Comparison Study between the Mechanisms of Allergic Asthma Amelioration by a Cysteinyl-Leukotriene Type 1 Receptor Antagonist Montelukast and Methylprednisolone

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ABSTRACT

We investigated the effects of cysteinyl-leukotriene (cysLT) type 1 receptor antagonist montelukast (MK) and compared them with those of methylprednisolone (MP) in an allergic asthma model. Rats sensitized to ovalbumin (OVA) received repeated intratracheal exposure to OVA for up to 3 consecutive days. Pretreatment with MK or MP before OVA exposure inhibited late airway response (LAR) and reduced cellular infiltration into the bronchial submucosa after the triple OVA. The amount of N-acetyl-leukotriene E$_4$ in the bile was significantly reduced by pretreatment with MK or MP, suggesting that both drugs reduced the production of cysLTs in the lungs. In the in vitro study, when the fragments of lungs that had been repeatedly pretreated with MK or MP and exposed to OVA were removed and incubated with OVA, the coaddition of either drug significantly reduced cysLT production. In contrast, the cysLT production following the addition of OVA to the lung fragments that had not received in vivo pretreatment with either drug was inhibited by MK but not by MP. These results indicate that MK and MP inhibit LAR by suppressing the infiltration of inflammatory cells into the bronchial submucosa, thereby inhibiting the production of cysLTs in the lungs, and that MK but not MP may inhibit cysLT production directly. The different effects on cysLT production between the two drugs may provide a rationale for the use of combination therapy with cysLT$_1$ receptor antagonists and steroids for the treatment of asthma.

Asthma, one of the most prevalent disorders among industrialized nations, is characterized by reversible bronchoconstriction, increased mucous secretion, and complex airway inflammation (Busse and Rosenwasser, 2003). Inhalation of a specific antigen in allergic subjects usually results in dual responses, an immediate airway response (IAR) and a late airway response (LAR) (Nagy et al., 1982). The mechanisms for LAR are considered to be causally related to the infiltration of eosinophils and other inflammatory cells into the bronchial submucosa following the IAR (Bousquet et al., 1990). Recent basic and clinical studies indicate that cysteinyl-leukotrienes (cysLTs) play an important role in both responses of bronchial asthma (Smith, 1996) via the following effects on the airway system: induction of profound bronchoconstriction (Dahlen et al., 1980), enhancement of vascular leakage (Dahlen et al., 1981), enhancement of mucous secretion in the bronchi (Coles et al., 1983), and induction of chemotactic activity of eosinophils (Laitinen et al., 1993; Henderson et al., 1996). The cellular origins of cysLTs in the lungs are considered to be mast cells, eosinophils, basophils, monocytes-macrophages, and cell-cell interactions, such as those between neutrophils and platelets (Samuelsson et al., 1987; Maclouf and Murphy, 1988). Several new drugs known as “leukotriene modifiers” have been developed to modulate the actions of cysLTs (Busse, 1998; Drazen et al., 1999). Namely, cysLT type1 receptor antagonists (cysLT$_1$RAs) and 5-lipoxygenase inhibitors block the effects of cysLTs on airway tissue and decrease the generation of cysLTs, respectively. On the basis of clinical studies, cysLT$_1$RAs have been shown to be as effective at reducing asthma symptoms (Reiss et al., 1997) and inflammatory cell infiltration into the bronchial submucosa as 5-lipoxygenase inhibitors (Nakamura et al., 1998). The cysLT$_1$RA has been shown to inhibit airway eosinophilia, hyper-responsiveness, and microvascular leak-

ABBREVIATIONS: IAR, immediate airway response; LAR, late airway response; cysLT, cysteinyl-leukotriene; cysLT$_1$RA, cysteinyl-leukotriene type 1 receptor antagonist; MK, montelukast; A-LTE$_4$, N-acetyl-LTE$_4$; MP, methylprednisolone; EIA, enzyme immunoassay; OVA, ovalbumin; HPLC, high-performance liquid chromatography; BALF, bronchoalveolar lavage fluid.
Molecular conditions and methods. All experimental protocols were approved by the institutional animal care and use committee of the School of Medicine, Fukuoka University. The cysLT1RA (MK sodium) was donated by Merck & Co., Inc. (Rahway, NJ). MP sodium succinate and Bordetella pertussis vaccine (50 μl) containing 6 × 109 heat-killed bacilli was given intraperitoneally as an adjuvant. Three days later, sterile normal saline (1 ml) containing 1 mg of OVA and 200 mg of aluminum hydroxide was subcutaneously injected for a booster effect. All animals selected for these studies were used from 14 to 28 days after the first injection.

Evaluation of the Effects of MK and MP. Sensitized rats were divided into groups by the number of OVA exposures and the different schedules of drug administration, as shown in Fig. 1. With respect to the number of OVA exposures, the sensitized rats were challenged daily by inhalation of OVA aerosol for two successive days (days 1 and 2) in the triple OVA exposure experiment. For this purpose, the inhalation of 0.25% OVA aerosol was accomplished by placing the rats for 20 min on each occasion in a 10-L Plexiglas chamber connected to an ultrasonic nebulizer known as the “Comfort-mini” (model-10; Sin-Ei Industries, Inc., Ageo, Japan). The next day (day 3), the final OVA challenge was performed by i.t. administration of 0.1 ml of a 1.7% OVA solution, as shown in Fig. 1, A and B. Ovalbumin grade V (Sigma-Aldrich) was used for OVA exposure (Abe et al., 2001). In the double OVA exposure, the sensitized rats were challenged by inhalation of OVA aerosol only for 1 day, and the final OVA challenge was performed by i.t. on the next day (OVA day 2). In the single OVA exposure, the rats were challenged by i.t. administration without any previous inhalation of OVA aerosol (Fig. 1C). The control indicates the OVA-sensitized rats received triple administration of saline. Administration of MK or MP was performed according to two different regimens on OVA Day 3; in one schedule, the drug was administered before every OVA exposure (triple pretreatment), whereas in the other schedule, the drug was administered only before the third OVA exposure (single pretreatment), as shown in Fig. 1, A and B, respectively. MK was dissolved in sterile saline, and the rats received the drugs gastrically at a rate of 10 mg/kg 1 h before the start of the i.t. OVA challenge. MP was dissolved in the dissolving solution supplied by the manufacturer (Pfizer Puurs) and injected into the rats intramuscularly at a rate of 10 mg/kg 1 h before the start of the i.t. challenge.

Measurement of Pulmonary Resistance ($R_L$). The rats were anesthetized by i.p. injection with urethane (1 g/kg, 25% (w/v)). The tip of the tracheal tube (a 5-cm length of PE-240 polyethylene tubing) was inserted into the trachea through an open tracheostomy. The next chamber connected to an ultrasonic nebulizer known as the “Comfort-mini” (model-10; Sin-Ei Industries, Inc., Ageo, Japan). The transpulmonary pressure was determined by monitoring the difference between the pressure in the external end of the tracheal cannula and esophageal cannula using a Statham DP-45 differential transducer (Validyne Engineering Corp., Northridge, CA). The intrapleural pressure was measured through a water-filled cannula (PE-240) that was placed in the lower third of the esophagus and connected to a Validyne transducer (Validyne Engineering Corp., Northridge, CA). The transpulmonary pressure was determined by monitoring the difference between the pressure in the external end of the tracheal cannula and esophageal cannula using a Statham DP-45 differential transducer (Validyne Engineering Corp., Northridge, CA). The intrapleural pressure was measured through a water-filled cannula (PE-240) that was placed in the lower third of the esophagus and connected to a Validyne transducer (Validyne Engineering Corp., Northridge, CA). The transpulmonary pressure was determined by monitoring the difference between the pressure in the external end of the tracheal cannula and esophageal cannula using a Statham DP-45 differential transducer (Validyne Engineering Corp., Northridge, CA). The intrapleural pressure was measured through a water-filled cannula (PE-240) that was placed in the lower third of the esophagus and connected to a Validyne transducer (Validyne Engineering Corp., Northridge, CA).
connected to one port of a DP-45 differential pressure transducer (Validyne Engineering Corp.). A Fleisch pneumotachograph and a differential transducer were used to monitor the respiratory flow rate (PULMOS-II system; MIPS, Osaka, Japan). \( R_L \) was estimated under artificial ventilation with a Harvard Apparatus Rodent Respirator (Millis, Bedford, MA) at a respiration rate of 70 breaths/min and a tidal volume of 3.5 ml (Abe et al., 2001). The \( R_L \) was measured before the challenge (baseline value). After challenge with OVA, the \( R_L \) was measured at 1, 5, 10, 15, 30, 45, and 60 min, and thereafter, \( R_L \) was examined every 30 min for 6 h.

### Histological and Cytological Examination

Six hours after the i.t. administration of OVA, the rats were exsanguinated by cutting the abdominal aorta. The trachea was joined to a tube with a three-way stopcock connected to a reservoir containing the fixative. The lungs were fixed in situ by the i.t. administration of 8% formaldehyde solution given at a pressure of 15 cm of H\(_2\)O. The lungs were then stained with hematoxylin-eosin to assess the degree of inflammation.

Bronchoalveolar lavage was performed via the tracheal cannula using 2 \( \times \) 10 ml of saline containing 1 mM EDTA. The bronchoalveolar lavage fluid (BALF) was centrifuged at 300g for 5 min at 4°C, and the cell pellet was resuspended in 1.0 ml of sterile saline with 0.2% rat serum. The total cell count was determined by adding 50 μl and the cell pellet was resuspended in 1.0 ml of sterile saline with the addition of OVA solution (100 μg/ml; Sigma) for 5 min at 37°C and further incubated for 30 min at 37°C after the addition of OVA solution (100 μg/ml; Sigma). For incubation in the controls, saline was added instead of OVA. After terminating the reaction by the addition of cold ethanol, LTB\(_4\) was partially purified through a Sep-Pak cartridge (Waters Corporation). After evaporation of methanol eluates under reduced pressure and resuspension with HPLC, solvent A, LTB\(_4\), LTD\(_4\), and LTE\(_4\) were separated with the Novapak C18 column and each fraction was collected. LTC\(_4\), and LTD\(_4\) fractions were assayed using a cysLT-EIA kit (Cayman Chemical, Ann Arbor, MI), and the LTE\(_4\) fraction was assayed using an LTE\(_4\)-EIA kit (Cayman Chemical). The sum of the amounts of LTC\(_4\), LTD\(_4\), and LTE\(_4\) was considered the cysLT amount.

### HPLC

The HPLC system consisted of a model 600 controller, a 717 autosampler (Waters Corporation), and the Novapak C18 column (Waters Corporation, Milford, MA). We used solvent A [acetonitrile/methanol/water acetic acid, 30:12:58.03 (v/v/v)] containing 0.03% EDTA-free acid (Dojindo, Kumamoto, Japan) and solvent B, which consisted of acetonitrile/methanol/water/acidic acid [68:12:20:0.01 (v/v/v)] containing 0.001% EDTA. All solvents were adjusted to pH 5.6 with ammonia solution (Nacalai Tesque, Kyoto, Japan). The mobile phase began with solvent A and then was changed to solvent B at 20 min. The flow rate was 1 ml/min. The retention times for LTC\(_4\), A-LTE\(_4\), LTD\(_4\), and LTE\(_4\) were approximately 4.2, 9.1, 13.1, and 15.1 min, respectively.

### Statistical Analysis

Data are reported as the means ± S.E.M. The statistical analysis was performed using the General Linear Models Procedure in Statistical Analysis System. A p value of less than 0.05 was considered to be statistically significant.

### Results

#### Time Course for Changes of \( R_L \)

Figure 2 shows the time course for changes of \( R_L \) after the third OVA challenge. Although control rats given 0.1 ml of saline i.t. did not show any significant changes in \( R_L \) up to 6 h after the challenge, rats that received the triple OVA exposure showed prominent LAR. As shown in Fig. 2A, triple pretreatment with MK or MP significantly suppressed LAR, but the intervention with MP seemed to be more potent than that with MK. The control indicates the OVA-sensitized rats that received triple i.t. administration of saline. Single pretreatment with MK or MP only before the third OVA exposure also significantly inhibited LAR, but the inhibition by either drug was less than that by the triple pretreatment (Fig. 2B).

Table 1 shows the peak height of IAR and LAR after the third OVA exposure with or without pretreatment with MK or MP. Although triple pretreatment with MP significantly suppressed IAR, both single and triple pretreatment with MK tended to suppress IAR, but not significantly. On the other hand, both drugs with either administration schedule significantly suppressed LAR.

#### Cytological Studies in BALF

Cytological studies were performed to examine the changes of total leukocyte number and the recovery of cellular differentiation in BALF and to evaluate the effects of MK and MP on the infiltration of inflammatory cells into airway space. The results are shown in Fig. 3. Alveolar macrophages made up more than 90% of recovered cells in BALF after the i.t. saline challenge (control). The triple OVA exposure resulted in significantly more leukocytes in BALF than in the controls and showed a diathesis toward increase in leukocyte number compared with the double exposure (OVA day 2). Concerning cellular differentiation, eosinophils and neutrophils were the predominant cells, and the lymphocyte number also increased significantly. As shown in Fig. 3, A and B, repeated pretreatment before every OVA exposure with either drug suppressed the accumulation of all types of leukocytes in BALF, but the effect of the single pretreatment only before the third exposure was weaker than that of the triple pretreatment. The single pretreatment with MK or MP did not significantly
inhibit infiltration of eosinophils in the airway space after
the third exposure.

**Histological Studies.** When the rats received double or
triple OVA exposures, histological findings in bronchial tis-
sue were examined at 6 h after the last exposure. As shown
in Fig. 4, A and B, an extremely high infiltration of inflam-
matory cells including eosinophils and neutrophils was rec-
novized in the bronchial submucosa after the third exposure
compared with the double exposure. Although triple pre-
treatment with MK suppressed the infiltration of inflamma-
tory cells into the bronchial submucosa, as shown in Fig. 4C,
that with MP almost completely inhibited the cellular infil-
tration (Fig. 4D). On the other hand, when the other admin-
istration schedule, single pretreatment only before the third
OVA exposure, was used to evaluate the effects of both drugs,
pretreatment with either MK or MP also moderately sup-
pressed the infiltration of inflammatory cells into the bron-
chial submucosa, and these suppressions were less potent
than those by triple pretreatment (Fig. 4, E and F).

**N-Acetyl-LTE₄ Level in Bile.** To examine the time
course of the generation of cysLTs in the lungs, we measured
A-LTE₄ excretion in biliary fluid after the third exposure to
OVA. Saline administration did not change the level of A-
LTE₄ (control). The third OVA challenge resulted in significa-
cantly greater biliary excretion of A-LTE₄ up to 6 h after
challenge. As shown in Fig. 5A, triple pretreatment with MK
or MP significantly reduced A-LTE₄ excretion in biliary fluid,
but pretreatment with MP seemed to be more potent than
that with MK. On the other hand, the single pretreatment
with either drug only before the third challenge also signifi-
cantly reduced A-LTE₄ excretion in bile (Fig. 5B), suggesting
that these two drugs suppressed cysLT production in the
lungs after antigen challenge.

**Relationship between N-Acetyl-LTE₄ Level and Leu-
koocyte Number in BALF.** When the A-LTE₄ level in bile
and leukocyte number in BALF from the individual rats were
plotted, a significant correlation was observed between the
two parameters, as shown in Fig. 6, A and B. The correlation
coefficient for this relationship (OVA day 3 + saline) was
0.849 \((p = 0.0051)\). When rats were pretreated with MK or
MP, the relationship between the A-LTE₄ level in bile and
the leukocyte number in BALF was well correlated under the
single pretreatment regimen (Fig. 6B) but not under the
double pretreatment regimen (Fig. 6A). The correlation coef-
ficients in the former treatment were 0.719 \((p = 0.0266)\) for
the single pretreatment with MK and 0.862 \((p = 0.0092)\) for
that with MP, respectively.
In Vitro Production of cysLTs from Chopped Lung Fragments. To examine the influence of MK or MP on the production of cysLTs in lung tissue after stimulation with the antigen, the chopped-sensitized lung fragments without previous OVA exposure were incubated with OVA in Tyrode's buffer in the presence or absence of either drug at various concentrations for 30 min at 37°C. An approximately 3-fold higher amount of cysLT was produced in the chopped lung fragments supplemented with OVA compared with those supplemented with saline (control), as shown in Fig. 7. When MK was added at various concentrations (1–100 μg/ml), the production of cysLTs was significantly suppressed at the highest concentration (100 μg/ml). In contrast, the addition of MP at 1 to 100 μg/ml seemed to increase the production of cysLTs but not to a significant extent compared with that by OVA alone. The cysLT amount in the presence of MK (100 μg/ml) was significantly lower than that in the presence of MP (100 μg/ml). Next, the influence of MK or MP on cysLT production was evaluated in the lungs that were removed after repeated exposures to the antigen (Fig. 8). The lungs were removed from rats that were sequentially exposed to OVA for the last 2 days without pretreatment of MK or MP (Fig. 8A). The lungs were chopped into small pieces and then incubated with OVA in the buffer with or without the coaddition of either 100 μg/ml MK or 100 μg/ml MP for 30 min at 37°C. Whereas the amount of cysLT from the chopped lung fragments was significantly greater by the addition of OVA than by the addition of saline (control), the coaddition of MK but not MP significantly inhibited...
cysLT production. The cysLT amount in the presence of MK was significantly lower than that in the presence of MP (Fig. 8A). In another trial, rats were daily challenged by the inhalation of OVA aerosol for 2 successive days with or without repeated pretreatment of 10 mg/kg MK or 10 mg/kg MP before every challenge. On the next day, the lungs were removed and chopped into small pieces. When the chopped lung fragments were incubated with the OVA solution for 30 min at 37°C, the coaddition of either 100 μg/ml MK or 100 μg/ml MP significantly inhibited cysLT production (Fig. 8B).

Table 2 summarizes the percent ratios of LTC₄, LTD₄, and LTE₄ in cysLTs produced from each incubation mixture containing the chopped lung fragments. LTC₄ was a major metabolite and occupied 55 to 70% of cysLTs produced by incubation for up to 30 min.

**Discussion**

This study indicates that either cysLT₁R antagonist or steroid suppresses LAR and infiltration of inflammatory cells into the bronchial submucosa following repeated antigen challenge. In a previous study, Henderson et al. (1996) reported that a 5-lipoxygenase inhibitor inhibited the infiltration of eosinophils into the bronchial wall following antigen challenge in a murine asthma model. Equivalent effects have been observed using cysLT₁R antagonists in similar models (Muñoz et al., 1997). It has already been reported that LTE₄ shows chemotactic activity toward eosinophils (Laitinen et al., 1993). In the present study, a cysLT₁R antagonist and a steroid each inhibited the accumulation of inflammatory cells in the bronchial submucosa and airway space in parallel with a decrease of A-LTE₄ excretion into bile. We speculate that...
the decrease in the number of cells accumulated in the lung, especially in the bronchial submucosal tissues, contributed to the decreased excretion of A-LTE₄ into the bile, suggesting a reduction in the generation of cysLTs in the lungs (Powell et al., 1995). In support of this idea, we observed a linear relationship between the number of leukocytes in BALF and the A-LTE₄ levels in bile. Pretreatment with either drug suppressed bronchoconstriction while maintaining the linear relationship between these two parameters. The single pretreatment with MP or MK significantly suppressed A-LTE₄ in the bile but did not inhibit the number of eosinophils in BALF at the A-LTE₄ levels in bile. Pretreatment with either drug suppressed bronchoconstriction while maintaining the linear relationship between these two parameters. The single pretreatment with MP or MK significantly suppressed A-LTE₄ in the bile but did not inhibit the number of eosinophils in BALF at the A-LTE₄ levels in bile. Pretreatment with either drug suppressed bronchoconstriction while maintaining the linear relationship between these two parameters. The single pretreatment with MP or MK significantly suppressed A-LTE₄ in the bile but did not inhibit the number of eosinophils in BALF. These results suggest that the cellular origin of cysLTs may come from macrophages rather than from eosinophils during LAR, as previously reported (Yu et al., 1995). These results may suggest that the suppression in the infiltration of leukocytes into the airway tissues by MP or MK contributes to the reduced production of cysLTs in the lungs. However, whether or not MK and MP directly reduce cysLT production from the sensitized lungs after antigen challenge remained unclear in these in vivo experiments.

To further analyze the mechanisms by which the two drugs reduce the generation of cysLTs, the effects of either drug on cysLT production in the sensitized chopped lungs were evaluated in vitro. The two drugs had different effects on the production of cysLTs induced by incubation with the antigen (see Fig. 7). Although MK reduced cysLT production at the high dose, MP showed a diastase to increase cysLT production from the chopped lungs after antigen challenge. This relationship between the two drugs was also similar in the experiment using chopped lung fragments after repeated antigen exposure (see Fig. 8A). Namely, the cooadition of MK at the high dose reduced cysLT production from the lungs with or without previous OVA exposure following the addition of OVA, but this effect was not observed with MP. This result suggests that MK is able to directly suppress the generation of cysLTs in the lung tissue, but MP is not. On the other hand, when we performed a similar experiment using the lung fragments from rats subjected to repeated OVA exposure and repeated in vivo pretreatment with either drug (see Fig. 8B), both drugs suppressed the in vitro generation of cysLTs after the third OVA exposure, and the suppression of cysLTs by MP was similar to that by MK. Consequently, it is concluded that MP does not directly inhibit cysLT generation from the lung tissue following antigen challenge, but MP is able to suppress cysLT production in the case of repeated treatment through broad anti-inflammatory effects, including inhibition of cellular infiltration into the bronchial submucosa after repeated antigen exposure. We could not propose an explanation of how cysLT production from the chopped lungs was inhibited by MK. On the other hand, Ramírez et al. (2003) reported that montelukast directly inhibited 5-lipoxygenase activity in mast cells at the lower micromolar ranges when stimulated by calcium ionophore A23187. However, this inhibition required cellular integrity, because MK did not inhibit 5-lipoxygenase activity in the homogenates from the cells. The dose of MK (100 μg/ml) used in the in vitro study is much higher than the concentrations required to block CysLT₁R in human lung preparations (Fregonese et al., 2002). However, in our in vivo experiments, MK was administered to rats at 10 mg/kg, which is approximately 50 times more than the usual clinical dosage. Concerning the in vivo dose of MK used in the present animal studies, other groups have used similar doses (10–25 mg/kg) (Wu et al., 2003; Leick-Maldonado et al., 2004). A blood concentration of 100 μg/ml may hardly be achievable after the administration of the in vivo dose at 10 mg/kg. However, this concentration may be achievable if used at high doses of 25 mg/kg or more, because the dose at 25 mg/kg raised the blood concentration nearly up to 80 μg/ml as shown by Wu et al. (2003). The dose of MK needed to block LTC₄ is much higher than that needed to block LTD₄ (Jones et al., 1995). The ratios of production of LTC₄ to LTD₄ in the rat lungs in the present study suggests that conversion of LTC₄ to LTD₄ was slower in this species than in humans (see Table 2). Concerning human chopped lung, Kumlin and Dahlen (1990) reported that LTC₄ was rapidly converted to LTD₄ and LTE₄, and only 10% of LTC₄ remained intact after 30 min of incubation at 37°C. Consequently, the discrepancy in γ-glutamyl transpeptidase activity between the two species may be one reason that the dose of cysLT₁RA required to ameliorate asthma in rats is higher than that in humans (Shi et al., 2001).

With respect to the two administration schedules used in this study, both were effective at inhibiting late bronchoconstriction, cellular infiltration into the bronchial submucosa, and cysLT production in the lungs, but the triple pretreatment regimen resulted in more complete suppression than the single pretreatment regimen. These results suggest that both drugs are also effective for treatment in the later advanced stages of the disease, when inflammation is already present. MP seemed to show similar but more potent effects than MK did. These results may be compatible with the previous clinical observation that severe asthma attack was not always ameliorated by cysLT₁R antagonists alone but required the coadministration of steroids (Tomari et al., 2001). This result provides further evidence for the effectiveness of steroids and cysLT₁RAs on allergic disorders as anti-inflammatory therapy. Concerning anti-inflammatory effects, Wu et al. (2003) reported that high doses of MK exerted anti-inflammatory effects in an animal model of acute asthma by inhibiting cytokine production. Since steroids have various anti-inflammatory and immunosuppressive effects on allergic reactions, including inhibition of cytokine gene induction, inhibition of
chemokine synthesis, repression of genes encoding cell surface receptors, and repression of adhesion molecules involved in leukocyte activation, migration, and recruitment (Karlin, 1998), the present finding that the effects of the cysLT1R antagonist were almost equal to those of the steroid suggests that the percent ratios of LTC4, LTD4, and LTE4 in cysLTs produced from the chopped lung fragments in the presence or absence of 100 μg/ml MK or 100 μg/ml MP and then in vitro-challenged with OVA (final concentration = 100 μg/ml) and incubated for 30 min at 37°C. Saline was used in place of OVA solution for the controls. B, sensitized rats exposed to the antigen by inhalation of OVA aerosol for the last 2 consecutive days without pretreatment. The next day, the lungs were removed and cut in small pieces (chopped lung). Each incubation mixture containing 300-mg chopped lung fragments are shown. The sensitized lung fragments (each 300 mg) without coaddition with MK or MP. A, sensitized rats exposed to the antigen by inhalation of OVA aerosol for the last 2 consecutive days without pretreatment. The next day, the lungs were removed and cut in small pieces (chopped lung). Each incubation mixture containing 300-mg chopped lung fragments in the presence or absence of 100 μg/ml MK or 100 μg/ml MP was similarly incubated as in A. Values are presented as the means ± S.E.M. (n = 4–8), *, p < 0.05 and **, p < 0.01 compared with the controls.

**TABLE 2**

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<tr>
<th></th>
<th>LTC4</th>
<th>LTD4</th>
<th>LTE4</th>
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<tr>
<td>Control</td>
<td>68.0±5.0</td>
<td>6.8±3.7</td>
<td>25.1±4.7</td>
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<td>OVA day 1 + saline</td>
<td>54.7±9.7</td>
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<td>OVA day 3 + saline</td>
<td>70.8±3.6</td>
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In conclusion, this study revealed that MK may have the novel effect of directly inhibiting cysLT generation when administered at a high dose in addition to the previously reported ameliorative effects of cysLT1R antagonists on bronchoconstriction, recruitment of inflammatory cells into loci, and inflammation (Wu et al., 2003). The finding that MK and MP had different effects on cysLT production may provide a further rationale for the use of combination therapy with cysLT1RAs and steroids for treatment of asthma.

**References**


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