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Involvement of thromboxane A_2 (TXA_2) in the early stages of oleic acid-induced lung injury and the preventive effect of ozagrel, a TXA_2 synthase inhibitor, in guinea-pigs

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Abstract

An intravenous injection of oleic acid into animals can produce a lung injury with hypoxaemia and pulmonary vascular hyper-permeability. Although oleic acid lung injury is used as a model of acute respiratory distress syndrome (ARDS), the precise mechanisms of the lung injury are still unclear. We have investigated whether thromboxane A_2 (TXA_2) participated in the lung injury and have evaluated the efficacy of ozagrel, a TXA_2 synthase inhibitor, on the lung injury in guinea-pigs. Oleic acid injection increased the plasma level of TXB_2, a stable metabolite of TXA_2, and the time-course of plasma TXB_2 was similar to that of the decreased partial oxygen pressure of arterial blood (Pao_2) induced with oleic acid. Ozagrel administered intravenously 30 min before oleic acid injection prevented the decrease in Pao_2 and pulmonary vascular hyper-permeability. It also prevented increases in lactate dehydrogenase activity, a measure of lung cell injury, TXB_2 and its weight ratio to 6-keto prostaglandin $F_{1\alpha}$ in bronchoalveolar lavage fluid. Although ozagrel administered simultaneously with oleic acid ameliorated the decrease in Pao_2, post treatment showed little effect. We suggest that TXA_2 participated in the oleic acid lung injury, as an "early phase" mediator, and rapidly-acting TXA_2 synthase inhibitors were effective in the prevention of acute lung injury.

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Introduction

Acute respiratory distress syndrome (ARDS) or acute lung injury (ALI) is a severe and complicated lung inflammation, seen in patients with sepsis, severe trauma, fat embolism and so on (Petty & Ashbaugh 1971; Ware & Matthay 2000). ARDS/ALI is characterized by pulmonary oedema and severe hypoxaemia, which are originally induced by an increase in pulmonary vascular permeability. Hypoxaemia is a critical state in patients which can result in brain damage and/or death. In addition, the mortality of ARDS/ALI is high (approximately 40%) (Nuckton et al 2002; Vincent et al 2003) because of lack of effective drugs. Therefore, it is extremely important to improve the hypoxaemia in patients with ARDS/ALI and to find effective drugs.

An intravenous injection of oleic acid can produce lung injury. Since pathophysiological changes induced by oleic acid are similar to those in patients with ARDS/ALI, the lung injury is known as one of the models of these diseases (Schuster 1994). Oleic acid-induced lung injury resembles specific forms of ARDS/ALI that follow pancreatitis (Maclver et al 1977), long bone fractures (Halilton et al 1964) and meconium aspiration (Clark et al 1987), all of which are thought to be caused from the toxicity of fatty acids. We have developed a system with the decrease in arterial oxygen tension (Pao_2) and the increase in pulmonary vascular permeability induced by a low dose (15 μL kg^{-1}) of oleic acid to screen drugs against ARDS/ALI (Moriuchi & Yuizono 1994; Moriuchi et al 1995a). An injection of 15 μL kg^{-1} oleic acid causes an immediate decrease in Pao_2 within 10 min. Using this screening system, we have found some candidate drugs against ARDS/ALI and have shown that reactive oxygen species, proteases and the fibrinolytic system were involved in the development of oleic acid-induced lung injury (Moriuchi & Yuizono

1994, 1995; Moriuchi et al 1995a, b, 1998). Nevertheless, the mechanism of rapid decrease in Pao_2 induced with oleic acid remains unclear.

Thromboxane A_2 (TXA_2) seems to play an important role in oleic acid lung injury (Schuster 1994; Furue et al 1999) as well as other experimental lung injury models (Bureau et al 1992; Schmeck et al 1998), including lipopolysaccharide-induced lung injury (Nagase et al 2000), and ARDS/ALI (Bulger & Maier 2000; Schilling et al 1998). It is suggested that TXA_2 participates in oleic acid lung injury through platelet aggregation, increasing both the airway and pulmonary vascular resistance, inducing pulmonary hypertension, and an enhancement of oedema formation (Schuster 1994). Although Thies et al (1996) and Goff et al (1997) demonstrated that a TXA_2-receptor blocker ameliorated the oleic acid-induced oxygenation dysfunction in their ex-vivo blood perfusion/ventilation system, little is known about whether an inhibition of TXA_2 could prevent or improve in-vivo oleic acid-induced lung injury, especially the decrease in Pao_2. In this study, this has been investigated using ozagrel.

Ozagrel is a TXA_2 synthase inhibitor, and is widely used in the treatment of asthma, cerebral thrombosis and vasospasm. The drug is reported to attenuate the pulmonary oedema in phorbol myristate acetate-induced lung injury in animals (Allison et al 1986; Sprague et al 1992). Therefore, we expected ozagrel to attenuate the oleic acid-induced lung injury.

This study was performed to clarify whether TXA_2 played an important part in the decrease in Pao_2, pulmonary vascular hyperpermeability and lung cell damage induced with oleic acid. The efficacy of ozagrel on the lung injury in guinea-pigs was examined.

Materials and Methods

The study was approved by the Animal Care and Use Committee of the Kumamoto University, and was performed in accordance with National Institutes of Health guidelines of the care and handling of animals.

The operation

An operation was performed as described by Moriuchi & Yuizono (1994). Briefly, Hartley strain guinea-pigs were anaesthetized with pentobarbital sodium (Nembutal, Dainabott Co., Osaka, Japan) (25 mg kg^{-1}, i.p.) and procaine (Sigma, St Louis, MO) was used for local anaesthesia. Catheters (1.1 mm outer diameter) were inserted into the subclavian vein and artery for injections of reagents and for blood sampling, respectively.

Measurement of plasma levels of TXB_2 after the oleic acid injection

Plasma levels of TXB_2 after an intravenous injection of 15 μL kg^{-1} oleic acid in guinea-pigs were determined. Arterial blood (800 μL) was collected 5 min before, and 6, 10, 15, 35, 55, and 75 min after the oleic acid or saline injection. Indometacin (sigma) and ethylenediaminetetraacetic acid disodium (EDTA; sigma) (final concentration of 5 μg mL^{-1} and 1 mg mL^{-1}, respectively) were added to the blood. The samples were centrifuged at 1800 g at 4 °C for 20 min and the plasma was recovered. The plasma was stored at −80 °C until assayed. The concentration of TXB_2 was analysed by an enzyme immunoassay kit (Cayman Chemical Co, Ann Arbor, MI).

To examine the effects of oleic acid on the plasma levels of TXB_2, 10 guinea-pigs (male, 560 ± 110 g) were divided into two groups. Group 1 received saline (15 μL kg^{-1}) (n = 5). Group 2 received oleic acid (15 μL kg^{-1}) (n = 5). Arterial blood was collected 5 min before, and 6, 10, 15, 35, 55 and 75 min after the oleic acid or saline injection.

Measurement of blood gases

To examine the effect of ozagrel on oleic acid-induced hypoxaemia, we measured arterial blood gas parameters (Pao_2, $Paco_2$ and pH). Briefly, 24 guinea-pigs (male, 660 ± 93 g) were divided into four groups. Group 1 received saline (1 mL kg^{-1}) plus oleic acid (15 μL kg^{-1}) (n = 6). Groups 2, 3 and 4 received ozagrel (Ono pharmaceutical company, Osaka, Japan) 20, 40 or 80 mg kg^{-1}, respectively, plus oleic acid (15 μL kg^{-1}) (n = 6 each group). Ozagrel or saline was administered 30 min before the oleic acid injection. Arterial blood (200 μL) was collected 5, 10 and 15 min before, and 6, 10, 15, 35, 55, and 75 min after the oleic acid injection and analysed with a blood gas analyser (ABL 300, Radiometer Ltd, Copenhagen, Denmark). The mean value of the blood gas parameters before the oleic acid injection was defined as the values at 0 min.

Determination of pulmonary vascular permeability

To examine effects of ozagrel on oleic acid-induced pulmonary vascular hyperpermeability, we measured pulmonary extravasation of Evans blue. Thirty-five guinea-pigs (male, 600 ± 110 g) were divided into five groups. Group 1 received saline (1 mL kg^{-1}) plus saline (15 μL kg^{-1}) (n = 7). Group 2 received saline (1 mL kg^{-1}) plus oleic acid (15 μL kg^{-1}) (n = 7). Groups 3, 4 and 5 received ozagrel (20, 40 or 80 mg kg^{-1}, respectively) plus oleic acid (15 μL kg^{-1}) (n = 7 each group). Ozagrel or saline was administered 30 min before the oleic acid injection and Evans blue (Sigma) (30 mg kg^{-1}) was administered 1 min before the oleic acid injection. Ninety minutes after the oleic acid injection, the chest cavity was opened. Pulmonary intravascular Evans blue was washed out by perfusing saline. This procedure was accomplished by inserting a 13-gauge blunt cannula through the right ventricle into the pulmonary artery, and perfusate outflow came out from the dissected left atrium. The lungs were perfused with 100 mL saline at a rate of 3 mL min^{-1} using a pump (EYELA Micro Tube Pump MP-3, Rikakikai Co., Tokyo, Japan). After the perfusion, the airways and lungs were removed and weighed. The right lung was cut into some sections (approximately 10-mm thick, cubic). Evans blue was extracted with 20 mL 100% formamide solution at 37 °C for 18 h. Their concentrations were

determined by light absorbance at 620 nm with a spectrophotometer (U 3200, Hitachi, Tokyo, Japan). Interpolation of the data was performed using a standard curve for absorbance from 100 ng mL^{-1} to 5 μg mL^{-1}. The amount of Evans blue from the tissue was then expressed as ng (mg wet weight tissue)$^{-1}$.

Determination of TXB_2, 6-keto $PGF_{1\alpha}$ and LDH activity in BALF

To examine the inhibitory effect of ozagrel on TXA_2 generation and pulmonary cell injury by oleic acid, we measured the concentration of TXB_2, a stable metabolite of TXA_2, and activity of lactate dehydrogenase (LDH), a marker of pulmonary cell injury, in bronchoalveolar lavage fluid (BALF). We also measured 6-keto $PGF_{1\alpha}$, a stable metabolite of prostacyclin (PGI_2). Fourteen guinea-pigs (male, 630 ± 85 g) were divided into three groups. Group 1 received saline (1 mL kg^{-1}) plus saline (15 μL kg^{-1}) (n = 4). Group 2 received saline (1 mL kg^{-1}) plus oleic acid (15 μL kg^{-1}) (n = 5), whilst group 3 received ozagrel (80 mg kg^{-1}) plus oleic acid (15 μL kg^{-1}) (n = 5). A catheter was inserted into the subclavian vein after the animals were anaesthetized with pentobarbital sodium (25 mg kg^{-1}, i.p.). Ozagrel or vehicle was administered 30 min before the oleic acid injection. To perform bronchoalveolar lavage, animals received a further 50 mg kg^{-1} pentobarbital sodium intraperitoneally 10 min before the lavage. Ninety minutes after the oleic acid injection, 10 mL cold saline cooled by ice with 0.7 mM EDTA was injected and withdrawn slowly through the trachea twice. Indometacin (final concentration 10 μg mL^{-1}) was added to the BALF to inhibit further metabolism of arachidonic acid to thromboxanes. The BALF was centrifuged at 250 g at 4 °C for 10 min and the supernatant was recovered. The supernatant was divided into samples and stored at −80 °C until assayed. The concentrations of TXB_2 and 6-keto $PGF_{1\alpha}$ were analysed using an enzyme immunoassay kit (Cayman Chemical Co.). The activity of LDH was analysed using a bio-analyser (Hitachi 7600, Hitachi, Tokyo, Japan).

Time-window of the effect of ozagrel on the hypoxaemia induced with oleic acid

We examined the effects of ozagrel administered simultaneously with oleic acid, and administered after the oleic acid injection on oleic acid-induced hypoxaemia. Twenty-four guinea-pigs (male, 560 ± 10 g) were divided into four groups. Group 1 received ozagrel (80 mg kg^{-1}) (n = 6) whilst group 2 received saline (1 mL kg^{-1}) (n = 6), both administered concomitantly with oleic acid (15 μL kg^{-1}). Groups 3 and 4 received ozagrel (80 mg kg^{-1}) (n = 6) or saline (1 mL kg^{-1}) (n = 6), respectively, 10 min after the oleic acid (15 μL kg^{-1}) injection. The blood gas parameters were measured in the same manner as described in Measurement of blood gases.

Statistical analysis

Multiple comparisons were made to examine the statistical significance of the data. When uniform variance of data was identified by Bartlett's analysis ($P < 0.05$), one-way analysis of variance was used to test for statistical differences. When significant differences ($P < 0.05$) were identified, the data were further analysed by Dunnett's or Tukey's multiple range test for significant differences among the values. If uniform variance of data was not identified, non-parametric multiple comparisons were made. After confirming significant differences ($P < 0.05$) by using Kruskal-Wallis' analysis, the differences were then examined by applying Dunnett's test. As to the comparison between unpaired two values, unpaired Student's t-test was performed.

Results

Changes in plasma levels of TXB_2 and blood gases

An intravenous injection of 15 μL kg^{-1} oleic acid caused a significant increase in the plasma level of TXB_2, a stable metabolite of TXA_2, compared with the saline group (Figure 1). The maximum increase, approximately 2.5-fold of the saline group, was observed 6 min after the oleic acid injection, corresponding with the time course of the decrease in Pao_2 (Figure 2). The maximum decrease in Pao_2, approximately 45% of the value at 0 min, was observed 6 min after the oleic acid injection. Seventy-five minutes after the oleic acid injection, Pao_2 had recovered to approximately 90% of the value at 0 min (Figure 2 and Table 1). A slight increase in $Paco_2$ was observed after the oleic acid injection, and arterial blood pH was little affected by oleic acid.

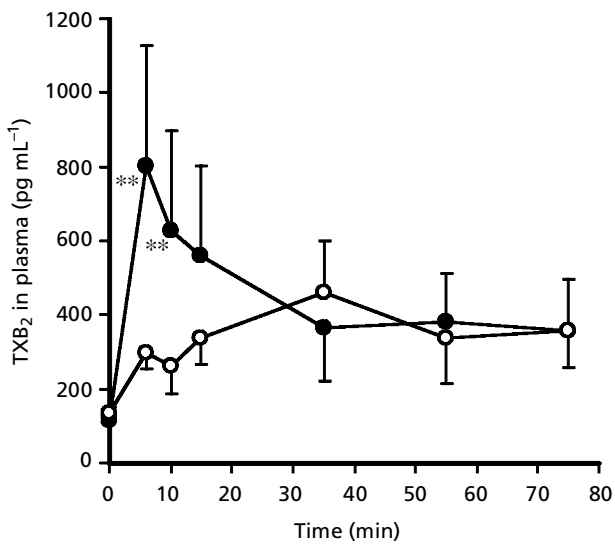

Figure 1 Time-course of the plasma TXB_2 levels after oleic acid injection. Each point represents mean ± s.e.m. Oleic acid (15 μL kg^{-1}, ●) caused a significant increase in TXB_2 compared with the saline group (○). The time of the maximum increase in TXB_2 seemed to correspond to the time of maximum decrease in Pao_2. **$P < 0.01$ compared with the saline group, n = 5.

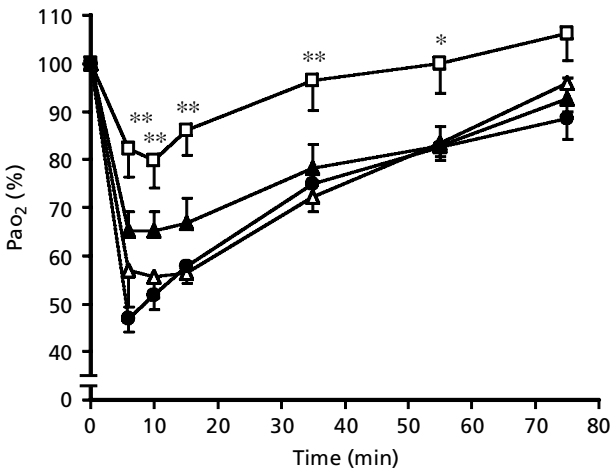

Figure 2 Preventive effects of ozagrel administered 30 min before oleic acid injection on the decrease in Pao_2. Each point represents mean ± s.e.m. Saline (●) or ozagrel (20 △, 40 ▲ or 80 mg kg^{-1} □) was administered intravenously 30 min before oleic acid injection (15 μL kg^{-1}). Ozagrel significantly reduced the decrease in Pao_2 induced by oleic acid in a dose-dependent manner. *$P < 0.05$, **$P < 0.01$ compared with the vehicle group, n = 6.

When 80 mg kg^{-1} ozagrel was administered intravenously 30 min before the oleic acid injection, it prevented the decrease in Pao_2 induced with oleic acid. The Pao_2 value was normalized as early as 35 min. Ozagrel at lower doses such as 20 and 40 mg kg^{-1} did not produce any significant preventive effect on oleic acid-induced decrease in Pao_2.

Changes in pulmonary vascular permeability

As shown in Figure 3, oleic acid significantly increased the vascular permeability in the lungs, as indicated by the extravasation of Evans blue given intravenously. In addition, distinctive patches of intense haemorrhage with Evans blue on the surface of the lungs were observed in the oleic acid injection group, but there were few patches of haemorrhage in the group administered oleic acid with ozagrel (80 mg kg^{-1}) pretreatment. When administered 30 min before the oleic acid injection, ozagrel prevented the oleic acid-induced increase in pulmonary vascular permeability in a dose-dependent manner (Figure 3).

Changes in TXB_2, 6-keto $PGF_{1\alpha}$ and LDH activity in BALF

The level of TXB_2 in BALF was markedly increased by oleic acid injection (Figure 4A), while the level of 6-keto $PGF_{1\alpha}$ in the BALF was increased to a lesser extent (Figure 4B), giving a significant increase in the weight ratio of TXB_2 to 6-keto $PGF_{1\alpha}$ (Figure 4C). When 80 mg kg^{-1} ozagrel was administered intravenously 30 min before the oleic acid injection, it reduced the oleic acid-induced increase in TXA_2 and its weight ratio to 6-keto $PGF_{1\alpha}$. LDH activity was markedly increased by the oleic acid injection and such an increase tended to be ameliorated by ozagrel ($P = 0.0502$) (Figure 5).

Time-dependent effect of ozagrel on the hypoxaemia induced with oleic acid

When 80 mg kg^{-1} ozagrel was administered intravenously 30 min before and simultaneously with the oleic acid injection, it attenuated the oleic acid-induced decrease in Pao_2 (Figure 6A and B). However, ozagrel at the same dose, when administered 10 min after the oleic acid injection, did not show any significant restorative effect on oleic acid-induced decrease in Pao_2 (Figure 6C).

Table 1 The blood gas parameters of animals administered ozagrel 30 min before oleic acid injection.

Treatment	Parameter	Time (min)						
		0	6	10	15	35	55	75
Oleic acid	Pao_2	88.3 ± 3.3	41.2 ± 2.3†	45.6 ± 3.0†	50.9 ± 2.4†	65.7 ± 2.5†	72.9 ± 5.2†	81.6 ± 5.2
	$Paco_2$	40.0 ± 1.8	43.0 ± 1.7	43.6 ± 1.6*	42.2 ± 1.5	39.1 ± 1.1	40.4 ± 1.2	39.1 ± 1.7
	pH	7.425 ± 0.020	7.400 ± 0.018	7.390 ± 0.026	7.393 ± 0.027	7.418 ± 0.020	7.426 ± 0.014	7.443 ± 0.014
Oleic acid + ozagrel (20 mg kg^{-1})	Pao_2	88.2 ± 3.4	50.6 ± 8.1†	48.7 ± 3.3†	49.6 ± 2.3†	63.2 ± 2.0†	73.3 ± 2.8*	83.7 ± 2.9
	$Paco_2$	39.4 ± 0.8	40.4 ± 1.2	40.8 ± 1.5	39.5 ± 1.7	39.8 ± 1.6	39.4 ± 1.6	40.1 ± 1.8
	pH	7.455 ± 0.007	7.438 ± 0.013	7.427 ± 0.012	7.430 ± 0.012	7.447 ± 0.010	7.459 ± 0.009	7.469 ± 0.009
Oleic acid + ozagrel (40 mg kg^{-1})	Pao_2	86.7 ± 3.5	55.7 ± 4.9†	55.5 ± 5.5†	57.0 ± 5.5†	67.1 ± 3.9†	71.8 ± 4.9	79.7 ± 3.3
	$Paco_2$	42.0 ± 1.9	42.0 ± 1.9	41.5 ± 2.2	41.3 ± 2.2	41.5 ± 1.5	41.1 ± 0.6	40.5 ± 1.2
	pH	7.409 ± 0.013	7.412 ± 0.021	7.406 ± 0.023	7.404 ± 0.022	7.397 ± 0.032	7.398 ± 0.030	7.404 ± 0.032
Oleic acid + ozagrel (80 mg kg^{-1})	Pao_2	86.3 ± 2.7	70.6 ± 4.5*	68.7 ± 4.0†	74.0 ± 4.1	82.8 ± 5.5	86.3 ± 4.1	91.1 ± 1.8
	$Paco_2$	41.0 ± 1.5	42.1 ± 2.1	42.6 ± 2.3	42.0 ± 2.5	41.1 ± 1.4	42.3 ± 1.6	42.7 ± 1.3
	pH	7.407 ± 0.019	7.422 ± 0.019	7.418 ± 0.018	7.424 ± 0.019	7.419 ± 0.025	7.426 ± 0.021	7.423 ± 0.023

Each value represents mean ± s.e.m. Pao_2, $Paco_2$ (mmHg). *$P < 0.05$, $^{\dagger}P < 0.01$ compared with the value at 0 min, n = 6.

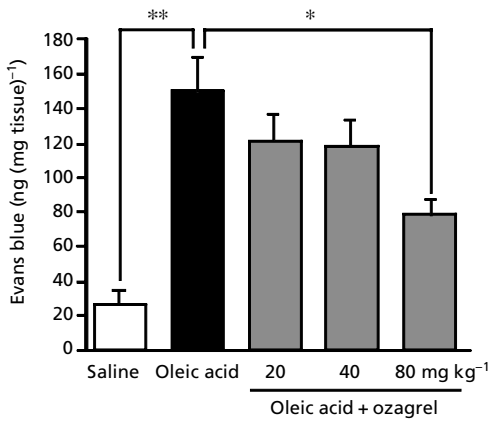

Figure 3 Preventive effects of ozagrel administered 30 min before oleic acid injection on the increase in pulmonary vascular permeability. Each bar represents mean ± s.e.m. There was a significant increase of Evans blue extravasation in the lungs in the oleic acid (saline + oleic acid) group (black bar) compared with the control (saline + saline) group (white bar). The increase in Evans blue extravasation induced by oleic acid was significantly reduced by ozagrel (grey bars) in a dose-dependent manner. *$P < 0.05$, n = 7.

Discussion

Results indicated that TXA_2 played an important part in the decrease in Pao_2 induced by oleic acid. The in-vivo findings were closely related to the ex-vivo results reported by Goff et al (1997) and Thies et al (1996), that a TXA_2-receptor blocker ameliorated the oleic acid-induced oxygenation dysfunction.

Ozagrel administered 30 min before or simultaneously with the oleic acid injection significantly prevented the decrease in Pao_2, while the TXA_2 synthase inhibitor given 10 min after the oleic acid injection did not affect the recovering process of Pao_2. This indicated that the synthesis of TXA_2, which responded to the oleic acid injection, was almost completed within 10 min after the oleic acid injection, and that therefore, post-treated ozagrel did not work. These results strongly suggested that TXA_2 was an important "early phase" mediator, which caused the decrease in Pao_2 induced by oleic acid. It should be noted that the simultaneous administration of ozagrel with oleic acid ameliorated the oleic acid-induced hypoxaemia. Ozagrel acted immediately against the oleic acid-induced lung injury.

The rapid appearance of plasma TXB_2 associated with the oleic acid injection may not be explained either by the direct activation of TXA_2 synthase or by the expression of cyclooxygenase (COX)-2. If oleic acid could directly activate the TXA_2 synthase, the level of PGI_2 might decrease with the increase in TXA_2, because they are derived from the same resource. In fact, the level of 6-keto $PGF_{1\alpha}$, a stable metabolite of PGI_2, in BALF was increased by the oleic acid injection to some extent. These findings are in accordance with those reported by Cui (1989) and Littner & Lott (1989). The expression of COX-2, which is controlled by the transcription of the messenger RNA, was unlikely to be up-regulated within 10 min after the oleic acid injection. Therefore, it seemed reasonable to assume that the TXA_2 generation by oleic acid was regulated by some upper stream events of arachidonic cascade, such as release of arachidonic acid by phospholipase A_2 (PLA_2) activation and stimulation of arachidonic acid metabolism by COX-1. TXA_2 plays a role in the expression of adhe-

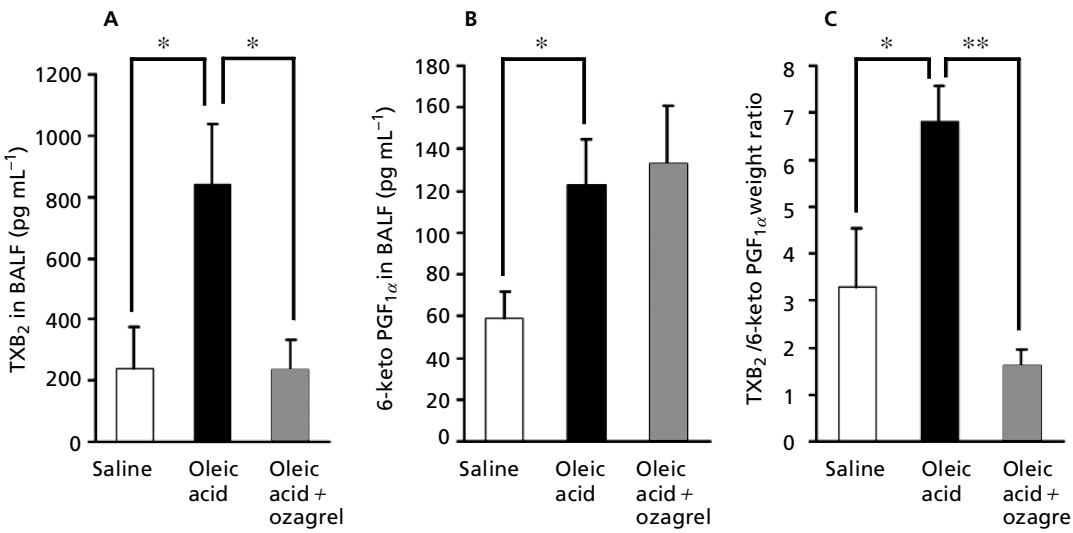

Figure 4 Effects of ozagrel administered 30 min before oleic acid injection on the TXB_2 (A) and 6-keto $PGF_{1\alpha}$(B) production and TXB_2/6-keto $PGF_{1\alpha}$ ratio (C) in BALF. Each bar represents mean ± s.e.m. There was a significant increase of TXB_2 and TXB_2/6-keto $PGF_{1\alpha}$ ratio in BALF in the oleic acid (saline + oleic acid) group (black bar) compared with the control (saline + saline) group (white bar). The increase in TXB_2 and TXB_2/6-keto $PGF_{1\alpha}$ ratio in BALF induced by oleic acid was significantly reduced by ozagrel (80 mg kg^{-1}) (grey bars). *$P < 0.05$, n = 4–5.

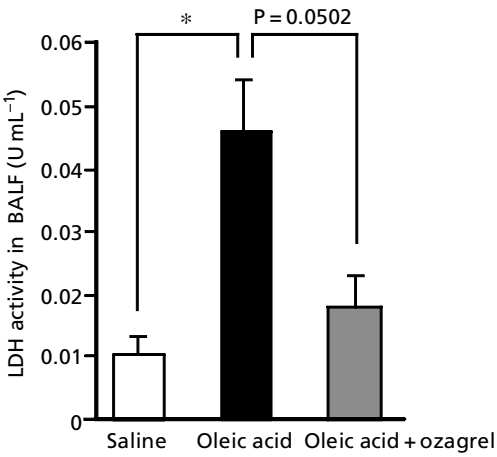

Figure 5 Effects of ozagrel administered 30 min before oleic acid injection on the LDH activity in BALF. Each bar represents mean ± s.e.m. There was a significant increase in LDH activity in BALF in the oleic acid (saline + oleic acid) group (black bar) compared with the control (saline + saline) group (white bar). The increase in LDH activity in BALF induced by oleic acid was reduced ($P = 0.0502$) by ozagrel (80 mg kg^{-1}) (grey bars). *$P < 0.05$, n = 4–5.

sion molecule (Ishizuka et al 1998) and interleukin-8 (Keelan et al 2000). Additionally, the inhibition of $sPLA_2$ and the knockout of the cytosolic PLA_2 gene are reported to be effective in the protection against oleic acid and lipopolysaccharide-induced-lung injury, respectively (Furue et al 1999; Nagase et al 2000). These findings indicate that the lipid mediators including TXA_2 are crucial in the development of ARDS/ALI.

Other studies described that ozagrel could stimulate PGI_2 synthesis, as a result of the inhibition of TXA_2 synthase (Nakazawa et al 1994). PGI_2 is known to attenuate oleic acid-induced lung injury through an inhibition of platelet aggregation and also through a pulmonary vasodilation (Miyazawa et al 1982; Nakazawa et al 2001). In this study, ozagrel did not increase 6-keto $PGF_{1\alpha}$ in BALF, while the drug decreased the weight ratio of TXB_2 to 6-keto $PGF_{1\alpha}$ in BALF. The increased TXB_2/6-keto $PGF_{1\alpha}$ ratio by oleic acid leads to a dysfunction of pulmonary circulation in oleic acid-induced lung injury (Stephenson et al 1992). Therefore, the effect of ozagrel against the lung injury may be partly due to the correction of such an imbalance. This effect may be beneficial and unique in TXA_2 synthase inhibitors in ARDS/ALI, compared with other drug candidates including non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids, because they can not improve the TXB_2/6-keto $PGF_{1\alpha}$ ratio. In fact, NSAIDs and glucocorticoids have shown no beneficial effects against ARDS/ALI (Kopp et al 2002).

The inhibition of TXA_2 synthase might increase COX-derived endoperoxides, such as PGH_2, which has a potential as a TXA_2 receptor agonist (Vezza et al 2002). Although little is known about whether COX-derived endoperoxides participate in oleic acid-induced lung injury, it would be interesting to examine the effect of a simultaneously-administered TXA_2-receptor antagonist with ozagrel on the lung injury or the effects of a dual inhibitor of TXA_2 synthase/receptor.

Ketoconazole, an imidazole derivative antifungal agent, is known to inhibit generation of TXA_2. Based on this property, Slotman et al (1988) and Yu & Tomasa (1993) examined the usefulness of ketoconazole as a prophylactic drug against ARDS/ALI. They demonstrated that the drug significantly decreased TXA_2 generation, the incidence of ARDS/ALI and mortality in patients with risk factors of ARDS/ALI, such as sepsis. However, ketoconazole did not

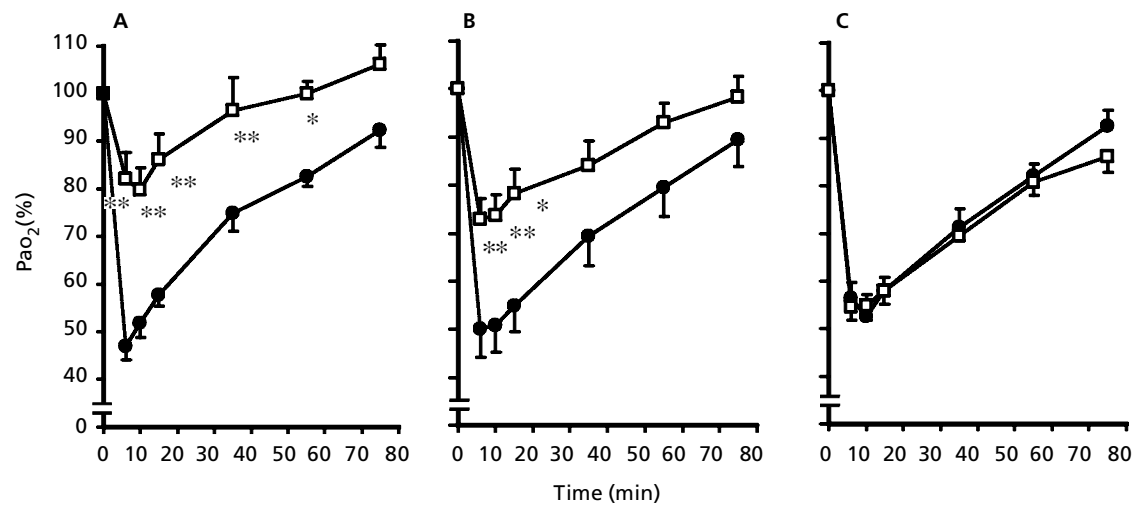

Figure 6 Time-window of the effects of ozagrel on the hypoxaemia induced with oleic acid. Ozagrel was administered 30 min before oleic acid injection (A), administered simultaneously with the oleic acid injection (B) or administered 10 min after oleic acid injection (C). Each point represents mean ± s.e.m. Saline + oleic acid (●), ozagrel 80 mg kg^{-1} + oleic acid (□). Ozagrel administered 30 min before and simultaneously administered with oleic acid significantly reduced the decrease in Pao_2 induced by oleic acid, while ozagrel administered 10 min after the oleic acid injection did not reduce the decrease. *$P < 0.05$, **$P < 0.01$ compared with the vehicle group, n = 6.

inhibit TXA_2 generation and death in the patients whose ARDS/ALI had already progressed (The ARDS Network 2000). The ARDS Network clinical reports suggested that if TXA_2 generation had been blocked during the early stage of ARDS/ALI, some beneficial clinical outcomes could have been observed. In addition, the reports were consistent with our hypothesis that TXA_2 was an important "early phase" mediator.

In this study, ozagrel prevented the increase in pulmonary vascular permeability induced with oleic acid. Some reports suggested that TXA_2 should be one of the mediators which could increase the pulmonary vascular permeability and induce a lung oedema (Lotvall et al 1992; Schulman et al 2002), through an increase in pulmonary arterial pressure and pulmonary vascular hypertension (Schuster 1994). In our previous reports, we described that one of the main mechanisms of the decrease in Pao_2 in our system was an increase in pulmonary vascular permeability (Moriuchi et al 1995a, b). Therefore, the preventive effect of ozagrel on the decrease in Pao_2 by the oleic acid injection, at least in part, may be through the inhibition of the increase in pulmonary vascular permeability.

We showed that ozagrel decreased LDH activity in the BALF, indicating that ozagrel prevented the oleic acid-induced pulmonary cell injury (Lu et al 2002). An increase in pulmonary vascular permeability by oleic acid is known to be associated with the inflammatory mediators such as elastase and superoxides released from inflammatory cells such as neutrophils (Moriuchi et al 1998). TXA_2 may facilitate the neutrophil accumulation at the site of inflammation in the lungs, because TXA_2 has a potential to stimulate the expression of intercellular adhesion molecule-1 (Ishizuka et al 1998) and that of interleukin-8 (Keelan et al 2000). Both of these are important factors for the migration and chemoattractants of neutrophils. Therefore, the cause of the pulmonary cell damage and pulmonary vascular hyperpermeability can be ascribed to the activation of neutrophils which are concerned with TXA_2.

Conclusion

TXA_2 is an important early phase mediator which caused the decrease in Pao_2 induced by oleic acid. We suggest that rapidly-acting TXA_2 synthesis inhibitors, such as ozagrel, would be promising candidates for preventing hypoxaemia and pulmonary vascular hyperpermeability in acute lung injuries that share some common mechanisms with oleic acid-induced lung injury (e.g. ARDS/ALI).

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