Imatinib ameliorates renal morphological changes in Cyp1a1-Ren2 transgenic rats with inducible ANG II-dependent malignant hypertension

Miguel L. Graciano and Kenneth D. Mitchell

Department of Physiology, Hypertension and Renal Center of Excellence, Tulane University Health Sciences Center, New Orleans, Louisiana

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Graciano ML, Mitchell KD. Imatinib ameliorates renal morphological changes in Cyp1a1-Ren2 transgenic rats with inducible ANG II-dependent malignant hypertension. Am J Physiol Renal Physiol 302: F60–F69, 2012. First published October 5, 2011; doi:10.1152/ajprenal.00218.2011.—The present study was performed to assess the effects of the platelet-derived growth factor (PDGF) receptor kinase inhibitor imatinib mesylate on the renal morphological changes occurring during the development of malignant hypertension in transgenic rats with inducible expression of the Ren2 gene [TGR(Cyp1a1Ren2)]. Arterial blood pressure was measured by radiotelemetry in male Cyp1a1-Ren2 rats during control conditions and during dietary administration of indole-3-carbinol (I3C; 0.3%) for 14 days to induce malignant hypertension. Rats induced with I3C (n = 5) had higher mean arterial pressures (178 ± 4 vs. 109 ± 2 mmHg, P < 0.001) and increased urinary albumin excretion (Ualb; 13 ± 5 vs. 0.6 ± 0.2 mg/day) compared with noninduced rats (n = 5). Chronic administration of imatinib (60 mg·kg−1·day−1 in drinking water, n = 5) did not alter the magnitude of the hypertension (176 ± 8 mmHg) but prevented the increase in Ualb (1.6 ± 0.3 mg/day). Quantitative analysis of proliferating cell nuclear antigen using immunohistochemistry demonstrated increased proliferating cell number in cortical tubules and interstitium (40 ± 7 vs. 13 ± 6 cells/mm2) of hypertensive rat kidneys. Renal cortical fibrosis evaluated by picrosirius red staining showed increased collagen deposition in kidneys of the hypertensive rats (1.6 ± 0.1 vs. 0.4 ± 0.1% of cortical area). Imatinib attenuated the increase in proliferating cell number in cortical tubules and interstitium (22 ± 5 vs. 38 ± 5 and 22 ± 6 vs. 40 ± 7 cells/mm2, respectively) and reduced the degree of collagen deposition (0.8 ± 0.2 vs. 1.6 ± 0.1%) in the kidneys of hypertensive rats. These findings demonstrate that the renal pathological changes that occur during the development of malignant hypertension in Cyp1a1-Ren2 rats involve activation of PDGF receptor kinase.

kidney; immunohistochemistry; renin-angiotensin system; renal pathology; peptide hormones

ANG II-DEPENDENT HYPERTENSION is characterized by increases in intrarenal ANG II levels, renal functional derangements, augmented tubular sodium reabsorptive capability, and development of progressive injury to several organs and tissues, including heart, kidney, and blood vessels (11, 12, 22, 30, 35, 41, 49, 56, 64, 70). In contrast, elevated intrarenal ANG II levels alone in the absence of elevated arterial pressure are not associated with perceptible renal injury. Thus, ANG II-induced renal functional and morphological derangements usually occur in a setting of elevated arterial pressure or associated with other pathophysiological conditions such as diabetes mellitus (22, 27, 30, 48, 49, 59, 60, 64, 66, 70). These findings indicate that elevated intrarenal ANG II levels alone are insufficient to cause derangements in renal hemodynamic function and renal injury. Additional factors such as growth factors, cytokines, and other paracrine agents, such as platelet-derived growth factor (PDGF), directly augmented by the elevated arterial blood pressure may contribute to the renal derangements in ANG II-dependent hypertensive states. Such elevated PDGF levels directly stimulated by the elevated arterial pressure may synergize with the elevated intrarenal ANG II levels to elicit renal functional and morphological derangements. In this regard, elevated arterial blood pressure increases PDGF expression as well as the expression of PDGF receptors in various tissues (8, 9, 19, 26, 28, 36, 42, 43, 50, 68). PDGF is a 28- to 35-kDa peptide present in platelets and is secreted from vascular smooth muscle cells (VSMCs), endothelial cells, macrophages, and fibroblasts (17, 47). The binding of PDGF induces dimerization of the PDGF receptors, leading to their activation via autophosphorylation of tyrosine residues in the PDGF receptor kinase domain (51, 55). This triggers phosphorylation events involving sequential activation of mitogen-activated protein kinase (MAPK) cascades, which then play a pivotal role in cell proliferation and differentiation, particularly of VSMCs (3). Specifically, tyrosine-phosphorylated PDGF receptors interact with several other cytoplasmic proteins that constitute Src homology 2 domains, including phospholipase Cγ, ras guanine 5’-triphosphatase-activating protein, phosphatidylinositol-3-kinase, and tyrosine phosphatase SHP-2 (5). These signaling molecules mediate cellular activities, including proliferation, migration, and differentiation in response to PDGF (5). Furthermore, PDGF transmits its signals to the intracellular space through activation of AKT and MAPKs (5). MAPKs phosphorylate JNKs, which in turn results in the phosphorylation of many transcription factors, including the c-Jun component of the activator protein-1 transcription family (5). C-Jun is known to be required for PDGF-induced VSMC migration and proliferation, and JNK knockdown can attenuate cell migration and proliferation in PDGF-stimulated VSMCs (5).

Although ANG II can directly activate PDGF receptors and induce PDGF expression (14, 26, 29, 34, 57), increases in arterial blood pressure directly induce PDGF expression and activate the PDGF receptor (8, 19, 36, 37, 42, 43, 50, 54, 68). Increased aortic steady-state mRNA levels of PDGF receptors were shown to be induced in DOCA salt-hypertensive rats (50). The increased PDGF receptor expression was partially reversed when normotension was reestablished by a low-salt diet and treatment with a thiazide diuretic (50). In this study, aortic PDGF receptor expression was increased in 10 wk-old spontaneously hypertensive rats. Collectively, these findings suggest an association between blood pressure increases and...
changes in aortic PDGF receptor gene expression and PDGF expression. In essence, these data indicate that hypertension can directly increase aortic steady-state mRNA levels for the PDGF receptor and increase PDGF expression. Such increased activation of PDGF expression and PDGF receptors likely contributes to cell proliferation and differentiation, particularly of VSMCs, leading to vascular hypertrophy in hypertensive states. Accordingly, arterial pressure-induced activation of PDGF chain expression and PDGF receptors may contribute to the proliferative responses that lead to renal functional and morphological derangements in ANG II-dependent hypertensive states. However, uncertainty exists regarding the contribution of PDGF to the renal morphological derangements that occur in ANG II-dependent malignant hypertension.

Transgenic rats with inducible activation of extrarenal renin gene expression [TGR(Cyp1a1Ren2)] were generated using a renin transgene under the transcriptional control of the cytochrome P-450 (Cyp1a1) promoter (23). This transgenic rat line was created by inserting a mouse Ren2 renin gene, fused to an 11.5-kb fragment of the cytochrome P-450 1a1 (Cyp1a1) promoter, into the genome of the Fischer 344 rat (23). Rats transgenic for the Cyp1a1-Ren2 construct do not constitutively express the Ren2 renin gene. Rather, the Ren2 gene is expressed, primarily in the liver, only upon induction of the Cyp1a1 promoter by aryl hydrocarbons such as indole-3-carbinol (I3C) (23). In essence, induction of the Cyp1a1 promoter by I3C is used to drive hepatic expression of the Ren2 renin gene. In this transgenic rat model, induction of the Cyp1a1 promoter by dietary administration of I3C results in a fixed level of expression the Ren2 renin gene and in the development of ANG II-dependent hypertension (23, 32). We demonstrated that at a dose of 0.3% (wt/wt), chronic dietary administration of I3C induces malignant hypertension in Cyp1a1-Ren2 transgenic rats (18, 31–33, 38, 40, 44, 45, 63). Malignant hypertension is a severe form of hypertension characterized by rapidly increasing blood pressure, severe renal vasoconstriction and ischemia, activation of the renin-angiotensin system, and renal injury that consists of glomerulosclerosis, myointimal proliferation, dilated tubules, inflammation, cellular proliferation in the cortical vessels and tubulointerstitium, and fibrinoid necrosis (12, 24, 32, 61, 62).

The present study was performed to determine the effects of the PDGF receptor-related kinase inhibitor imatinib mesylate on the renal morphological changes that occur in Cyp1a1-Ren2 transgenic rats with ANG II-dependent malignant hypertension, especially those related to proliferating cell number in cortical tubules and interstitium, and renal cortical fibrosis as they might relate to the pathophysiology of this severe form of ANG II-dependent hypertension.

MATERIALS AND METHODS

Experimental groups and blood pressure measurement. The experimental procedures used in this study conform to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of Tulane University Health Sciences Center. Experiments were performed on adult (14–24 wk old) male Cyp1a1-Ren2 transgenic rats bred at Tulane University Health Sciences Center from stock animals supplied by Professor John J. Mullins, University of Edinburgh (Edinburgh, Scotland). In one group, male Cyp1a1-Ren2 transgenic rats (n = 5) were fed a normal diet containing the aryl hydrocarbon I3C (0.3% wt/wt; diet TD 05381; Harlan-Teklad, Madison, WI) for 14 days to induce ANG II-dependent malignant hypertension. In a second group (n = 5), age-matched male Cyp1a1-Ren2 rats were fed a normal diet containing 0.3% I3C (Harlan-Teklad) for 14 days and simultaneously treated chronically with the PDGF receptor kinase inhibitor imatinib mesylate (Novartis Pharma AG, Basel, Switzerland). Imatinib was administered via the drinking water at a dose of 60 mg·kg⁻¹·day⁻¹, a dose that has previously been shown to reduce vascular hypertrophy and matrix deposition in ANG II-infused hypertensive rats (25). A third group of Cyp1a1-Ren2 rats fed a normal rat diet (n = 5) not containing I3C (diet TD 99414; Harlan-Teklad) served as controls.

The rats’ arterial blood pressures were monitored continuously using radiotelemetry. Radiotelemeters (PA-C40; DSI, St. Paul, MN) were surgically implanted in the abdominal cavity with the catheter end inserted into the descending aorta and fixed in place with a nylon patch and surgical adhesive (39, 40). Animals were allowed 5 days to recover from the surgery before initiation of the study. Upon initiation of the study, rats were placed in individual cages and transducers were activated with a magnet to initiate blood pressure measurements for the duration of the study.

Urinary albumin concentrations. Urinary albumin concentrations were measured using a commercially available ELISA kit for rat albumin (Cayman Chemical, Ann Arbor, MI). Following completion of the 14-day I3C induction protocol, the rats were decapitated and trunk blood was collected into chilled tubes containing 5 mM EDTA for determination of plasma renin activity (PRA). PRA was measured using a commercially available RIA kit (DiaSorin, Stillwater, MN). The abdominal cavity was opened via a midline incision and the kidneys were excised, fixed in buffered formalin (10% vol/vol), and embedded in paraffin for immunohistochemical identification of proliferating cell activity and determination of collagen deposition in the cortical interstitium.

Immunohistochemistry. Interstitial and tubular proliferating cells and interstitial collagen deposition were analyzed in 3-μm-thick paraffin-embedded sections obtained after 10 days of treatment. Sections were mounted on glass slides, deparaffinized in xylen, and rehydrated through graded ethanol and in deionized water in the final step. Sections were then subjected to microwave irradiation in citrate buffer to enhance antigen retrieval and preincubated with 5% normal horse serum in Tris-buffered saline or in PBS to prevent nonspecific protein binding. Negative control experiments for all antigens were performed by omitting the incubation with the primary antibody.

To detect proliferating cells, renal sections were analyzed using a monoclonal mouse antibody (Dako) specific for the proliferating cell nuclear antigen (PCNA) and an indirect streptavidin-biotin alkaline phosphatase technique. Sections were preincubated with avidin and biotin solutions to block nonspecific binding of these compounds and then with normal horse serum (Vector Labs, Burlingame, CA) as mentioned. The incubation with the primary antibody was carried out overnight at 4°C in a humidified chamber. After being washed, sections were then incubated with rat-absorbed biotinylated anti-mouse IgG (Vector Labs) for 45 min at room temperature, followed by incubation with the streptavidin-biotin-alkaline phosphatase complex (Dako) for 30 min at room temperature. Sections were incubated with a freshly prepared substrate, consisting of naphthol AS-MX Phosphate (Sigma, St. Louis, MO) and fast red dye (Sigma), counterstained with Mayer’s hemalaun (Merck, Darmstadt, Germany) and covered with glycergel (Dako). The quantification of cortical interstitial and tubular PCNA-positive cells was carried out in a blind fashion under ×400 magnification and expressed as cells per millimeter squared. For each section, 30 microscopic fields, each corresponding to an area of 0.13 mm², were examined.
RESULTS

A shown in Fig. 1, dietary administration of 0.3% I3C for 14 days resulted in the development of hypertension (178 ± 4 vs. 109 ± 2 mmHg, \(P < 0.001\)). Mean arterial blood pressure increased from 98 ± 3 to 108 ± 2 mmHg \((P < 0.05)\) by day 2 and continued to increase to 178 ± 4 mmHg \((P < 0.001)\) by day 13. The hypertensive animals also exhibited severe lethargy, piloerection, and assumption of hunched posture, which are clinical manifestations of malignant hypertension in the rodent (23, 24, 32, 61, 62). Thus, as previously observed (18, 31–33, 38, 40, 44, 45, 63), dietary administration of 0.3% I3C induced malignant hypertension in Cyp1a1-Ren2 rats. Rats induced with I3C and treated chronically with imatinib similarly developed hypertension over the 14-day induction period (Fig. 1). Mean arterial blood pressure increased from 105 ± 4 to 114 ± 3 mmHg \((P < 0.05)\) by day 2 and continued to increase to 176 ± 8 mmHg \((P < 0.001)\) by day 13. The magnitude of the increase in mean arterial blood pressure in the rats induced with I3C and treated chronically with imatinib was not different from that observed in rats induced with I3C and not treated with imatinib [178 ± 4 vs. 176 ± 8 mmHg, not significant (NS); Fig. 1]. Similarly, the time course of the increase in mean arterial blood pressure in the rats induced with I3C and treated chronically with imatinib was not different from that observed in rats induced with I3C and not treated with imatinib (Fig. 1). Thus, chronic administration of the PDGF receptor kinase inhibitor imatinib did not alter the either the time course or the magnitude of the hypertension induced by dietary administration of 0.3% I3C. The mean arterial blood pressure of the control Cyp1a1-Ren2 rats not induced with I3C remained unaltered over the course of the study (108 ± 3 vs. 109 ± 2 mmHg, NS). Thus, the rats not induced with I3C did not develop malignant hypertension.

As shown in Fig. 2, rats induced with I3C for 14 days exhibited a pronounced increase in PRA (43 ± 10 vs. 8 ± 2 ng ANG I·ml⁻¹·h⁻¹, \(P < 0.001\)). Chronic administration of imatinib did not alter the magnitude of the increase in PRA elicited by dietary administration of I3C (44 ± 8 vs. 8 ± 2 ng ANG I·ml⁻¹·h⁻¹, \(P < 0.001\); Fig. 2), indicating that blockade of PDGF receptors does not interfere with the hepatic expression of the Cyp1a1-Ren2 transgene. Figure 3 demonstrates that rats induced with I3C but not treated with imatinib exhibited
pronounced albuminuria (13 ± 5 vs. 0.6 ± 0.2 mg/day, P < 0.001). Chronic imatinib treatment substantially attenuated the magnitude of the albuminuria in Cyp1a1-Ren2 rats induced with 0.3% I3C (1.6 ± 0.3 vs. 13 ± 5 mg/day, P < 0.001; Fig. 3), suggesting that PDGF receptor blockade ameliorates glomerular injury in Cyp1a1-Ren2 rats with malignant hypertension.

To evaluate the presence of proliferating cells, immunohistochemistry was performed to stain cells containing PCNA. Representative pictures of proliferating tubular cells can be seen in Fig. 4A (noninduced rats), Fig. 4B (rats induced with I3C), and Fig. 4C (rats induced with I3C and treated chronically with imatinib). Quantitative analysis of renal PCNA expression demonstrated increased proliferating cell number in cortical tubules of hypertensive rats compared with normotensive rats (38 ± 5 vs. 18 ± 1 cells/mm², P < 0.001) and was particularly observed in dilated tubules (Fig. 5). As shown in Figs. 4C and 5, imatinib markedly attenuated the increase in proliferating cell number in cortical tubules (22 ± 5 vs. 38 ± 5 cells/mm², P < 0.001). Similarly, and as depicted in Figs. 6B and 7, there was also an increased number of proliferating cells in the cortical interstitium (40 ± 7 vs. 13 ± 6 cells/mm², P < 0.001). As shown in Figs. 6C and 7, imatinib treatment substantially attenuated the increase in proliferating cells in the cortical interstitium (22 ± 6 vs. 40 ± 7 cells/mm², P < 0.001). Renal cortical fibrosis evaluated by picrosirius red staining demonstrated increased collagen deposition in the cortex of kidneys of hypertensive rats (1.6 ± 0.1 vs. 0.4 ± 0.1% of cortical area, P < 0.001; Figs. 8B and 9). As with proliferating cell number in cortical tubules and interstitium, chronic imatinib treatment reduced the degree of collagen deposition (0.8 ± 0.2 vs. 1.6 ± 0.1% of cortical area, P < 0.001; Figs. 8C and 9). Collectively, these findings demonstrate that chronic blockade of PDGF receptors with imatinib markedly ameliorates the renal injury in Cyp1a1-Ren2 transgenic rats with ANG II-dependent malignant hypertension.

DISCUSSION

The present study evaluated the effects of chronic administration of the PDGF receptor-related kinase inhibitor imatinib on the renal morphological changes that occur in Cyp1a1-Ren2 transgenic rats with ANG II-dependent malignant hypertension. In a previous study (12), we demonstrated that the renal pathological changes in Cyp1a1-Ren2 rats with malignant hypertension included myointimal hyperplasia and tubular dilation, glomerulosclerosis, and tubulointerstitial inflammation and proliferation, particularly in the perivascular areas. Thus, the results of this previous study demonstrated that the renal pathological changes that occur following induction of malignant hypertension in Cyp1a1-Ren2 rats primarily consist of inflammation and cellular proliferation in cortical vessels and tubulointerstitium. Consistent with these previous findings, the present study demonstrated that Cyp1a1-Ren2 transgenic rats induced with 0.3% I3C for 14 days exhibited markedly elevated mean arterial blood pressures, increased PRA and urinary albumin excretion, increased proliferating cell number in cortical tubules and cortical interstitium, and increased renal collagen deposition. Chronic administration of imatinib did not alter the magnitude of the hypertension or the magnitude of the increase in PRA, but it prevented the increase in urinary albumin excretion. In addition, imatinib administration markedly abrogated the increase in proliferating cell number in cortical tubules and cortical interstitium and substantially reduced the degree of collagen deposition in the kidneys of the hypertensive rats.
The present finding that hypertensive Cyp1a1-Ren2 transgenic rats exhibited substantially elevated PRA is consistent with previous observations that the development of ANG II-dependent malignant hypertension in this model is mediated by increased PRA secondary to induced expression of the Cyp1a1-Ren2 transgene in the liver (23, 32, 39). Such hepatic expression of the Ren2 renin gene is not subject to the normal physiological feedback mechanisms regulating the activity of the renin-angiotensin system. In the present study, chronic administration of imatinib did not alter the magnitude of the elevated PRA in Cyp1a1-Ren2 rats induced with 0.3% I3C, indicating that blockade of PDGF receptors does not interfere with the hepatic expression of the Cyp1a1-Ren2 transgene. In light of this observation, one would predict that chronic imatinib treatment did not alter the elevated circulating and intrarenal ANG II levels that occur following induction of malignant hypertension in Cyp1a1-Ren2 rats (32, 39). Such a likelihood is supported by the fact that imatinib treatment did not prevent or reduce the magnitude of the I3C-induced increases in arterial blood pressure. As previously observed (31), rats induced with I3C but not treated with imatinib exhibited pronounced albuminuria, which is indicative of severe glomerular injury. The present finding that imatinib markedly attenuated the magnitude of the albuminuria in the induced rats without reducing arterial blood pressure is indicative of a major role of PDGF and activation of PDGF receptors in mediating the early glomerular damage occurring during the development and progression of ANG II-dependent malignant hypertension. Although the specific mechanisms by which imatinib reduced the albuminuria remain unclear, changes of glomerular size, fibrosis, cell number, and proliferation of glomerular regions could certainly have played an important role in the observed effect of imatinib to reduce albuminuria. Moreover, despite the recent controversy regarding the tubular vs. the glomerular origin of albuminuria, as discussed by Comper et al. (6), albuminuria is usually considered a surrogate marker of glomerular damage (16) and was used here as such. Nevertheless, further studies are required to address the specific mechanisms by which chronic imatinib administration attenuates the albuminuria in hypertensive Cyp1a1-Ren2 rats.

The present findings that chronic imatinib administration markedly attenuated the renal injury and substantially reduced the degree of albuminuria in Cyp1a1-Ren2 rats with malignant hypertension suggest that blockade of PDGF receptors is renoprotective in these hypertensive rats. However, it should be recognized that the effects of chronic imatinib treatment on the derangements in renal hemodynamics and excretory function that occur in Cyp1a1-Ren2 rats with malignant hypertension.
were not determined in the present study. Thus, the present study does not allow definitive conclusions to be drawn as to whether chronic imatinib administration elicits similar beneficial effects on renal hemodynamics and excretory function in Cyp1a1-Ren2 rats with malignant hypertension. Additional studies are required to evaluate the renoprotective effects of chronic blockade of PDGF receptors with imatinib in these hypertensive rats.

ANG II-dependent malignant hypertension is characterized by rapidly increasing arterial blood pressure, marked elevations of PRA, and circulating and intrarenal ANG II levels, and a proliferative response leading to renal injury (12, 32, 39). The observations that in the absence of elevated arterial pressure increased circulating and intrarenal ANG levels alone are not associated with perceptible renal injury and that ANG II-induced renal injury usually occurs in a setting of elevated arterial pressure suggest that elevated intrarenal ANG II levels alone are insufficient to cause renal injury. Accordingly, additional factors such as growth factors, cytokines, and other paracrine agents augmented by the biomechanical stimulus caused by increased arterial pressure likely contribute to the proliferative response. In view of the evidence that increases in arterial pressure directly induce PDGF expression and activate PDGF receptors (8, 19, 36, 37, 42, 43, 50, 54, 68), increased activation of PDGF expression and PDGF receptors likely contributed to the cortical tubular cell and interstitial cell proliferation and increased collagen deposition in the renal cortical interstitium observed in the present study. Thus, arterial pressure-induced activation of PDGF expression and PDGF receptors may contribute to the proliferative responses that lead to renal morphological derangements and renal injury in ANG II-dependent malignant hypertension. In essence, the increase in blood pressure elicited by elevated circulating and intrarenal ANG II levels elicits chronic elevations of PDGF expression and PDGF receptors that contribute to the proliferative response associated with ANG II-dependent malignant hypertension. Consistent with such a possibility, we observed that chronic administration of the PDGF receptor-related kinase inhibitor imatinib markedly abrogated the increase in proliferating cell number in cortical tubules and interstitium...
and substantially reduced the degree of collagen deposition in the kidneys of hypertensive rats. Because imatinib treatment did not reduce the magnitude of the increase in blood pressure in induced Cyp1a1-Ren2 rats, the present findings also indicate that PDGF signaling rather than the direct baromechanical actions of the elevated arterial pressure contributes importantly to the hypertensive-related renal injury in ANG II-dependent malignant hypertension. Similarly, imatinib administration did not reduce PRA, which would suggest that the elevated circulating and intrarenal ANG II levels were similarly unaffected by imatinib administration. These findings indicate that increases in ANG II levels even in the presence of elevated arterial blood pressure are insufficient to cause cortical tubule cell proliferation, cortical interstitial cell proliferation, or increased renal cortical collagen deposition under conditions in which PDGF receptors are blocked.

In the present study, inhibition of PDGF-related kinase prevented the development of interstitial fibrosis in hypertensive Cyp1a1-Ren2 transgenic rats. It should be noted that interstitial fibrosis is the final common endpoint of any chronic kidney disease and a good predictor of future renal insufficiency (67) and it has been also used as such marker on clinical grounds. Indeed, it was used in the Oxford classification of IgA nephropathy (65), a very common kidney disease as well as in human kidney transplantation (15). Interestingly, the technique we employed in the current study to evaluate renal cortical interstitial fibrosis, unpolarized picrosirius red, showed high interassay reproducibility and provided one of the best correlations with glomerular filtration rate in a human study comparing several techniques to analyze cortical interstitial fibrosis (10).

The participation of PDGF in fibrosis formation has been subject of investigation in several different studies. In a study involving the Anti-Thy 1.1 model of inflammatory glomerulonephritis, the administration of a neutralizing antibody to PDGF-D reduced tubulointerstitial matrix accumulation and retarded the progression of renal failure (2). In a study evaluating cardiac fibrosis in a murine model of uremic heart disease induced by 5/6 nephrectomy, it was demonstrated that imatinib was able to reduce interstitial cardiac fibrosis (1). In addition, imatinib was shown to diminish the formation of fibrosis both in the heart and the kidneys and attenuated the loss of cardiac function in hypertensive homozygous TGR(mRen2)27 rats in the absence of a significant decrease in arterial blood pressure (53). Similarly, in the present study, chronic imatinib administration did not alter the magnitude of the hypertension but markedly decreased the degree of renal cortical fibrosis in Cyp1a1-Ren2 rats with inducible ANG II-dependent malignant hypertension. Interestingly, imatinib proved very efficacious in preventing fibrosis in chronic allograft nephropathy, a well-known pathology prone to fibrosis in a model of rat kidney transplant (52). Additionally, imatinib improved renal damage in chronic ureteral obstruction, the paradigm to study renal fibrosis (58). Finally, the ability of imatinib to decrease fibrosis formation was particularly reinforced by the observation that this drug improved skin fibrosis in a clinical case of nephrogenic systemic fibrosis, a devastating disease induced by the use of the MRI contrast agent gadolinium in patients with reduced renal function (4).

The proposed mechanisms through which imatinib constraints fibrogenesis involve the inhibition of TGF-β, the known final trigger of fibrosis formation. Actually, it has been shown that imatinib diminishes the expression of TGF-β, the most important inducer of matrix formation, in an experimental model of nephritis (69). Complementarily, imatinib may block directly Abelson (Abl) tyrosine kinase that mediates matrix deposition caused by TGF-β release (7, 46). Interestingly, nilotinib, a novel Bcr-Abl, c-Kit, PDGF receptor-associated tyrosine-kinase inhibitor that is more active against Bcr-Abl without losing PDGF-blocking properties, is also protective against kidney injury (20). Although the current study did not address the mechanisms by which imatinib ameliorated renal fibrosis, the benefit shown here does not involve amelioration of hypertension and, therefore, is likely related to inhibition of the intrinsic mechanisms of collagen deposition or of the activation of the cellular network involved in this mechanism.

The amelioration of renal damage by imatinib might involve more proximal events besides matrix deposition. Accordingly, it may diminish renal inflammation as confirmed in experiments that are primarily inflammatory such as glomerulonephritis induced by the administration of anti-glomerular basement membrane antibody (21) and Anti-thy 1 model of glomerulonephritis (2). It is important to emphasize that all models mentioned so far, diabetes, chronic transplant nephropathy, ureteral obstruction, hypertension, always involve a certain degree of inflammatory damage to the kidneys.

Although PDGF is expressed primarily by platelets, PDGF is also expressed in and secreted from VSMCs, endothelial cells, macrophages, and fibroblasts (17, 47). Thus, it is possible that in the present study increased arterial pressure-mediated increase in PDGF expression and secretion by renal VSMCs, endothelial cells, macrophages, and fibroblasts contributed to the increase in proliferating cell number in cortical tubules and interstitium and the elevation of the degree of collagen deposition in the kidneys of the hypertensive rats. However, it is also possible that platelets within the renal interstitial environment also contributed to the renal injury by secreting increased amounts of PDGF in response to the ANG II-mediated increase in arterial pressure. In this regard, it has been shown that clodigrel, an inhibitor of platelet activity, protects the kidney...
against renal parenchymatous injury in ANG II-infused hypertensive rats (13). Regardless of the source of the PDGF, the present findings indicate that PDGF receptors are located within the kidney and that activation of PDGF receptors plays an important role in mediating hypertensive-related renal injury.

It is worth noting that imatinib is not a specific inhibitor of the PDGF receptor and that it also inhibits Abl (the Abelson proto-oncogene) and c-kit. Thus, it is likely that the dose of imatinib used in the present study not only inhibited PDGF receptors, but it also inhibited Abl and c-kit and that inhibition of Abl and c-kit may have contributed, in part, to the ability of imatinib to ameliorate the renal injury in Cyp1a1-Ren2 rats with malignant hypertension. Clearly, the present data do not allow one to discriminate between these possibilities, and future studies will be required to address this particular issue. Similarly, the present data do not allow determination of whether PDGF receptors are activated in the kidneys of Cyp1a1-Ren2 rats with malignant hypertension or provide definitive evidence that the dose of imatinib used in the present study was sufficient to inhibit renal PDGF receptors. Nevertheless, the present findings that chronic imatinib administration markedly ameliorated the renal injury in Cyp1a1-Ren2 rats with malignant hypertension clearly indicate that the dose of imatinib used was sufficient to elicit substantial and effective inhibition of tyrosine kinases. As imatinib is specific for the tyrosine kinase domain in Abl (the Abelson proto-oncogene), c-kit, and the PDGF receptor, the present findings suggest that imatinib-mediated inhibition of tyrosine kinase domain of the PDGF receptor contributed, at least in part, to the marked amelioration of the renal injury in Cyp1a1-Ren2 rats with malignant hypertension. Additional studies are required to address this issue.

In summary, the results of the present study confirm that the renal morphological changes that occur in Cyp1a1-Ren2 transgenic rats with ANG II-dependent malignant hypertension are characterized by increased proliferating cell number in cortical tubules and cortical interstitium and increased collagen deposition in the renal interstitium. Such renal pathological changes that occur during the development of malignant hypertension in Cyp1a1-Ren2 rats involve activation of PDGF receptor-related kinase, and blocking this pathway ameliorates the renal morphological abnormalities observed in this model of ANG II-dependent malignant hypertension.

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Present address of M. L. Graciano: Departamento de Medicina Interna, Hospital Universitário Antônio Pedro, Universidade Federal Fluminense, Rua Marquês de Paraná 303, 6° andar, CEP 24033-900, Niterói, Rio de Janeiro, Brazil.

REFERENCES


