Losartan, a selective inhibitor of subtype AT1 receptors for angiotensin II, inhibits neutrophil recruitment in the lung triggered by fMLP

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Abstract: We have shown that losartan, a selective inhibitor of AT1 receptors for angiotensin II (AII), inhibits the binding of [^{3}H]fMLP to neutrophil receptors (FPR). Here, we analyze, in Wistar rats, the effect of losartan on neutrophil recruitment in the lung triggered by fMLP. We found that i.v. infusion of losartan (0.4–20.0 μg/kg/min) inhibits neutrophil recruitment induced by i.t. instillation of fMLP, without affecting the responses induced by other stimuli, such as aggregated human IgG (aIgG), precipitating immune complexes (IC), or zymosan. Histological evaluation of lungs as well as the analysis of lung hemorrhage indices showed that losartan prevents tissue injury partially in fMLP-challenged rats. We also analyzed the effect of losartan on lung-neutrophil recruitment triggered by i.t. instillation of *Pseudomonas aeruginosa*. Not only was there a marked decrease in neutrophil recruitment but also a significant increase in the survival of rats instillated with *Pseudomonas aeruginosa*, as a consequence of losartan treatment. Our results support the notion that losartan may be useful in the treatment of certain lung inflammatory disorders associated with bacterial infectious diseases. *J. Leukoc. Biol.* 68: 700–706; 2000.

Key Words: *chemotaxis · angiotensin · Pseudomonas aeruginosa*

INTRODUCTION

The renin-angiotensin system is a bioenzymatic cascade in which renin acts on angiotensinogen to form angiotensin I (AI), which is then converted by an angiotensin-converting enzyme (ACE) to AII [1, 2]. All known effects of the renin-angiotensin system, i.e., vasoconstriction, aldosterone stimulation, and salt and water homoestasis, seem to be mediated via stimulation of the G protein-coupled AT1 receptor by AII [3, 4]. Losartan (2-*n*-butyl-4-chloro-5-hydroxymethyl-1-[(1H-tetrazol-5-yl biphenyl-4-yl)methyl] imidazole, potassium salt) is the prototype of AT1 receptor antagonists and was the first such drug available for clinical use since 1990. Actually, it is widely used to manage hypertension [5, 6]. We recently demonstrated [7] that losartan inhibits neutrophil shape change, adherence, and chemiluminescence responses triggered by *N*-formylmethionyl-leucyl-phenylalanine (fMLP) markedly, without affecting responses induced by other stimuli such as immune complexes (IC), zymosan (Zy), and concanavalin A (Con A). Neither saralasin, another antagonist of AII receptors, nor captopril, an ACE inhibitor, reproduced the effects of losartan, suggesting that they do not involve an action exerted through the AT1 receptor. Further studies revealed that losartan inhibits competitively the binding of [^{3}H]fMLP to neutrophil receptors (FPR) [7]. The mechanisms that enable losartan to inhibit binding fMLP to FPR remain undefined. However, it is noteworthy that AT1 receptors for AII and FPR belong to the class of G protein-coupled seven-transmembrane domain receptors and share 25–30% sequence identity [8].

The current study was undertaken to analyze the effect of losartan *in vivo* in rat models of lung injury triggered by fMLP or *Pseudomonas aeruginosa*. In both models, we observed that losartan markedly prevents neutrophil recruitment in the lung.

MATERIALS AND METHODS

Reagents

The chemotactic peptide fMLP, Zy, and saralasin were purchased from Sigma Chemical Co. (St. Louis, MO). Human recombinant C5a was from Fluka Chemical Corp. (Ronkonkoma, NY). The inhibitor of ACE captopril was obtained from Squibb Laboratory (Paris, France). Losartan was from Dupont (Boston, MA). Human heat-aggregated immunoglobulin G (aIgG) and precipitating IC were prepared, as we previously described [9].

Preparation of neutrophils

Citrated blood samples were obtained from adults male Wistar rats, and neutrophils were isolated by dextran sedimentation and Histopaque gradient centrifugation, as previously described [10]. Contaminating erythrocytes were removed by hypotonic lysis. After washing, the cells (>88% neutrophils on May Grunwald-Giemsa-stained Cytopreps) were resuspended at the desired

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concentration in RPMI 1640 medium (Gibco, Detroit, MI) and supplemented with 1% heat-inactivated fetal calf serum (FCS; Gibco).

Chemiluminescence (CL) assay

Neutrophils were suspended at 2.5×10^6/ml in RPMI 1640 medium supplemented with 1% FCS. Luminiscence responses were measured with a Lumi-aggregometer (Chrono-Log Corp., Haverton, PA) at 1000 revolutions/min and 37°C in the presence of luminol (0.1 μM), as we previously described [7]. In all cases, light emission was registered continuously for 10 min. Data are expressed as the maximum response observed during this period in relative CL units. One CL unit was defined as 1-cm shifting of the light-emission signal on the paper recorder.

Adherence assay

Neutrophils were suspended in RPMI 1640 medium supplemented with 1% FCS and were labeled with Na_2CrO_4 (1 μCi/10^6 cells) for 1 h at 37°C. The cells were then washed four times with saline and resuspended in RPMI 1640 medium supplemented with 10% FCS to a density of 4×10^6/ml. This suspension (100 μL) was added to each well in 96-well, flat-bottomed, polystyrene plates. Neutrophils were incubated in the presence or absence of different stimuli for 30 min at 37°C in 5% CO_2/95% humidified air and were washed three times with culture medium to remove nonadherent neutrophils. Adherent neutrophils were then lysed with 1 N NH_4OH, and the radioactivity present in the lysates was measured. Cell adherence was expressed as the number of neutrophils that remained adherent to the plastic surface after washing.

Assessment of lung myeloperoxidase activity

Neutrophil infiltration into the lung was quantified by measuring myeloperoxidase (MPO) activity in lungs, as demonstrated previously [11]. Briefly, lungs were homogenized and treated with Triton X-100 in potassium phosphate buffer, pH 6.0. After centrifugation at 2000 *g* for 30 min, the supernatant fluids were reacted with H_2O_2 (30% stock diluted 1:100; Sigma) in the presence of *0*-dianisidine hydrochloride (1 mg/ml; Sigma), and the MPO content was shown as a change in optical density (OD) at 460 nm.

Histopathologic studies

Rat lung tissue was fixed with 10% buffered formalin, pH 7.2, dehydrated in graded alcohols, embedded in paraffin, and cut into 6 μm sections. Mounted sections were stained for light microscopy with hematoxylin and eosin. Sections were examined for features of lung injury, including congestion, alveolar edema, and accumulation of inflammatory cells. All morphologic studies were done by a pathologist blinded, with respect to the different experimental groups studied.

Animal models

Adults male Wistar rats weighing ~250 g were used in all experiments. Animals were housed under standard lighting (lights on from 6.00 to 18.00 h) and temperature (23°C) conditions. Food and water were available *ad libitum*. Rats were anesthetized i.p. with urethane (1.2 g/kg body weight), and the trachea was exposed. Then, 50 μl fMLP (10^{-6} M), aIgG (500 μg/ml), IC (100 μg/ml), Zy (200 μg/ml), or human recombinant C5a (100 μg/ml) was instillated via an intratracheal catheter during inspiration. The concentration of each stimulus was selected on the basis of preliminary experiments (unpublished results). Immediately thereafter, losartan (0.4–20 μg/kg/min), saralasin (20 and 200 μg/kg/min), captopril (20 and 200 μg/kg/min), or saline (controls) were administered by continuous i.v. infusion. Rats were sacrificed 4 h after challenging, and lung MPO content was evaluated as described above.

In another set of experiments, rats were challenged by intratracheal (i.t.) instillation of live *Pseudomonas aeruginosa* isolated from a bacteremic patient. Bacteria were maintained in peptone broth containing 25% glycerol. The strain was propagated on tryptone soy agar plates for 24 h at 37°C. Then, one colony was transferred to tryptone soy broth for an additional period of 24 h at 37°C. After this time, the bacteria were centrifuged at 3000 *g* for 20 min, and the bacterial pellet was washed twice with saline. Finally, the pellet was resuspended in saline at a concentration of 10^9 colony-forming units (CFU)/ml, and 50 μl of this suspension was i.t. instillated. Immediately thereafter, losartan (20 μg/kg/min) or saline (controls) was administered by continuous i.v. infusion. In both groups of animals (untreated and losartan-treated), we performed two different experiments: 1) Animals were sacrificed 4 h after challenging, and the increase in lung MPO activity was analyzed as described above. 2) The survival of the animals of each group was analyzed at 8 and 18 h after i.t instillation of *Pseudomonas aeruginosa*.

Hemorrhage index

Anesthetized rats were challenged by i.t. instillation of fMLP (50 μl, 10^{-6} M). Immediately thereafter, they received red blood cells (injected i.v.) from wild-type rats labeled with ^{51}chromium (^{51}Cr-RBC). Losartan (20 μg/kg/min) or saline (controls) was then administered by continuous i.v. infusion. Rats were killed 4 h later, and a blood sample (1 ml) was obtained from the inferior vena cava. Following exsanguination, the pulmonary circulation was flushed with 5 ml sterile saline. The lungs were assayed for ^{51}Cr radioactivity as was the venuos blood obtained at death. The hemorrhage index was then determined for each animal as follows: index = lungs cpm/1 ml blood cpm.

Statistical analysis

Results are expressed as means ± SE. Statistical significance was determined using Student's *t*-test. A probability level of $p < 0.05$ was considered statistically significant.

RESULTS

Losartan inhibits rat neutrophil activation triggered by fMLP

We have shown previously that losartan inhibits human neutrophil activation triggered by fMLP selectively (7). Here, we analyzed in a first set of experiments whether losartan exerts similar effects in rat neutrophils. To this aim, we determined the effect of losartan on chemiluminescence and adherence responses induced by different agonists. In agreement with our observations in human neutrophils, we found that losartan inhibited chemiluminescence emission and adherence triggered by fMLP markedly, without affecting those responses triggered by other stimuli such as IC, Zy, and Con A (**Fig. 1**).

Losartan inhibits lung-neutrophil recruitment and attenuates lung injury induced by i.t. instillation of fMLP

Next, we analyzed whether losartan prevents lung-neutrophil recruitment triggered by fMLP in adults male Wistar rats by measuring the lung MPO content. Preliminary studies showed that high levels of neutrophil recruitment in the lungs were observed 4 h after i.t. instillation of fMLP (50 μl, 10^{-6} M; unpublished results). Therefore, the effects of losartan were analyzed at this time in subsequent experiments. Losartan did not modify lung MPO content in saline-instillated rats: absorbance change = 0.46 ± 0.08, 0.41 ± 0.11, 0.47 ± 0.08, 0.49 ± 0.13, and 0.49 ± 0.10 (untreated rats and rats treated with 10, 2.5, 0.5, and 0.1 μg/kg/min of losartan, respectively, n=3–6). By contrast, losartan inhibited neutrophil recruitment markedly in the lungs, triggered by i.t. instillation of fMLP, even when it was used at concentrations as low as 0.4 μg/kg/min (**Fig. 2**). Light microscopic analysis of lung tissue from fMLP-treated animals showed thickened alveolar septae with increased cellularity (**Fig. 3**), primarily because of an increased number of neutrophils (unpublished results). These signs of tissue injury were much less evident in losartan-

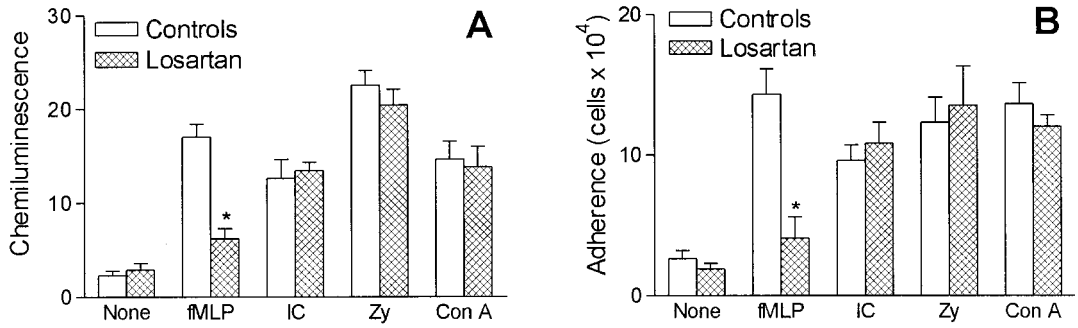

Fig. 1. Effect of losartan on neutrophil chemiluminescence and adherence responses triggered by different stimuli. Chemiluminescence emission (A) and adherence (B) were assessed as described in Materials and Methods, in the absence (open bars) or presence (cross-hatched bars) of losartan (10 μg/ml), which was added 5 min before the addition of stimuli. Results are expressed in relative chemiluminescence units (RCLU) and number of adherent cells, respectively. The following stimuli were used: fMLP (25 nM), IC (50 μg/ml), Zy (50 μg/ml), and Con A (20 μg/ml). Data are expressed as the arithmetic mean ± SE of four to seven experiments performed in duplicate. $*P < 0.005$ vs. neutrophils stimulated by fMLP in the absence of losartan.

treated rats (Fig. 3). Further experiments were performed to evaluate the effect of losartan on lung hemorrhage indices (HI) in rats challenged with fMLP (50 μl, 10^{-6} M). It was found that losartan (20 μg/kg/min) induced a reduction of 55% in HI: 0.26 ± 0.04 vs. 0. 11 ± 0.02, $p < 0.05$ (HI from saline-treated vs. losartan-treated rats, mean±SE, $n=6$).

Next, we performed additional experiments to analyze the time course of the anti-inflammatory effect of losartan. Losartan (10 μg/kg/min) was administered as a continuous i.v. infusion for 1 h. Then, fMLP (50 μl, 10^{-6} M) was i.t. instillated immediately or 2, 4, or 6 h after losartan infusion was completed. In all cases, rats were sacrificed 4 h after fMLP instillation, and lung MPO content was evaluated as described above. The results obtained (**Fig. 4**) showed that losartan inhibited the increase in lung MPO content triggered by fMLP significantly, even when it was given 6 h before challenge with fMLP.

Could losartan exert an anti-inflammatory effect when given after the onset of inflammation? To answer this question, fMLP (50 μl, 10^{-6} M) was i.t. instillated, and after 2 h, losartan (10 μg/kg/min) or saline (controls) was administered by continuous i.v. infusion for an additional 2 h. Then, rats were sacrificed and lung MPO content was evaluated. The results obtained showed that losartan inhibited lung-neutrophil recruitment significantly: absorbance change = 1.75 ± 0.19 vs. 1.18 ± 0.14 for saline- and losartan (10 μg/kg/min)-treated rats, respectively (mean±SE, $n=6$, $p<0.05$). The degree of inhibition observed under these experimental conditions, however, was less than that found when losartan was given immediately after fMLP instillation (see Fig. 2).

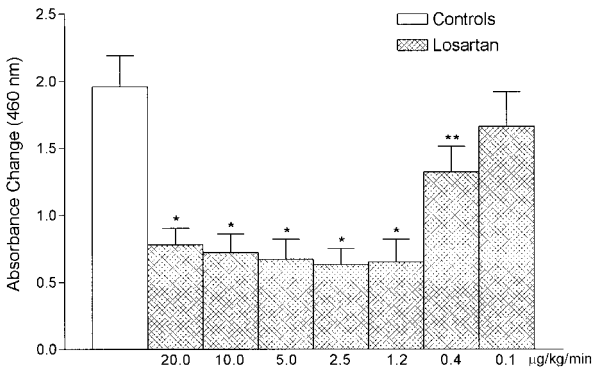

Fig. 2. Losartan inhibits lung-neutrophil recruitment triggered by fMLP. Neutrophil infiltration was quantified by measuring MPO activity in lungs 4 h after i.t. instillation of fMLP (50 μl, 10^{-6} M). Data are expressed as changes in absorbance at 460 nm. Open bar, saline-treated rats challenged by fMLP; cross-hatched bars, losartan-treated rats challenged by fMLP. Each bar represents the arithmetic mean ± SE of five to nine rats. Statistical significance, $*P < 0.01$, $**P < 0.05$, compared with saline-treated rats challenged by fMLP.

Losartan does not inhibit neutrophil recruitment in the lung induced by i.t. instillation of aIgG, IC, Zy, or C5a

To determine if losartan was able to inhibit lung-neutrophil recruitment triggered by other stimuli, in another set of experiments, we examined the effect of losartan on lung inflammatory responses triggered by i.t. instillation of aIgG, IC, Zy, and the chemotactic peptide C5a. As expected, losartan did not prevent the increase in MPO content induced by these stimuli (**Fig. 5**).

The anti-inflammatory effect of losartan does not involve the inhibition of AT1 receptors for AII

Further experiments were performed to analyze whether the anti-inflammatory activity of losartan could be attributed, at least in part, to the inhibition of AT1 receptors for AII expressed by inflammatory cells. To this aim, we analyzed whether other antagonists of the renin-angiotensin system, such as the inhibitor of ACE, captopril, and the peptidic inhibitor of AT1 receptors saralasin [3, 4], were able to reproduce the effect of losartan. As shown in **Figure 6**, these inhibitors did not modify lung MPO content in saline-instillated rats. Moreover, they did not prevent the increase in lung MPO content triggered by fMLP.

Losartan inhibits neutrophil recruitment in the lung triggered by i.t. instillation of *Pseudomonas aeruginosa*

Pulmonary infections with *Pseudomonas aeruginosa* remain a serious problem in patients with a variety of underlying dis-

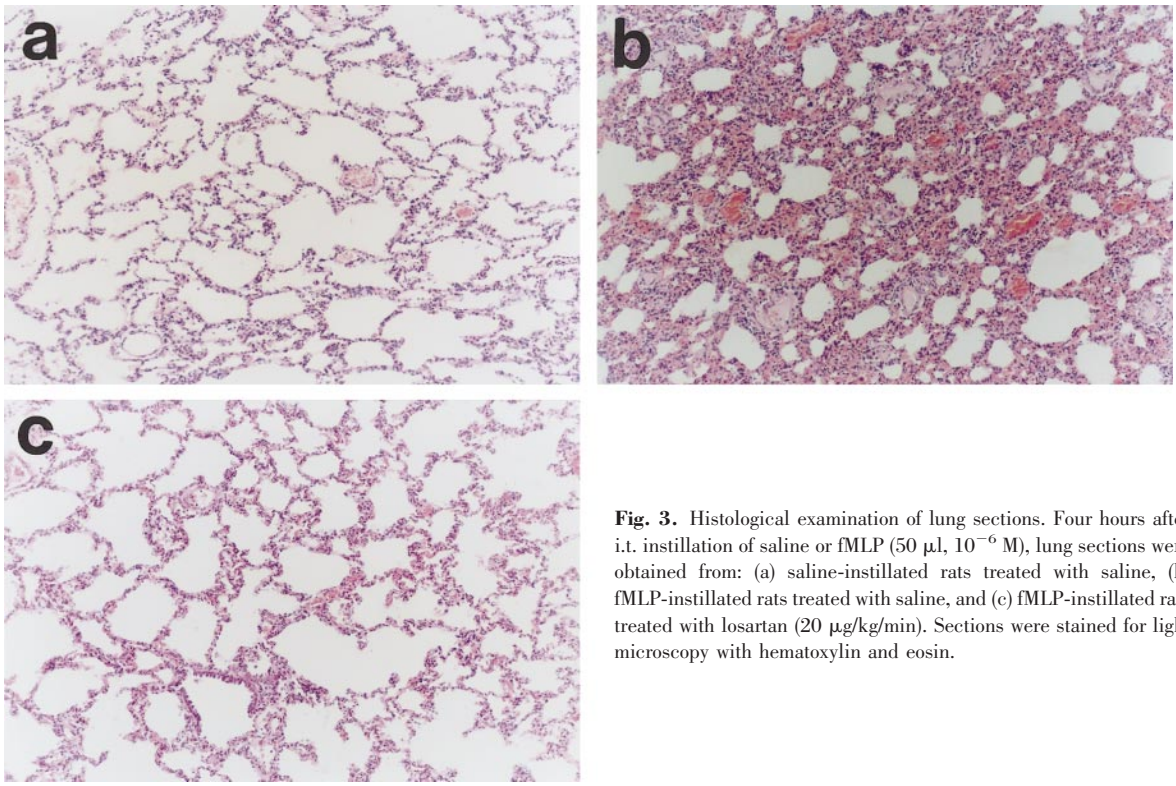

Fig. 3. Histological examination of lung sections. Four hours after i.t. instillation of saline or fMLP (50 μl, 10^{-6} M), lung sections were obtained from: (a) saline-instillated rats treated with saline, (b) fMLP-instillated rats treated with saline, and (c) fMLP-instillated rats treated with losartan (20 μg/kg/min). Sections were stained for light microscopy with hematoxylin and eosin.

eases [12–14]. It is well known that the presence of *Pseudomonas aeruginosa* in the lungs triggers the recruitment of neutrophils, which play a critical role in the clearance of bacteria, from the bloodstream [15]. Although the mechanisms responsible for the initiation of acute inflammation by *Pseudomonas aeruginosa* are still not well defined, formyl peptides appear to be involved [16, 17]. Taking this into account, we evaluated the effect of losartan on neutrophil recruitment in the lungs induced by i.t. instillation of *Pseudomonas aeruginosa*. Our results showed that losartan inhibits dramatically the increase in MPO content in infected lungs (**Fig. 7**).

Losartan improves survival of rats challenged by i.t. instillation of *Pseudomonas aeruginosa*

Recruitment and activation of neutrophils into the lungs constitute a critical host-defense mechanism against airborne bacteria such as *Pseudomonas aeruginosa* [18, 19]. However, massive infiltration of neutrophils may also induce acute lung injury, as observed in different models of acute respiratory

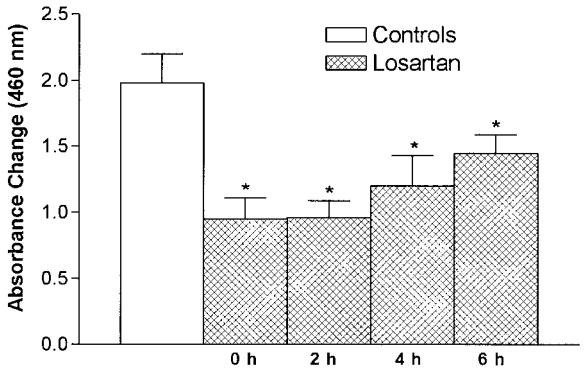

Fig. 4. Time-course study of the inhibitory effect of losartan on lung-neutrophil recruitment triggered by fMLP. Losartan (10 μg/kg/min) was administered as a continuous i.v. infusion for 1 h. After this time, fMLP (50 μl, 10^{-6} M) was i.t. instillated immediately or 2, 4, or 6 h after losartan infusion was completed. Neutrophil infiltration was quantified by measuring MPO activity in lungs 4 h after instillation of fMLP. Data are expressed as changes in absorbance at 460 nm. Open bar, saline-treated rats challenged by fMLP; cross-hatched bars, losartan (10 μg/kg/min)-treated rats challenged by fMLP. Each bar represents the arithmetic mean ± SE of four to six rats.

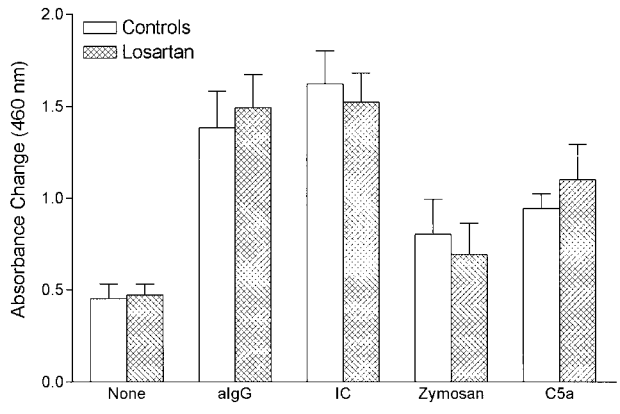

Fig. 5. Losartan does not inhibit lung-neutrophil recruitment triggered by aIgG, IC, Zy, or C5a. Neutrophil infiltration was quantified by measuring MPO activity in lungs 4 h after i.t. instillation (50 μl) of aIgG (500 μg/ml), IC (100 μg/ml), Zy (200 μg/ml), or C5a (100 μg/ml). Data are expressed as changes in absorbance at 460 nm. Open bars, saline-treated rats; cross-hatched bars, losartan (20 μg/kg/min)-treated rats. Each bar represents the arithmetic mean ± SE of four to eight rats.

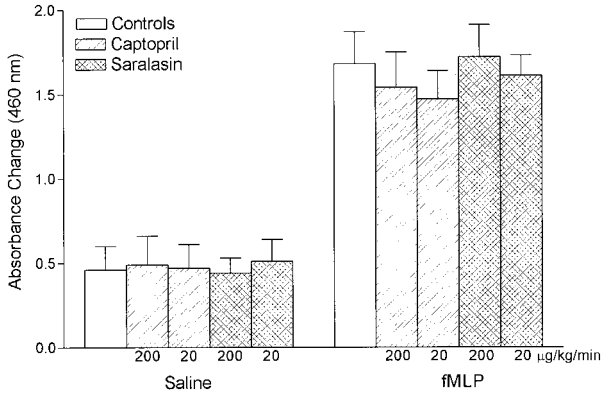

Fig. 6. Captopril and saralasin do not inhibit lung-neutrophil recruitment triggered by fMLP. Neutrophil infiltration was quantified by measuring MPO activity in lungs 4 h after i.t. instillation of fMLP (50 μl, 10^{-6} M). Data are expressed as changes in absorbance at 460 nm. Open bars, saline-treated rats; hatched bars, captopril-treated rats; cross-hatched bars, saralasin-treated rats. Each bar represents the arithmetic mean ± SE of four to five rats.

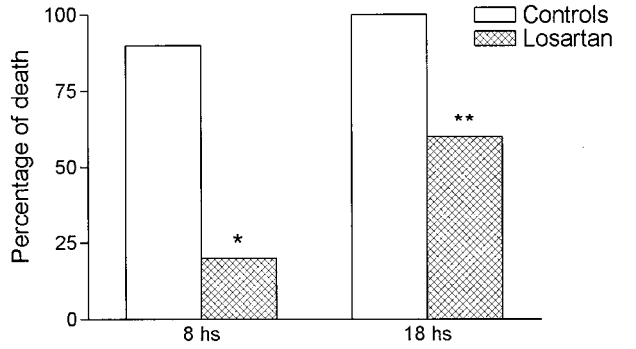

Fig. 8. Losartan improves survival of *Pseudomonas aeruginosa*-instillated rats. Animal survival was analyzed at 8 h and 18 h postinstillation with *Pseudomonas aeruginosa*. Open bars, saline-treated rats; cross-hatched bars, losartan (20 μg/kg/min)-treated rats. Data are expressed as percentage of death (n=10 for each group).

distress syndrome (ARDS) [20, 21]. In our experimental model, instillation of *Pseudomonas aeruginosa* induces massive neutrophil recruitment and ARDS (unpublished results). Taking this into account, next we analyzed whether inhibition of neutrophil recruitment by losartan might improve survival of rats instillated with *Pseudomonas aeruginosa*. As shown in **Figure 8**, 9 out of the 10 control animals died 5–8 h after instillation. In contrast, only two animals of the losartan group died during this period ($p<0.01$, untreated vs. losartan-treated rats). A significant improvement in the survival of losartan-treated animals was also observed at 18 h postinstillation, with the percentages of death for untreated and losartan-treated animals 100% and 60%, respectively ($p<0.05$).

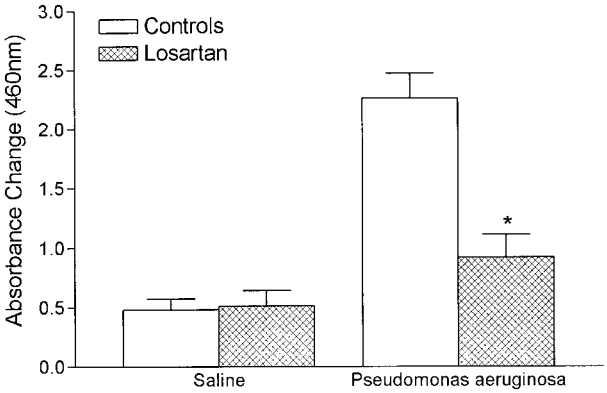

Fig. 7. Losartan inhibits lung-neutrophil recruitment triggered by *Pseudomonas aeruginosa*. Neutrophil infiltration was quantified by measuring MPO activity in lungs 4 h after i.t. instillation of *Pseudomonas aeruginosa* (50 μl, 10^9 CFU/ml). Data are expressed as changes in absorbance at 460 nm. Open bars, saline-treated rats; cross-hatched bars, losartan (20 μg/kg/min)-treated rats. Each bar represents the arithmetic mean ± SE of five rats. Statistical significance, $*P < 0.01$, compared with saline-treated rats challenged by *Pseudomonas aeruginosa*.

DISCUSSION

Recent data have indicated that AII is able to trigger inflammatory responses through AT1 receptors expressed on leukocytes and vascular smooth muscle. Thus, AII stimulates neutrophil migration [22], induces cytosolic calcium changes in monocytes [23], and also induces the activation of the proinflammatory transcription nuclear factor-κB (NF-κB) in phagocytes [24]. Further observations indicated that inhibition of AII activity prevents monocyte chemoattractant protein-1 expression and macrophage infiltration in a rabbit model of early accelerated atherosclerosis [25], as well as the development of renal injury induced by immune complexes in mice [26, 27]. Taken together, these findings suggest that AII plays an important role in certain inflammatory responses and supports the possibility that losartan and other inhibitors of AT1 receptors represent a useful tool in the treatment of these processes.

In the present work, we show that losartan inhibits efficiently neutrophil recruitment in the lungs triggered by i.t. instillation of fMLP. Histological evaluation of lungs and the analysis of hemorrhage indices showed that losartan prevented the induction of lung injury associated with neutrophil infiltration. It seems likely that the mechanism(s) through which losartan exerts these anti-inflammatory effects depends on its ability to inhibit FPR, a property that we have recently described [7]. In support of this hypothesis, we found that: 1) losartan inhibited rat neutrophil activation triggered by fMLP markedly, without affecting the responses induced by other stimuli, such as IC, Zy, and Con A; and 2) losartan did not prevent lung-neutrophil recruitment induced by aIgG, IC, Zy, or C5a. Conversely, the fact that neither captopril, an ACE inhibitor, nor saralasin, a peptidic inhibitor of AT1 receptors, were able to prevent lung-neutrophil recruitment in rats challenged with fMLP strongly suggests that the anti-inflammatory activity of losartan cannot be attributed to its ability to antagonize AT1 receptors for AII expressed on inflammatory cells.

It is well known that neutrophil recruitment in response to gram-negative infection involves bacterial-derived chemotactic factors such as *N*-formylpeptides, as well as endogenous mediators produced in response to lipopolysaccharide (LPS) and other bacterial products, such as C5a, LB4, platelet-activating

factor (PAF), and interleukin (IL)-8 [16, 17, 28–31]. A key finding in our study is that lung-neutrophil recruitment triggered by i.t instillation of *Pseudomonas aeruginosa* is inhibited markedly in losartan-treated rats. Moreover, we found that losartan improves survival of rats challenged with *Pseudomonas aeruginosa*. The most likely explanation for these results is that *N*-formylpeptides account, at least in part, for lung-neutrophil recruitment triggered by *Pseudomonas aeruginosa*. In this regard, it is important to note that although pulmonary infections with *Pseudomonas aeruginosa* remain a serious problem in patients with a variety of underlying diseases as a result of their ability to induce a vigorous and ultimately toxic neutrophil-inflammatory response [12–14], the chemotactic factors responsible for lung-neutrophil recruitment remain undefined. Studies performed in C5a receptor-deficient mice challenged with *Pseudomonas aeruginosa* showed not only a lack of inhibition but rather a marked increase in lung-neutrophil influx compared with their wild-type littermates [32]. Conversely, recent results published by Skerrett *et al.* [33] showed that mice lacking type 1 receptors for tumor necrosis factor α (TNF-α) exhibited an impaired pulmonary inflammatory response to inhaled LPS but an augmented response to live *Pseudomonas aeruginosa*, despite impaired chemokine responses to both stimuli. Support for the involvement of *N*-formylpeptides in the development of acute lung inflammation triggered by *Pseudomonas aeruginosa* has been provided by two previous findings showing that: 1) supernatants of *Pseudomonas aeruginosa* cultures exhibit chemotactic activity for neutrophils, and this activity was because of, almost exclusively, the presence of *N*-formylmethionyl peptides [17]; and 2) *N*-formylpeptides constitute one of the major sources of chemotactic activity found in bronchial secretions, which were colonized with *Pseudomonas aeruginosa* usually [34], of cystic fibrosis patients [35]. Studies in FPR-deficient mice will provide clear insights into the role of *N*-formylpeptides in lung-neutrophil recruitment triggered by *Pseudomonas aeruginosa* infection.

The FPR is a high-affinity receptor that mediates phagocyte-chemotactic responses to *N*-formylpeptides. Recently, Hartt *et al.* [36] showed that the gene Fpr-rs2 encodes a second mouse neutrophil FPR, FPR2, which recognizes *N*-formylpeptides with low affinity [the dissociation constant (K_d) of FPR2 is >100× higher compared with FPR]. The expression of both receptors is consistent with the ability of *N*-formylpeptides to induce two distinct concentrations optimum for chemotaxis of mouse neutrophils. Whether rat phagocytes express high- and low-affinity receptors for *N*-formylpeptides also remains to be determined. However, our results showing that losartan inhibits *in vitro* activation of rat neutrophils triggered by nanomolar concentrations of fMLP strongly suggest that *in vivo* anti-inflammatory effect of losartan is mediated, at least in part, by the inhibition of high-affinity FPR.

Pharmacological modulation of lung inflammation aimed at decreasing the recruitment of potentially harmful cells, without impairing antibacterial lung defenses, is an alternative to be considered in patients with bacterial pneumonia. Our results support the notion that losartan may be useful in treating certain bacterial pulmonary infections in which inflammatory responses mediated by neutrophils exert deleterious effects. In this regard, it is noteworthy that low doses of losartan are able to prevent the development of respiratory distress in septic rats (unpublished results). Further studies are being undertaken currently to define whether the anti-inflammatory effects of losartan observed in our experimental models could be explained solely by its ability to inhibit FPR.

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REFERENCES

1. Regoli, D., Park, W. K., Rioux, F. (1974) Pharmacology of angiotensin. Pharmacol. Rev. 26, 69–81.
2. Peach, M. J. (1977) Renin-angiotensin system: biochemistry and mechanisms of action. Physiol. Rev. 57, 313–348.
3. Timmermans, P. B., Wong, P. C., Chiu, A. T., Herblin, W. F., Benfield, P., Carini, D. J., Lee, R. J., Wexler, R. R., Saye, J. A., Smith, R. D. (1993) Angiotensin II receptors and angiotensin II receptor antagonists. Pharmacol. Rev. 45, 205–251.
4. Clauser, E., Curnow, K. M., Davies, E., Conchon, S., Teutsch, B., Vianello, B., Monnot, C., Corvol, P. (1996) Angiotensin II receptors: protein and gene structures, expression and potential pathological involvement. Eur. J. Endocrinol. 134, 403–408.
5. Johnston, C. I. (1995) Angiotensin receptor antagonists: focus on losartan. Lancet 346, 1403–1407.
6. Ardaillou, R. (1999) Angiotensin II receptors. J. Am. Soc. Nephrol. 10 (Suppl. 11), S30–S38.
7. Raiden, S., Giordano, M., Andonegui, G., Trevani, A.S., López, D. H., Nahmod, V., Geffner, J. R. (1997) Losartan, a selective inhibitor of subtype AT1 receptors for angiotensin II, inhibits the binding of N-formylmethionyl-leucyl-phenylalanine to neutrophil receptors. J. Pharmacol. Exp. Ther. 281, 624–628.
8. Bernstein, K. E. , Alexander, R. W. (1992) Counterpoint: molecular analysis of the angiotensin II receptor. Endocr. Rev. 13, 381–386.
9. Schattner, M., Lazari, M., Trevani, A., Malchiodi, E., Kempfer, A., Isturiz, M. A., Geffner, J. R. (1993) Activation of human platelets by immune complexes prepared with cationized human IgG. Blood 82, 3045–3051.
10. Reinhardt, P. H., Ward, C. A., Giles, W. R., Kubes, P. (1997) Emigrated rat neutrophils adhere to cardiac myocytes via α_4 integrin. Circ. Res. 81, 196–201.
11. Shanley, T. P., Schmal, H., Warner, R. L., Schmid, E., Friedl, H. P., Ward, P. A. (1997) Requirements for C-X-C chemokines (macrophage inflammatory protein-2 and cytokine-induced neutrophil chemoattractant) in IgG immune complex-induced lung injury. J. Immunol. 158, 3439–3448.
12. Speer C. P. (1999) Inflammatory mechanisms in neonatal chronic lung disease. Eur. J. Pediatr. 158 (Suppl. 1), S18–S22.
13. Kinoshita, M., Mochizuki, H., Ono, S. (1999) Pulmonary neutrophil accumulation following human endotoxemia. Chest 116, 1709–1715.
14. Dinwiddie, R. (2000) Pathogenesis of lung disease in cystic fibrosis. Respiration 67, 3–8.
15. Doring, G., Dauner, H. M. (1988) Clearance of *Pseudomonas aeruginosa* in different rat lung models. Am. Rev. Respir. Dis. 138, 1249–1253.
16. Cerquetti, N.C., Sordelli, D. O., Bellanti, J. A., Hooke, A. M. (1986) Lung defenses against *Pseudomonas aeruginosa* in C5-deficient mice with different genetic backgrounds. Infect. Immun. 52, 853–857.
17. Fontán, P. A., Amura, C. R., García, V. E., Cerquetti, M. C., Sordelli, D. O. (1992) Preliminary characterization of *Pseudomonas aeruginosa* peptide chemotactins for polymorphonuclear leukocytes. Infect. Immun. 60, 2465–2469.
18. Rehm, S. R., Gross, G. N., Pierce, A. K. (1980) Early bacterial clearance from murine lungs. Species-dependent phagocyte response. J. Clin. Invest. 66, 194–199.
19. Ozaki, T., Maeda, M., Hayashi, H., Nakamura, Y., Moriguchi, H., Kamei, T., Yasuoka, S., Ogura, T. (1989) Role of alveolar macrophages in the neutrophil-dependent defense system against *Pseudomonas aeruginosa*

infection in the lower respiratory tract. Amplifying effect of muramyl dipeptide analog. Am. Rev. Respir. Dis. 140, 1595–1601.
20. Weiland, J. E., Davis, W. B., Holter, J. F., Mohammed, J. R., Dorinsky, P. M., Gadek, J. E. (1986) Lung neutrophils in the adult respiratory distress syndrome: clinical and pathophysiologic significance. Am. Rev. Respir. Dis. 133, 218–225.
21. Abraham, E. (2000) What role does neutrophil apoptosis play in acute respiratory distress? Crit. Care Med. 28, 253–254.
22. Elferink, J. G., de Koster, B. M. (1997) The stimulation of human neutrophil migration by angiotensin II: its dependence on Ca^{2+} and the involvement of cyclic GMP. Br. J. Pharmacol. 121, 643–648.
23. Lijnen, P., Fagard, R., Petrov, V. (1997) Cytosolic calcium changes induced by angiotensin II in human peripheral blood mononuclear cells are mediated via angiotensin II subtype 1 receptors. J. Hypertens. 15, 871–876.
24. Kranzhofer, R., Browatzki, M., Schmidt, J., Kubler, W. (1999) Angiotensin II activates the proinflammatory transcription factor nuclear factor-kappaB in human monocytes. Biochem. Biophys. Res. Commun. 257, 826–834.
25. Hernández-Presa, M., Bustos, C., Ortego, M., Tuñon, J., Renedo, G., Ruiz-Ortega, M., Egido, J. (1997) Angiotensin-converting enzyme inhibition prevents arterial nuclear factor-κB activation, monocyte chemoattractant protein-1 expression, and macrophage infiltration in a rabbit model of early accelerated atherosclerosis. Circulation 95, 1532–1539.
26. Suzuki, Y., Shirato, I., Okumura, K., Ravetch, J. V., Takai, T., Tomino, Y., Ra, C. (1998) Distinct contribution of Fc receptors and angiotensin II-dependent pathways in anti-GBM glomerulonephritis. Kidney Int. 54, 1166–1174.
27. Hisada, Y., Sugaya, T., Yamanouchi, M., Uchida, H., Fujimura, H., Sakurai, H., Fukamizu, A., Murakami, K. (1999) Angiotensin II plays a pathogenic role in immune-mediated renal injury in mice. J. Clin. Invest. 103, 627–635.
28. Cardozo, C., Edelman, J., Jagirdar, J., Lesser, M. (1991) Lipopolysaccharide-induced pulmonary vascular sequestration of polymorphonuclear leukocytes is complement independent. Am. Rev. Respir. Dis. 144, 173–178.
29. Kunkel, S. L., Standiford, T., Kasahara, K., Strieter, R. M. (1991) Interleukin -8 (IL-8): the major neutrophil chemotactic factor in the lung. Exp. Lung Res. 17, 17–23.
30. Hashimoto, S., Pittet, J. F., Hong, K., Folkesson, H., Bagby, G., Kobzic, L., Frevert, C., Watanabe, K., Tsurufuji, S., Wiener-Kronish, J. (1996) Depletion of alveolar macrophages decreases neutrophil chemotaxis to *Pseudomonas* airspace infections. Am. J. Physiol. 270, L819–L828.
31. Goncalves de Moraes, V. L., Boris Vargaftig, B., Lefort, J., Meager, A., Chignard, M. (1996) Effect of cyclo-oxygenase inhibitors and modulators of cyclic AMP formation on lipopolysaccharide-induced neutrophil infiltration in mouse lung. Br. J. Pharmacol. 117, 1792–1796.
32. Hopken, U. E., Lu, B., Gerard, N. P., Gerard, C. (1996) The C5a chemoattractant receptor mediates mucosal defense to infection. Nature 383, 86–89.
33. Skerrett, S. J., Martin, T. R., Chi, E. Y., Peschon, J. J., Mohler, K. M., Wilson, C. B. (1999) Role of type 1 TNF receptor in lung inflammation after inhalation of endotoxin or *Pseudomonas aeruginosa*. Am. J. Physiol. 276, L715–L727.
34. Hoiby, N. (1982) Microbiology of lung infections in cystic fibrosis patients. Acta Paediatr. Scand. Suppl. 301, 33–54.
35. Dayer-Pastore, F., Schlegel-Hauter, E. E., Belli, D. C., Rochat, T., Dudez, T. S., Suter, S. (1998) Chemotactic factores in bronchial secretions of cystic fibrosis patients. J. Infect. Dis. 177, 1413–1417.
36. Hartt, J. K., Barish, G., Murphy, P. M., Gao, J. L. (1999) N-formylpeptides induce two distinct concentration optima for mouse neutrophil chemotaxis by differential interaction with two N-formylpeptide receptor (FPR) subtypes: molecular characterization of FPR2, a second mouse neutrophil FPR. J. Exp. Med. 190, 741–747.