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The Effect of the Prostaglandin I_2 Analogue OP-2507 on Adrenaline-Induced Pulmonary Edema in Rabbits and Analysis of Hemodynamic Changes

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ABSTRACT—This study was carried out to understand the onset mechanism of adrenaline (ADR)-induced pulmonary edema (PE) and the effect of drugs related to the arachidonate cascade in a rabbit model. ADR was administered intravenously by a bolus injection to the rabbits at 50, 75 and 100 μg/kg. To evaluate the severity of PE, the lung-water ratio (LWR) was calculated as a ratio of the difference between wet and dry lung weight to dry lung weight. The PE incidence and LWR exhibited a dose-dependent increase, and LWR correlated with the left atrial pressure (LAP). The involvement of the arachidonate cascade was evaluated by the co-administration of flurbiprofen, a cyclooxygenase inhibitor; ozagrel, a thromboxane synthase inhibitor; and OP-2507 (15-*cis*-(4-*n*-propylcyclohexyl)-16,17,18,19,20-pentanor-9-deoxy-6,9-α-nitriloprostaglandin F_1 methyl ester), a prostaglandin I_2 analogue. Co-treatment of the rabbits with ADR and flurbiprofen resulted in an increase in LAP and the incidence of PE, whereas co-administration of ozagrel did not exhibit any significant changes in the measured parameters. Conversely, OP-2507 reduced the LAP, PE incidence and LWR when co-administered with ADR. Rabbits co-treated with OP-2507 displayed an improved cardiac function. The results of these studies demonstrated the effectiveness of OP-2507 in protecting the lung and cardiac function from the ADR-induced PE.

Keywords: Adrenaline, Pulmonary edema, Heart failure, Cyclooxygenase inhibitor, Prostaglandin I_2 analogue

The mechanisms involved in pulmonary edema (PE) have been the topic of investigation of several researchers (1, 2). The majority of such studies was aimed at the characterization of the biochemical and histological nature of PE. A number of studies attempted to elucidate some of the possible biochemical mediators such as histamine, 5-hydroxytryptamine and bradykinin. Such studies have demonstrated the vast complexity of this condition and the involvement of several important biochemical pathways. However, the key mechanism of this disorder is still largely open to further investigation.

In spite of the recent remarkable progress in anesthesiology and resuscitation techniques, several reported cases of acute PE have been documented (3). In most instances adrenaline (ADR) has been identified as the culprit in inducing such PE. ADR is one of the most powerful cardiac stimulants, and it is typically administered in the treatment of heart failure, anesthesia and upon certain surgical procedures. Several cases of PE have been encountered when ADR was used and attributed to a possible overdose administration of ADR. Several studies that investigated this phenomenon demonstrated the ADR-induced increase in pulmonary capillary pressure would directly or indirectly damage the vasculature, leading to the resultant PE (4 – 7).

Prostaglandins (PG) I_2 and thromboxane (TX) A_2 are two powerful bioactive agents that exhibit a wide variety of effects on various body organs and tissues (8 – 10). Both agents are derived from PGH_2, which is generated from arachidonic acid by the action of PGH_2-synthase. For example, exposure of endothelial cells to PGI_2 results in a significant increase in the intracellular cAMP levels, thereby leading to vasodilation (8, 9). Conversely TXA_2 has a vasoconstrictive effect (10). The use of a cyclooxygenase (COX) inhibitor or TXA_2 synthase inhibitors to lower the levels of TXA_2 would therefore be expected to decrease the pulmonary capillary pressure. In turn, such a decrease may be useful in preventing the ADR-induced PE.

The aims of the present these studies were to elucidate the mechanisms of PE using the rabbit model. Initially we sought to verify whether the ADR-mediated increase in the

pulmonary capillary pressure was an important factor in inducing PE. Moreover, we investigated the effects obtained by manipulating the levels of bioactive prostaglandins and thromboxanes during ADR administration. The manipulation of the levels of bioactive agents was accomplished using the PGI_2 analogue OP-2507, the TXA_2 synthase inhibitor ozagrel and the COX inhibitor flurbiprofen against ADR-induced PE.

MATERIALS AND METHODS

Chemicals and laboratory animals

Adrenaline (Bosmin®) was obtained from Daiichiseiyaku (Tokyo), and the COX inhibitor flurbiprofen axetil (Lopion®) was obtained from Kakenseiyaku (Tokyo). The TX synthase inhibitor ozagrel hydrochloride (Vega®) and the PGI_2 analogue OP-2507 (15-*cis*-(4-*n*-propylcyclohexyl)-16,17,18,19,20-pentanor-9-deoxy-6,9-α-nitriloprostaglandin F_1 methyl ester) were obtained from Ono Pharmaceutical Co. (Osaka). All chemicals were prepared fresh as solutions in saline. Albino male rabbits (2.5 – 3.5 kg) were purchased from Saitama Animal Labs (Saitama-ken) and housed under controlled conditions for one week prior to any surgical procedures.

Surgical procedures

All animal handling techniques were carried out according to the guiding principles governing the care and use of laboratory animals approved by The Japanese Pharmacological Society. The rabbits were anesthetized with pentobarbital sodium in a dose of 50 mg/kg injected into the ear vein and restrained on their backs. Supplemental doses of pentobarbital were administered as required. The individual difference of rabbit's body temperature was so large that we kept the body temperature at 38 – 39°C measured in the rectum, using a heating blanket during the duration of the experiment to obtain a constant condition. A polyethylene tube was cannulated into the ear vein for the injection of test compounds.

The systemic blood pressure (SBP) was recorded from the left femoral artery by means of a polyethylene tube, connected to a transducer (PEZ-10; Nihon Kohden, Tokyo). The heart rate (HR) was recorded by a cardiotachograph triggered by a signal from the arterial pressure pulse recording. Other polyethylene tubes were inserted into the tips of the left and right atrial appendage and connected to the transducer (PEZ-10; Nihon Kohden) to record the left atrial pressure (LAP) and right atrial pressure (RAP), respectively. In addition, the data were represented by calculating the value of the area under the curve (AUC), which approximated the area surrounded by the blood pressure (BP)-time curve and the line representing the BP before the ADR administration (1). The AUC values were determined by subtracting the BP value obtained prior to ADR administration (baseline BP) from the systolic BP obtained following ADR injection. The BP values were recorded every min while systolic BP remained above the baseline BP value. The values were subsequently integrated and the value obtained defined as AUC. BP values below the baseline BP were regarded as a zero.

Surgery was performed by a left thoracotomy cutting the first to fourth ribs at the middle of the sternum. This was done after cutting the mammary artery ligated in the second intercostal space. The bilateral pleurae were maintained intact and the aortic blood flow was measured by a square wave electromagnetic flowmeter (MF-25, Nihon Kohden). A probe (5 mm in diameter) was applied to the ascending aorta and the recording obtained was ascribed as the cardiac output (CO). The CO was measured at 1-min intervals.

Evaluation of PE

The initial evaluation of the induced PE was accomplished by a bolus injection of ADR. Following a 15-min induction period, the rabbits were euthanized by exanguination via the femoral artery. The degree of PE was then classified into four groups as reported previously (11): Grade 0, no change; Grade 1, the group in which small amounts of edema froth were recognized in the bronchi with compression of the removed lungs; Grade 2, the group in which froth ran out spontaneously from the trachea upon tracheotomy; Grade 3, the group in which the froth ran out from the nostril within 15 min. The PE was considered negative if a Grade 0 or 1 was observed. After the classification of PE grades, the individual lungs were removed and the attached tissues were trimmed away. The lungs were weighed to obtain the wet lung weight. The individual lungs were then dried at 70°C for 48 h and weighed again to obtain the dry lung weight. The lung-water ratio (LWR) was calculated as a ratio of the difference between wet and dry lung weight to dry lung weight. The LWR was used to gauge the severity of the PE and the amount of excess fluid in the lung.

Study 1 – Hemodynamic changes after ADR injection

Twenty-eight rabbits were randomly divided into 4 groups to obtain an ADR-induced PE dose-response relationship. Rabbit groups 1 – 4 randomly received 0, 50, 75 and 100 μg/kg ADR in saline (1 ml), respectively. The volume of the material was administered continuously at 1 min.

After performing the surgical procedure and the confirmation of stable baseline conditions, 5 ml of saline was injected intravenously in 1 min. ADR was administered via the ear vein by a bolus injection 5 min after the saline administration. In each group, hemodynamic changes

were analyzed and the degree of the PE was evaluated as described previously.

Study 2 – Plasma ADR concentration after ADR injection

Five rabbits were used to measure the plasma ADR concentration following administration of a 100 μg/kg dose. Arterial blood samples (2.5 ml) were collected at 0, 0.5, 5 and 8 min after ADR injection. Plasma ADR concentration was measured by high-performance liquid chromatography separation (12).

Study 3 – The effects of COX inhibitor, TX synthase inhibitor and PGI_2 analogue on ADR-induced PE

Forty-nine rabbits were divided into 7 groups. ADR (75 μg/kg) was administered to the rabbits after the treatment with the following drugs, which were randomly allocated to each group:

1. ADR only (as a control)
2. COX inhibitor (flurbiprofen)
   (a-1: 5 mg/kg, a-2: 10 mg/kg)
3. TX synthase inhibitor (ozagrel)
   (b-1: 10 mg/kg, b-2: 30 mg/kg)
4. PGI_2 analogue (OP-2507)
   (c-1: 100 ng/kg per minute, c-2: 200 ng/kg per minute)

In the control group, 5 ml saline was given to the rabbits 5 min prior to ADR administration. The other drugs were administered to the rabbits as follows:

The effect of COX inhibitor and TX synthase inhibitor: Flurbiprofen or ozagrel was each administered 5 min prior to ADR injection. Each of the inhibitors was administered to the rabbits in 2 doses (injected volumes were kept at 5 ml) in 1 min, intravenously.

The effect of PGI_2 analogue: OP-2507 was dissolved in saline in 2 doses (injected volumes were kept at 5 ml). The continuous infusion of OP-2507 was started 10 min before ADR injection and finished 5 min after ADR injection.

Study 4 – Ventilatory effects in ADR-induced PE treated with OP-2507

Twelve rabbits were randomly divided into 2 groups. ADR at 75 μg/kg was administered to the one group without OP-2507 pretreatment. The blood samples were drawn from the femoral artery and arterial PaO_2 and $PaCO_2$ were measured (ABL 5; Radiometer Medical, Copenhagen, Denmark), before and after thoracotomy, 5 and 1 min before ADR injection and 0.5, 1, 3, 5, 10 and 15 min after ADR injection. ADR at 75 μg/kg was administered to another group treated with OP-2507. The blood gas was analyzed before and after the thoracotomy, 5 and 1 min before ADR injection, and 0.5, 1, 3, 5, 10 and 15 min after ADR injection. Respiratory rates were monitored with a polygraph (CP-602G, Nihon Kohden).

Statistical analyses

All data were expressed as the means ± S.E.M., and differences were analyzed by one-way analysis of variance (ANOVA) with multiple comparison tests. Significance was accepted for $P<0.05$ unless otherwise indicated. Scheffe's method was used to compare the two relevant data sets. The differences of the PE incidence were tested by Fisher's exact probability test, and the differences of the grade of PE were tested by the chi-square test. To establish a relationship between two variables, correlation coefficients were determined.

RESULTS

Study 1 – Hemodynamic changes following ADR administration

A set of typical records of hemodynamic changes derived from group 3 is shown in Fig. 1. The changes were initiated by the injection (bolus) of ADR (75 μg/kg). The traces indicate increases in the SBP and HR with a concomitant decrease in the CO. The figure also shows an immediate elevation in the LAP and RAP following ADR injection. In some cases rabbits exhibited arrhythmic heart beats that included supraventricular or ventricular ectopic beats, all of which disappeared within 1 min.

The dependence of PE incidence on the given ADR dose

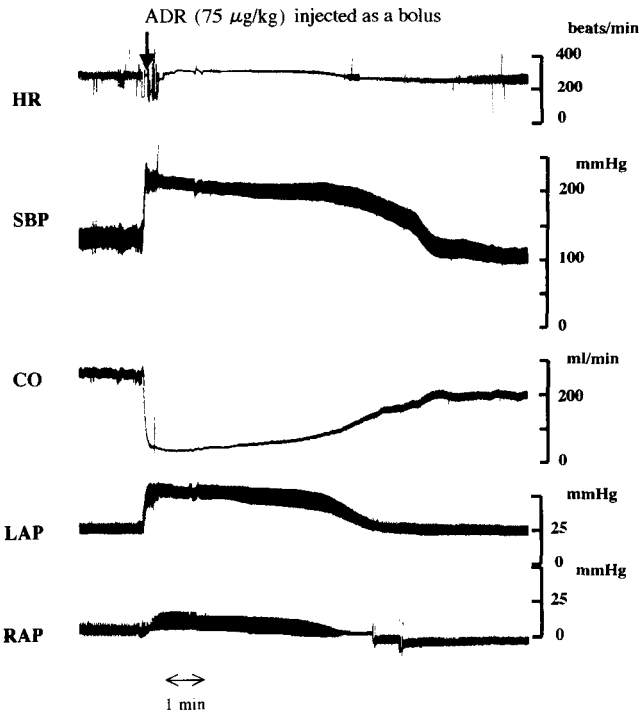

Fig. 1. A typical set of records of hemodynamic changes in rabbits of group 3 after the injection of ADR (75 μg/kg). The abbreviations used are: heart rate, HR; systemic blood pressure, SBP; cardiac output, CO; left atrial pressure, LAP; right atrial pressure, RAP.

Table 1. The severity of ADR-induced PE: Study 1

Group	ADR (μg/kg)	Pretreatment	Incidence of PE	Grade 0	1	2	3	LWR
1	0	null	0% (0)	7	0	0	0	3.7 ± 0.2
2	50	null	0% (0)	5	2	0	0	4.0 ± 0.1
3	75	null	86% (6)**	1	0	3	3**	5.0 ± 0.3**
4	100	null	86% (6)**	0	1	3	3**	5.0 ± 0.2**

Each group consisted of 7 rabbits. The incidence of pulmonary edema (PE) is described as the rate and the number of PE developed in the group. Grade represents the number of each stage of PE in the group. LWR is the lung-water ratio, and all data are expressed as the mean ± S.E.M. The differences of the means among the each group are tested by one-way analysis of the variance (ANOVA) and Scheffe's test. The differences of the incidence of PE were tested by Fisher's exact probability test, and the differences of the grade were tested by the chi-square test. **$P<0.01$.

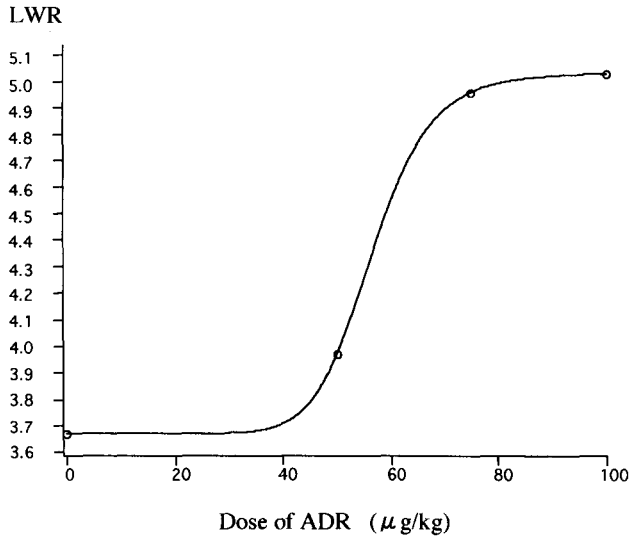

Fig. 2. The relationship between the dose of ADR and the LWR in ADR-induced pulmonary edema. The lung-water ratio (LWR) was calculated as a ratio of the difference between wet and dry weight to dry lung weight. The generated curve was fitted to a sigmoidal function and the resultant equation is $Y = (a-b) / (1 + (c / x)^d) + e$, $a=35.3$, $b=33.9$, $c=56.6$, $d=10.2$ and $e=3.67$. The equation fitted the observed data with a correlation coefficient of 1.00.

is shown in Table 1. The grade of PE was described as the number of each grade of the individually obtained PE in the groups. The elevation of PE grade depended on the given dose of ADR. As expected, the LWR was elevated with increasing doses of ADR. When the LWR was plotted as a function of the administered ADR, a sigmoidal curve was obtained as shown in Fig. 2. This curve reached near-maximal level at a dose range of 75 μg/kg and then it plateaued.

The observed SBP, LAP and RAP are shown in Table 2. As shown in Fig. 3, the LWR displayed significant correlation with the AUC of the LAP value ($r=0.660$, $P<0.01$). In the figure, the LWR also shows significant correlations with the maximum LAP produced by ADR ($r=0.684$, $P<0.01$) and the LAP increase produced by ADR ($r=0.668$, $P<0.01$).

Concerning the CO, the measured CO was affected by the body weight of the rabbit and exhibited large inter-individual differences. The observed CO values prior to ADR injection were for Group 1: 230 ± 43.8 ml/min, Group 2: 203 ± 57.9 ml/min, Group 3: 252 ± 55.5 ml/min, and Group 4: 306 ± 53.3 ml/min. The CO ratio was used in order to exclude the body weight influence and minimize the fluctuations of the cardiac output produced by ADR. Using this approach, the CO just before ADR administration was de-

Table 2. The various blood pressures in the ADR-induced PE: Study 1

Group	ADR (μg/kg)	Pretreatment	Systemic blood pressure (mmHg) pre	maximum	AUC	Left atrial pressure (mmHg) pre	maximum	AUC	Right atrial pressure (mmHg) pre	maximum
1	0	null	113 ± 3	120 ± 3	34 ± 12	9 ± 1	10 ± 1	3 ± 2	9 ± 1	10 ± 1
2	50	null	108 ± 9	214 ± 8**	308 ± 57*	9 ± 1	36 ± 4**	63 ± 11*	11 ± 1	15 ± 2
3	75	null	99 ± 5	196 ± 8**	393 ± 28**	8 ± 1	45 ± 2**	122 ± 12**	6 ± 1	15 ± 1
4	100	null	111 ± 7	209 ± 10**	370 ± 57*	11 ± 1	48 ± 2**	145 ± 20**	13 ± 1	18 ± 2*

All data are expressed as the mean ± S.E.M. Each group consisted of 7 rabbits. Blood pressures just before ADR administration are regarded as the pre blood pressure. Maximum means the maximum pressure produced by ADR. AUC is defined as the area under the time BP curve. The differences of the means among groups 1, 2, 3 and 4 were tested by one-way analysis of variance(ANOVA) and Scheffe's test. *$P<0.05$, **$P<0.01$.

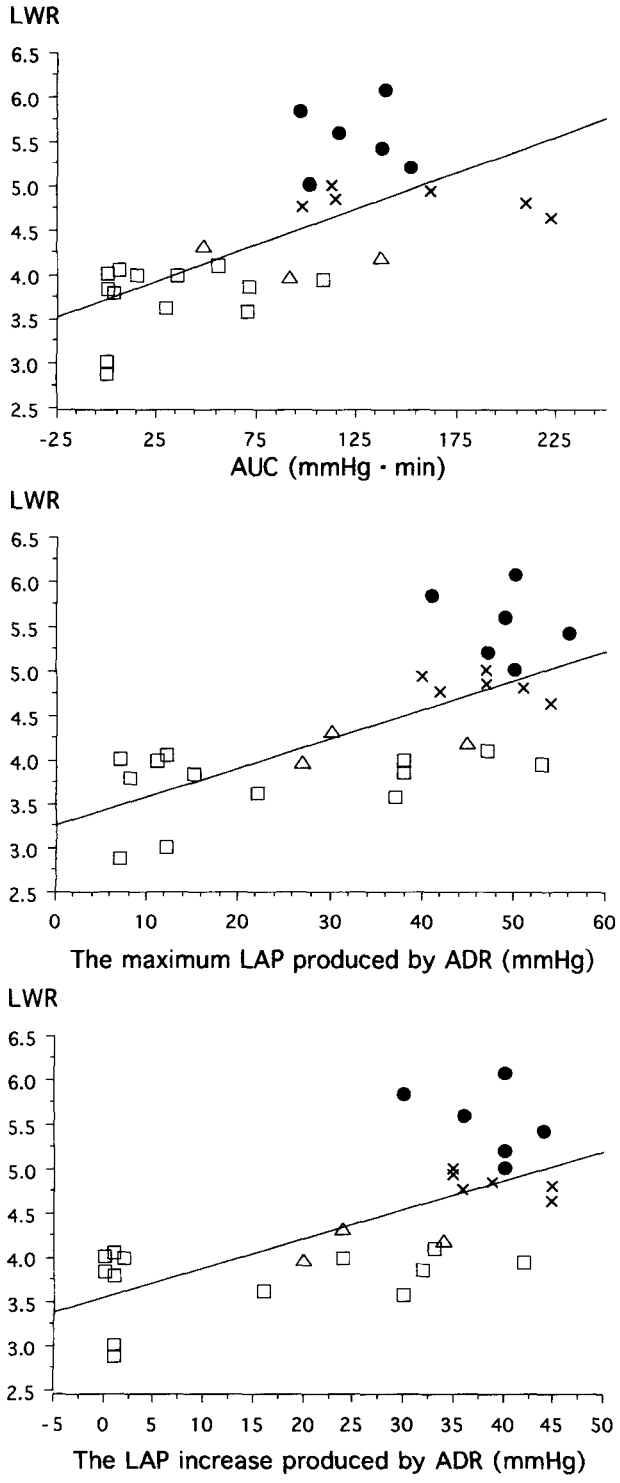

Fig. 3. The relationships among the LWR, the area under the LAP-time curve (AUC), the maximum LAP produced by ADR, the LAP increase produced by ADR and the PE grade. The severity and incidence of PE are prone to increase with AUC, the maximum LAP and the LAP increase produced by ADR. LWR correlated with AUC ($r=0.660$, $P<0.01$), the maximum LAP ($r=0.684$, $P<0.01$) and the LAP increase produced by ADR ($r=0.668$, $P<0.01$). Each point on the graph indicates the PE grade. □: group 1, △: group 2, ×: group 3, ●: group 4.

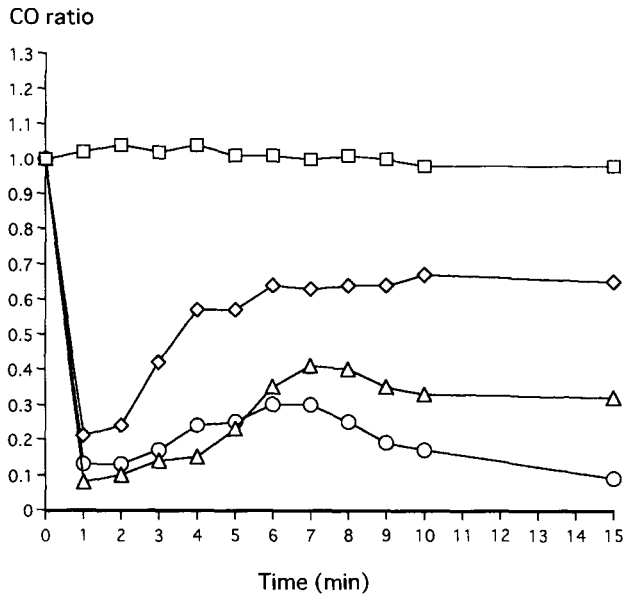

Fig. 4. The time course of the CO ratio observed in study 1. The CO ratio is defined as follows: The cardiac output (CO) just before ADR administration is defined as baseline CO. Taking the baseline CO as 1, the CO ratio at each time is represented as the ratio to the baseline CO. The points are expressed as the means among the group. Each group consisted seven rabbits. □: control, ◇: 50 μg/kg, △: 75 μg/kg, ○: 100 μg/kg.

fined as baseline CO. Moreover, the CO ratios were calculated as ratios of the CO values to the baseline CO. The CO ratio just before ADR injection was assumed as 100%. Figure 4 shows the time course of the CO ratio in study 1. The CO ratio decreased as the dosage of ADR increased.

Study 2 – Plasma ADR concentration after ADR injection

The time course of plasma ADR concentration and the changes of LAP are shown in Fig. 5. The plasma ADR concentration and the LAP began to rise immediately following ADR injection and attained maximum level 30 s subsequent to ADR injection. They decreased at a faster rate (approximately 8 min) after they reached the maximum level and returned to the initial level.

Study 3 – The effects of COX inhibitor, TX synthase inhibitor and PGI_2 analogue on ADR-induced PE

The PE was induced by a bolus injection of ADR at 75 μg/kg. This dose of ADR was chosen based on the data derived from the first study.

The effect of COX inhibitor and TX synthase inhibitor: The incidence of PE and LWR derived from study 3 are shown in Table 3. Upon pretreatment with flurbiprofen, the observed PE incidence and the calculated LWR were not significantly different from those obtained in the control group, which did not receive flurbiprofen prior to

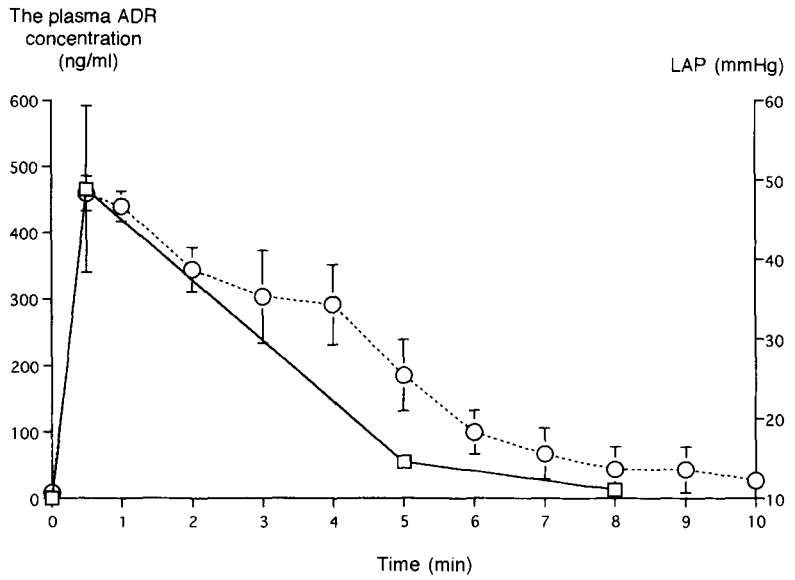

Fig. 5. The time course of the plasma ADR concentration and left atrial pressure. To observe the changes in plasma ADR concentration, 5 rabbits were given ADR at 100 μg/kg intravenously and blood samples were collected at the indicated time intervals. Plasma ADR concentration was measured by the HPLC-separation method. The time course of the plasma ADR concentration (□) was similar to that of left atrial pressure (LAP) (○).

Table 3. The severity of ADR-induced PE: Study 3

Group		ADR (μg/kg)	Pretreatment	Incidence of PE	Grade 0	1	2	3	LWR
1 control		75	null	71% (5)	1	1	1	4	5.1 ± 0.4
2	a-1	75	Flurbiprofen, 5 mg/kg	86% (6)	0	1	1	5	5.7 ± 0.4
	a-2	75	Flurbiprofen, 10 mg/kg	100% (7)	0	0	2	5	5.7 ± 0.3
3	b-1	75	Ozagrel, 10 mg/kg	86% (6)	0	1	0	6	5.4 ± 0.4
	b-2	75	Ozagrel, 30 mg/kg	86% (6)	0	1	1	5	5.4 ± 0.4
4	c-1	75	OP-2507, 100 ng/kg per minute	28% (2)	5	0	2	0*	4.0 ± 0.2*
	c-2	75	OP-2507, 200 ng/kg per minute	14% (1)*	6	0	1	0*	4.1 ± 0.1*

Each group consisted of 7 rabbits. The incidence of pulmonary edema (PE) is described as the rate and the number of PE developed in the group. Grade represents the number of each stage of PE in the group. LWR is the lung-water ratio, and all data are expressed as the mean ± S.E.M. The differences of the means among each groupweretestedby onewayanalysisofthe variance (ANOVA) and Scheffe's test. The differences of the incidence of PE were tested by Fisher's exact probability test, and the differences of the grade were tested by the chi-square test. *$P<0.05$.

Table 4. The various blood pressures in the ADR-induced PE: Study 3

Group		ADR (μg/kg)	Pretreatment	Systemic blood pressure (mmHg) pre	maximum	AUC	Left atrial pressure (mmHg) pre	maximum	AUC	Right atrial pressure (mmHg) pre	maximum
1 control		75	null	125 ± 8	234 ± 7	389 ± 55	10 ± 1	48 ± 3	113 ± 10	7 ± 1	13 ± 1
2	a-1	75	Flurbiprofen, 5 mg/kg	113 ± 5	204 ± 9*	311 ± 27*	10 ± 1	56 ± 6	134 ± 14	6 ± 1	12 ± 2
	a-2	75	Flurbiprofen, 10 mg/kg	116 ± 4	213 ± 5	311 ± 36	8 ± 1	56 ± 3	153 ± 21	8 ± 1	13 ± 1
3	b-1	75	Ozagrel, 10 mg/kg	108 ± 5	209 ± 5*	345 ± 47	9 ± 1	52 ± 5	124 ± 20	8 ± 1	14 ± 1
	b-2	75	Ozagrel, 30 mg/kg	112 ± 4	208 ± 9*	309 ± 50	11 ± 1	50 ± 4	145 ± 28	7 ± 1	15 ± 1
4	c-1	75	OP-2507, 100 ng/kg per minute	101 ± 4*	211 ± 9	346 ± 29	9 ± 1	41 ± 3	78 ± 6*	8 ± 1	13 ± 1
	c-2	75	OP-2507, 200 ng/kg per minute	101 ± 6*	213 ± 10	340 ± 60	10 ± 1	34 ± 3*	57 ± 11*	9 ± 1	12 ± 1

All data are expressed as the mean ± S.E.M. Each group consisted of 7 rabbits. Blood pressures just before ADR administration are regarded as the pre blood pressure. Maximum means the maximum pressure produced by ADR. AUC is defined as the area under the time BP curve. The differences of the means among each group were tested by one-way analysis of variance (ANOVA) and Scheffe's test. *$P<0.05$.

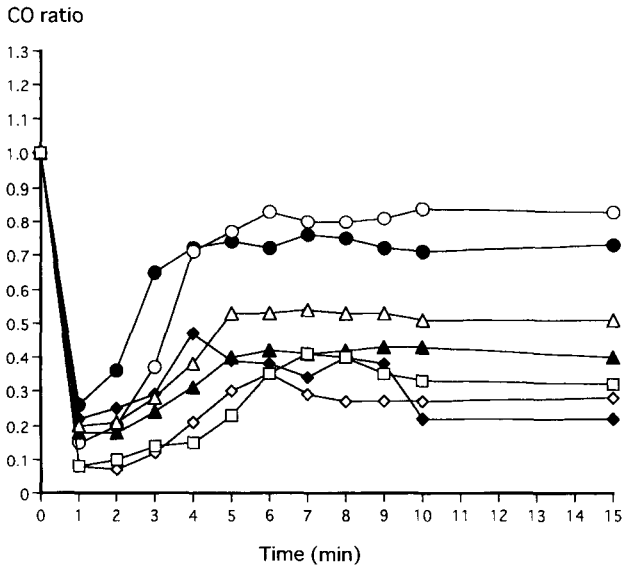

Fig. 6. The time course of the CO ratio in study 2. The CO ratio is as follows: The cardiac output (CO) just before ADR administration is defined as baseline CO. Taking the baseline CO as 1, the CO ratio at each time is represented as the ratio to the baseline CO. The points are expressed as the means among the group. Each group consisted of seven rabbits. ADR at 75 μg/kg was administered to the rabbits after the treatment with the following drugs. □: control; ◇: flurbiprofen, 5 mg/kg; ◆: flurbiprofen, 10 mg/kg; △: ozagrel, 10 mg/kg; ▲: ozagrel, 30 mg/kg; ○: OP-2507, 100 ng/kg per minute; ●: OP-2507, 200 ng/kg per minute.

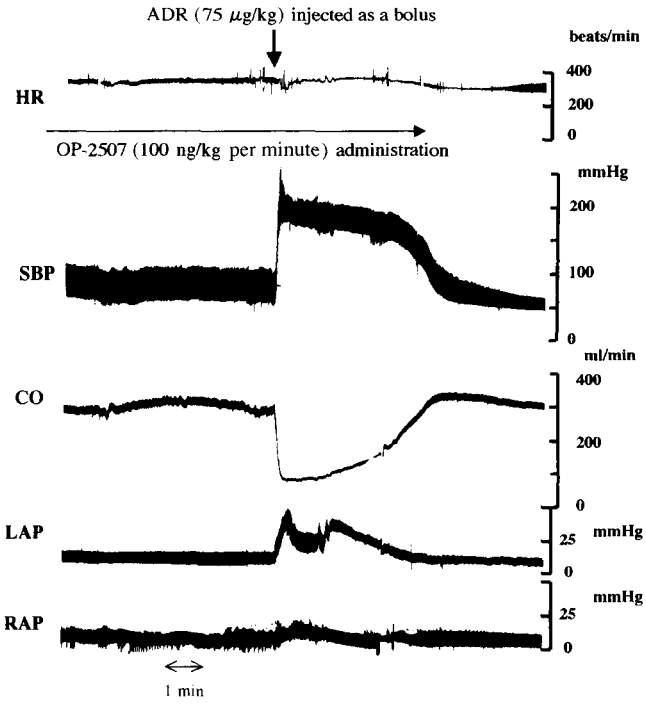

Fig. 7. Typical records of the hemodynamic changes after ADR (75 μg/kg) injection in animals treated with OP-2507. The abbreviations are follows: heart rate (HR), systemic blood pressure (SBP), cardiac output (CO), left atrial pressure (LAP) and right atrial pressure (RAP).

ADR treatment. Table 4 summarizes the measured blood pressures. The maximum blood pressure produced by ADR and AUC in the SBP were lower than those in the control group. However, in the other measured parameters, there were no significant differences compared with the control group. Figure 6 shows the time course of the CO ratio and there were no differences among the groups.

In the pretreatment groups b-1 and b-2 that received the TX synthase inhibitor ozagrel, no differences were observed in the measured parameters obtained prior and subsequent to ozagrel administration. Following the injection of ADR, the maximal attained SBP was lower than that of the control group. However, the PE incidence and LWR did not show any significant differences from the control group (Table 3). There were no significant differences in the other measured parameters, compared with those of the control group (Table 4).

The effect of PGI_2 analogue: Figure 7 shows one of the typical records of the hemodynamic changes in group c, to which 100 ng/kg per minute of OP-2507 was given by continuous infusion. The continuous infusion of OP-2507 was started 10 min before ADR injection and finished 5 min after ADR injection. A slight effect on the systemic circulation before ADR injection was observed at a high dose of OP-2507. In the pretreatment groups (c-1 and c-2), the SBP was reduced during OP-2507 infusion (Table 4).

The PE incidence and LWR were reduced compared with those of the control group (Table 3). In addition, after ADR injection, the maximum value and AUC of the LAP produced by ADR were lower than those of the control group (Table 4).

In the pretreatment groups (c-1 and c-2), the CO ratio recovered to initial levels at a faster rate compared with that of the control group (Fig. 6). However, after ADR injection, the other measured parameters such as HR, SBP and RAP showed no significant differences compared with those of the control group (Table 4).

*Study 4 – Ventilat*ory effects in ADR-induced PE treated with OP-2507

Figure 8 shows the time course of the arterial blood gas analysis in ADR-induced PE. PaO_2 and $PaCO_2$ showed no significant differences before and after thoracotomy in 2 groups. PaO_2 decreased during OP-2507 administration before ADR injection. PaO_2 at 1 min before ADR injection in the OP-2507-treated group was lower than that in the non-treated group. PaO_2 significantly decreased just after ADR injection and did not return to the initial level in the non-treated group. However, PaO_2 returned to the initial level in the OP-2507-treated group. In addition, $PaCO_2$

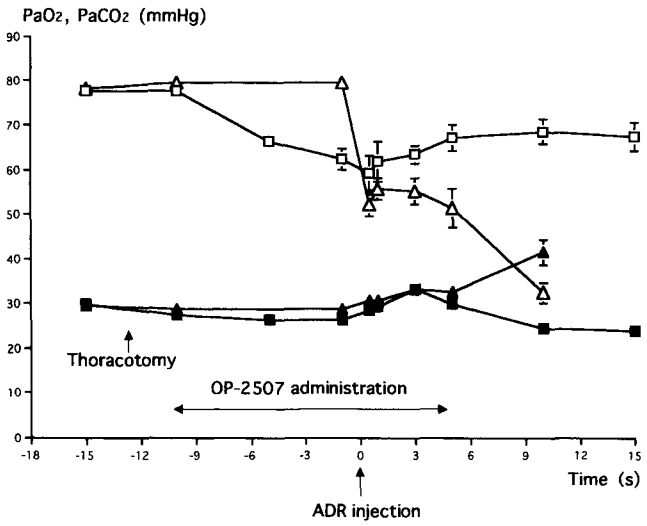

Fig. 8. The time course of the means of arterial PaO_2 and $PaCO_2$. Twelve rabbits were randomly divided into 2 groups. ADR (75 μg/kg) was administered to two groups with and without OP-2507 pretreatment. □: PaO_2 pretreated with OP-2507, △: control (PaO_2), ■: $PaCO_2$ pretreated with OP-2507, ▲: control ($PaCO_2$).

increased in the non-treated group after ADR injection. $PaCO_2$ of the non-treated group was higher than that of the OP-2507-treated group at 10 min after ADR injection.

DISCUSSION

Previous results from our laboratory examining the ADR-induced PE in the rat model demonstrated the beneficial effects that might be obtained when a PGI_2 analogue was co-administered with ADR. To further understand the hemodynamic changes associated with ADR administration and the beneficial effects exerted by the PGI_2 analogue, we carried out the present experiments using rabbits.

In the first study, hemodynamic changes persisted for more than 5 min subsequent to ADR (75 or 100 μg/kg) administration. To evaluate whether the increase of the LAP was affected by the plasma ADR concentration, study 2 was carried out (Fig. 5). In those sets of experiments, the maximum ADR concentration was 466 ± 281 ng/ml, which was measured 30 s following ADR injection. The rate of change in the plasma ADR concentration closely paralleled that of the LAP, suggesting that the hemodynamic changes depended on the plasma ADR.

The degree of PE was classified into the four groups described previously (11). In addition, we used LWR to estimate the severity of PE. The LWR is a reliable index for estimating the degree of fluid retention in addition to being an easily obtainable index (1, 11, 13). It is also reported that the LWR of 4.6 is a critical point. The probability that edematous lungs may be mistaken for non-edema and vice versa is 0.15 (11). In our study, all cases over 4.6 were Grades 2 and 3. In addition, we used a parameter, AUC, which reflected the extent of increasing BP and persistent period (1).

The incidence and grade of PE and LWR increased in a dose-dependent fashion. The data presented in Table 2 indicated that the maximum value and AUC produced by ADR in the SBP and RAP were independent of the ADR dose. However, the maximum value and AUC produced by ADR in the LAP showed some dependence, especially with the higher dose of ADR. In addition, Fig. 3 shows the relationship between the LWR and LAP. The onset of PE was related to the increase of the maximum value and AUC in the LAP produced by ADR.

Regarding the pulmonary capillary pressure, the elevation in hydrostatic pressure was shown to damage the vascular structure and induce PE in various experimental models (4–7). The elevation in the pulmonary vascular pressure had a direct damaging effect on the pulmonary vasculature (4). The ultrastructural damages were seen at pulmonary capillary mural pressures of 40 mmHg and above (5) and pulmonary arterial pressures of 70 mmHg and above in a rabbit model (6). It is also reported that LAP values higher than 35 mmHg would trigger the onset of PE in a rabbit model with continuous infusion of ADR (7). In this study, PE was induced at 40 mmHg or more above the maximum LAP (12/15) and at 120 mmHg·min or more above the AUC (6/7) in most of the rabbits. Maintenance of persistent high LAP is the essential factor for inducing ADR PE. These results suggest that the AUC of LAP may serve as one of the useful indices to detect the onset level of PE.

In study 3, a pretreatment with flurbiprofen (COX inhibitor) prior to ADR was evaluated for its ability to prevent the incidence of PE. Using this approach, the elevation of LAP and lung weight gain were not significantly different from those of the control group that did not receive flurbiprofen. However, in the preliminary studies that utilized rabbits that were treated with ADR at a dose of 50 μg/kg and flurbiprofen at a 10 mg/kg dose, the AUC of LAP and the PE incidence increased in comparison with those of the non-pretreatment groups. Table 5 summarized these results in the preliminary study. These results suggested that the elevation in the AUC produced by ADR was due to the inhibition of PG synthesis following treatment with flurbiprofen. In addition, these findings suggest that no differences were observed in study 3 because the ADR dose-LWR curve was flattened at ADR concentrations of 75 μg/kg and higher. Additional evidence to support this hypothesis comes from the work of Rothchild and Castania (14), which demonstrated COX inhibitor-mediated suppression of the ADR-induced consumption of kininogen. The study also indicated a reduction in peripheral pulmo-

Table 5. The effect of cyclooxygenase inhibitor on the ADR-induced PE

ADR (μg/kg)	Pretreatment	Left atrial pressure (mmHg)			Incidence of PE (%)	LWR
		pre	maximum	AUC (mmHg·min)		
50	Flurbiprofen, 10 mg/kg	12 ± 2	52 ± 8	115 ± 14*	57% (4/7)*	4.5 ± 0.3
50	null	9 ± 1	36 ± 4	63 ± 11	0% (0/7)	4.0 ± 0.1

All data are expressed as the mean ± S.E.M. Each group consisted of 7 rabbits. Blood pressure just before ADR administration is regarded as the pre blood pressure. Maximum means the maximum pressure after ADR administration. AUC is defined as the area under the time-BP curve. The incidence of pulmonary edema (PE) is described as the rate and the number of PE developed in the group. LWR is the lung-water ratio. The differences of the means among each group were tested by one-way analysis of variance (ANOVA) and Scheffe's test. The differences of the incidence of PE were tested by the chi-square test. *$P<0.05$.

nary vascular permeability by released kinin is not linked to the blockade of prostaglandin synthesis. In this study, flurbiprofen showed no preventive effects against ADR-induced PE. It is suggested that the elevation of the LAP produced by ADR seems to be a more important factor than the decline in the permeability caused by flurbiprofen.

The TX synthase inhibitor ozagrel is often used to treat bronchial asthmatic attacks by preventing the bronchoconstrictive effect of TX. Ozagrel was also shown to prevent platelet aggregation and to counteract the vasoconstrictive effects of TX. Based on such evidences, we anticipated ozagrel would be effective in preventing ADR-induced PE. Ozagrel was administered 5 min prior to the ADR injection. We chose this particular timing because 50% of the vasodilative effects of ozagrel were observed 3 – 10 min after its administration (15). In this study, ozagrel did not prevent the increase in the lung weight or the LAP elevation produced by ADR. In this regard, pretreatment with ozagrel did not exhibit any protective effect against ADR-induced PE.

PGI_2 has been used as a protective agent in various experimental ischemic models (16 – 20). For example Okuda et al. (16) showed that the PGI_2 analogue OP-2507 (a stable analogue of PGI_2) had a direct cytoprotective effect on ischemia and reperfused lung tissue. This cytoprotective effect was independent of its inhibitory effects on platelet aggregation and its vasodilative action. However, no reports have been published that examined OP-2507 effects on the acute ADR-induced PE. In this report, we examined the effect of OP-2507 on acute ADR-induced PE in the rabbit model.

OP-2507 is a less potent hypotensive agent than prostacyclin (17). In this study, OP-2507 inhibited the elevation of the LAP and lung weight gain as compared with those of the control group. The preventive effects against ADR-induced PE were observed only at higher doses of OP-2507. Such preventive effects that were observed at higher doses of OP-2507 were mainly due to its suppression of the LAP elevation. In support of our findings, Palmer et al. (18) showed that at high doses of PGI_2, the pulmonary capillary wedge pressure decreased and prevented PE in patients with primary pulmonary hypertension and pulmonary veno-occlusive disease.

Many studies reported the lack of improvement in the PaO_2 following PGI_2 treatment (19). In this study, the PaO_2 decreased during OP-2507 administration (Fig. 8). Immediately after ADR injection, PaO_2 decreased from the initial level of 63.3 ± 2.0 mmHg to 59.1 ± 3.9 mmHg. However it returned to the same initial level, at a faster rate in the OP-2507-treated group. Devitt et al. (19) showed that PGI_2 was not reflected inan improved PaO_2, because the venous admixture was increased by increasing blood flow through a fixed shunt. Based on such evidence, we speculate that a significant portion of the effects of OP-2507 we observed in the lung and heart is a result of its vasodilative effect and not relevant to any ventilatory effects.

The rate of recovery of the cardiac function in ADR-induced PE was faster upon OP-2507 treatment. This result suggested that the afterload was decreased mainly by the direct vasodilative effects of OP-2507. The cardiac output recovery was also aided indirectly following the prevention of PE and the resultant improvement of the PaO_2. However, PaO_2 was not improved sufficiently by OP-2507 treatment. Oguchi et al. (20) reported that OP-2507 increased coronary flow, cardiac output and myocardial ATP content in myocardial ischemia. This result suggested the possibility that such direct effects on the myocardium were related to the prompt improvement of the cardiac output that we observed.

In conclusion, our studies showed that the elevation and increase in duration of the LAP produced by ADR are some of the most important factors determining the onset of ADR-induced PE. We showed that the onset mechanism was not relevant to SBP and RAP. Ozagrel (TXA_2 synthase inhibitor) and flurbiprofen (COX inhibitor) showed no preventive effect against ADR-induced PE. However, an adequate pretreatment with OP-2507 (PGI_2 analogue) prevented the ADR-induced PE. Our findings indicate that PGI_2 analogues may have a beneficial use for lung preser-

vation in various circumstances. Finally, this experimental rabbit model, in which the hemodynamic changes could be monitored under spontaneous breathing, may serve as a useful tool for studying cardiovascular drugs.

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