EFFECT OF THE LEUKOTRIENE RECEPTOR ANTAGONIST ONO-1078 ON EXTRAVASCULAR LUNG WATER AFTER CORONARY LIGATION IN DOGS

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To determine whether or not leukotrienes are associated with pulmonary edema after coronary ligation in dogs, we studied the effects of a specific leukotriene C4/D4 antagonist, ONO-1078, on extravascular lung water, as measured by the thermal-dye double indicator dilution method (Extravascular thermal volume, ETV).

Six dogs served as a sham control (group 1). The proximal left anterior descending coronary artery was ligated for 45 min in 6 dogs (group 2), and in 6 others the coronary artery was ligated after pretreatment with ONO-1078 ($1 \mu\text{g/kg/min}$) (group 3). ETV and pulmonary arterial pressure were measured at control and 45 min after coronary ligation.

In group 1, no significant change was found in ETV. Although there was no significant increase in pulmonary arterial pressure after coronary ligation, ETV significantly increased in groups 2 and 3. ETV increased more in groups 2 and 3 than in group 1, but there was no significant difference between groups 2 and 3.

Thus, ONO-1078 did not effectively prevent the accumulation of extravascular lung water after coronary ligation, indicating that leukotrienes are not important mediators of pulmonary edema after myocardial ischemia.

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PULMONARY edema during the acute phase of myocardial infarction is generally believed to result from high pulmonary microvascular pressure. However, some experimental studies have suggested that microvascular hypertension does not fully explain the pulmonary edema seen in animals after coronary ligation^{1–5}. These latter studies suggest that some of the pulmonary edema after myocardial infarction results from increased pulmonary vascular permeability.

Key words:

Leukotriene
Coronary ligation
Pulmonary edema

Richeson et al observed that pulmonary protein and fluid leakage were minimized by infusion of indomethacin, and speculated that the direct and indirect effects of prostaglandin and prostaglandin metabolites may have provoked this condition⁵. However, the role of 5-lipoxygenase products in lung injury after myocardial ischemia has not yet been elucidated. Specific leukotriene C4/D4 receptor antagonists have been shown to decrease pulmonary edema after endotoxemia^{5–9} and to reduce myocardial injury after myocardial ischemia^{10,11}. To evaluate the role of leukotrienes on extravascular lung water accumulation and

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hemodynamic indices after myocardial ischemia, we studied the effects of ONO-1078^{12,13} a leukotriene C4/D4 receptor antagonist, following coronary ligation in dogs.

METHODS

General preparation

Twenty six mongrel dogs weighing between 13 and 30 (16 ± 2 , mean $\pm$ SD) kg were anesthetized with intravenous pentobarbital (25 mg/kg) and supplemented as necessary during the experiment. The dogs were ventilated through a cuffed orotracheal tube by a Harvard animal respirator with ambient air at a tidal volume of 15 ml/kg and a rate of 13 to 15 breaths/min. The dogs' lungs were inflated to 20 cmH₂O every 15 min to minimize microatelectasis.

A left thoracotomy was then performed through the fifth intercostal space. A 7F Swan-Ganz catheter was placed into the pulmonary artery from the femoral vein. The position of the catheter was determined from the pressure recordings. A 5F lung water catheter (Edwards Laboratory, Model 96-020) was advanced into the descending aorta from the femoral artery.

Measurements

Extravascular thermal volume (ETV) measurements were taken by injecting 10 ml of cold (0 °C) saline containing 5 mg of indocyanine dye into the right atrium. Simultaneously, blood was withdrawn by a syringe pump through a densitometer cuvette (Waters, DC-410) attached to the femoral lung catheter. A dye signal proportional to concentration was generated in the densitometer and a thermal signal proportional to temperature was generated from the thermistor on the end of the lung water computer. Both signals were fed into a computer (Edwards Laboratory, Model 9310) which simultaneously determined the mean transit time of the 2 indicators and the cardiac output. ETV was derived from these values as follows: $ETV = \text{Cardiac output} \times (\text{thermal mean transit time} - \text{dye mean transit time})$.

Blood pressure and heart rate were measured by a transducer connected to the femoral arterial catheter. Pulmonary arterial pressure was obtained from a transducer connected to the pulmonary arterial catheter.

The limb leads and epicardial leads of the electrocardiogram were monitored continuously throughout the experiment.

Protocol 1 (n=18)

To determine the effects of surgical invasion on ETV and hemodynamic indices, 6 animals served as a sham control. After a suture was placed around the proximal left anterior descending coronary artery above the first major diagonal branch, the control data were recorded for 45 min without coronary artery ligation (group 1). Thirty minutes before coronary ligation, the 6 dogs in group 2 received vehicle injection (i.e., 0.9% NaCl) and the 6 dogs in group 3 received continuous intravenous infusion of ONO-1078 (1 μ g/kg/min). Ligation of the coronary artery was maintained for 45 min in groups 2 and 3. Since ONO-1078 is water-insoluble, a solution consisting of 5% ethanol, 1% HCO40 [polyoxyethylene hydrogenated castor oil (40EO)] and saline was used as the vehicle. Myocardial ischemia was confirmed by a change in the color of the myocardium from its normal red to a dusky blue, by a change of the ST segment in the epicardial electrocardiogram and by abnormal regional contraction in the distribution of the ligated vessel. Measurements of the hemodynamic indices and ETV recordings were obtained at control and 45 min after ligation.

Protocol 2 (n=8)

To determine the effects of ONO-1078 on the hemodynamic response to leukotriene D4, another group of 8 dogs was studied. After baseline measurements of pressures, heart rate, cardiac output and ETV, leukotriene D4 (1 μ g/kg, Wako Pure Chemical Industries, Osaka) was administered by intravenous injection over 1 min in 4 dogs. The effects of ONO-1078 were tested in 4 other dogs by administering leukotriene D4 (1 μ g/kg) after continuous intravenous infusion of ONO-1078 (1 μ g/kg/min). Hemodynamic indices were recorded 45 min after leukotriene D4 injection. All experimental studies conformed to the animal use guidelines in our university.

Test drug

ONO-1078 was developed and supplied

TABLE I CHANGES IN HEMODYNAMIC PARAMETERS

		Group 1 (n=6)	Group 2 (n=6)	Group 3 (n=6)	$\Delta G1$ vs $\Delta G2$	$\Delta G1$ vs $\Delta G3$	$\Delta G2$ vs $\Delta G3$
CO (ml/min/kg)	Control	142±29	154±43	161±34	NS	NS	NS
	After	139±33	118±33*	143±25	NS	NS	NS
HR (/min)	Control	147±12	147±15	150±8	NS	NS	NS
	After	147±14	146±20	152±8	NS	NS	NS
PAP (mmHg)	Control	21±5	21±3	19±4	NS	NS	NS
	After	19±2	20±3	21±4	NS	NS	NS
PWP (mmHg)	Control	11±5	14±2	12±2	NS	NS	NS
	After	11±4	14±3	13±2	NS	NS	NS
BP (mmHg)	Control	89±19	100±22	87±10	NS	NS	NS
	After	84±15	100±19	82±12	NS	NS	NS

Abbreviations: CO, cardiac output; HR, heart rate; PAP, pulmonary arterial pressure (mean); PWP, pulmonary artery wedge pressure; BP, blood pressure (mean); Group 1, sham ligation; Group 2, coronary ligation; Group 3, pre-treatment with ONO-1078 and coronary ligation; $\Delta G1$, changes of hemodynamic parameters in group 1; $\Delta G2$, changes of hemodynamic parameters in group 2; $\Delta G3$, changes of hemodynamic parameters in group 3; NS, not significant; * $p < 0.005$ vs control value.

Fig.1. Individual changes in extravascular thermal volume (ETV) in 3 groups. Note that while ETV increased after coronary ligation in groups 2 and 3, the magnitudes of these changes were not significantly different. ETV increased significantly more in groups 2 and 3 than in group 1.

Statistical analysis

Values are expressed as the mean±SD. Comparable points in each group were compared with a two-way analysis of variance followed by Scheffe's test. A $p < 0.05$ was considered statistically significant.

RESULTS

Protocol 1

Changes in hemodynamic parameters after coronary ligation

The hemodynamic data are presented in Table I. There were no significant differences between the hemodynamic indices of the 3 groups at control. There were no significant changes in hemodynamic parameters during the 45 min in groups 1 and 3. Cardiac output declined after coronary ligation in group 2 ($p < 0.005$) without significant changes in pulmonary arterial pressure or pulmonary artery wedge pressure. However, there were no significant differences between the magnitude of the changes in cardiac output of the 3 groups.

Changes in ETV after coronary ligation

The changes in ETV are shown in Fig. 1 and Table II. There was no significant difference between the ETV of the 3 groups at control. In the sham ligation group (group 1), ETV did not significantly change during

by Ono Pharmaceutical Co., Ltd., Osaka, Japan.

TABLE II CHANGES IN ETV AFTER CORONARY LIGATION

		Group 1 (n=6)	Group 2 (n=6)	Group 3 (n=6)	$\Delta G1$ vs $\Delta G2$	$\Delta G1$ vs $\Delta G3$	$\Delta G2$ vs $\Delta G3$
ETV	Control	7.2±0.8	7.0±0.5	6.8±0.8	p<0.05	p<0.01	NS
(ml/kg)	After	7.5±0.9	9.2±1.1**	10.0±2.2*			

Abbreviations: ETV, extravascular thermal volume; Group 1, sham ligation; Group 2, coronary ligation; Group 3, pre-treatment with ONO-1078 and coronary ligation; $\Delta G1$, change of ETV in group 1; $\Delta G2$, change of NS, not significant; **p<0.01, *p<0.05 vs control value.

TABLE III EFFECTS OF ONO-1078 ON HEMODYNAMIC PARAMETERS AFTER LTD4 INJECTION

		LTD4 (n=4)	ONO+LTD (n=4)	$\Delta LTD4$ vs $\Delta ONO+LTD4$
CO	Before	155±36	155±10	
(ml/min/kg)	45 min	140±38***	151±5	NS
HR	Before	134±19	137±19	
(/min)	45 min	140±14	139±16	NS
PAP	Before	23±4	22±4	
(mmHg)	45 min	28±2*	22±3	p<0.05
PWP	Before	13±1	12±2	
(mmHg)	45 min	15±1*	13±3	p<0.005
BP	Before	99±9	100±9	
(mmHg)	45 min	98±8	102±4	NS
ETV	Before	7.1±0.2	7.2±0.2	
(ml/kg)	45 min	7.8±0.2**	7.3±0.3	p<0.005

Abbreviations: CO, cardiac output; HR, heart rate; PAP, pulmonary arterial pressure (mean); PWP, pulmonary artery wedge pressure; BP, blood pressure (mean); ETV, extravascular thermal volume; LTD4, leukotriene D4 injection; ONO+LTD4, leukotriene D4 injection after pretreatment with ONO-1078; $\Delta LTD4$, changes of hemodynamic parameters after leukotriene D4 injection; $\Delta ONO+LTD4$, changes of hemodynamic parameters after pretreatment with ONO-1078; NS, not significant; ***p<0.005, **p<0.01, *p<0.05 vs baseline.

the 45 min. However, ETV increased from 7.0±0.5 ml/kg to 9.2±1.1 ml/kg (p<0.01) in group 2 and from 6.8±0.8 ml/kg to 10.0±2.2 ml/kg (p<0.05) in group 3 after coronary ligation. The increase in ETV in groups 2 and 3 was significantly larger than that in group 1. However, there was no significant difference between the increase in ETV of groups 2 and 3 after coronary ligation. In 3 additional dogs, we verified that vehicle of ONO-1078 had no effect on hemodynamic parameters and ETV.

Protocol 2

Effects of ONO-1078 pre-treatment on leukotriene D4

Administration of 1 µg/kg of leukotriene D4 reduced cardiac output, and increased pulmonary artery pressure, pulmonary artery

wedge pressure and ETV (Table III). However, leukotriene D4 did not induce significant changes in hemodynamic indices following pre-treatment with ONO-1078. There were significant differences between the change of pulmonary artery pressure, pulmonary artery wedge pressure and ETV in the pre-treated and non-pre-treated groups (p<0.05, p<0.005, p<0.005, respectively). Therefore, pre-treatment with ONO-1078 inhibited the hemodynamic changes which had been induced by leukotriene D4 injection.

DISCUSSION

In this study the thermal-dye double indicator dilution method was used to measure extravascular lung water. We found that

ONO-1078 ($1 \mu\text{g/kg/min}$), a specific leukotriene C4/D4 antagonist, did not prevent the accumulation of lung water after coronary artery ligation in dogs. This amount of ONO-1078 has been shown to antagonize leukotriene C4/D4 receptors^{12,13}. In protocol 2, leukotriene D4 infusion produced an increase in pulmonary artery pressure, pulmonary artery wedge pressure and ETV. These results were consistent with previous studies which described pulmonary hypertension and an increase in ETV in dogs and sheep¹⁴⁻¹⁷. Pre-treatment with ONO-1078 ($1 \mu\text{g/kg/min}$) inhibited the hemodynamic changes which had been observed following leukotriene D4 injection.

The thermal-dye technique has been tested in experimental animals with normal and edematous lungs by comparing ETV with gravimetric estimates of lung water content and an excellent correlation has been found between the 2 methods¹⁸⁻²⁰. In our laboratory, postmortem gravimetric determinations of lung water in 10 dogs (4 dogs received sham ligation and 6 dogs received coronary ligation) showed an excellent correlation with ETV obtained by the thermal-dye method²¹. Thus, this method was an accurate method for determining trends in extravascular lung water accumulation in this study.

Pulmonary edema in some patients with myocardial infarction can occur without an increase in pulmonary artery wedge pressure²²⁻²⁵. This type of pulmonary edema is believed to be associated with the increased permeability of the pulmonary vascular bed, and this suggestion has been confirmed in several animal models¹⁻⁵. Richeson et al observed that pulmonary protein and fluid leakage were minimized by infusion of indomethacin, and speculated that the direct and indirect effects of prostaglandin and prostaglandin metabolites may have provoked this condition⁵.

We recently examined a canine model of pulmonary edema after coronary ligation and suggested that platelet-activating factor may play an important role²⁶. Voelkel et al suggested that the effects of platelet-activating factor may be partially due to the action of leukotriene C4/D4²⁷. Peptidoleukotrienes, 5-lipoxygenase products, have many pathophysiological actions includ-

ing enhancement of pulmonary vascular permeability, induction of pulmonary vasoconstriction¹⁴⁻¹⁷ and aggravation of myocardial ischemia^{10,11}. Since several leukotriene antagonists have been shown to attenuate or block endotoxin-induced pulmonary edema, it is generally believed that leukotrienes play an important role in endotoxin-induced lung injury⁶⁻⁹. However, the role of leukotrienes in pulmonary edema after myocardial ischemia has not yet been elucidated. In this study, ETV increased after coronary ligation in the absence of increased pulmonary artery wedge pressure. Our data support the suggestion that some of the pulmonary edema following myocardial ischemia results from increased pulmonary vascular permeability¹⁻⁵. However, the ETV increase after coronary ligation was not inhibited in group 3, indicating that ONO-1078 did not effectively prevent the accumulation of extravascular lung water caused by coronary artery ligation. Our findings suggest that leukotrienes are not an important mediator in pulmonary edema after myocardial ischemia.

Since leukotriene C4/D4 did not exhibit protective effects in this model, our previous observation that a platelet-activating factor receptor antagonist blocked pulmonary edema after coronary ligation becomes even more significant. Our present data suggest that the contribution of platelet-activating factor to pulmonary edema after myocardial ischemia does not require further mediation by leukotrienes.

In conclusion, extravascular lung water increased after coronary ligation in dogs, and a leukotriene C4/D4 antagonist did not prevent this accumulation. Thus, leukotriene C4/D4 does not play an important role in pulmonary edema after myocardial ischemia.

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