PREVENTION OF ADRENALINE-INDUCED PULMONARY EDEMA BY STEM BROMELAIN IN RABBITS AND ANALYSIS OF THE HEMODYNAMIC CHANGES

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In the course of our researches on the preventive action of stem bromelain (BRM), a protease obtained from pine apple plant, against the adrenaline induced pulmonary edema in rats, it was noticed that a pretreatment with this enzyme also inhibited the occurrence of the circulatory and respiratory changes caused by adrenaline which were remarkable in the nontreated animals (1). This suggested that further analysis of the differences in the hemodynamic pattern might throw light on the mechanism of the pulmonary edema and the mode of action of BRM. In the present study, we made an investigation of the effects of BRM in rabbits and analyzed the data to locate the sites of action from the hemodynamic point of view.

MATERIALS AND METHODS

Male rabbits, weighing 2.5 to 3.0 kg were used. The rabbits were anesthetized with pentobarbital sodium, 25 mg/kg, injected intravenously and were fixed on their backs. Supplemental doses of pentobarbital were administered as required. The body temperature was maintained between 38.5-40°C by a heating blanket.

The systemic blood pressure was recorded from the femoral artery by means of a polyethylene tube (PE 160) connected to a pressure transducer (Nihon Kohden, LPU-0.5). The heart rate was recorded by a cardio-tachograph (Nihon Kohden, RT-2A) triggered by the signal from the arterial pressure pulse recording. Another polyethylene tube (PE 160) was inserted into the femoral vein for injection. For measurements of the left atrial pressure and the aortic flow, a left thoracotomy was performed by cutting the 1st, 2nd and 3rd ribs at the left margin of the sternum after ligating the mammary artery in the first intercostal space. The pleura could be left intact in the rabbit by this procedure. So the animals were breathing spontaneously. The left atrial pressure was recorded by means of a polyethylene tube (PE 160) placed in the tip of the atrial appendage, and connected to a pressure transducer (Nihon Kohden, LPU-0.5). The aortic flow was measured by the square wave electromagnetic flowmeter (Nihon Kohden, MF-25). The flowmeter

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probe, which had a lumen size of 5 mm in diameter for a snug fit, was applied to the ascending aorta. The respiration of the animal was recorded with a thermister (Nihon Electric Company, BT-15) placed in the tracheal cannula or by means of a strain-gauge transducer (Nihon Kohden, SB-1T) connected to the skin of the thoracic wall with string.

The pulmonary edema was induced by an intravenous infusion of l-adrenaline, 250 µg/ml, at a rate of 0.12 ml/min for 10 minutes. This dose of adrenaline was chosen based on the data of preliminary experiments, in which the incidence of pulmonary edema was 70-100%. Five minutes elapsed between ceasing of the infusion and exanguination via the femoral artery, then the lungs were taken out and examined for pulmonary edema. Only those lungs whose lower cut trachea produced no froth when squeezed gently were regarded as negative.

The pulmonary blood volume of the heparinized rabbit was measured after ligation of the blood vessels to and from the lungs. The atrioventricular groove was ligated quickly, and the heart and the lungs were taken out. Then the blood was collected as thoroughly as possible from the left auricle through the polyethylene tube. This was regarded as the pulmonary blood volume. In some experiments, the total blood volume was also measured by using Evans blue dye. Two mg/kg of the dye was intravenously injected, and 10 minutes later, 2 ml of the blood was taken. Plasma was separated by centrifuging for 40 minutes at 6,000 rpm, and the absorbance was measured at 620 mµ.

Crude stem bromelain (BRM) (Dainippon Pharmaceutical Co., Ltd., P-741 powder) and l-adrenaline hydrochloride (Sankyo, Adrenalin) were used for the experiment. The powder of BRM was dissolved in 10 mg/ml in saline and after centrifugation the supernatant was used for injection. The dose of adrenaline is expressed in terms of the base.

RESULTS

Prevention of pulmonary edema

Preliminary experiments showed that, different from the experiments with rats, the effect of preadministration of 10 to 20 mg/kg of BRM was uncertain. It was also noted that BRM caused much less hypotension than in rats. However, when injected rapidly a little after the start of adrenaline infusion, BRM proved to be very effective in protecting

<p>| Table 1. The incidence of pulmonary edema, lung weight (LW) and left atrial pressure (LAP) in three groups of rabbits. |
|-----------------|--------|--------|------|--------|</p>
<table>
<thead>
<tr>
<th>Drugs</th>
<th>N</th>
<th>ED(+)</th>
<th>ED(-)</th>
<th>LW (g)</th>
<th>LAP (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adrenaline</td>
<td>8</td>
<td>7</td>
<td>1</td>
<td>15.9±6.5</td>
<td>38±3.3</td>
</tr>
<tr>
<td>Group 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adrenaline+Bromelain (5 min before)</td>
<td>8</td>
<td>8</td>
<td>0</td>
<td>16.2±2.7</td>
<td>25±2.7</td>
</tr>
<tr>
<td>Group 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adrenaline+Bromelain (1 min after)</td>
<td>8</td>
<td>0</td>
<td>8</td>
<td>8.9±1.1</td>
<td>17±3.2</td>
</tr>
</tbody>
</table>

N = Number of experiments. ED(+) = Number of animals in which pulmonary edema was produced. ED(-) = Number of animals in which pulmonary edema was not produced. The values of LW and LAP are the means±S.D. (n=7 in group 1, n=8 in groups 2 and 3). The ED(-) case of group 1 was omitted from the calculation.
FIG. 1. Lung weights (crossed columns) and left atrial pressures (striated columns) of three groups of rabbits.

Top: Rabbits received adrenaline alone, and pulmonary edema occurred in 7 out of 8. Middle: BRM was injected slowly 5 minutes prior to adrenaline infusion. Pulmonary edema occurred in all the animals. Bottom: BRM was injected rapidly 1 minute after the start of adrenaline infusion. Pulmonary edema was prevented. Values shown are the mean ± S.D. of 7 (in Top group) and 8 (in Middle and Bottom groups) rabbits.

the animals against pulmonary edema. This was confirmed by the following experiment.

Twenty-four rabbits were divided into three groups. Each group consisted of 8 rabbits. Group 1: This control was given adrenaline alone. Group 2: Each rabbit received 10 mg/kg of BRM intravenously 5 minutes prior to the start of infusion of adrenaline. The injection of BRM was made slowly enough (over a period of 2 minutes) not to cause hypotension. Group 3: To the animals of this group, 10 mg/kg of BRM was injected rapidly (within 15 seconds) into the marginal ear vein one minute after the start of infusion of adrenaline. The results are summarized in Table 1 and graphically shown in Fig. 1. While pulmonary edema developed in 7 and 8 rabbits in group 1 and group 2, respectively, it was completely prevented in group 3. As shown in the Table 1, the weight of the edematous lungs were much heavier than that of the nonedematous lungs.

Hemodynamic changes

Fig. 2, A shows the recordings of the systemic arterial pressure (BP), heart rate (HR), cardiac output (CO) or mean aortic flow and the left atrial pressure (LAP) of an animal of group 1. Similar recordings of an animal of group 3 are shown in Fig. 2, B. The hemodynamic pattern of the animals of group 2 was essentially the same as that of group 1.

a) Systemic arterial pressure: Adrenaline caused a sustained rise in the systemic arterial pressure throughout the period of infusion. As shown in Fig. 2, B, when BRM was injected one minute later, as indicated by the arrow, a slight and transient fall of the
arterial pressure was observed.

b) Heart rate: Sinus bradycardia and atroventricular block accompanied the initial phase of the rise in the arterial pressure. Normal sinus rhythm was then recovered. Fig. 3 indicated the course of these changes in cardiac rhythm which was observed in a preliminary experiment. Sometimes paroxysmal ventricular tachycardia of short duration took place, being accompanied by a precipitous fall in the arterial pressure.

c) Cardiac output: As shown in Fig. 2, A, a marked and sustained reduction of cardiac output was regularly observed. Usually, a slight and transient increase in cardiac output preceded the reduction (Fig. 2, B). In the tracings of the phasic flow of aorta (Fig. 3), the volume of a pulsatile flow was larger in B than in A, but in spite of the marked bradycardia the flow was not increasing in C. Finally it became very small in D.

Upon injection of BRM (Fig. 2, B), the cardiac output increased promptly, exceeding the initial value, and then gradually declined to a steady level which was still relatively high. In the animals of group 3 and group 1, the cardiac outputs 5 and 9 minutes after the start of infusion of adrenaline were measured. In group 3, the values were $51.9 \pm 5.1\%$ and $57.0 \pm 4.8\%$ of the preinfusion level (mean \pm S.E., n=6), while those of group 1 in which adrenaline caused pulmonary edema were $36.0 \pm 4.6\%$ and $37.7 \pm 3.9\%$ (mean \pm S.E., n=6). The difference of these values between group 1 and group 3 is of a statistical significance. Two experiments of each group were excluded from the calculation, since the recordings of cardiac output were either lacking or unsatisfactory.
Fig. 3. Changes in the systemic arterial pressure (BP), the left atrial pressure (LAP) and the aortic flow (AF) induced by intravenous infusion of adrenaline in a rabbit. A, B, C and D are high speed recordings taken at the corresponding time points which are shown in the low speed recording (upper, left). At the arrow, infusion of adrenaline started. The recording speed is different before and after E. Note that, in C, the pulsatile flow of the aorta is not much larger than it is in the control period (A), despite the great aortic pressure pulse.

**d) Left atrial pressure:** A striking elevation of the left atrial pressure was regularly observed when adrenaline caused pulmonary edema (Fig. 2, A). It reached as high as 38±3.3 mmHg in group 1 and 35±2.7 mmHg in group 2 (Table 1). The rise was suppressed by the injection of BRM in group 3 (Fig. 2, B), the pressure being 17±3.2 mmHg (mean ±S.D.).

**Respiration**

In most cases the respiratory movement was regular, and no remarkable change was noticed. In some cases, however, transient apnea was observed.

**Pulmonary blood volume and total blood volume**

The effects of adrenaline and BRM on the pulmonary blood volume were examined in the following experiment.

Fifteen rabbits were divided into three groups of same size. 1) Group A: After the operative procedures were finished, the atrioventricular groove of the heart was ligated quickly, and the blood in the lungs was drawn from the left appendage in the way as described in Materials and Methods section. No drug was given to the animals of this group. 2) Group B: Ligation of the atrioventricular groove was performed 3.5 to 4 minutes after
TABLE 2. Lung blood volume (LBV), lung weight (LW) and left atrial pressure (LAP) of three groups of rabbits.

<table>
<thead>
<tr>
<th>Drugs</th>
<th>N</th>
<th>ED(+)</th>
<th>ED(-)</th>
<th>LBV(ml)</th>
<th>LW(gram)</th>
<th>LAP(mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>5</td>
<td>0</td>
<td>5</td>
<td>8.2±1.1</td>
<td>11.8±1.9</td>
<td>near zero</td>
</tr>
<tr>
<td>Group B Adrenaline</td>
<td>5</td>
<td>5</td>
<td>0</td>
<td>17.4±5.0</td>
<td>14.0±2.9</td>
<td>35±4.1</td>
</tr>
<tr>
<td>Group C Adrenaline+Bromelain 1 min after</td>
<td>5</td>
<td>0</td>
<td>5</td>
<td>9.0±2.8</td>
<td>9.6±1.1</td>
<td>16±2.2</td>
</tr>
</tbody>
</table>

Values of LBV, LW and LAP are means±S.D. In groups B and C, measurements were made 3.5 to 4 minutes after the start of adrenaline infusion.

**FIG. 4.** Lung blood volume (open columns), lung weights (crossed columns) and left atrial pressure (striated columns) of three groups of rabbits. ADR: Adrenaline alone was infused, and pulmonary edema was produced. ADR+BRM: Stem bromelain was administered intravenously 1 minute after the start of adrenaline-infusion. Pulmonary edema was not produced in this group. Values shown are the means±S.D. (n=5).

In this regard, the total blood volume was measured in 7 animals, after the operative procedures were finished. The value obtained was 211±28 ml or 73.1±11.7 ml/kg (mean ±S.D.). These data and those in Table 2 indicate that in animals in which pulmonary edema occurred, the pulmonary blood volume was doubly increased, that is from 4% to 8% of the total blood volume approximately.
DISCUSSION

The results of the present study clearly indicate that BRM can prevent the adrenaline induced pulmonary edema in rabbits if it is administered in an appropriate way, and that some hemodynamic factors are involved in the mechanism of action of BRM.

The main hemodynamic changes which associated with the adrenaline induced pulmonary edema were; a rise in the systemic arterial pressure, a marked and sustained reduction in the left ventricular output, a striking elevation of the left atrial pressure and an increase of the lung blood volume. These observations confirm what was stated by earlier authors (2–4). A common interpretation of these events is as follows: Adrenaline causes an increase in the peripheral arterial resistance and a shift of blood from the systemic to the pulmonary vascular bed. Failure of the overloaded left ventricle and a high left atrial pressure take place, the latter exceeding the oncotic pressure. The attendant increase of pressure in the proximal pulmonary venocapillary system accompanied by a larger filtering area results in pulmonary edema.

When BRM was administered one minute after the start of infusion of adrenaline, all the above mentioned changes were suppressed or reversed and pulmonary edema was prevented. Two possible mechanisms of action of BRM might then be suggested: 1) Cardiotonic action of BRM, and 2) reduction of the peripheral arterial resistance, although relative significance of these two factors in preventing the edema is not yet certain.

Cardiotonic action of BRM was also noticed when the enzyme was injected intravenously before the administration of adrenaline. As shown in Fig. 5, a larger pulsatile flow was recorded from the ascending aorta. In addition, when BRM was tested in the isolated frog's heart (Straub's preparation), it demonstrated a positive inotropic effect. As reported previously, the edema preventive action of BRM in rats depends on its pro-

![Fig. 5. The change of aortic flow caused by stem bromelain (BRM). BP = systemic arterial pressure. RR = respiration. Inspiration downstroke. SV = stroke volume or aortic flow. The aortic flow increased after intravenous injection of BRM.](image-url)
teolytic activity (5). So these findings offer an interesting problem, "possible cardiotonic action of proteases". Recently some evidence along this line has been reported by Imai et al. (6).

Following the injection of BRM, the systemic arterial pressure fell transiently despite a marked increase in the cardiac output, indicating that the peripheral arterial resistance was reduced by BMR. It was found in this laboratory that BRM activates plasma kallikrein and releases plasma kinin in rats, causing a fall in the systemic arterial pressure [Katori et al. (7, 8)]. A similar action of BRM was also observed in rabbit plasma in vitro (Katori, unpublished data). The kinin release in the circulating blood of the rabbit by the enzyme is under study.

A striking difference was seen between the effects of BRM injected prior to adrenaline and those of BRM injected 1 minute after the start of infusion of adrenaline. Further analyses are needed in order to correlate the time relationship with the efficacy of BRM in preventing pulmonary edema. In this regard, however, it is noteworthy that trypsin was reported to be very effective in preventing the adrenaline induced pulmonary edema in rats when it was given immediately before adrenaline [Hiramatsu et al. (9)], although a pretreatment with trypsin proved noneffective in our previous experiment (1).

With regard to the mechanism of the remarkable left ventricular depression caused by adrenaline, the following information deserves consideration, besides a simple overloading due to increased peripheral resistance. Experiments on the negative inotropic action of noradrenaline were reported by Cotten et al. (10), and Nakashima et al. (11). On the other hand, Worthen et al. discussed about a particular nature of the left ventricular failure, a decrease of the compliance, which is associated with the adrenaline induced pulmonary edema in the dog (12). Possible myocardial anoxia and elevation of the plasma potassium concentration might be additional factors.

Whether or not the hemodynamic changes observed in the present study sufficiently explain the occurrence and development of the pulmonary edema is another problem. In the dog's lung, Guyton estimated the rate of edema formation, which occurred after the left atrial pressure exceeded about 25 mmHg, the protein osmotic pressure of plasma, to be 0.065 g of fluid per min per mmHg per 100 g wet lung tissue (13). Later a value of 0.07 was obtained in the isolated perfused preparation [Gaar et al. (14)]. These figures seem to be too small to account for the weight increase observed in the present study. On the other hand, a previous communication from this laboratory on the ultrastructure of edematous lungs of rat reported that the main site of lesion was found to be the alveolar epithelium, the capillary endothelium being intact, when the edema was induced by adrenaline (15). These facts seem to imply that some essential factors other than hemodynamic or simple physicochemical ones still remain to be elucidated.

At present, the authors are of the opinion that the hemodynamic changes caused by adrenaline are the requirements for the occurrence and development of this type of pulmonary edema, and that the improvement of those hemodynamic events is an important part of the mechanism of the edema preventing action of BRM.
SUMMARY

1. Pulmonary edema was induced in rabbits by an intravenous infusion of adrenaline. The marked hemodynamic changes observed were: a rise in the systemic arterial pressure, a reduction of the left ventricular output, an elevation of the left atrial pressure and an increase of the pulmonary blood volume.

2. When stem bromelain (BRM) was injected intravenously 1 minute after the start of the infusion of adrenaline, the hemodynamic changes were suppressed or reversed, and pulmonary edema was prevented very effectively.

3. When BRM was given 5 minutes before adrenaline, however, the hemodynamic changes caused by adrenaline were not appreciably modified, and pulmonary edema occurred.

4. With regard to the possible mechanism of BRM in the prevention of the adrenaline induced pulmonary edema, the following possibilities are considered: a) The cardiotonic action of BRM, b) the reduction of the peripheral resistance due to plasma kinin which was supposed to be released by BRM.

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REFERENCES