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## The Relevance of Leukotriene B<sub>4</sub> to the Development of Acute Lung Injury Induced by Lipopolysaccharide

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Acute lung injury (ALI) induced by lipopolysaccharide (LPS) develops by the activation of leukocytes via various mediators. Leukotriene B<sub>4</sub> (LTB<sub>4</sub>) has a strong effect on activation and migration of leukocytes. We investigated the role of LTB<sub>4</sub> in the chain leading to the development of ALI induced by LPS, by observing how an LTB<sub>4</sub> receptor antagonist, ONO-4057, suppresses or mitigates leukocyte activation and migration. The 36 rabbits used in the experiment were divided into 3 groups: C group (control group of 12 rabbits treated with physiological saline solution only); L group (of 12 rabbits treated with 20 µg/kg LPS) and L-O group (of 12 rabbits treated with, first, 10 mg/kg ONO-4057, then LPS). Blood samples were taken before, 3 h after and 6 h after the injection of drugs; then the rabbits were exsanguinated. The right and left lungs were removed for wet/dry weight ratio and bronchoalveolar lavage fluid (BALF) measurements, respectively. We measured: the leukocyte counts in the peripheral blood, the chemiluminescence (CL) intensity to measure the amount of oxygen free radical species (active oxygen species) production, the LTB<sub>4</sub> concentration in the blood, the complement activity levels (CH<sub>50</sub>), the polymorphonuclear neutrophil elastase (PMN-E) and myeloperoxidase (MPO) levels in BALF, and the wet/dry weight ratio of the right lung. The leukocyte counts in L and L-O rabbits decreased significantly 3 h after LPS injection, then were regained by the 6th h. Regarding CL (with and without zymosan stimulation), there was no significant difference over time for C group. For L group, the zymosan-stimulated CL showed a significant increase at the 6th h, whereas the non-stimulated CL showed significant increases at the 3rd and 6th h. For L-O group, the zymosan-stimulated CL showed a significant increase at the 6th h, whereas the non-stimulated CL increased after 3 h, then slightly decreased after 6 h. The LTB<sub>4</sub> levels showed significant increases at the 6th h for both L and L-O groups. The CH<sub>50</sub> showed significant decreases at 6th h for both L and L-O groups. The MPO activity in the BALF was significantly high for both the L and L-O groups. There was a tendency for a high PMN-E level in the BALF for L group. The mean wet/dry weight ratio of the right lung was significantly high for L group, compared to both C and L-O groups. Although an inhibitory effect on LTB<sub>4</sub> receptors by ONO-4057 failed to prevent leukocyte migration, it successfully suppressed the activity of non-stimulated CL, MPO and PMN-E, and, as a result, prevented the wet/dry weight ratio from increasing.

**Key words:** ALI; LPS; LTB<sub>4</sub>; LTB<sub>4</sub> receptor antagonist

Acute lung injury (ALI) or acute respiratory distress syndrome (ARDS) is caused, to a large extent, by infections or endotoxemia by the endotoxin, lipopolysaccharide (LPS) (Knaus et al., 1994). Finding possible treatments for them is a subject of paramount clinical importance. LPS

stimulates macrophages, resulting in production of humoral factors such as tumor necrosis factor (TNF), interleukin-1 (IL-1), interleukin-8 (IL-8), leukotriene B<sub>4</sub> (LTB<sub>4</sub>) and so on (Said and Hussein, 1989). These factors further stimulate lymphocytic activity, resulting in the production

of various mediators, which then form networks of their own by interacting with one another (Schlag and Redl, 1996). These mediator networks further aggravate inflammations, which cause activation of neutrophils. Also, LPS directly activates alternative complement pathways (Said and Hussein, 1989). The neutrophils activated by LPS accumulate in the lungs (Pawe et al., 1982), then produce polymorphonuclear neutrophil elastase (PMN-E) and oxygen free radical species via myeloperoxidase (MPO) activity (Idell et al., 1985; Said and Hussein, 1989). Neutrophils normally act in defense of the body but, if activated excessively, migrate into the interstitium and alveoli and cause tissue damage, and ultimately ALI. In order to prevent or minimize the ill effects of neutrophils, therefore, the chain of neutrophil activation (described above) must be broken (Fisher et al., 1994). Among the mediators, the complement C5a, TNF- $\alpha$ , IL-1, IL-8, platelet activating factor (PAF), LTB<sub>4</sub>, etc. are considered to be the major causes of ALI because of their strong effect on neutrophil activation and migration (Van Zee et al., 1991; Vandermeer et al., 1995; Belcastro et al., 1996). However, the role played by LTB<sub>4</sub>, which is a metabolic product of arachidonic acid and 5-lipoxygenase, in LPS-induced ALI (Palmlblad et al., 1981; Yoshimura et al., 1994) has not been adequately studied so far. ONO-4057 was developed as an LTB<sub>4</sub> receptor antagonist. In vitro and in vivo, ONO-4057 has been shown to be effective in inhibiting leukocyte migration and degranulation; and in dermatitis experiments, it was shown to have an inhibitory effect on MPO activity (Kishikawa et al., 1992).

In this paper, we report on the results of our experiment designed to investigate the role played by LTB<sub>4</sub> in the chain leading to the development of ALI triggered by LPS, by observing how an LTB<sub>4</sub> receptor antagonist, ONO-4057, suppresses or mitigates leukocyte activation and migration.

## Materials and Methods

The animal experiment reported here was done in conformity with the ethical guidelines per-

taining to animal experiments, observed by the Faculty of Medicine, Tottori University. We used 36 rabbits (Japanese White rabbits; female; range of weight, 1.7-2.9 kg), which were randomly divided into 3 groups: C group (control group of 12 rabbits to be treated with physiological saline solution only); L group (of which 12 rabbits were to be injected with LPS) and L-O group (of which 12 rabbits were to be treated with ONO-405 first, and then LPS). Initially, a sample of 7 mL of blood was taken from the ear vein of each rabbit in the 3 groups before the administration of the drugs. The C group rabbits, then, were given 12 mL of physiological saline solution. The L group rabbits were given 7 mL of physiological saline solution and 5 mL of LPS solution (*E.coli* B8., Difco, Detroit, MI; LPS 20  $\mu$ g per each kg of rabbit weight,  $\mu$ g/kg, dissolved into physiological saline solution). The L-O group rabbits were given, first, 5 mL of ONO-4057 (10 mg/kg) solution (20 mg/mL, dissolved into 7% NaHCO<sub>3</sub>, to which physiological saline solution was added to make the total to 5 mL); then, they were given 2 mL of physiological saline solution for 2 min intravenously; and finally, they were given 5 mL of LPS solution (20  $\mu$ g/kg) through the ear vein. We let the rabbits free in the cages until the next blood sampling time. Three h after the initial drug administration, another sample of 2 mL of blood was taken from the ear vein; then the rabbits were given 2 mL of physiological saline solution intravenously. We let them free again. Six hours after the LPS injection, a sample of 7 mL of blood was taken from the ear vein. The rabbits, then, were given 20 mg/kg of pentobarbital intravenously, and were anesthetized by 50 mg/kg of ketamine via muscle injection. Then we initiated tracheotomy on the rabbits locally anesthetized with 1% lidocaine. Breathing was controlled by inserting a tube endotracheally and using a ventilator for small animals (Model 681, Harvard Apparatus, South Natic, MA) set at 20 respirations/min, 6 mL of room air per kg body weight, and 2 cm H<sub>2</sub>O of positive end-expiratory pressure. The sternum (locally anesthetized with 1% lidocaine) was cut in the middle and, with the thorax open, the pericardium was opened,

and the right ventricle was incised for exsanguination. The heart and the lungs (with the respiration tube inserted) were taken outside the body as a unit. The right hilum was ligated, and the right lung was removed for wet/dry weight measurement. Twenty milliliters of physiological saline solution (5 mL over 4 times) were infused into the left lung through the main trachea; then a sample of 12–15 mL of bronchoalveolar lavage fluid (BALF) was taken from the left lung.

The measurements were the leukocyte counts in the peripheral blood; the chemiluminescence (CL)/leukocyte ( $n = 6$  with zymosan stimulation and  $n = 6$  without, for each group) to measure the amount of oxygen free radical species production; the LTB<sub>4</sub> levels ( $n = 8$  for C group;  $n = 9$  for L group and  $n = 9$  for L-O group); the complement activity levels (CH<sub>50</sub>); the PMN-E and MPO activity levels, from the supernatants of the BALF, centrifuged at 1000 rpm, 10 min. The leukocytes were counted by Celltac (MEK-5158, Nihon Kohden, Tokyo, Japan). For CL, a Luminescence Reader (BLR-201, ALOKA, Tokyo) was used to measure the 50-min luminescence of the 0.1 mL of heparin-added blood in 1.2 mL of luminol-HEPES buffer solution (14 mM HEPES, pH 7.4, containing

66 mM of luminol), stirred at 37°C. Zymosan-stimulated CL was measured when 0.1 mL of 40 mg/mL non-opsonized zymosan (zymosan A: Sigma Chemical Co., St. Louis, MO) was added; and non-zymosan-stimulated CL, without it. LTB<sub>4</sub> was measured by the Radio Immuno Assay method (Mitsubishi Chemical BCL, Tokyo). Complement activity was measured by the CH<sub>50</sub> method (SRL Inc., Tokyo). PMN-E activity in the BALF (0.5 mL) was measured by the hydrolysis method of Yoshimura and co-workers (1994): the hydrolysis of the substrate, 250 μM of L-hydroglutamyl-L-propyl-L-valin-*p*-nitroanilide (Dai-ichi Chemicals, Tokyo), was measured by a Multiskan MS (Labsystems, Basingstroke, Helsinki, Finland) at 405 nm, following the incubation with a Tris buffer (25 mM, pH 8.3) at 37°C for 4 h. MPO activity in the BALF (0.5 mL) was measured by the methods described previously (Henson et al., 1978; Belcastro et al., 1996): The absorption density of *o*-dianisidine (96 μg/mL) was measured by the Multiskan MS at 480 nm (extinction coefficient:  $1.13 \times 10^4/M$ ), following incubation with phosphorous buffer (38 mM, pH 6.2) and 0.0038% H<sub>2</sub>O<sub>2</sub> at 37°C for 15 min.

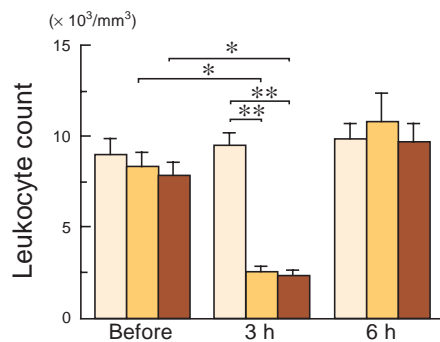
The measured values are expressed as mean and SEM. The statistical analysis was performed by an analysis of variance, followed by Scheffé's test (for differences among groups) and a paired Student's *t*-test (for differences within groups). A *P* value of less than 0.05 was considered statistically significant.

## Results

There was no statistically significant difference in the weights of the rabbits among all groups.

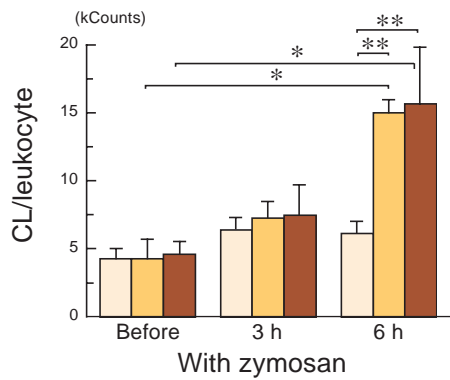
There was no significant difference over time in leukocyte counts for C group, whereas the leukocyte counts for L and L-O groups decreased significantly after 3 h, but they regained after 6 h (Fig. 1).

Regarding CL (with and without zymosan stimulation), there was no significant difference

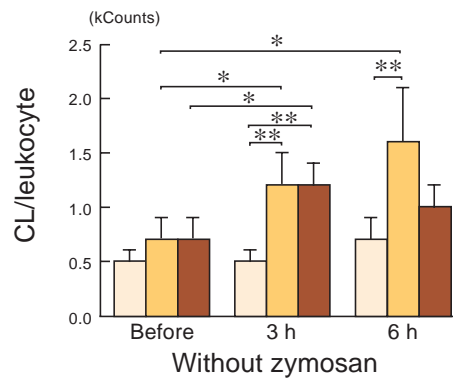


**Fig. 1.** Changes in leukocyte counts for the 3 groups ( $n = 12$ , mean  $\pm$  SEM, for each group). \*Versus the previous counts of the same group ( $P < 0.05$ ); \*\*versus the counts in the control group at each time ( $P < 0.05$ ). □, C group (control group); ■, L group (20 μg/kg LPS); ■, L-O group (20 μg/kg LPS and 10 mg/kg ONO-4057).

Abbreviations: ALI, acute lung injury; ARDS, acute respiratory distress syndrome; BALF, bronchoalveolar lavage fluid; CH<sub>50</sub>, complement activity levels; CL, chemiluminescence; IL, interleukin; LPS, lipopolysaccharide; LTB<sub>4</sub>, leukotriene B<sub>4</sub>; MPO, myeloperoxidase; PAF, platelet activating factor; PMN-E, polymorphonuclear neutrophil elastase; TNF, tumor necrosis factor



**Fig. 2.** Changes in chemiluminescence (CL) intensity with zymosan stimulation for the 3 groups ( $n = 6$ , mean  $\pm$  SEM, for each group). \*Versus the previous intensity of the same group ( $P < 0.05$ ); \*\*versus the intensity of the control group at each time ( $P < 0.05$ ). □, C group (control group); ■, L group (20  $\mu$ g/kg LPS); ■, L-O group (20  $\mu$ g/kg LPS and 10 mg/kg ONO-4057).

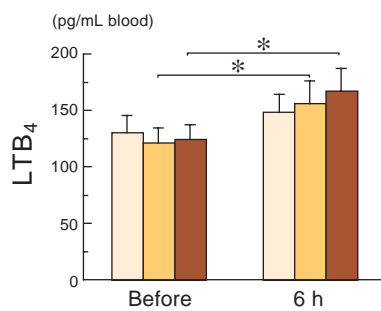


**Fig. 3.** Changes in chemiluminescence (CL) intensity without zymosan stimulation for the 3 groups ( $n = 6$ , mean  $\pm$  SEM, for each group). \*Versus the previous intensity of the same group ( $P < 0.05$ ); \*\*versus the intensity of the control group at each time ( $P < 0.05$ ). □, C group (control group); ■, L group (20  $\mu$ g/kg LPS); ■, L-O group (20  $\mu$ g/kg LPS and 10 mg/kg ONO-4057).

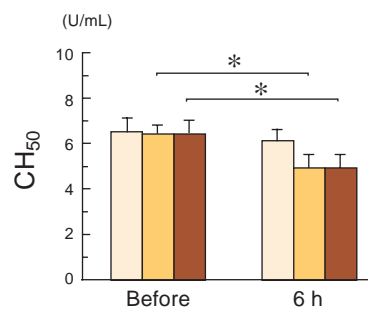
over time for C group. The zymosan-stimulated CL for L group showed a significant increase from  $4.214 \pm 1.397$  kCounts/leukocyte count (before LPS injection) to  $14.952 \pm 0.965$  (6 h after), whereas the non-stimulated CL showed a significant increase from  $0.728 \pm 0.210$  (before LPS injection) to  $1.097 \pm 0.320$  (3 h after), then to  $1.554 \pm 0.467$  (6 h after) (Figs. 2 and 3). Similarly, the zymosan-stimulated CL for L-O group showed a significant increase from  $4.556 \pm 0.934$  (before LPS infusion) to  $15.648 \pm 4.168$  (6 h after) (Fig. 2), whereas the non-stimulated CL increased from  $0.732 \pm 0.234$  (before LPS injection) to  $1.170 \pm 0.245$  (after 3 h), but it de-

creased to  $0.963 \pm 0.235$  (after 6 h) (Fig. 3). The intergroup comparisons show that the zymosan-stimulated CL for L and L-O groups (6 h after LPS injection) was significantly higher than that for C group, whereas the non-stimulated CL for L group (3 and 6 h after) was significantly higher than that for C group, and the non-stimulated CL for L-O group (3 h after) was significantly higher than that for C group (Figs. 2 and 3).

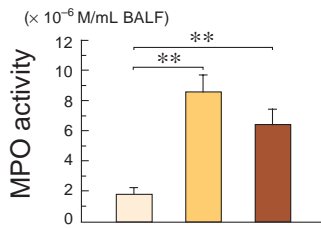
While there was no significant difference in  $LTB_4$  (before and 6 h after LPS injection) among all groups, the  $LTB_4$  for L and L-O groups showed significant increases from



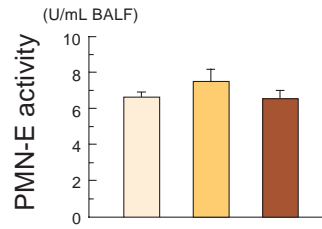
**Fig. 4.** Changes in leukotriene B<sub>4</sub> ( $LTB_4$ ) concentration for the 3 groups ( $n = 12$ , mean  $\pm$  SEM, for each group). \*Versus the previous concentration of the same group ( $P < 0.05$ ). □, C group (control group); ■, L group (20  $\mu$ g/kg LPS); ■, L-O group (20  $\mu$ g/kg LPS and 10 mg/kg ONO-4057).



**Fig. 5.** Changes in complement activity for the 3 groups ( $n = 12$ , mean  $\pm$  SEM, for each group). \*Versus the previous activity of the same group ( $P < 0.05$ ). □, C group (control group); ■, L group (20  $\mu$ g/kg LPS); ■, L-O group (20  $\mu$ g/kg LPS and 10 mg/kg ONO-4057).



**Fig. 6.** Myeloperoxidase (MPO) activity of bronchoalveolar lavage fluid (BALF) for the 3 groups ( $n = 12$ , mean  $\pm$  SEM, for each group). \*\*Versus the control group ( $P < 0.05$ ). □, C group (control group); ■, L group (20 µg/kg LPS); ■, L-O group (20 µg/kg LPS and 10 mg/kg ONO-4057).



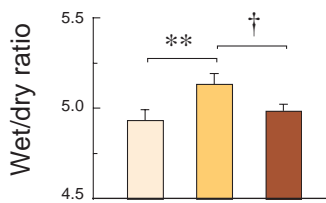
**Fig. 7.** Polymorphonuclear neutrophil elastase (PMN-E) activity of bronchoalveolar lavage fluid (BALF) for the 3 groups ( $n = 12$ , mean  $\pm$  SEM, for each group). □, C group (control group); ■, L group (20 µg/kg LPS); ■, L-O group (20 µg/kg LPS and 10 mg/kg ONO-4057).

121.12  $\pm$  12.91 and 123.91  $\pm$  12.88 pg/mL blood (before LPS injection) to 155.86  $\pm$  19.89 and 166.89  $\pm$  20.11 (6 h after), respectively (Fig. 4).

Regarding the CH<sub>50</sub> for C group, there was no significant difference between the time before physiological saline solution was given (6.5  $\pm$  0.6 U/mL) and the time 6 h later (6.1  $\pm$  0.5), whereas for L and L-O groups, CH<sub>50</sub> showed significant decreases from 6.4  $\pm$  0.4 and 6.4  $\pm$  0.6 (before LPS injection) to 4.9  $\pm$  0.6 and 5.0  $\pm$  0.8 (6 h after), respectively (Fig. 5).

The MPO level in the BALF for C group was 1.77  $\pm$  1.50  $\times 10^{-6}$  M/mL BALF, whereas those for L and L-O groups were significantly higher at 8.54  $\pm$  1.11 and 6.38  $\pm$  1.03, respectively; and the MPO of L-O group tended to be lower ( $P = 0.17$ ) than that for L group (Fig. 6).

Although there was no significant difference in PMN-E in the BALF among groups, the PMN-E for L group tended to be higher (7.22  $\pm$  2.36 U/mL BALF) in contrast with those for C group (6.85  $\pm$  0.98,  $P = 0.26$ ) and L-O group (6.76  $\pm$  1.63,  $P = 0.25$ ) (Fig. 7).



**Fig. 8.** Wet/dry ratios of the 3 groups ( $n = 12$ , mean  $\pm$  SEM, for each group). \*\*Versus the control group ( $P < 0.05$ ). †Versus the L-O group ( $P < 0.05$ ). □, C group (control group); ■, L group (20 µg/kg LPS); ■, L-O group (20 µg/kg LPS and 10 mg/kg ONO-4057).

The mean wet/dry weight ratio for L group was significantly higher (5.13  $\pm$  0.06) than those for C group (4.93  $\pm$  0.06) and L-O group (4.98  $\pm$  0.04) (Fig. 8).

## Discussion

Our experiment showed that: i) ONO-4057 did not block leukocyte migration caused by LPS; ii) while ONO-4057 did not have an effect on the zymosan-stimulated CL of leukocytes (6 h after LPS injection), it inhibited the non-stimulated CL; iii) there was a tendency for ONO-4057 to inhibit PMN-E and MPO activities in the BALF; and iv) ONO-4057 successfully inhibited the increase in wet/dry weight ratio.

The experiments using guinea pigs by Kishikawa and coworkers (1992) showed that ONO-4057 inhibited the LTB<sub>4</sub>-induced leukopenia in the peripheral blood in a dose-dependent manner, effective when 10 mg/kg or more were ingested and when 0.3 mg/kg or more were given intravenously. In our experiment, the rabbits received intravenous injection of 10 mg/kg of ONO-4057 2 min before LPS was injected intravenously. The reason for our decision to use 10 mg/kg of ONO-4057 this time is because there were 3 rabbits which were given 5 mg/kg of ONO-4057, but which did not show any effect of ONO-4057 to inhibit the LPS-induced decrease in leukocyte count; and the rabbits which were only given 10 mg/kg of ONO-4057 did not die nor did they show any signs of abnormality such as weakness or a lowering of their activity level. From these observations,



we decided to try 10 mg/kg of ONO-4057, expecting sure signs of the effectiveness of ONO-4057.

We decided on the dosage of LPS to be 20 µg/kg, which was the dosage inferred from our preparatory experiments, to be maximally effective without adversely affecting the design of our experiment. We also decided that our observation time should be 6 h, on the basis of the significant results of other experiments in which observation time was 6 h, to measure the effectiveness of certain drugs in inhibiting the activation of neutrophil elastase (Kubo et al., 1994; Nishina et al., 1997), and by taking into consideration the time it takes for various kinds of cytokines to be produced (Sakamoto et al., 1994; Salvemini et al., 1995; Nishina et al., 1997).

It has been reported that the concentration level of LTB<sub>4</sub> after LPS injection is inferred to have 2 phases of change: the peak of LTB<sub>4</sub> at an earlier stage (1 h after) is induced by macrophages in the blood, whereas the LTB<sub>4</sub> peak at a later stage (4 h after) is due to the leukocytes accumulated in the lungs (Kobayashi et al., 1993). In our experiment, the LTB<sub>4</sub> concentration levels for L and L-O groups showed increases at 6 h, thus not showing the effectiveness of ONO-4057 in inhibiting the production of LPS-induced LTB<sub>4</sub>. We speculate that this was due to the fact that although ONO-4057 might have had an effect on LTB<sub>4</sub> receptors, neutrophils could still have produced LTB<sub>4</sub> by other means (such as via complements, PAF, concentrated IgG, TNF, etc.) (Fogh et al., 1992).

It has been reported that with the rabbits injected with LPS intravenously, there was an accumulation of leukocytes in the lungs and liver (after 2 h) and decreases in the leukocyte count in the peripheral blood, the bone marrow, and the spleen (Toft et al., 1994). From our preparatory experiments we knew that the injection of LPS into rabbits decreased their leukocyte count in the peripheral blood after 5 min; this proved to be true for the L group in our present experiment. Also, the leukocyte count even for L-O group, like L group, significantly decreased 3 h after LPS injection, and ONO-4057 failed to inhibit leukocyte migration.

The amount of complement activity for L

and L-O groups significantly decreased 6 h after LPS injection. We conjecture that this was due to the fact that the leukocytes accumulated in the liver as well as in the lungs, and damaged the liver; and the production of complements in the liver could not catch up with the consumption of LPS-induced complements (Arai et al., 1989).

The non-stimulated CL intensity for L-O group (as well as that for L group) showed a high value at 3 h; but it became lower after 6 h. This, together with the fact that the LTB<sub>4</sub> for L-O group (as well as that for L group) was high at 6 h, indicates that there was a factor other than LTB<sub>4</sub> that contributed to the production of an oxygen free radical species. LPS, directly and indirectly, stimulates and produces various mediators and cytokines. It also stimulates C3 directly, and activates alternative complement pathways (Said and Hussein, 1989). We think that the higher non-stimulated CL intensity levels of L and L-O groups, compared to the level of C group, at 3 h, were due to the fact that complements were directly stimulated by LPS and the neutrophil activity increased because of other mediators and cytokines.

Zymosan activates complement components (after C3) via properdin-related factors. The zymosan-stimulated CL intensity levels for all groups before treatments and C group at 3 and 6 h were about 10 times higher than their non-stimulated CL counterparts. This can be explained by saying that the complements in the blood reacted to zymosan.

The zymosan-stimulated CL intensity levels for L and L-O groups were significantly high at 6 h, and the non-stimulated CL level of L-O group decreased after 6 h. This can be explained in terms of the Second Attack Theory (Ogawa, 1996). According to the theory, we can say that the LPS in L and L-O groups heightened the responsiveness of the neutrophils, which zymosan further stimulated as a second attack, resulting in more production of oxygen free radical species and, consequently, an increase in CL. The amount of oxygen free radical species production in the peripheral blood in L-O group, when no additional zymosan-induced stimulation was present, can be known

from the amount of oxygen free radical species production without zymosan stimulation at 6 h. We can attribute to the effectiveness of ONO-4057 the fact that the production capacity of oxygen free radical species from leukocytes in the peripheral blood is lower for L-O group than for L group. From these facts, we inferred that ONO-4057 had an inhibitory effect on LTB<sub>4</sub> receptor activity and, thus, helped lower the production of oxygen free radical species from neutrophils; but ONO-4057 failed to control the LPS-heightened responsiveness of neutrophils.

The levels of the MPO and PMN-E for L-O group tended to be lower than those for L group, indicating a weak positive effect of ONO-4057. The amount of oxygen free radical species increases and decreases, depending on whether PMN-E is present or not, respectively (Kusner and King, 1989). Also, one of the PMN-E inhibitors,  $\alpha$ 1-protease inhibitor ( $\alpha$ 1-PI), becomes inactivated in the presence of oxygen free radical species (Tanaka et al., 1991). Thus, oxygen free radical species and PMN-E influence each other. The wet/dry weight ratio indicates an increase in vascular permeability (Suzuki et al., 1994) due to the damage done to endothelial cells (Tate and Repine, 1983) jointly by oxygen free radical species and PMN-E. The results of our experiment indicate that ONO-4057 must have had an inhibitory effect (up to 6 h) on the LPS-induced increase in vascular permeability in the lungs.

From the above discussion, we can say that ONO-4057 was effective against lung injuries caused by LPS, but with a few limitations. First, the intravenous injection of ONO-4057 caused our rabbits to show signs of pain, and death in some cases with LPS after ONO-4057 injection. We used ONO-4057 diluted in physiological saline solution and injected it slowly, but there is a possibility that the pain caused by ONO-4057 injection might have led to the production of various mediators, cytokines, etc. Also, while LPS 20  $\mu$ g/kg alone did not kill any rabbits, pretreatment with ONO-4057 killed 3 out of 15 rabbits within 1.5 h to 2 h. However, the rabbits given LPS alone were weak, suffering from diarrhea, whereas the rabbits which

were pretreated with ONO-4057 and which survived 6 h until the end of the experiment, exhibited no sign of diarrhea or any abnormality. We know that the lethal dose of ONO-4057 given intravenously for rats is 300 mg/kg\*; but we lack similar data pertaining to rabbits. It might be that for rabbits, the 50% effective dose of ONO-4057 is very close to the 50% lethal dose. Also, this might indicate the complexity of mediator networks. We speculate that in our dead rabbit cases, the antagonistic effect of ONO-4057 was so strong on LTB<sub>4</sub> receptors that LTB<sub>4</sub>, which is necessary for normal physiological functioning, was inhibited, and consequently, parts of the mediator networks were blocked, which caused a malfunction in homeostasis in those dead rabbits.

### Conclusions

Although ONO-4057, as an LTB<sub>4</sub> receptor antagonist, did not prevent leukocyte migration caused by LPS, it tended to control the non-stimulated CL level of leukocytes in the peripheral blood, and suppressed the MPO and PMN-E activity in the BALF, as a result, preventing the wet/dry ratio from increasing. From these results, we conclude that LTB<sub>4</sub> must be playing a role in LPS-caused lung injuries. However, our experiment also suggests the possibility that an inhibitory effect on LTB<sub>4</sub> receptors might adversely lead to the suppression of the natural protective mechanism by LTB<sub>4</sub> against inflammation, and might further lead to the breakdown of mediator networks.

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