGMP-mediated nitric oxide bioactivity. Second, the observed beneficial effects could be...
due to the antiapoptotic effect of sildenafil [29], as discussed earlier. In addition, as shown by Valente et al [38], PDE inhibitors reduce the expression of collagen I and α-smooth muscle cell actin; therefore, it is possible that sildenafil could directly ameliorate tubulointerstitial fibrosis and epithelial-mesenchymal transition. Finally, the control of blood pressure may offer an additional beneficial effect, particularly in this experimental model [22, 23].

As expected, AT1 receptor blockade ameliorated proteinuria and attenuated severity of the glomerular and tubulointerstitial injury in the 5/6 nephrectomy animals. In addition to their antihypertensive action, AT1 blockers attenuate oxidative stress, inflammation, and matrix accumulation. Moreover, AT1 receptor blockade results in up-regulation of eNOS and nNOS via angiotensin-II-mediated activation of AT2 receptor. It is of note that in the remnant kidney intrarenal mRNA and protein expression of inducible (iNOS), eNOS, and nNOS are all reduced [3, 4, 39]. Thus, improved nitric oxide production capacity with AT1 blockade may have contributed to the beneficial effects observed in our losartan-treated animals. The results of the present studies are in concert with investigations that show that maneuvers that enhance nitric oxide availability are renoprotective [40–42]. In the renal ablation model administration of a nitric oxide donor in association with lisinopril resulted in amelioration of the nephropathy [43]. Yet, nitric oxide donors may exert cytotoxicity by facilitating the formation of peroxynitrite from the interaction of nitric oxide with superoxide [44–47] which can limit their potential benefit in the treatment of renal disease [48]. Thus, our study has identified a novel approach to the prevention and amelioration of chronic renal damage that merits further investigation.

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