Nonpeptide Antagonists of AT1 Receptor for Angiotensin II Delay the Onset of Acute Respiratory Distress Syndrome

SILVINA RAIDEN, KAREN NAHMOD, VÍCTOR NAHMOD, GUILLERMO SEMENIUK, YANINA PEREIRA, CLARISA ALVAREZ, MIRTA GIORDANO, and JORGE R. GEFFNER

Laboratory of Immunology, Institute of Hematologic Research, National Academy of Medicine (S.R., K.N., M.G., J.R.G.); Institute of Medical Research “Alfredo Lanari” (V.N., G.S., Y.P., C.A.); and Department of Microbiology, Buenos Aires University School of Medicine (M.G., J.R.G.), Buenos Aires, Argentina

Received April 12, 2002; accepted June 25, 2002

ABSTRACT

We have previously reported that losartan, a selective antagonist of AT1 receptors for angiotensin II (AII), strongly suppresses the activation of neutrophils by N-formylmethionyl-leucyl-phenylalanine (fMLP) through a mechanism that does not involve inhibition of AT1 receptors. Herein, we analyze whether losartan would prevent the development of the acute respiratory distress syndrome (ARDS) triggered by i.t. instillation of Bordetella bronchiseptica. Although this effect was associated with a significant inhibition of lung-neutrophil recruitment, lung bacterial clearance was not impaired but rather, it was significantly improved. We also found that another nonpeptide AT1 receptor blocker, irbesartan, exerted similar effects to losartan, i.e., it was also able to inhibit neutrophil activation by fMLP and to delay the onset of ARDS in B. bronchiseptica-challenged rats. Neither the inhibitor of angiotensin-converting enzyme captopril, nor the nonselective peptide inhibitor of AT1 receptors saralasin reproduced these effects. Our data are consistent with the possibility that nonpeptide AT1 receptor blockers delay the onset of ARDS triggered by bacterial infection through a mechanism dependent, at least in part, on their ability to prevent neutrophil activation by N-formyl-peptides.

Angiotensin II (AII) is an important molecule controlling blood pressure and volume in the cardiovascular system. Most of the known effects of AII seem to be mediated via stimulation of the G protein-coupled AT1 receptor (Timmermans et al., 1993; Clauser et al., 1996). Losartan (2-n-butyl-4-chloro-5-hydroxymethyl-1-(1H-tetrazol-5-yl)biphenyl-4-yl) methyl imidazole, potassium salt) is the prototype of the antagonists of AT1 receptor for angiotensin II. It was the first such drug available for clinical use since 1990 and actually it is widely used to manage systemic arterial hypertension (Johnston, 1995; Ardaillou, 1999; Timmermans, 1999).

We have previously reported that losartan impairs neutrophil activation triggered by N-formylmethionyl-leucyl-phenylalanine (fMLP) through a mechanism that does not involve blockade of AT1 receptors and depends, at least in part, on the inhibition of fMLP binding to neutrophil receptors for fMLP (FPRs) (Raiden et al., 1997, 2000). Taking this into account, and considering that bacteria induce neutrophil chemotaxis by releasing N-formyl peptides, we analyzed whether losartan was able to prevent lung-neutrophil recruitment in rats challenged by i.t. instillation of Pseudomonas aeruginosa. We found that losartan markedly decreases neutrophil accumulation in infected lungs (Raiden et al., 2000).

The acute respiratory distress syndrome (ARDS) is a devastating clinical syndrome of acute lung injury of high mortality rate (40–60%) despite intensive care using currently available drugs (Wyncoff and Evans, 1999; Ware and Matthay, 2000). It is the most severe form of a wide spectrum of pathological processes designated as acute lung injury. Lung injury in ARDS is caused by damage to the pulmonary vessels and alveoli mediated, at least in part, by activated neutrophils, resulting in massive pulmonary edema, neutrophil infiltration, and surfactant dysfunction (Weiland et al., 1986; Gattinoni et al., 1994; Wyncoff and Evans, 1999; Ware and Matthay, 2000).

In this work, we report that losartan delays the onset of ARDS in Wistar rats challenged by i.t. instillation of Bordetella bronchiseptica. Although this effect was associated with

ABBREVIATIONS: AII, angiotensin II; fMLP, N-formylmethionyl-leucyl-phenylalanine; FPR, N-formylmethionyl-leucyl-phenylalanine receptor; ARDS, acute respiratory distress syndrome; IL, interleukin; ZAS, zymosan-activated serum; aIgG, human heat-aggregated IgG; [Ca$^{2+}$], intracellular Ca$^{2+}$ concentration; AM, acetoxymethyl ester; CFU, colony-forming unit; TxA$_2$, thromboxane A$_2$.
a significant inhibition of lung-neutrophil recruitment, lung bacterial clearance was not impaired but rather, it was significantly improved. In addition, we found that another non-peptide AT1 receptor blocker, irbesartan, exerted similar effects to those of losartan, i.e., it was also able to inhibit neutrophil activation by fMLP and to delay the onset of ARDS in *B. bronchiseptica*-challenged rats.

**Materials and Methods**

**Reagents.** Zymosan, fMLP, captopril, saralasin, and IL-8 were purchased from Sigma-Aldrich (St. Louis, MO). Zymosan-activated serum (ZAS), used as a source of C5a, was prepared by incubating 15 mg of zymosan with 1 ml of fresh serum with end-over-end rotation for 1 h at 37°C. Then serum was heat-inactivated for 30 min at 56°C. After spin at 1000g for 15 min at 4°C, the supernatant was collected and stored at -70°C. Human heat-aggregated IgG (alG) was prepared by heating human IgG at a concentration of 5 mg/ml for 12 min at 63°C. Then alG was centrifuged at 10,000g for 5 min, and the precipitate was discarded.

**Preparation of Neutrophils.** Citrated blood samples were obtained from adult male Wistar rats, and neutrophils were isolated by dextran sedimentation and Histopaque gradient centrifugation, as described previously (Reinhardt et al., 1997). Contaminant erythrocytes were removed by hypotonic lysis. After washing, the cells (>98% neutrophils on May Grunwald-Giemsa-stained Cytotrips) were resuspended at the desired concentration in RPMI 1640 medium (Invitrogen, Carlsbad, CA) and supplemented with 1% heat-inactivated fetal calf serum (Invitrogen).

**Measurement of Fluctuations in Intracellular Ca2+ Concentrations [Ca2+]i.** Changes in [Ca2+]i were monitored using fluo-3/AM, as described previously (Kao et al., 1989). Briefly, neutrophils, suspended at a concentration of 5 × 106 cells/ml in RPMI 1640 medium were incubated with 4 μM fluo-3/AM for 30 min at 30°C. Then loaded cells were washed twice and resuspended at 5 × 106 cells/ml in RPMI 1640 medium supplemented with 5% heat-inactivated fetal calf serum. Aliquots of 50 μl of this cell suspension were then added to 450 μl of RPMI 1640 medium containing 5% fetal calf serum and warmed at 37°C. The samples were immediately loaded onto the flow cytometer, and the basal fluorescence (FL1) was recorded during 15 s. Then cells were activated by different stimuli, in the absence or presence of AT1 inhibitors, and the fluorescence was recorded during an additional 100 s. Acquisition of samples was performed at 37°C. Fluctuations in cytoplasmic free calcium concentrations were recognized as alterations in fluo-3 fluorescence intensity over time. Data were analyzed by using CellQuest software (BD Biosciences, Mountain View, CA). A gate based on forward and side scatter was used to exclude debris. To determine the percentage of cells responding to the stimuli, several nonoverlapping 10-s-wide time gates were used to create one-parameter histograms of the logarithmic fluo-3/AM intensity. A control histogram was created during the first 10 s of acquisition before the addition of the stimulus. Histograms from gates corresponding to 10 to 20 s after the addition of each stimulus were compared with the control histogram to determine the percentage of cells demonstrating increased fluorescence. This percentage corresponds to the proportion of cells that responds with Ca2+ flux to stimulation.

**Assessment of Lung Myeloperoxidase Activity.** Neutrophil infiltration into the lung was quantified by measuring myeloperoxidase activity in lungs 7 h after challenging (Shanley et al., 1997). Briefly, lungs were homogenized and treated with Triton X-100, in potassium phosphate buffer, pH 6.0. After centrifugation at 2000g for 30 min, the supernatant fluids were reacted with H2O2 (30% stock diluted 1:100; Sigma-Aldrich) in the presence of O-dianisidine hydrochloride (1 mg/ml) (Sigma-Aldrich), and the myeloperoxidase content was reported as change in optical density at 460 nm.

**Histopathological 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 morphological studies were done by a pathologist blinded with respect to the different experimental groups studied.

**Bacteriological Cultures of Lung Homogenates.** Rats were challenged by i.t. instillation of live *B. bronchiseptica* (50 μl, 106 colony-forming units (CFU/ml). Immediately thereafter, losartan (20 μg/kg/min) or saline (control) was administered by continuous i.v. infusion. Animals were sacrificed 7 h after challenging, the lungs were exposed aseptically, removed, and homogenized. The concentration of bacteria was quantified by placing successive 10-fold dilutions of the suspension on tryptone soy agar plates and scoring visible colonies after 24 h of incubation at 37°C. Results were expressed as CFU per lung.

**Animal Models.** Adult male Wistar rats weighing about 250 g were used in all experiments. Animals were housed under standard light (lights on from 6:00 AM to 6:00 PM) and temperature (23°C) conditions. Food and water were available ad libitum. Rats were anesthetized i.p. with urethane (1.2 g/kg of body weight), and the trachea was exposed. Then 50 μl of a *B. bronchiseptica* suspension (106 CFU/ml) was instilled via an intratracheal catheter during inspiration. Immediately thereafter, losartan (0.2–200 μg/kg/min) or saline (control) was administered by continuous i.v. infusion through the jugular vein. Losartan infusion was maintained throughout the experiment. Rats were allowed to breathe spontaneously during the experiment without oxygen therapy. The measurements of PaO2, PaCO2, and pH were then made at different times to assess the extent of respiratory failure, using a 280 blood gas analyzer (Ciba-Corning Co., Tarrytown, NY), and a sample volume of 0.3 ml from carotid artery. Acute lung injury was considered to be present if PaO2/FiO2 was <300 and ARDS if PaO2/FiO2 was ≤200 (FiO2 ≥ 0.21).

**Statistical Analysis.** Results are expressed as means ± S.E.M. Statistical significance was determined using Student’s t test. A probability level of p < 0.05 was considered statistically significant.

**Results**

**Losartan Prevents the Drop in PaO2/FiO2 Values Triggered by Instillation of *B. bronchiseptica*.** Instillation (i.t.) of *B. bronchiseptica* in adult Wistar rats induced deterioration of gas exchange, which was not observed in the saline-treated group (Fig. 1). Acute lung injury (PaO2/FiO2 ≤ 300) and ARDS (PaO2/FiO2 ≤ 200) were observed at 5 and 7 h after challenging, respectively. Sixteen rats were originally instilled with *B. bronchiseptica*, but two animals died before 7 h and, consequently, were excluded from analysis.

We next examined whether losartan could prevent lung injury triggered by instillation of *B. bronchiseptica*. The results obtained are shown in Fig. 2. Losartan (0.2–200 μg/kg/min) did not modify PaO2/FiO2 ratio in saline-instilled rats, whereas it markedly prevented the decrease in PaO2/FiO2 values triggered by instillation of *B. bronchiseptica*. Significant effects were observed at doses of 2 to 200 μg/kg/min. Therefore, the dosages of losartan were kept to 100 μg/kg/min or less.

Having shown that losartan prevented the decrease in PaO2/FiO2 values, we sought to analyze its effect on pCO2, pH, and the recruitment of neutrophils in the lung, measured as the increase in lung myeloperoxidase content. Table 1 shows that losartan prevented the decrease in pH values, the...
increased in pH CO₂ as well as significantly inhibited lung neutrophil influx.

The typical histopathology of the lungs from rats challenged by *B. bronchiseptica* is depicted in Fig. 3, which shows thickened alveolar septae and a marked increase in cellularity dominated by polymorphonuclear leukocytes. As expected, these signs of inflammation were much less evident in losartan-treated rats.

We then performed additional experiments to establish whether losartan could prevent the progressive deterioration of gas exchange when given 4 h after the instillation of *B. bronchiseptica*, time at which a significant decrease in PaO₂/FiO₂ values was observed (Fig. 1). As shown in Fig. 4, there was a much more slow decrease in PaO₂/FiO₂ values in rats treated with losartan than in those treated with saline.

**Losartan Improves Survival of Rats Challenged with *B. bronchiseptica***. Fig. 5 shows that losartan delayed mortality of infected rats. In fact, at 10 h after instillation of *B. bronchiseptica* none of saline-treated animals were alive, whereas none of the 10 animals treated with losartan died at this time point. However, at 18 h after challenging, there was only one survivor in the losartan-treated group. As observed for untreated rats challenged with *B. bronchiseptica*, the death of losartan (20 µg/kg/min)-treated rats was preceded by an increase in lung-neutrophil recruitment. Thus, myeloperoxidase content, measured as absorbance change at 460 nm, increased from 0.58 ± 0.22 at 7 h to 0.96 ± 0.24 at 11 h after challenging (n = 5, p < 0.05). Moreover, a progressive deterioration of gas exchange was also observed: PaO₂/FiO₂ = 337 ± 28 versus 225 ± 33, mean ± S.E.M. (n = 6) for losartan (20 µg/kg/min)-treated rats at 7 and 11 h after instillation of *B. bronchiseptica*.

**Losartan Does Not Impair but Rather It Improves Lung Bacterial Clearance.** We then analyzed the effect of losartan treatment on the bacterial burden. Quantitative bacteriology was performed on lung homogenates from rats killed 7 h after instillation of *B. bronchiseptica*. Surprisingly, we observed that the amount of *B. bronchiseptica* recovered from the lungs was not higher, but rather it was significantly lower in the losartan-treated group (mean CFU/lung = 2.4 ± 0.8 × 10⁷, n = 6) compared with the saline-treated group (8.1 ± 1.2 × 10⁷, n = 6, p < 0.05). This unexpected finding led us to analyze whether losartan would be able to exert a bacteriostatic or bactericidal effect. Experiments were performed by culturing *B. bronchiseptica* in tryptic soy broth for 24 h at 37°C. We observed no differences in the number of bacteria harvested from cultures performed in the absence or presence of losartan (1–200 µg/ml) (data not shown).

**Effect of Other Antagonists of the Renin-Angiotensin System on Development of ARDS Triggered by Instillation of *B. bronchiseptica***. We next analyze whether other antagonists of the renin-angiotensin system were also able to delay the onset of ARDS in infected rats. To this aim, we used captopril, an inhibitor of the angiotensin-converting enzyme, and saralasin, a nonselective peptide inhibitor of AT₁ receptors (Regoli et al., 1974; Timmermans et al., 1993; Clauer et al., 1996). None of these compounds were able to prevent the drop in PaO₂/FiO₂ values in rats challenged by *B. Bronchiseptica* (Fig. 6). In contrast, it was markedly prevented by irbesartan, a nonpeptide blocker of AT₁ receptors.

### Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>PaO₂/FiO₂ (mm Hg)</th>
<th>PaCO₂ (mm Hg)</th>
<th>pH</th>
<th>Lung MPO (Abs 460 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline i.t. + Saline i.v. (Controls)</td>
<td>448 ± 29</td>
<td>38 ± 5</td>
<td>7.38 ± 0.05</td>
<td>0.32 ± 0.13</td>
</tr>
<tr>
<td><em>B. bronchiseptica</em> i.t. + Saline i.v.</td>
<td>151 ± 29*</td>
<td>59 ± 6*</td>
<td>7.19 ± 0.04*</td>
<td>1.37 ± 0.3*</td>
</tr>
<tr>
<td><em>B. bronchiseptica</em> i.t. + Losartan i.v.  (20 µg/kg/min)</td>
<td>345 ± 19**</td>
<td>42 ± 4**</td>
<td>7.31 ± 0.02**</td>
<td>0.69 ± 0.23**</td>
</tr>
</tbody>
</table>

Statistical significance: *p < 0.01 compared with controls; **p < 0.01 compared with rats challenged by *B. bronchiseptica* and treated with saline.
based on modifications of losartan’s prototypic chemical structure (Timmermans, 1999).

Nonpeptide AT1 Receptor Blockers Irbesartan, Candesartan, and Valsartan Share with Losartan the Ability to Selectively Inhibit Neutrophil Activation Triggered by fMLP. Taking into account that irbesartan prevented lung injury in a similar manner to losartan, we next examined whether it was also able to inhibit neutrophil activation by fMLP. Studies were performed in isolated neutrophils by measuring rises in intracellular Ca\(^{2+}\) concentrations triggered by fMLP. Irbesartan almost completely inhibited Ca\(^{2+}\) transients triggered by fMLP without affecting those responses triggered by other stimuli such as ZAS, aIgG (Fig. 7) or IL-8 (data not shown). Interestingly, similar inhibitory effects were observed using two additional nonpeptide blockers of AT1 receptors (5, 16), candesartan and valsartan (10 \(\mu\)g/ml), as indicated by the percentage of cells activated by fMLP of 75\(\%\), 14, 16\(\%\), and 7\(\%\) (control, candesartan, and valsartan, respectively; mean \(\pm\) S.E.M., n = 5, p < 0.001, candesartan and valsartan versus controls). In agreement with the observations made with losartan and irbesartan, Ca\(^{2+}\) transients triggered by aIgG, ZAS, or IL-8 were unmodified by candesartan and valsartan (data not shown).

Discussion

We have previously reported that losartan, a nonpeptide blocker of AT1 receptors for AII, inhibits neutrophil activation triggered by fMLP (Raiden et al., 1997). Studies performed in vitro showed that neutrophil responses triggered by fMLP such as adherence, shape change, and the production of oxygen-reactive intermediates were markedly sup-
receptor is an asparagine residue located in position 294 (Hunyady et al., 1998), also conserved in FPR, in a region (291–299) in which seven of the nine residues are identical in both FPR and AT1 receptor. Further studies are required to determine whether residues 108 and 294 are involved in the binding of losartan to FPR. 

The experiments described in this report were performed to test the hypothesis that losartan would exert a protective effect in an animal model of ARDS. This hypothesis is based on our recent findings showing that losartan improves survival of P. aeruginosa-infected rats (Raiden et al., 2000). In the present work, we report that losartan delays the onset of ARDS in Wistar rats challenged by i.t. instillation of B. bronchiseptica. We also show that another nonpeptide blocker of AT1 receptors, irbesartan, exerts similar effects to those of losartan; it is also able to selectively inhibit neutrophil activation by fMLP, as well as to delay the onset of ARDS in infected rats.

Increasing evidence indicates that phagocytic cells express AT1 receptors, and that activation of these receptors by angiotensin II triggers a variety of inflammatory responses such as cytosolic calcium changes (Lijnen et al., 1997), activation of nuclear factor-κB (Kranzhofer et al., 1999), and production of tumor necrosis factor-α in mononuclear phagocytes (Nahmod et al., 1992), as well as neutrophil chemotaxis (Elferink and de Koster, 1997). However, although local production of AII increases at least 5-fold during the course of acute lung injury induced by B. bronchiseptica (S. Raiden, unpublished data), the mechanisms through which nonpeptide blockers of AT1 receptors delay the onset of ARDS do not seem to involve the inhibition of AT1 receptors, because neither captopril, an ACE inhibitor, nor saralasin, a nonselective inhibitor of AII receptors (Regoli et al., 1974; Timmermans et al., 1993, 1996), was able to prevent the drop in PaO2/FiO2 values in infected rats. Our results are consistent with the possibility that losartan delays the onset of ARDS triggered by lung-bacterial infection by virtue of its ability to antagonize fMLP, decreasing neutrophil accumulation in infected lungs.

In a study directed to analyze the role of formyl peptide (pathogen-derived) and chemokine (host-derived) chemoattractants in lung-leukocyte recruitment, in a mouse model of pneumococcal pneumonia, Fillion et al. (2001) found that both types of chemoattractants contribute to the recruitment of either neutrophils or mononuclear phagocytes. In regard to neutrophils, it was found that treatment of mice with a formyl peptide receptor antagonist (Boc-PLPLP) resulted in 40% reduction in neutrophil counts recovered in bronchoalveolar lavage fluid, compared with infected mice receiving placebo. Interestingly, despite the differences between experimental models used in Fillion’s study and in our work, we found a similar inhibition in neutrophil recruitment to infected-lungs, as a consequence of losartan-treatment (Table 1). However, it is important to note that even though losartan was continuously administered to infected rats, its ability to prevent lung neutrophil recruitment declined 7 h after challenging, supporting a change in the mechanisms responsible for neutrophil accumulation in the lungs from a fMLP-dependent to a fMLP-independent pathway. This change could be related to the local production of C-X-C chemokines, which may act as potent chemoattractants for neutrophils. In fact, in the model of pneumococcal pneumonia described by Fillion pressed by losartan, whereas the responses triggered by other stimuli such as immune complexes, lectins, zymosan, and C5a were unmodified (Raiden et al., 1997). Studies performed in Wistar rats, on the other hand, indicated that losartan prevented lung-neutrophil recruitment triggered by i.t. instillation of fMLP, without affecting neutrophil accumulation induced by immune complexes, zymosan, and C5a (Raiden et al., 2000). The ability of losartan to antagonize fMLP-mediated responses does not involve inhibition of AT1 receptors and could be explained, at least in part, by its capacity to inhibit the binding of fMLP to FPR (Raiden et al., 1997, 2000). Interestingly, both AT1 and FPR belong to the class of G protein-coupled seven-transmembrane domain receptors (Murphy, 1994; Timmermans et al., 1997) and share 31% sequence identity (Bernstein and Alexander, 1992). Because the ligand binding sites on these receptors are not well defined, we cannot determine the degree of homology in their binding pockets. Based on studies using chimeric receptors, site-directed mutagenesis, and inhibition assays using FPR-derived peptides, possible domains for fMLP binding have been identified in extracellular loops and all transmembrane segments (Perez et al., 1993, 1994; Quehenberger et al., 1993). In a more recent study, Miettinen et al. (1997), using site-directed mutagenesis, identified 10 putative transmembrane amino acids that may participate in binding of formylated peptides, which are located in the second, third, fourth, fifth, sixth, and seventh transmembrane domains. Regarding AT1 receptor, it also seems that the binding of both angiotensin II and nonpeptide antagonists that block angiotensin binding to this receptor (i.e., losartan) is dependent on several interactions that involve different receptor domains (Feng et al., 1995; Noda et al., 1995; Hunyady et al., 1998). Interestingly, Hoe and Saavedra (2002) have recently shown that the most important amino acid for losartan binding to AT1 receptor is a valine located in position 108, which is conserved in FPR. This position corresponds to the third transmembrane domain, which has 32% identity in both FPR and AT1 receptor. Another amino acid that seems to be involved in the binding of nonpeptide antagonists to AT1...
et al. (2001), the production of MIP-2 and KC, two C-X-C chemokines, peaked in the lung at 4 h, and seemed to plateau from 8 to 24 h after the infection.

The mechanisms through which losartan delay the onset of ARDS could also involve additional pathways unrelated to its ability to antagonize fMLP-triggered responses. A number of reports have shown that nonpeptide AT1 receptor blockers such as losartan, its active metabolite EXP3174, and irbesartan, are also able to competitively block the thromboxane A2 (TxA2)/prostaglandin endoperoxide H2 receptor (Liu et al., 1992; Bertolino et al., 1994; Li et al., 1997, 1998; Li et al., 2000). These results should be taken into account because thromboxane A2 seems to be involved in the development of acute lung injury and ARDS. Experiments performed ex vivo, in rabbit heart-lung preparations, showed that TxA2 receptor blockade ameliorates acute lung injury triggered by oleic acid (Thies et al., 1996; Goff et al., 1997). Studies carried out in a sheep model of ARDS showed that TxA2 receptor blockade successfully blunted the early pulmonary hypertension seen after endotoxin administration but did not affect the subsequent increase in pulmonary capillary permeability (Wisner et al., 1988). Moreover, three clinical studies have suggested that ketoconazole, an inhibitor of thromboxane synthase, may be effective in preventing the development of ARDS in high-risk critically ill patients (Slotman et al., 1988; Yu and Tomasa, 1993; Sinuff et al., 1999). In contrast, a recent large multicenter trial did not confirm these promising initial reports and found no improvement in survival, ventilator-free days, organ failure-free days, or any measure of lung function, in ketoconazole-treated patients (ARDS Network Authors, 2000). Further studies are required to define whether the antagonistic actions of losartan and irbesartan on TxA2 receptors play any role in the delay of the onset of ARDS observed in our experimental model.

We were surprised to find that pulmonary clearance of bacteria was augmented in losartan-treated rats, despite the reduced recruitment of neutrophils in the lung. Two hypotheses should be considered to explain this unexpected result. First, inflammatory response in ARDS seems to be excessive relative to the burden of bacterial infection (Weiland et al., 1986; Gattinoni et al., 1994; Wyncoll and Evans, 1999; Ware and Matthay, 2000). Although the phagocytic capacity afforded by neutrophil influx into the lung in response to bacteria is essential to defense capabilities against invading bacteria, an excessive inflammatory reaction causes a high degree of tissue destruction that may result in the impairment of bacterial clearance (Doring and Dauner, 1988; Chmiel et al., 1999). Thus, the possibility exists that losartan may improve bacterial clearance by virtue of its ability to transiently protect against tissue injury after intrapulmonary deposition of bacteria. Alternatively, the possibility exists that the improvement of bacterial clearance induced by losartan may be related to an action exerted on alveolar macrophages, cells that play a critical role in host defense mechanisms against lung infection (Broug-Holub et al., 1997; Kooguchi et al., 1998; Zhang et al., 2000). Although we have no direct evidence in vivo supporting this possibility, we have recently found that culture of rat peritoneal macrophages with losartan increases the ability of macrophages to ingest bacteria (S. Raiden, unpublished data).

We demonstrate herein for the first time that nonpeptide blockers of AT1 receptors delay the onset of ARDS triggered by bacterial infection. However, it should be pointed out that even though dramatic improvement was observed at early time points, there was no long-term protection. Our results support the notion that AT1 receptor antagonists might prove to be useful tools to delineate early and late mechanisms of ARDS.