Angiotensin II-Induced Pulmonary Edema in a Rabbit Model

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ABSTRACT—We conducted the present study to propose a rabbit model of pulmonary edema (PE) induced by angiotensin II (All) and to test the preventive effect of losartan on this form of PE. All was administered to rabbits intravenously at 50, 100, 150 or 300 µg/kg, either by continuous infusion (10 min) or by bolus injection (30 sec). Continuously administered All (150 µg/kg) induced PE in most cases, while a bolus injection of the same dosage did not. Additionally, the incidence of PE increased with higher dosages of All when it was infused continuously. A newly established parameter, the area under the systolic blood pressure-time curve corrected by baseline (cAUC), was prone to rise as the incidence of PE increased. Moreover, cAUC significantly correlated with the wet-dry lung weight ratio (r = 0.66, P<0.05). Subsequently, 0.5 or 3.0 mg/kg of losartan was given before continuous infusion of 150 µg/kg of All. The higher dosage of losartan prevented PE completely, while the lower one did so moderately. We concluded that intravenous administration of All induces PE, probably as a result of increasing afterload. Furthermore, an adequate dosage of losartan can prevent PE because it reduces the pressor effect of All.

Keywords: Angiotensin II, Pulmonary edema, Heart failure, Afterload, Losartan

Pulmonary edema (PE) is defined as a state with excessive extravascular lung fluid, generally resulting from increasing hydrostatic pressure or hyperpermeability in the pulmonary capillary bed (1). The disorder sometimes appears as a manifestation of heart failure. Although many common causes of PE are due to combinations of both mechanisms, cardiogenic PE is mainly attributable to hydrostatic etiology.

Key factors that contribute to the onset of heart failure are excess activity of the sympathetic nervous system and that of the renin-angiotensin system (RAS). The former contribution has been well-documented by numerous studies including those of adrenaline-induced PE (2–7). Also concerning RAS, recent studies have implied its importance in the development of heart failure. In particular, angiotensin II (All) is considered to play a leading role in its onset, since it produces strong vasoconstriction and increases systemic arterial pressure. However, most studies relating to All have shown only an indirect connection, such as efficacy of angiotensin converting enzyme inhibitors against heart failure (8). Our literature survey found little direct evidence proving the induction of heart failure by All. In 1995, Shimakura et al. first revealed that intravenous All injection can produce PE in rats, possibly as a result of increasing afterload (9), but the relationship between this form of PE and heart failure has not yet been fully elucidated. Additionally, because the vasoconstrictive response to All is widely different among species (10), it remains unclear whether such PE occurs after All administration in other animals besides rats. To our knowledge, no studies using rabbits have reported the development of PE by All (11, 12). We therefore undertook the present study in order to: 1) propose an experimental procedure for inducing PE by intravenous administration of All in rabbits, 2) find key factors that indicate the connection between the PE and heart failure and 3) examine the effect of pretreatment with losartan, a nonpeptide type-1 All receptor antagonist, on the PE model.

The data presented show that the incidence of PE increases with a higher dose of All that is given continuously to rabbits and that this form of PE is attributable to increasing afterload and following heart failure. Additionally, losartan can prevent the induction of PE, probably by a suppressive effect on the BP elevation that is
induced by AII.

MATERIALS AND METHODS

Protocol and general procedures

Male rabbits (n = 63), weighing 2.5–3.0 kg (Kanamaru Animal Lab., Saitama), were housed under controlled conditions for at least one week before the experiments. The rabbits were randomly divided into 9 groups of 7 rabbits each. Animals in the first 7 groups were assigned to receive various doses of AII, while those in the other 2 groups were used to test the effect of losartan on AII-induced PE.

Each rabbit was anesthetized with pentobarbital sodium (30 mg/kg, i.v.) via the ear vein and then fixed on its back. A polyethylene tube (PE-190) was inserted into the left femoral artery, for monitoring arterial blood pressure (BP) (blood pressure transducer, E10-PZ; Nihon Kohden, Tokyo) and its differentiated value (dP/dt) (differentiator, ED-601G; Nihon Kohden). The left femoral vein was also catheterized by another tube (PE-50) for injections. For monitoring respiratory movement (RM), a thermister pickup (Nihon Kohden) was attached to the nose which detects respiratory air flow. BP, dP/dt, and RM were simultaneously recorded with a thermister array recorder (RM-7000, Nihon Kohden). Heart rate and respiratory rate were counted by the resulting records. Using a heating blanket, the body temperature of the rabbit was maintained at 39–40 °C throughout the experiment.

For the first experiment, AII was dissolved in saline at the concentrations of 125, 250, 375, and 750 μg/ml before use (pH 6–7). By means of either continuous i.v. (1.2 ml/10 min, c.i.v.) or bolus i.v. (1.2 ml/30 sec), each solution was given at a rate of 0.4 ml/kg to groups of rabbits at the indicated concentration and by the indicated route: 50 μg/kg, c.i.v.; 100 μg/kg, c.i.v.; 150 μg/kg, c.i.v.; 300 μg/kg, c.i.v.; 50 μg/kg, bolus i.v.; and 150 μg/kg, bolus i.v. As a control, the same volume of saline alone was given to another group by c.i.v.

In the experiment for evaluating the preventive effect, losartan was dissolved in saline to prepare solutions of 2 mg/ml and 12 mg/ml. Ten minutes before AII administration, solutions at the volume of 0.25 ml/kg (0.5 mg/kg and 3 mg/kg, respectively) was injected as a bolus to each of 7 rabbits. The animals received the edematogenic dose of AII that was determined according to the dose-incidence relationship in the initial study. The other procedures were same as those in the first experiment.

Measurements of indices and evaluation of pulmonary edema

When AII was given alone, systolic BP just before AII administration was regarded as baseline. After the introduction of AII, the difference between the peak systolic BP and the baseline BP (Dmax) was obtained as an index of an increase in BP. Additionally, the area under the systolic BP-time curve corrected by baseline BP (cAUC) was determined as a parameter that reflects both the degree of a rise in BP and its persistent time, according to the trapezoidal rule (Fig. 1). The cAUC actually approximates the area surrounded by the systolic BP-time curve and the line representing the baseline BP. In the study of losartan, systolic BP was also recorded before and after losartan administration. The latter was regarded as the

![Fig. 1. Determination of the area under the systolic blood pressure (BP)-time curve corrected by baseline BP (cAUC). The solid curve shows the sequence of change in systolic BP after angiotensin II (AII) administration. The white arrow indicates when AII infusion was started. Systolic BP just before AII administration was regarded as the baseline (indicated by the broken line). The values determined by systolic BP minus baseline BP (—) were recorded every minute while systolic BP stayed above the baseline, and these were all integrated as cAUC. BP values below the baseline were regarded as zero.](image-url)
baseline BP.

Ten minutes after the end of the administration of All, every rabbit was euthanatized by exanguination via the femoral artery; then the lung was immediately excised, and the attached tissues were trimmed away. The development of PE was judged by froth or liquid running through the bronchi when the lungs were removed (severe PE) or when they were squeezed gently (mild PE). Only lungs whose bronchi produced no froth when squeezed were regarded as negative. After measurement of the wet lung wt., the lungs were dried at 70°C for 48 hr to obtain the dry lung wt. The wet-dry lung wt. ratio (wet/dry wt., WDR) was calculated in every rabbit to quantify the excess water retention in the lung.

Test agents

Losartan (losartan sulfate) was a gift of Banyu Pharmaceutical Co., Ltd. (Tokyo). Angiotensin II (angiotensin II, human) was purchased from Nacalai Tesque (Kyoto).

Statistical analyses

All data are expressed as the mean±S.D. The level of significance used was 0.05, excluding those cases with special notes in parentheses. To compare the means of several groups, one-way analysis of variance was used at first. When a significant difference was found, then Scheffe’s method was used to compare the two relevant sets of data. The differences of BPs between before and after pretreatment were tested by the paired t-test. The significance of different probabilities were examined by Fisher’s exact probability test. The relationship between two variables was assessed by calculating the correlation coefficient (r).

RESULTS

Hemodynamic changes during angiotensin II administration

Figure 2a shows changes of BP, dP/dt and RM in a rabbit given 150 µg/kg of All continuously. Similar

![Hemodynamic changes after angiotensin II administration](image)

**Fig. 2.** Typical records of hemodynamic changes after intravenous angiotensin II (All) administration. Panel a shows the changes when All was infused continuously (10 min), and panel b demonstrates those when All was injected as a bolus (30 sec). Continuous All infusion caused sustained elevation in blood pressure (BP), while bolus injection of All raised BP temporarily. The changes of differentiated BP (dP/dt) also differed between the two methods of administration. Typically, there were few effects on respiratory movement (RM).
recordings of an animal injected with 150 μg/kg of All are shown in Fig. 2b. In these records, the characteristic feature is the prompt elevation of BP; this change appeared in all the animals that received All alone. However, the reduction patterns of BPs differed widely among the groups. Typically, BP fell more rapidly when All was given a bolus i.v. than it did with c.i.v. administration. In addition, the reduction of BPs were likely to become slower as a higher dose of All was infused. Concerning heart beat, most of the rabbits had arrhythmias including supraventricular or ventricular ectopic beats and atrioventricular block in the administration period, all of which disappeared within 30 sec. Sinus bradycardia was sometimes observed just after the introduction of All. Typical changes of dP/dt in cases that received c.i.v. administration were as follows: at first, dP/dt decreased immediately after All administration; then it increased until it reached the peak value, and it decreased again gradually. All administrations sometimes depressed the respiratory movement. The respiratory rate showed little change just after the administration of All, but it increased slowly as BP decreased, in most cases.

Relation between the incidence of pulmonary edema and the dose of angiotensin II

Table 1 summarizes the results in the first experiment. When All was given by c.i.v. (1.2 ml/10 min), the incidence of PE increased in a dose-dependent manner. The dose-incidence relationship was sigmoid-shaped rather than linear (Fig. 3). This curve flattened in the dose range of 150 μg/kg and more. Also, cAUCs and WDRs seemed larger with a higher dose of All. Moreover, at a dose of 150 μg/kg, both WDR and the incidence of PE were significantly smaller when All was injected bolus i.v. than when it was given by c.i.v. Similarly, cAUCs of the animals that received All administrations bolus i.v. were smaller than those of the animals given by c.i.v. This difference between the two administration methods seems to reflect the differences in the reduction pattern of BP. On the other hand, Dmax did not differ significantly among the groups (Table 1).

Based on the results shown in Table 1, it was determined that All would be given by c.i.v. (1.2 ml/10 min) at a concentration of 150 μg/kg in the following experiment.

Effect of losartan on pulmonary edema

Figure 4 represents the actions of losartan on BP, dP/dt and RM in the rabbits. A slight but significant reduction of BP was noted after losartan injection (6±3 mmHg, P <0.05). The higher dose of losartan markedly suppressed the elevation of BP that would be induced by All alone and prevented PE completely. Also in rabbits given a lower dose of losartan, the pretreatment with it reduced the incidence of PE moderately but significantly (P<0.05) (Table 2). With regards to dP/dt and RM, the changes observed in animals without the pretreatment were also suppressed, in most cases.

Evaluation of the indices

Figure 5 shows the relationships in cAUC, WDR and the development of PE. The severity of PE seemed to be

### Table 1. Administration of angiotensin II and the incidence of pulmonary edema

<table>
<thead>
<tr>
<th>Dose of All (μg/kg, method)</th>
<th>Body weight (kg)</th>
<th>Systolic BP at baseline (mmHg)</th>
<th>Dmax (mmHg)</th>
<th>cAUC (mmHg min)</th>
<th>WDR</th>
<th>The incidence of PE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Null</td>
<td>3.0±0.2</td>
<td>141±6</td>
<td>0</td>
<td>0</td>
<td>4.78±0.07</td>
<td>0/7</td>
</tr>
<tr>
<td>50, c.i.v.</td>
<td>3.1±0.1</td>
<td>142±9</td>
<td>75±17**</td>
<td>296±86*-</td>
<td>4.85±0.10</td>
<td>1/7</td>
</tr>
<tr>
<td>100, c.i.v.</td>
<td>2.9±0.2</td>
<td>142±13</td>
<td>70±10**</td>
<td>375±109**</td>
<td>5.05±0.29</td>
<td>3/7</td>
</tr>
<tr>
<td>150, c.i.v.</td>
<td>3.0±0.1</td>
<td>143±9</td>
<td>70±6**</td>
<td>474±176**</td>
<td>5.23±0.35*</td>
<td>6/7*</td>
</tr>
<tr>
<td>300, c.i.v.</td>
<td>3.0±0.1</td>
<td>146±8</td>
<td>72±6**</td>
<td>420±90**</td>
<td>5.20±0.34*</td>
<td>7/7</td>
</tr>
<tr>
<td>50, bolus i.v.</td>
<td>3.1±0.2</td>
<td>139±8</td>
<td>81±15**</td>
<td>242±134**</td>
<td>4.87±0.15</td>
<td>1/7</td>
</tr>
<tr>
<td>150, bolus i.v.</td>
<td>3.0±0.1</td>
<td>141±9</td>
<td>83±11**</td>
<td>202±197**</td>
<td>4.82±0.31</td>
<td>1/7</td>
</tr>
</tbody>
</table>

All of the groups consisted of 7 rabbits. Systolic blood pressures (BPs) measured just before angiotensin II (All) administrations were regarded as the baseline, and the differences between the peak and baseline BPs (Dmax) were calculated after All administrations. The incidence of pulmonary edema (PE) is described as the number of rabbits in which PE developed/the number of rabbits in the group. The other abbreviations are as follows: continuous intravenous infusion for 10 min (c.i.v.), 30 sec of bolus intravenous injection (bolus i.v.), the area under the time-systolic BP curve corrected by the baseline (cAUC) and the wet-dry lung weight ratio (WDR). The differences of the indices was body weight, baseline BP, Dmax, cAUC and WDR were tested by one-way analysis of variance. Those in body weight and baseline BP were not significant. *P<0.05, **P<0.01, compared to the control group if not indicated, by post hoc Sheffe's test; †P<0.05, compared to the control group, by Fisher's exact probability test.
well-indicated by WDR. If we judge the results from a critical WDR ratio of 4.9, the probability that edematous lungs may be mistaken for nonedema is 1/19 (5.3%) and vice versa, 3/30 (10%). Concerning cAUC and the PE, both the severity and the incidence of PE were likely to increase as cAUC becomes larger. No cases with cAUCs of less than 300 mmHg·min developed edematous lungs, while most cases with cAUCs of over 500 mmHg·min developed severe PE. The correlation between cAUC and WDR was statistically significant (r=0.66, P<0.05). The contribution rate (r²) was 0.44, which means that 44% of a variation in WDR can be explained by the discrepancies in cAUC.

**DISCUSSION**

All infused intravenously to rabbits is a very powerful pressor agent. It produces a prompt increase in systemic arterial pressure, most likely as a result of systemic arterial constriction. In the present study, we successfully produced PE following an elevation in systemic arterial pressure. Concerning this type of PE model, one notable problem is that administration duration of All differs among individuals in proportion to their body weight. However, we think the differences have little influence on the present results because the differences of mean body weights among the groups were not significant and because BPs in most rabbits had returned to the baseline within 10 min in this study.

The present results revealed that both the dosage and duration of All given to the rabbits are major deter-
Table 2. Preventive effect of pretreatment with losartan on angiotensin II-induced pulmonary edema

<table>
<thead>
<tr>
<th>Losartan (dose)</th>
<th>Body weight (kg)</th>
<th>Systolic BP (mmHg) before losartan</th>
<th>Systolic BP (mmHg) baseline</th>
<th>$D_{max}$ (mmHg)</th>
<th>cAUC (mmHg·min)</th>
<th>WDR</th>
<th>The incidence of PE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Null (Control, 150, c.i.v.)</td>
<td>3.0±0.1</td>
<td>143±9</td>
<td>—</td>
<td>70±6</td>
<td>474±176</td>
<td>5.23±0.35</td>
<td>6/7</td>
</tr>
<tr>
<td>Low dose (0.5 mg/kg)</td>
<td>2.9±0.1</td>
<td>141±9</td>
<td>135±12</td>
<td>74±17</td>
<td>484±157</td>
<td>4.88±0.17$^*$</td>
<td>2/7$^*$</td>
</tr>
<tr>
<td>High dose (3.0 mg/kg)</td>
<td>3.0±0.2</td>
<td>139±8</td>
<td>134±8</td>
<td>42±18$^*$</td>
<td>266±119$^*$</td>
<td>4.78±0.19$^{**}$</td>
<td>0/7$^*$</td>
</tr>
</tbody>
</table>

All of the groups consisted of 7 rabbits. The group of "150, c.i.v." in Table 1 was taken as the control group. Angiotensin II (All) was given to rabbits by the same method as used for the control group. Systolic blood pressures (BPs) measured just before losartan injections were described as those before losartan, and those just before All administrations were used as the baseline. The differences between the peak and baseline BPs ($D_{max}$) were calculated after All administrations. The incidence of pulmonary edema (PE) is described as the number of rabbits in which PE developed/the number of rabbits in the group. The other abbreviations are as follows: the area under the time-systolic BP curve corrected by baseline (cAUC), the wet-dry lung weight ratio (WDR) and continuous intravenous infusion for 10 min (c.i.v.). The differences of the means among the groups in systolic BP before losartan, $D_{max}$, cAUC and WDR, were tested by one-way analysis of variance. $^*P<0.05$, $^{**}P<0.01$, compared to the control group if not indicated, by post hoc Sheffe's test; $^{*}P<0.05$, by the paired t-test; $^{**}P<0.05$, compared to the control group, by Fisher's exact probability test.

Fig. 5. The relationships among the area under the systolic blood pressure-time curve corrected by baseline (cAUC), wet-dry lung weight ratio (WDR) and the development of pulmonary edema (PE). The closed circles show the cases with severe PE, the half-tone circles show those with moderate PE, and the open circles show those without PE. Generally, both the severity and the incidence of PE are prone to increase with cAUC. WDR seems to reflect the severity of PE, and it moderately correlates with cAUC ($r=0.66$, $P<0.05$).
minants for whether or not PE was induced. When AII was infused continuously for about 10 min, the incidence of PE and cAUC increased in relation to the dosage, as shown in Table 1. Additionally, the duration of AII had a great impact on the onset of PE. Concerning the two administration methods of c.i.v. (10 min) and bolus i.v. (30 sec), the former induced PE more frequently than the latter did. Although we did not try other methods, we can speculate on what would be the results of other administration durations according to the present data. For instance, the administration dose per time of "300 µg/kg/10 min" is substantially equal to that of "150 µg/kg/5 min". Supposing that AII was given at the rate of "150 µg/kg/5 min", the BP-time curve within the initial 5 min must be similar to that of "300 µg/kg/10 min". Then, BP will fall soon after the end of AII infusion because the half-life of exogenous AII in vivo is short (about 70 sec) (13). Consequently, the cAUC of this method will be smaller than that of "300 µg/kg/10 min" and so will the incidence of PE. Similarly, the dose per time of "150 µg/kg/15 min" and "150 µg/kg/30 min" are the same as that of "100 µg/kg/10 min" and "50 µg/kg/10 min", respectively. Since BPs in most of the animals given "100 µg/kg/10 min" and "50 µg/kg/10 min" had fallen below the baseline BP within 10 min, an administration duration of longer than 10 min will have little influence on the cAUC. With this speculation, AII administration of 150 µg/kg/10 min is thought to be most appropriate as edematogenic method.

Some of the observations herein are highly indicative of a significant connection between the increasing systemic BP and the development of PE. One such finding is that WDRs significantly correlate with cAUCs. As shown in Fig. 5, WDR is an easily obtainable index that reflects the degree of lung fluid retention to some extent. Although the absolute value of WDR may be influenced by the experimental procedures such as the squeezing process, WDR would be reliable for estimating the degree of fluid retention of the lung if the same procedures are performed. Ishikawa et al. have documented in their work that 85% of the judgements of PE by critical WDR value correspond with the judgement of PE by the edema froth (14), which is consistent with this study. This similarity would indicate the acceptable reproducibility and responsibility of WDR. On the other hand, the area under the systemic BP-time curve, a parameter used for evaluating the antihypertensive effect (15), reflects both the extent of increasing BP and its persistent period. In comparison with D_max or the peak BP, which represents the degree of an increase in BP alone, cAUC seems more suitable for explaining the diversity of the incidence of PE between the groups. Thus, WDR and cAUC well-indicate the severity of PE and the changes of BP in this study, respectively.

Because continuing hypertension suggests an increment of cardiac afterload, the significant correlation between the two parameters makes us think that heart failure makes a large contribution to the development of PE.

One concept that may explain the development of PE after a rise of afterload is that of afterload mismatch (16). In short, an increase of afterload will expand the ventricular volume (=increasing preload) to maintain cardiac output. The ventricle can compensate for the added afterload when it is within the limit of the preload reserve, while left heart failure will develop if it exceeds the adaptable range. Failure of the left heart system can result in PE due to increasing hydrostatic pressure. This simple and understandable concept may explain some of the observations in this study. For example, the sequential change of dp/dt, a parameter indicating left ventricular contraction, may be understood as follows: it may decrease initially in response to increasing afterload; then increase gradually according to the recovery of cardiac output caused by the adaptation of preload; and finally, it may decrease again, probably due to the decompensation of the left ventricle. Otherwise, since the PE did not develop in cases that had cAUCs lower than 300 mmHg·min and the PE always occurred when the cAUC exceeded 500 mmHg·min, those values may imply the adaptable range of preload reserve. However, the above explanation does not include one essential factor: the change of the original ventricular contractility. The entire mechanism process that occurred after AII administration cannot be discussed by this concept alone. Baker et al. have reported that a small dosage of AII increases cardiac work due to not only increased afterload but also increased contractility of the left ventricle (17). The positive inotropic action of AII may also play some roles in the present study. From this point of view, additional research is required to understand the overall hemodynamic performance.

AII receptor inhibitors are currently attractive drugs for clinicians to treat patients with hypertension or heart failure. In particular, losartan is the initially developed, nonpeptide, AII type-1 receptor inhibitor that can be given p.o. Regardless of numerous studies, however, the effects of these drugs against the respiratory systems and pulmonary circulation remain to be elucidated. In this study, losartan is given 10 min before AII administration. The 10-min interval could be used to obtain a sufficient drug effect and stabilization of BP, because the elimination half-life of losartan is sufficiently long (41 min in dogs and 2.1 hr in humans) (18, 19). As a result of the pretreatment, the higher dose of losartan could completely prevent the development of PE. This preventive effect of losartan would be attributable to its suppression of the BP elevation in large part. However, the same ex-
planation can not explain why the lower dose of losartan also prevented PE moderately without apparent suppression of the elevation in BP. Because type-1 receptors exist not only in the smooth muscle of vessels but also widely in tissues of the circulatory and respiratory systems, mechanisms other than vasodilation may be involved in the action of losartan. In the literature, Yukioka et al. reported that one AII antagonist improved gas exchange in the lung and that the improvement of blood gas analysis may be attributed to the direct action of this drug against the smooth muscle of airway. On the other hand, Mookherjee et al. reported no change of gas analysis or airway resistance. Thus, whether these drugs would be effective on respiratory dysfunction or not is still controversial.

Another question that should be answered is the association of permeability factors with the onset of PE. Gil and McNiff pointed out that even a bolus administration of 10 μg/kg of AII to rabbits produces epithelial cell damage rather than endothelial cell damage, the former being commonly observed in lung injury with hyper-permeability. Concerning adrenaline-induced PE, eicosanoids or cytokines are thought to play some roles in its onset. This must be clarified by additional experiments.

In conclusion, we have proposed a rabbit model of PE that is induced by intravenous administration of AII, and also revealed the probable etiology of this form of PE. Moreover, an adequate pretreatment with losartan and we also revealed the probable etiology of this form of PE that is induced by intravenous administration of AII, and also revealed the probable etiology of this form of PE. The present model can be used to clarify the role of AII in heart failure and the effectiveness of losartan on the circulatory and respiratory systems.

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