

Effect of Leukotriene D₄ on Tracheal Mucociliary Transport Velocity in Quails

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ABSTRACT—We investigated the effect of leukotriene D₄ (LTD₄) on tracheal mucociliary transport in quails. Topical application of LTD₄ (0.2–2 ng) to tracheal mucosa dose-dependently increased mucociliary transport velocity (MCTV) in 5 or 10 min after application. Forty minutes after application of 2 ng of LTD₄, MCTV was decreased to about 84% of that in the control group. Both the transient increase and the subsequent decrease induced by 2 ng of LTD₄ were blocked by ONO-1078 (Pranlukast: 4-oxo-8-[4-(4-phenylbutoxy)-benzoylamino]-2-(tetrazol-5-yl)-4H-1-benzopyran) (0.03–3 mg/kg, i.m.), a specific leukotriene antagonist. These results suggest that LTD₄ possesses a biphasic effect on tracheal mucociliary transport through leukotriene receptors.

Keywords: Tracheal mucociliary transport, Leukotriene D₄, ONO-1078

Peptide leukotrienes, 5-lipoxygenase products of arachidonic acid metabolism, induce potent bronchoconstriction, increase vascular permeability leading to mucosal edema, and enhance mucus secretion (1). Therefore, peptide leukotrienes may be crucial to pathogenesis in allergic bronchial asthma (2). Although mucociliary transport is an important pulmonary defense mechanism, effects of leukotrienes on mucociliary transport remain inconclusive. Some findings showed that leukotriene D₄ (LTD₄), receptors of which are abundant in the lung (3), impaired mucociliary transport in allergic and nonallergic sheep (4). On the other hand, there are contradictory reports that LTD₄ increased tracheal ciliary beat frequency in sheep and dogs (5, 6). Accordingly, in the present study, we examined the effect of LTD₄ on tracheal mucociliary transport velocity (MCTV) using a newly-developed method (7) in which LTD₄ is directly applied to the tracheal mucosa in quails.

Male quails weighing 100–120 g (Kyudo Co., Ltd., Fukuoka) were anesthetized with urethane (1 g/kg, i.p.), and then they were fixed on their back and the feathers on the larynx were cut off. The skin of the front neck was ripped opened 3 cm along the median line with two pairs of forceps and the traumatic margins were clipped with two celfins to keep the traumatic opening slightly open. The local blood vessels and connective tissues were care-

fully separated from the trachea to expose the trachea. A thread was laid under the exposed trachea so that the part of the trachea used for the test could be kept level. An incision (approx. 2 cm) in the trachea was made with a hot knife. As soon as the operation was over, the quail was inserted into an observation box. In the observation box, the quail tracheal mucosa was kept under about 38°C and approximately 100% humidity by use of a humidifier (8, 9). Ash powders were placed on the caudal side of the tracheal mucosa to select the site where the powder was carried at the fastest speed, and MCTV was measured at the same site throughout the experiment. When MCTV became constant, the time taken for the powder to move 10 mm was measured.

LTD₄ was donated by Ono Pharmaceutical Co., Ltd. (Osaka). The LTD₄ stock, which was dissolved in 50% ethanol and stored at –60°C, was diluted with phosphate-buffered saline without Ca²⁺ and Mg²⁺ (PBS) to appropriate concentrations. In the observation box, the LTD₄ solutions were applied directly to the trachea according to the method developed in our laboratory (7). This method is advantageous because it is not only accurate and stable but also simple. A 1-μl aliquot of LTD₄ was splashed through a 21-gauge needle with a plastic sprayer on its tip, by applying 0.5 kg/cm² of air pressure for 30 msec. The switch to apply air pressure was conducted by an electro-magnetic valve controlled by an electronic stimulator (SEN-3201; Nihon Kohden, Co.,

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Ltd., Tokyo). In sham-treated animals, 1% ethanol in PBS was applied. Ethanol or LTD₄ was applied 15 min after the MCTV measurement started. ONO-1078 (Pranlukast: 4-oxo-8-[4-(4-phenylbutoxy)benzoylamino]-2-(tetrazol-5-yl)-4H-1-benzopyran; Ono Pharmaceutical Co., Ltd., Osaka), a specific leukotriene receptor antagonist (1), was dissolved freshly in 50% ethanol and then administered into the femoral muscle 15 min before ethanol or LTD₄ was applied. In each experiment, MCTV was measured for 75 min through a microscope. The results were calculated by the following equation: $V_T/V_0 \times 100\%$ (V_T =the average velocity in each 5 min and V_0 =the average velocity in the last 5 min before application). MCTV values are expressed as means \pm S.E. Statistical analyses were performed by Dunnett's *t*-test, and a *P* value < 0.05 was considered significant.

The application of 0.2 to 2 ng of LTD₄ significantly increased MCTV in a dose-dependent manner, whereas 1% ethanol had little effect on MCTV (Fig. 1). The LTD₄-in-

duced increase reached its maximum in the first 5 min after application and gradually returned to baseline values. In turn, 40 min after application of 2 ng of LTD₄, MCTV was decreased to about 84% of that in control group, while 0.2 and 0.6 ng of LTD₄ had a little effect on MCTV during that time period.

The increase induced by 2 ng of LTD₄ was dose-dependently inhibited by pretreatment with 0.03–3 mg/kg of ONO-1078, while ONO-1078 alone showed no effect on MCTV (Fig. 2). ONO-1078 also significantly reversed the LTD₄-induced delayed decrease in a dose-dependent fashion.

Impaired mucociliary clearance is a typical characteristic of asthmatic attack induced by allergen, which may be caused by release of autacoid mediators from the immunoglobulin (IgE)-antigen reaction with resident or inflammatory cells in the airway (2). The efficacy of ciliary action to propel inhaled particles and cellular debris in the airway toward the pharynx depends upon several

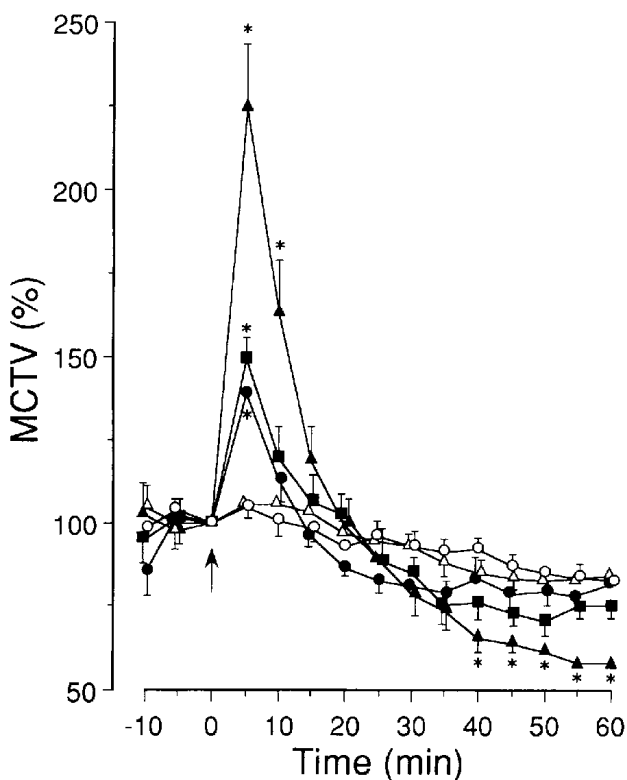


Fig. 1. Effect of topically applied LTD₄ on mucociliary transport velocity in quails. Values are each expressed as a percentage of the preapplication values. Each point and vertical bar represent a mean value and S.E. of six animals. ○: Control, △: Vehicle (1% ethanol), ●: 0.2 ng of LTD₄, ■: 0.6 ng of LTD₄, ▲: 2 ng of LTD₄. **P* < 0.01, statistically significant difference from the vehicle value (Dunnett's *t*-test).

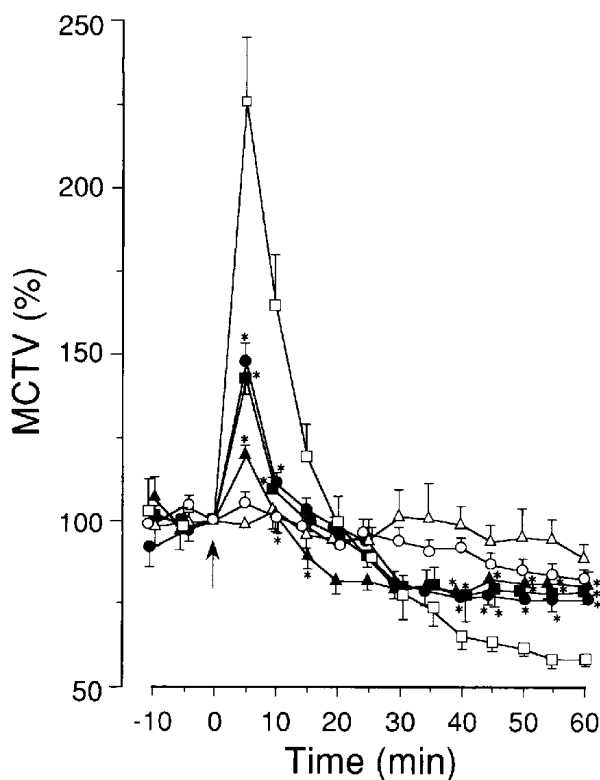


Fig. 2. Inhibitory effect of ONO-1078 on LTD₄-induced changes in mucociliary transport velocity. Values are each expressed as a percentage of the preapplication values. Each point and vertical bar represent a mean value and S.E. of six animals. ○: Control, △: 3 mg/kg of ONO-1078, □: 2 ng of LTD₄, ●: 2 ng of LTD₄ and 0.03 mg/kg of ONO-1078, ■: 2 ng of LTD₄ and 0.3 mg/kg of ONO-1078, ▲: 2 ng of LTD₄ and 3 mg/kg of ONO-1078. **P* < 0.05, statistically significant difference from the group given 2 ng of LTD₄ (Dunnett's *t*-test).

characteristics including the frequency and coordination of ciliary beat, rheological properties of the airway surface fluid and mucus secretion (5). It has been well recognized that LTD₄ can facilitate ciliary beat frequency (5, 6). In the present study, we observed that 2 ng of LTD₄ increased MCTV within the first 5 min and the increase gradually subsided in the next 5 min. The time course of this increase in MCTV was similar to that of the increase in ciliary beat frequency (5). Therefore, the transient increase in MCTV induced by LTD₄ may result from the LTD₄-induced increase of ciliary beat frequency.

We also found that 2 ng of LTD₄ induced a decrease in MCTV 40 min after application. This result is consistent with the finding that the earliest decrease in MCTV was observed 30 min after airway challenge with LTD₄ in allergic and nonallergic sheep (4). LTD₄ has been identified as a potent mucus secretagogue (4), affecting rheological properties of the airway surface fluid. Therefore, it is possible that the LTD₄-induced decrease in MCTV could be related to the mucus secretagogue effect of LTD₄ (4, 5). The syntheses and release of macromolecular mucous glycoproteins induced by LTD₄ are a series of relatively prolonged biological processes (5, 10) and the time-consuming processes may explain the time lag between LTD₄ application and appearance of the decreasing effect on MCTV.

ONO-1078 suppressed both the transient increase and the subsequent decrease induced by 2 ng of LTD₄, suggesting LTD₄-receptor-mediated actions on tracheal mucosa. LTD₄ receptors are known to be coupled to phospholipase C via a pertussis toxin-sensitive G-protein in airway smooth muscles (11). Consequently, LTD₄ will induce polyphosphoinositide hydrolysis and protein kinase C activation (11). Recently, we found that protein kinase C activation induced mucus secretion in hamster tracheal epithelial cells (12). At least in part, the excessive mucus secretion induced by protein kinase C activation may be involved in the subsequent decrease induced by LTD₄.

In conclusion, using a newly-developed method for topical application, we demonstrated that LTD₄ had a biphasic action on mucociliary transport function, consisting of an initial transient increase that may be caused by ciliostimulation and a subsequent prolonged depression that may be due to excessive mucus secretions (5). In addition, if LTD₄ can partly contribute to the impaired mucociliary transport function, ONO-1078 may possess some preventive roles in the impaired function without effects on normal mucociliary function. With respect to

human tracheobronchial diseases, however, the significance of the present results in quails remains to be tested by experiments involving patients and normal subjects.

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