Losartan attenuates bleomycin-induced lung fibrosis by increasing Prostaglandin-E₂ synthesis


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ABSTRACT

Background: The angiotensin system plays a role in the pathogenesis of pulmonary fibrosis. This study examines the molecular effect of losartan, an angiotensin-II type1 receptor antagonist, in bleomycin-induced lung fibrosis. Our objective was to investigate the antifibrotic effect of losartan and its possible implication in the regulation of prostaglandin-E2 synthesis and cyclooxygenase-2 expression. Methods: Rats were given a single intratracheal instillation of bleomycin (2.5 U/kg) at the beginning of the study. Losartan (50 mg/kg/day) was administrated orally, every day, starting one day prior to the lung fibrosis induction up to the conclusion of each experiment. Results: Losartan reduced the inflammation induced by bleomycin, as we found a lower myeloperoxidase activity and protein content in broncoalveolar lavage fluid. There was an inhibition of collagen deposition induced by bleomycin after losartan treatment, as observed by the reduction of hydroxyproline content and the amelioration of morphological changes. Prostaglandin-E2 levels from fibrotic lungs were lower than in normal lungs. Losartan significantly increased prostaglandin-E2 levels at both 3 and 15 days. We also observed a reduction of cyclooxygenase-2 expression by bleomycin at 3 days and this effect was relieved by losartan. Conclusions: Our results suggest that the antifibrotic effect of losartan appear to be mediated by its ability to stimulate the production of prostaglandin-E2. Losartan, already widely used clinically, could be assessed as a new therapy in lung fibrosis.

INTRODUCTION

Idiopathic pulmonary fibrosis (IPF) is defined as a specific form of chronic interstitial lung disease, associated with the histological appearance of usual interstitial pneumonia. The poor prognosis of IPF patients, with a mean survival period of 2-4 years, and the inefficacy of current therapy, based on corticosteroids and immunosuppressors, call for the investigation of alternative therapeutic strategies.[1] IPF is characterized by the loss of lung architecture through increased epithelial cell apoptosis and abnormal wound repair, leading to the formation of fibroblast-myofibroblast foci and extracellular matrix deposition.[2][3][4] This pathological process is related to the interactions of several cytokines, chemical mediators and growth factors, derived from epithelial and mesenchymal cells.[5][6] Experimental lung fibrosis induced by bleomycin is a well studied model of fibrogenesis supported by ample literature. This model of pulmonary fibrosis resembles that seen in humans and has been used to assess the effects of potential therapeutic agents.

Angiotensin-II (ANGII) is produced by proteolytic cleavage of its precursor angiotensin-I by angiotensin-converting enzyme (ACE). Experimental evidence suggests that ANGII is a regulator of the fibrotic response to tissue injury. It has been reported that ANGII plays an important role in cardiac, renal, hepatic and pancreatic fibrogenesis.[7][8][9][10] It has also been demonstrated that ANGII is a crucial mediator in the pathogenesis of pulmonary fibrosis. ANGII induces human
lungs fibroblasts proliferation and lung procollagen production via activation of type1 receptor (AT1).\[11]\[12]\[13]\nIt is known that losartan, a selective ANGII AT1 receptor antagonist, inhibits the proliferation of human lung fibrotic fibroblasts induced by ANGII in vitro.\[12]\[13]\nMoreover, losartan inhibits the deposition of lung collagen in the rat model of bleomycin-induced pulmonary fibrosis.\[11]\nThe mechanisms involved in the pulmonary antifibrotic effect of losartan have been widely investigated but are still not fully understood. One potential mechanism is the regulation of other inflammation mediators and growth factors. There is evidence that the antifibrotic effect of losartan is mediated through ANGII AT1 receptors and involves, at least, the downregulation of transforming growth factor beta (TGF-\(\beta\)), a profibrotic mediator.\[11]\nIt has been reported that prostaglandin-E\(_2\) (PGE\(_2\)) is a potent inhibitor of fibroblast proliferation, collagen synthesis and fibroblast to myofibroblast differentiation.\[4]\nIt has been shown that broncoalveolar lavage fluid (BALF) of IPF patients contains 50% less PGE\(_2\) than in normal individuals.\[14]\nFurthermore, alveolar macrophages and fibroblasts also have a reduced capacity to produce PGE\(_2\) in vitro.\[15]\[16]\nThe failure to synthesize PGE\(_2\) has been shown to be associated with a decreased capacity to upregulate cyclooxygenase-2 (COX-2).\[16]\[17]\[18]\nThe main objective of the present study was to investigate the antifibrotic effect of losartan and its possible implication in the regulation of PGE\(_2\) synthesis and COX-2 expression in a rat model of bleomycin-induced pulmonary fibrosis. Some of the results of this study have previously been reported in the form of abstracts.\[19]\[20]\nMETHODS

**Animals**
Adult male pathogen-free Sprague-Dawley rats, weighing 225-250g at the beginning of the studies, were obtained from Criffa (Iffa Credo, France). The animals were maintained in a controlled environment. They fed on rodent chow (A04; Panlab, Barcelona, Spain) and tap water ad libitum. This study was approved by the institutional Ethics Committee and complied with European Community regulation (Directive 86/609/EEC) and Spanish guidelines for laboratory animal care.

**Chemicals**
Bleomycin sulphate was obtained from Almirall-Prodesfarma (Barcelona, Spain), halothane (Fluothane) from Zeneca Farma (Pontevedra, Spain) and sodium pentobarbital from Normon (Madrid, Spain). Losartan was provided from Merk and Co. (West Point, PA, USA).

**Experimental model**
Animals were anaesthetized under halothane and a single dose of 2.5 U/kg bleomycin dissolved in sterile saline (0.9% NaCl) was instilled intratracheally, via the transoral route, by a little glass device performed specially for it.\[21]\nControl animals received the same volume of intratracheal saline solution. The dose of
bleomycin was selected from previous experiments to cause no mortality but consistent biochemical and histological damage. The duration of each experiment was 3 or 15 days post-instillation. The animals were sacrificed at the end of each experiment by a lethal injection of sodium pentobarbital (100 mg/kg, i.p.) followed by exsanguination from the abdominal aorta. Lung tissues were weighed and processed separately, for bronchoalveolar lavage, biochemical and histological studies as indicated below.

**Experimental groups**
Animals were randomly distributed into four groups in each experiment: sterile saline solution (vehicle for bleomycin) + water (vehicle for losartan) (n=4); sterile saline solution + losartan (n=4); bleomycin + water (n=8); bleomycin + losartan (n=8).
Losartan (50 mg/kg/day) or water was administered orally (at 9:00 a.m), starting one day prior to the instillation of bleomycin, up to the conclusion of the experiment. The treatment was dissolved in a final volume of 1-2 ml of distilled water. The experimental groups were repeated twice at 3 days and at 15 days. The oral administration was selected as usual in the clinical setting, and the dose level and schedule were based on previous studies.

**Biochemical studies**
Neutrophilic infiltration was assessed by measuring myeloperoxidase (MPO) activity. MPO was measured photometrically with 3,3′,5,5′-tetramethylbenzidine as a substrate. Lung samples were macerated with 0.5% hexadecyltrimethylammonium bromide in 50 mM phosphate buffer pH 6.0. Lung homogenates were disrupted for 30 s using a Labsonic (B.Braun) sonicator at 20% power and submitted to three cycles of snap freezing in dry ice and thawing before a final 30 s sonication. Samples were incubated at 60°C for 2 h and then spun down at 4000 x g for 12 min. Supernatants were collected for MPO assay. Enzyme activity was assessed photometrically at 630 nm. The assay mixture consisted of 20 µl supernatant, 10 µl tetramethylbenzidine (final concentration 1.6 mM) dissolved in DMSO and 70 µl H₂O₂ (final concentration 3.0 mM) diluted in 80 mM phosphate buffer pH 5.4. Results are expressed as units (U) of MPO activity per lung.
Lung hydroxyproline (HP) content was measured as indicator of collagen deposition by the method outlined by Woessner. Lung samples were homogenized and then hydrolyzed in 6 N HCl for 18 h at 110°C. The hydrolysate was neutralized with 2.5 M NaOH. Aliquots (2 ml) were analyzed for HP content after the addition of chloramine T (1 ml), perchloric acid (1 ml), and dimethylaminobenzaldehyde (1 ml). Samples were read for absorbance at 550 nm in a spectrophotometer. Results are expressed as µg of HP per lung.

**Bronchoalveolar Lavage Fluid**
BALF was obtained by cannulating the trachea and lavaging four times with 10 ml of NaCl 0.9%. The BALF was centrifuged (300 g, 10 min, 4°C) and the supernatant was used for biochemical studies. Total protein concentration was measured by a standard dye technique from BioRad (Munich, Germany). The levels of PGE₂ were
measured by a competitive enzyme-immunoassay kit, from Cayman Chemical (Ann Arbor, MI, U.S.A), following the manufacturer’s protocols.

**RNA preparation and RT-PCR of COX-2**

Total RNA was prepared with Trizol Reagent following the manufacturer’s instructions (GibcoBRL, Life Technologies) and RNA concentrations were calculated from A$_{260}$ determinations. RNA integrity and loading amounts were assessed by examining 18S and 28S ribosomal RNA banding of samples electrophoresed in 1% agarose gel under nondenaturing conditions and stained with ethidium bromide. Analysis of the COX-2 and β-actine (β-Act) mRNA expression was done by a semi-quantitative RT-PCR method. One µg total RNA was used and the sequences amplified by the Life Technologies One Step RT-PCR System according to the manufacturer’s protocol. The forward primer for COX-2 was: 5'-GCT-GTA-CAA-GCA-GTG-GCA-AA- 3' and the reverse primer was: 5'-ATG-GTG-GCT-GTC-TTG-GTA-GG-3'. β-Act was: 5'-TCA-TGA-AGT-GTG-ACG-TTG-ACA-TCC-GT-3' and the reverse primer was: 5'-CCT-AGA-AGC-ATT-TGC-GGT-GCA-CGA-TG-3'. Sequences were resolved by electrophoresis in denaturing 1.8% agarose gel and staining with ethidium bromide.

**Lung histological studies**

The histological evaluation was carried out on lungs that were not lavaged. The lungs were first perfused through their main bronchus with a fixative solution (10% neutral-buffered formalin), at a pressure of 25 cm H$_2$O, immersed in the fixative for 12-24h, and blocks were taken. Tissue paraffined blocks were cut into 4 µm-thick sections, and stained with haematoxylin-eosin and Masson’s trichrome to identify inflammatory cells, connective tissue and collagen deposition. The histological changes were evaluated by a descriptive method.

**Statistical analysis**

Data are expressed as mean ± standard error of the mean (SEM) with 95% confidence intervals (CI) of n experiments. Statistical analysis was carried out by analysis of variance (ANOVA) followed, when differences were significant, by appropriate pot hoc tests including the Newman-Keuls tests. Differences between groups were tested using the paired Student’s t test (GraphPad Software Inc, San Diego, CA, USA). Differences were considered statistically significant when p was less than 0.05.
RESULTS

Losartan treatment improved bleomycin-induced lung inflammation and collagen deposition

The lung/body weight ratio was significantly increased in fibrotic rats induced by bleomycin both at 3 (control group: 0.0045 (0.0001) (95% CI 0.0046 to 0.0047); bleomycin group: 0.0077 (0.0003) (95% CI 0.0071 to 0.0083)), and at 15 days (control group: 0.004 (0.0002) (95% CI 0.0037 to 0.0043); bleomycin group: 0.0084 (0.0012) (95% CI 0.0071 to 0.0098)). Losartan treatment did not significantly change this ratio (Fig. 1A,B).

An increase in MPO activity was observed in bleomycin-exposed rats compared with the control groups at 3 days (Fig. 2A) and 15 days (Fig. 2B) after bleomycin instillation (3 days: control group 146.8 (5.2) units/lung (95% CI 138.4 to 155.1); bleomycin group 399 (28.7) units/lung (95% CI 350.3 to 447.7), 15 days: control group 279.5 (103.9) units/lung (95% CI 177.6 to 381.4); bleomycin group 1000 (16.4) units/lung (95% CI 968 to 1032)). This increase was significantly reduced by losartan (3 days: bleomycin + losartan group 247.1 (28.6) units/lung (95% CI 198.5 to 295.7), 15 days: bleomycin + losartan group 869.4 (24.9) units/lung (95% CI 832.1 to 906.7)).

Typically, total fluid recovery in the BALF exceeded 80%, and the percentages of fluid recovered did not significantly differ among experimental groups. Protein in BALF was significantly increased after 3 days of bleomycin instillation, and this effect was inhibited by losartan treatment (Fig. 3A) (control group: 1.2 (0.1) mg/100g (95% CI 1 to 1.4); bleomycin group: 10.3 (0.5) mg/100g (95% CI 9.4 to 11.2); bleomycin + losartan group: 8 (0.9) mg/100g (95% CI 6.5 to 9.5)). Fifteen days after bleomycin instillation, the difference between the control group and bleomycin group was significant, but there were no differences between the bleomycin group and the losartan treatment group. (Fig. 3B) (control group: 0.7 (0.1) mg/100g (95% CI 0.6 to 0.8); bleomycin group: 1.3 (0.1) mg/100g (95% CI 1.1 to 1.5).

Lung hydroxyproline content, a marker of collagen deposition, was increased at 15 days after exposure to bleomycin. This increase was significantly blocked by losartan, although levels remained higher than those found in animals not exposed to bleomycin (Fig. 4)(control group: 2875 (71) µg/lung (95% CI 2794.5 to 2955); bleomycin group: 6564 (414) µg/lung (95% CI 5838.4 to 7290); bleomycin + losartan group: 5081 (43) µg/lung (95% CI 5021.6 to 5140.6).

Effect of losartan in PGE2 amount and COX-2 mRNA levels

The concentration of PGE2 in BALF (pg/lung) was significantly inhibited by bleomycin instillation at 3 days. This effect was abrogated by losartan treatment, which succeeded in achieving an increased amount of PGE2 (Fig. 5A) (control group: 33849.1 (2065.6) pg/lung (95% CI 30985.9 to 36712.2); bleomycin group: 24673.7 (1516.7) pg/lung (95% CI 22015 to 27332.5); bleomycin + losartan group: 35076.3 (3986.6) pg/lung (95% CI 28309.6 to 41843.1). There were no significant differences in the PGE2 amount between control group and BLM group 15 days after fibrotic induction. Losartan treatment significantly increased the PGE2 concentration at 15 days of the bleomycin-instillation (Fig. 5B) (control group:
63520.3 (9189.5) pg/lung (95% CI 47922.1 to 79118.4); bleomycin group: 64499.6 (2807) pg/lung (95% CI 59735 to 69264.1); bleomycin + losartan group: 81419.1 (5542.5) pg/lung (95% CI 71361.8 to 91476.4). The expression of COX-2 mRNA was generally lower at 3 days after bleomycin induction. Fibrotic rats treated with losartan significantly expressed an increased amount of COX-2 mRNA (Fig. 6A) (control group: 0.44 (0.05) (95% CI 0.35 to 0.52); bleomycin group: 0.38 (0.003) (95% CI 0.37 to 0.38); bleomycin + losartan group: 0.59 (0.03) (95% CI 0.54 to 0.64). There were no changes in COX-2 mRNA expression at 15 days after bleomycin instillation and we did not observe significant differences in losartan group (Fig. 6B) (control group: 0.53 (0.01) (95% CI 0.45 to 0.62); bleomycin group: 0.51 (0.06) (95% CI 0.37 to 0.64); bleomycin + losartan group: 0.45 (0.08) (95% CI 0.28 to 0.62).

Histological features
Haematoxylin-eosin and Masson’s trichrome-stained lung sections were examined by light microscopy. Lungs from control groups at 3 and 15 days were histologically normal (data not shown). The administration of bleomycin resulted in characteristic histological changes; these include areas of inflammatory infiltration, thickening of alveolar walls (Fig. 7A) and an increase in interstitial collagen deposition and fibroblastic appearance (Fig. 7B). Losartan treatment significantly reduced the alterations in lung morphology; there were fewer inflammatory infiltrates (Fig 7C), less collagen deposition and septal widening (7D).

DISCUSSION
In the present study, we have demonstrated that losartan, a selective ANGII AT1 antagonist, ameliorates experimental lung fibrosis induced by bleomycin and increases PGE2 synthesis. Our findings provide further evidence for the anti-fibrotic effect of losartan and support the potential role of PGE2 as a protective molecule in lung fibrosis.

ANGII has been demonstrated to be an important mediator in the pathogenesis of lung fibrosis and may influence the progression of lung injury via a number of mechanisms.[2] [5] It has been reported that there is an increase in lung ANGII concentration, which precedes increases in lung collagen, and an upregulation of AT1 receptor expression in lung parenchyma after bleomycin–induced lung injury.[11][12] [25] The presence of a pulmonary renin-angiotensin system within the lung has been suggested several times in the literature. It has been reported that angiotensinogen and ANGII type1 receptors are expressed in lung tissue.[26] Moreover, a high concentration of ACE in BALF has been observed in patients with sarcoidosis and IPF.[27]

The present study shows that losartan decreased lung inflammatory infiltration significantly, at both early (3 days) and late (15 days) time points after bleomycin exposure. The anti-inflammatory effect of AT1 receptor blockade has been reported in previous studies.[25] [28][29] Our findings agree with those of Otsuka et al, who demonstrated that candesartan cilexetil significantly decreases the concentration of neutrophils in BALF in the bleomycin model of pulmonary fibrosis, suggesting that ANGII may play a role in neutrophilic infiltration in the lung. Our
study also confirms that losartan is effective in reducing lung fibrosis induced by bleomycin.

Controversially, a recent study has been reported that losartan cannot attenuate the collagen deposition induced by bleomycin in mice.[30] Differences in the animal model, the dose of bleomycin and losartan, and the time point of hydroxyproline measurements can account for discrepancies between our results and the Keogh et al. study. Nevertheless, a large number of previous reports have demonstrated that AT1 receptor antagonists decrease lung collagen deposition, in accordance with our observations.[11] [25] [31]

The mechanisms of lung antifibrotic effect of ANGII AT1 receptor antagonists have been investigated in the last years.[11][12][13] [25] It is known that losartan inhibits the proliferation of human lung fibrotic fibroblasts induced by ANGII in vitro, through the activation of AT1 receptor and mediated by TGF-β.[12] Renzoni et al. have reported that ANGII receptor type1 gene is one of the most relevant targets of TGF-β in primary lung fibroblasts from patients with IPF.[32] In the bleomycin model of pulmonary fibrosis, the administration of losartan or candesartan cilexetil attenuates TGF-β expression and lung collagen deposition.[11] [25] Similarly, other observations suggest that the antifibrotic effect of losartan could involve the regulation of TGF-β expression, providing further insights into the links between profibrotic mediators in the pathogenesis of pulmonary fibrosis. The present study was designed to investigate whether losartan can also act through antifibrotic mediators.

The most relevant finding in our study was the effect of losartan on PGE2 synthesis and COX-2 expression. Losartan significantly reversed the decrease in PGE2 synthesis induced by bleomycin. A growing body of evidence supports the hypothesis that PGE2 has a crucial role in the modulation of tissue repair and lung fibrosis.[33] PGE2 inhibits fibroblast migration and proliferation in response to various mitogens and abrogates TGF-β-induced collagen production.[16] [34][35] In addition, fibrotic fibroblasts exhibit a marked reduction in the ability to upregulate PGE2 synthesis in response to TGF-β, with the consequent loss of the antiproliferative response to TGF-β, mediated by PGE2.[17] Moreover, lung epithelial cells are a major source of PGE2, and the capacity of these cells to inhibit fibroblast proliferation is related to their ability to produce PGE2.[36] It appears that these changes are important in transforming the fibroblast from a relatively passive cell into a crucial component of IPF pathogenesis.[2] We hypothesized that the increased amount of ANGII may suppress the induction of PGE2 synthesis, and this may result in an imbalance between profibrotic and antifibrotic mediators. Thus, the reduction of ANGII action would enhance the protective effect of PGE2 in the fibrotic process. Our results confirm that AT1 antagonism increases PGE2 and reduces collagen deposition. Taken together, these observations indicate that losartan acts on at least two of the most crucial mediators implicated in lung fibrosis, improving the balance between profibrotic (TGF-β1) and antifibrotic mediators (PGE2).

The analysis of COX-2 mRNA expression has shown that losartan reversed the reduction of COX-2 induced by bleomycin at 3 days, but no significant differences were found at 15 days. These results concur with studies of the kidney, in which it was demonstrated that ANGII inhibits COX-2 expression in rat renal cortex and
that the administration of either captopril or losartan increases this expression.[37] However, the mechanisms involved in the regulation of COX-2 by ANGII in pulmonary fibrosis have not been investigated. The relevance of COX-2 as a protective mediator of pulmonary fibrosis has been demonstrated in vivo and in vitro.[17] [38] COX-2 is the major source of the PGE₂ synthesized by alveolar epithelial cells.[36] The failure to synthesize PGE₂ in fibroblasts and lung tissue from patients with idiopathic pulmonary fibrosis has been shown to be associated with a decreased capacity to upregulate COX-2.[16] [17] [18] Interestingly enough, the increased PGE₂ level observed in lung fibrosis under losartan treatment did not correlate with a significant increase in COX-2 mRNA expression. This could be a consequence of the fact that AT1 antagonists also have an effect on other enzymes implicated in PGE₂ synthesis such as PGE₂ synthase, a hypothesis suitable for future investigation.

In summary, our study shows that losartan enhances PGE₂ synthesis in experimental lung fibrosis and provides new insights into how ANGII AT1 receptor antagonists act against lung fibrosis. In addition, these data throw new light on the importance of ANGII as a regulator of the other main molecules implicated in the fibrotic process. This is a new approach to the acknowledgment of the molecular mechanisms implicated in the lung fibrotic process which could provide new insights to future therapies in lung fibrosis.
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Legends of figures

Figure 1. Relationship between lung weight and body weight. There was a significant increase in the lung/body-weight relationship after bleomycin (BLM) administration at 3 days (Fig. 1A) (*p=0.001 versus group control) and at 15 days (Fig. 1B) (* p<0.001 versus group control). Losartan (LOS) had no effect on this relationship (3 days: p=0.84, 15 days: p=0.88). Statistical analysis was performed by one-way ANOVA followed by Student t test.

Figure 2. Effect of losartan on lung inflammation induced by bleomycin was assessed by MPO activity (U/lung) in lung tissue. Bleomycin induced a rise in MPO activity, at both 3 days (Fig 2A) and 15 days (Fig 2B), and this increase was inhibited by losartan (50mg/kg). Statistical analysis was performed by one-way ANOVA followed by Student t test. (*p < 0.001 versus group control, +p = 0.004 versus group BLM at 3 days, +p = 0.005 versus group BLM at 15 days).

Figure 3. Effect of losartan on lung inflammation induced by bleomycin was measured by protein content in BALF (mg/100g). Proteins in fluid from BALF increased after bleomycin instillation, on day 3 and 15 (at 3 days: *p < 0.001 versus group control, at 15 days: *p = 0.04 versus group control). At 3 days (Fig 3A), losartan reduced the total protein production initially induced by bleomycin (+p = 0.04 versus group BLM). No differences were found between the BLM group and BLM + LOS group at 15 days (Fig 3B) (p=0.11). Statistical analysis was performed by one-way ANOVA followed by Student t test.

Figure 4. Anti-fibrotic effect of losartan on bleomycin-induced lung fibrosis quantitatively measured using HP content (µg/lung) at 15 days. The increased amount of HP content after BLM induction was inhibited by LOS). Statistical analysis was performed using one-way ANOVA followed by Student t test (*p= 0.001 versus group control, +p < 0.001 versus group BLM).

Figure 5. Losartan upregulated PGE₂ (pg/lung) synthesis. At 3 days, bleomycin instillation reduced PGE₂ synthesis and this reduction was prevented by losartan (Fig 5A) (*p = 0.01 versus group control, +p = 0.01 versus group BLM). At 15 days, rats instilled with saline solution or BLM showed no differences in PGE₂ synthesis (p=0.9), but LOS treatment upregulated PGE₂ production (Fig 5B) (+p = 0.004 versus group BLM). Statistical analysis was performed using one-way ANOVA followed by Student t test.

Figure 6. Effect of Losartan on lung COX-2 mRNA expression. The reduction of COX-2 expression after 3 days of BLM instillation was not significant (Fig 6A). We observed an increase in COX-2 expression after 3 days of BLM instillation with LOS treatment (+p = 0.02 versus bleomycin group). At 15 days (Fig 6B), there were no differences between BLM and BLM+LOS group (p=0.3). Statistical analysis was done by one-way ANOVA followed by Student t test.
Figure 7. Histological examination of the anti-inflammatory and anti-fibrotic effects of losartan on bleomycin-instilled lungs. Rats were instilled by BLM or saline solution. After 3 or 15 days of treatment with LOS they were sacrificed. Removed lungs were stained with Hematoxilin-eosin (H-E) or Masson-trichrome stain. A: Fibrotic lung induced by bleomycin at 3 days and stained with H-E, presence of extensive interstitial inflammatory infiltration. B: Fibrotic lung induced by bleomycin at 15 days and stained with Masson, with patchy areas of interstitial collagen deposition. C: Fibrotic lung treated with losartan at 3 days and stained with H-E. There were fewer infiltrate of inflammatory cells. D: Fibrotic lung treated with losartan at 15 days and stained with Masson. Although multifocal parenchymal lesions were also present, there were less septal widening and less collagen deposition. All fields were examined in each group. (Original magnification: 1x40).
A) 3 days

B) 15 days
15 days

μg HP/lung

Control  Control LOS  BLM  BLM + LOS

0  2500  5000  7500