Pulmonary Edema Induced by Angiotensin II in Rats

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ABSTRACT—We performed this study to demonstrate the experimental procedure for inducing pulmonary edema by angiotensin II (AT II) in rats and to determine the mechanism of hemodynamic pulmonary edema. In the pilot study, 10 μg/ml of AT II was found to be adequate as the edematogenic dose for inducing pulmonary edema. The edematogenic dose of AT II was intravenously given to rats pretreated with 20 mg/kg of an AT II-receptor antagonist, E 4177 (3-[(2'-carboxybiphenyl-4-yl)methyl]-2-cyclopropyl-7-methyl-3H-imidazo[4,5-b]pyridine), and to rats given 10 mg/kg of an alpha-adrenergic blocker, phentolamine. Similarly, pulmonary edema was induced by 25 μg/ml of adrenaline in rats pretreated with E 4177 (20 mg/kg) and rats with no pretreatment. E 4177 completely suppressed the development of AT II-induced pulmonary edema, whereas phentolamine could not. On the contrary, E 4177 could not suppress the development of adrenaline-induced pulmonary edema. We concluded that AT II-induced pulmonary edema will develop via the specific AT II receptor without the indirect action of adrenaline.

Keywords: Pulmonary edema, Angiotensin II, Adrenaline, Angiotensin II-receptor antagonist, Phentolamine

Heart failure causes an increase in systemic vascular resistance and an accumulation of excess fluid as the compensatory adaptations of enhanced activities of the sympathetic nervous system and the renin-angiotensin system. The clinical features relating to pulmonary edema arise in the end-stage of the left ventricular failure due to the excessive compensatory adaptations. Angiotensin-converting enzyme inhibitors have currently attracted the attention of physicians as effective drugs that improve the prognostic outcomes of patients with heart failure (1). Since the drugs suppress the production of angiotensin II (AT II) which has a vigorously vasoconstrictive effect, they bring about a favorable clinical course in those patients. Although many studies have established experimentally that high doses of adrenaline, one of the most powerful cardiac stimulants, induce pulmonary edema as the result of increased afterload in systemic circulation which elevates the left atrial pressure (2), there are no reports to our knowledge, on the experimental induction of pulmonary edema by AT II. In the course of the study on pulmonary edema, we had been concerned with the vasoconstrictive effect of AT II and found that the bolus intravenous injection of AT II also induced pulmonary edema in rats. We therefore undertook the present study for the following reasons: 1) to propose the experimental procedure for inducing pulmonary edema by the administration of AT II in rats and 2) to determine the mechanism of AT II-induced pulmonary edema and the protective measures against it.

MATERIALS AND METHODS

Animals and outline of the experiment
Male albino rats of the Wistar strain (n=57) weighing 250-300 g were purchased from Saitama Animal Lab. (Saitama) and housed under controlled conditions for at least two weeks before the experiments. They were randomly divided into eight groups according to the experimental procedure described below: The present study was conducted in three parts. First, 18 rats were studied to determine the edematogenic dose of AT II. Secondly, to determine the key mechanism of AT II-induced pulmonary edema and the protective measures against it, 23 rats were given the edematogenic dose of AT II under each specific pretreatment with a nonpeptide AT II receptor antagonist, E 4177 (3) (n=7); an alpha-adrenergic blocker, phentolamine (n=8); and a vasodilator, nitroglycerin (n=8). Last, adrenaline-induced pulmonary edema was pro-
duced in eight rats under the pretreatment with E 4177 and in another eight rats that served as the control to determine if AT II indirectly affected adrenaline-induced pulmonary edema.

**Surgical procedures for the experiment**

Each rat was anesthetized with an intraperitoneal injection of 50 mg/kg pentobarbital sodium and then fixed on its back. After intubating a polyethylene endotracheal tube, the femoral vein and artery were catheterized to provide a route for administrations and to monitor arterial blood pressure through a high pressure transducer (Nihon Kohden, Tokyo; P 10 EZ), respectively. After administering AT II or adrenaline, the peak values of arterial blood pressure were recorded as the index of afterload in the systemic circulation. Respiratory movement was traced with a costabdominal pneumograph connected to a low pressure transducer (Nihon Kohden, AR-650 H). Heart rate, respiratory rate and blood pressure were recorded with a polygraph (Nihon Kohden, CP-602G). The body temperature of the rats was maintained at approximately 39°C during the experiment.

**Determination of the edematogenic dose of AT II**

AT II was dissolved in saline at various concentrations of 5, 10 and 15 µg/ml (pH 6 to 7) before use. As shown in Table 1, eighteen rats were divided into three different groups for administration of each different AT II solution (Group 1: five rats for 5 µg/ml of AT II solution, Group 2: eight for 10 µg/ml, and Group 3: five for 15 µg/ml). The bolus dose of 1 ml/kg AT II solution was administered through the venous cannula. The rats were killed at once to examine the lung when froth or pink liquid appeared in the tracheal tube, but otherwise, just after observing the vital signs for 10 min. After exanguination via the cut abdominal aorta, the lung was rapidly removed, and the attached tissues were trimmed away for gross observation. Then the net weight of the lung was measured to evaluate the degree of pulmonary edema. The development of pulmonary edema was judged by 1) macroscopic hemorrhage observed as hemorrhagic patches and spots in the lung and 2) froth or liquid running out of the tracheal tube. The lung was considered not to have pulmonary edema only when no froth came out from the cut lower trachea even by gently squeezing the lung (4). The lung body weight index (L.B.I. = lung weight × 100 /body weight) was calculated in every rat to quantify the degree of excess fluid retention in the lung. The edematogenic dose of AT II for our experiments was determined by evaluating the dose-incidence relationship.

**Effect of pretreatments with E 4177, phentolamine and nitroglycerin on AT II-induced pulmonary edema**

Twenty-three rats were subdivided into three groups for each specific pretreatment (Group 4: seven rats for E 4177, Group 5: eight for phentolamine and Group 6: eight for nitroglycerin) (Table 1). Twenty min before injecting the edematogenic dose of AT II, bolus doses of 20 mg/kg of E 4177 and 10 mg/kg of phentolamine were administered to rats in Groups 4 and 5, respectively. In the remaining rats, Group 6, constant infusion of nitroglycerin (concentration: 1 mg/ml, infusion rate: 0.1 ml/kg/min) was started just before administering AT II. In each rat, the development of pulmonary edema was assessed according to the same procedure described in the above section.

**Effect of pretreatment with E 4177 on adrenaline-induced pulmonary edema**

A bolus dose of 25 µg/kg adrenaline was injected to 16 rats in order to produce pulmonary edema (Table 1). Eight of them received no pretreatment as the control group (Group 7), and the other eight was given a bolus dose of 20 mg/kg E 4177 20 min prior to the administration of adrenaline (Group 8). The lung was investigated as previously done.

**Test agents**

The following agents were used: angiotensin II (Angiotensin II (Human); Peptide Institute, Inc., Osaka); E 4177 (3-[(2'-carboxybiphenyl-4-yl)methyl]-2-cyclopropyl-7-methyl-3H-imidazo[4,5-b]pyridine; Eizai Co., Ltd., Tokyo); phentolamine mesylate (Regitin; Ciba-Geigy, Hyogo); nitroglycerin (Millisrol; Nihonkayaku, Tokyo), adrenaline (Bosmin; Daiichiseiyaku, Tokyo).

**Statistical analyses**

All data are expressed as the mean ± S.D. The variances of the means were tested for homogeneity of distribution by the F-test. When the variances were found to be homogenously distributed, the differences between two means were compared by the two-tailed Student's t-test for paired or unpaired observations. The linear relationship between two variables was assessed by calculating the correlation coefficient (r).

**RESULTS**

**Determination of the edematogenic dose**

The incidences of AT II-induced pulmonary edema are summarized in Table 1. The incidence was more likely to closely correlate with the logarithmic value of the dose of AT II (r=0.985) than with the value of the dose itself (r=0.948), although both correlations did not reach a
Systolic BPs were measured just before AT II administrations, and peak values of systolic BPs were obtained after AT II administrations. Abbreviations are as follows: angiotensin II (AT II), blood pressure (BP), lung body weight index (L.B.I.) and pulmonary edema (PE). **Significant difference (P < 0.01) by the paired t-test.

significant level because of the small number of sample points (n=3). This suggested that the relationship between the dose and the incidence was curvilinear rather than linear. Figure 1 shows that the dose-incidence curve flattens out in the dose range of 10 μg/kg and over. This finding indicated that the doses of 15 μg/kg or more had an excessively strong effect to induce pulmonary edema. We thereby selected 10 μg/kg of AT II as the edematogenic dose. The administration of AT II produced a sustained rise in systolic and diastolic blood pressure with initial bradycardia, and it usually depressed the respiratory amplitude throughout the period of observation (Fig. 2a). Elevated blood pressure after AT II injection indicated an increased afterload in the systemic circulation due to the vasoconstrictive effect of AT II. However, despite no signs of developing pulmonary edema (no froth and no elevation in blood pressure) during the 10-min observation, the lungs in several cases obviously exhibited an edematous appearance, and froth came out upon transection of the lower trachea. These results suggested that the elevation in blood pressure was not necessarily related to the development of pulmonary edema. The larger the dose given, the larger was the value of L.B.I., indicating the dose-dependent severity of AT II-induced pulmonary edema (Table 1).

Effect of pretreatments
Table 2 shows the preventive effect of each pretreatment against pulmonary edema induced by the edematogenic dose (10 μg/kg) of AT II. Although every pretreatment somewhat decreased systolic blood pressure, AT II administrations significantly increased systolic blood pressure in all three groups, and the elevations of it were particularly remarkable in rats with phentolamine (Group 5) and in those with nitroglycerin (Group 6). Systolic blood pressure and L.B.I. were significantly lower in rats with E 4177 (Group 4) than in the control (Group 2). The electrocardiogram showed neither bradycardia nor arrhythmias, and the pneumogram did not show any remarkable irregularities in rats with E 4177 (Group 4) (Fig. 2b). Namely, the pretreatment with E 4177 could completely prevent the lung from AT II-induced pulmonary edema. An initial bradycardia, a respiratory irregularity and an elevated systolic blood pressure were still observed in rats with nitroglycerin (Group 6). Nonetheless, nitroglycerin was also judged as protective because of the lower incidence of pulmonary edema and significantly smaller value of L.B.I. in rats with nitroglycerin (Group 6) than in the control (Group 2). Conversely, the pretreatment with phentolamine failed to prevent AT II-induced pulmonary edema. Rats with phentolamine (Group 5)

Table 1. Determination of the edematogenic dose of angiotensin II

| Material group (n) | Dose of AT II (μg/kg) | Systolic BP (mmHg) | L.B.I. | Incidence of PE (%) |
|-------------------|----------------------|-------------------|--------|-------------------|
|                   |                      | before AT II | after AT II |                  |
| Group 1 (n=5)     | 5                    | 116.0±10.2     | 213.0±32.9 | 0.56±0.15 | 40            |
| Group 2 (n=8)     | 10                   | 115.8±9.7      | 238.0±19.6 | 0.79±0.20 | 87.5          |
| Group 3 (n=5)     | 15                   | 113.0±18.8     | 265.0±14.1 | 1.13±0.23 | 100           |

Systolic BPs were measured just before AT II administrations, and peak values of systolic BPs were obtained after AT II administrations. Abbreviations are as follows: angiotensin II (AT II), blood pressure (BP), lung body weight index (L.B.I.) and pulmonary edema (PE). **Significant difference (P < 0.01) by the paired t-test.

Fig. 1. The curvilinear relationship between the incidence of pulmonary edema and the dose of angiotensin II. The solid line indicates the curvilinear regression line between the incidence and the dose (y=56.11 Log, x + 47.99).
Fig. 2. Typical records for demonstrating the preventive effect of E 4177 on angiotensin II (AT II)-induced pulmonary edema. Panel a shows hemodynamic changes in AT II-induced pulmonary edema. Intravenous injection of AT II causes a remarkable elevation in blood pressure (BP) and irregularities in respiratory rate (RR) and heart rate (HR). On the contrary, as shown in panel b, pretreatment with E 4177 can completely suppress the elevation in BP and the irregularities in RR and HR.

Effect of E 4177 on adrenaline-induced pulmonary edema

The indirect effect of AT II on adrenaline-induced pulmonary edema was studied by using E 4177 (Fig. 3, a and b). As shown in Table 3, the systolic blood pressure after administering adrenaline significantly rose regardless of the pretreatment with E 4177 (Group 7) (Fig. 3b). Systolic blood pressure after administrations of adrenaline and L.B.I. values were not significantly different between rats with the pretreatment (Group 7) and those without (Group 8). Namely, E 4177 could not suppress the development of adrenaline-induced pulmonary edema.

DISCUSSION

AT II, regulating systemic vascular resistance and fluid balance, physiologically stimulates the secretion of adrenaline that can induce pulmonary edema almost exclusively by alpha-adrenergic action (4–8). A previous
Table 2. Effects of various pretreatments on angiotensin II-induced pulmonary edema

| Material group (n) | Pretreatment (dose) | Systolic BP (mmHg) before pretreatment(1) | before AT II(2) | after AT II(3) | L.B.I. | Incidence of PE (%) |
|-------------------|---------------------|-------------------------------------------|-----------------|---------------|-------|---------------------|
| Group 2 (n = 8)   | Null                | ---                                       | 115.8±9.7       | 238.0±19.6    | 0.79±0.2 | 87.5               |
| Group 4 (n = 7)   | E 4177 (20 mg/kg)   | 123.6±18.9                                | 110.7±33.3      | 131.0±32.6    | 0.39±0.03 | 0                  |
| Group 5 (n = 8)   | Phentolamine (10 mg/kg) | 109.5±23.9                             | 98.1±28.5       | 217.0±19.9    | 0.81±0.23 | 75.0               |
| Group 6 (n = 8)   | Nitroglycerin (1 mg/kg) | 122.6±11.0                               | 100.0±21.3      | 230.3±14.4    | 0.46±0.12 | 37.5               |

Group 2 is regarded as the control group. Systolic BPs were measured (1)just before each pretreatment and (2)just before AT II administrations. Peak values of systolic BP were obtained (3)after AT II administrations. Abbreviations are as follows: blood pressure (BP), angiotensin II (AT II), lung body weight index (L.B.I.) and pulmonary edema (PE). *Significant difference (P<0.05) by the paired t-test. § Significant difference (P<0.05) from Group 2.

Table 3. Effect of E 4177 on adrenaline-induced pulmonary edema

| Material group (n) | Pretreatment (dose) | Systolic BP (mmHg) before pretreatment(1) | before ADR(2) | after ADR(3) | L.B.I. | Incidence of PE (%) |
|-------------------|---------------------|-------------------------------------------|---------------|--------------|-------|---------------------|
| Group 7 (n = 8)   | Null                | ---                                       | 111.0±11.2    | 258.9±15.0   | 0.92±0.37 | 87.5               |
| Group 8 (n = 8)   | E 4177 (10 mg/kg)   | 113.7±12.5                                | 112.7±17.9    | 247.4±20.8   | 0.83±0.26 | 62.5               |

Systolic BPs were measured (1)just before each pretreatment and (2)just before ADR administrations. Peak values of systolic BP were measured (3)after ADR administrations. Abbreviations are as follows: blood pressure (BP), adrenaline (ADR), lung body weight index (L.B.I.) and pulmonary edema (PE). *Significant difference (P<0.05) by the paired t-test.

Study showed that an alpha-adrenergic blocker completely suppressed the development of adrenaline-induced pulmonary edema. Thereby, the indirect effect of adrenaline on AT II-induced pulmonary edema had to be studied using the alpha-adrenergic blocker phentolamine. Our results revealed that phentolamine could not sufficiently suppress AT II-induced pulmonary edema, whereas the AT II-receptor antagonist E 4177 could. This suggests that AT II-induced pulmonary edema develops as the result of the increased afterload via the specific AT II receptors without the indirect action of adrenaline. On the contrary, the indirect effect of AT II on adrenaline-induced pulmonary edema was also investigated using E 4177, which could not decrease the incidence of adrenaline-induced pulmonary edema. These indicate that AT II and adrenaline cause pulmonary edema by a different process respectively. Since nitroglycerin reduces the venous return (=preload) and directly decreases the vascular resistance of arteries (=afterload), it is theoretically reasonable that nitroglycerin has a preventive effect on the development of AT II-induced pulmonary edema (9, 10).

Since the development of PE is probably due not only to elevated afterload in the systemic circulation but also to hyperpermeability in the pulmonary vascular beds (11), it is reasonable to discuss the preventive effect of AT II receptor antagonists on the changes in the afterload (12, 13) and the pulmonary vascular permeability (14). As regards to the increased afterload caused by AT II, our experiments showed that only E 4177 could suppress the elevation of blood pressure after administering AT II. This implies that E 4177 prevents AT II-induced pulmonary edema by suppressing the elevation in the afterload. On the other hand, as regards to the pulmonary vascular hyperpermeability, the experiment of Yukioka et al. showed that AT II-receptor antagonists were also effective as therapeutic agents for pulmonary edema induced in sheep with oleic acid causing the hyperpermeability (15). This report suggests the additional suppressive effect...
of AT II-receptor antagonists on the pulmonary vascular permeability.

Gil and McNiff proposed the contradictory results that the administration of 10 μg/kg AT II to rabbits produced no signs of hemodynamic pulmonary edema, which were assessed as electron-microscopical findings of interstitial fluid accumulation and perivascular cuffs and increased weight of the lung (16). The reason for this contradiction is not obvious, but may partially depend on the differences in animal species.

Enhanced activities of the endogeneous catecholamine and renin-angiotensin system largely contribute to the incidence of acute pulmonary edema in the end-stage of the left ventricular failure, and thereby, further studies are needed to investigate the effect of adrenaline and AT II on pulmonary edema. For this purpose, we consider that our experimental models may play some role to elucidate the mechanism of pulmonary edema and establish the preventive means against it.

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