Rhinology, 33, 199-202, 1995

The effects of leukotrienes C_4 and D_4 on ciliary activity of human paranasal sinus mucosa *in vitro**†

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SUMMARY

The effects of leukotrienes C_4 and D_4 on ciliary activity of human paranasal sinus mucosa were investigated in vitro. Normal mucosa was surgically obtained from human paranasal sinuses and incubated in the form of tissue culture. Ciliated cells were magnified under an inverted microscope, and ciliary activity was photoelectrically measured. LTD_4 progressively inhibited ciliary activity, and showed a more potent effect on ciliary activity compared to LTC_4. The concentrations of LTC_4 and LTD_4 in the incubation medium were determined by radioimmunoassay when the mucosa was incubated with 10^{-8} M LTC_4. The concentration of LTD_4 gradually increased and after 90 min reached the maximum of $0.71x10^{-8}$ M, while that of LTC_4 was reduced to about 10% of its initial concentration within 60 min. These results suggested the possible conversion of LTC_4 to LTD_4 on the mucosa, and that LTC_4 can inhibit ciliary activity by means of LTD_4.

Key words: leukotriene C_4, leukotriene D_4, ciliary beats, nasal mucosa

INTRODUCTION

Leukotrienes C_4 (LTC_4) and D_4 (LTC_4) are potent chemical mediators, which play important roles in allergic diseases such as nasal allergy and asthma. LTC_4 and LTD_4 are detected in nasal secretion of patients with nasal allergy who undergo *in vivo* challenge with specific antigen (Cretricos et al., 1984). LTC_4 and LTD_4 also provoke clinical symptoms such as nasal discharge and obstruction when allergic patients are intranasally challenged (Terada et al., 1987). LTC_4 and LTD_4 have various biological effects such as increased vascular permeability (Ueno et al., 1981), hypersecretion of respiratory tracts (Coles et al., 1983; Johnson and McNee, 1983) and bronchoconstriction (Dahlen et al., 1980).

There are other chemical mediators involved in allergic pathogenesis. MBP and ECP are granule proteins, which are derived from eosinophils. PAF is a lipid mediator similar to leukotrienes. It has been reported that MBP (Frigas et al., 1980; Hisamatsu et al., 1990), ECP (Motojima et al., 1989) and PAF (Ganbo and Hisamatsu, 1990; Hisamatsu et al., 1991) induce mucosal dysfunction and damage *in vitro*. These chemical mediators decrease ciliary beat frequency and inhibit mucociliary transport system. However, there are conflicting reports about LTC_4 and LTD_4. Some have reported that ciliary inhibition was induced (Bisgaard and Pedersen, 1987; Weisman et al., 1990), whereas others have reported ciliary activation (Wanner et al., 1983, 1986; Tamaoki et al., 1991). Therefore, it is important to determine the effect of LTC_4 and LTD_4 on ciliary activity to understand the pathogenesis of allergic disorders. In the present study, we have investigated their effects on ciliary activity *in vitro* using ciliated cells in the form of tissue culture, which were obtained from human paranasal sinus mucosa.

MATERIAL AND METHODS

Preparation of LTC_4 and LTD_4 test solutions

LTC_4 and LTD_4 (Ultrafine Chemicals, Manchester, UK) were dissolved in 20% ethanol at a concentration of 10^{-4} M and then diluted with RPMI 1640 solution to a final concentration of 10^{-8} M. Each test solution consisted of 1.5 ml to which the mucosal specimens were exposed. The control solution without mediators was prepared in the same manner as the experimental solution.

Maintenance of human paranasal sinus mucosa

Normal human paranasal sinus mucosa was removed by surgical procedure from ethmoid sinuses of patients, who suffered from facial trauma. The mucosa was rinsed in RPMI solution to

* Received for publication June 29, 1994; accepted December 9, 1994
† Presented at the 15th Congress of the European Rhinologic Society, Copenhagen, June 19-23, 1994

remove blood cells and mucus, and then cut into pieces. The mucosal specimens were transferred onto a collagen layer in culture dishes, and incubated in RPMI solution containing 10% fetal calf serum (FCS). The culture medium was changed 48 h later to remove mucus and cellular debris.

Observation and recordings of ciliary activity

The experiments were performed at 37°C under an inverted microscope equipped with a thermoregulator and a humidified CO_2 chamber. By using a videocamera and a VTR system, the mucosal surface profile was magnified on the TV monitor and recorded on videotapes. More than 10 ciliated cells could be observed on the TV monitor. Ciliary activity of each ciliated cell on the mucosal surface was photo-electrically measured by placing a photo-cell on each beating bundle of cilia.

Statistical analysis

The significant difference between recorded values was statistically determined at $p < 0.05$ in the Student's t-test for unpaired data.

Radioimmunoassay of LTC_4 and LTD_4

LTC_4 and LTD_4 in the incubation medium were measured with a $LTC_4/LTD_4/LTE_4$ RIA kit (Amersham Japan Co. Ltd, Tokyo, Japan). The procedure was as follows. A 4-fold volume of ethanol was added to the medium obtained from the culture dishes, and this medium was immediately centrifuged at 2,000*g* for 15 min at 4°C. Ten ml of 0.1 M phosphate buffer (pH 7.2) and 30 ml of methylene dichloride were added to the supernatant. After this mixture shake was centrifuged at 2,000*g* for 10 min at 4°C, the upper layer was applied onto a Sep-Pak C18 column, which was previously conditioned with methanol and water. The column was washed with 3 ml of 60% methanol containing 0.1% acetic acid. Leukotrienes were eluted from the column with 80% methanol containing 0.1% acetic acid. The eluate was evaporated and dried using a centrifugal evaporator at a temperature of 37°C.

The specimens were dissolved with the mobile phase for leukotriene separation, and then LTC_4 and LTD_4 in the specimen were separated and collected using the reverse-phase HPLC technique (Anderson et al., 1983). HPLC conditions are shown in Table 1. After the specimens separated for the LTC_4 and LTD_4 assay were again dried using the centrifugal evaporator, they were used for LTC_4 and LTD_4 discriminative assay.

To each polystyrene test tube were added: 0.1 ml each of the specimen dissolved in 0.1 M phosphate buffer (pH 7.2), ^{3}H-labelled LTC_4 and antiserum for leukotrienes, and incubated at 4°C for 18 hrs. Dextran charcoal (0.2 ml) was added to the reaction mixture. After the reaction mixture was again incubated for 10 min at 4°C, they were centrifuged at 2,000*g* for 10 min at 4°C. The radioactivity in the supernatant was measured with scintillant for 4 min using a ß-scintillation counter.

Table 1. HPLC conditions.

column:	Nucleosil C18 (4.6×250 mm)
mobile phase:	methanol/acetonitrile/0.05% acetic acid (15:33:54), pH 5.2
flow rate:	1.0 ml/min
detection:	OD at 280 nm

RESULTS

Effect of LTC_4 and LTD_4

The effects of LTC_4 and LTD_4 on ciliary activity are illustrated in Figure 1. Baseline ciliary activity varied between 10.0 and 11.8 Hz (10.9±0.6 Hz; n=30); there were no significant differences in baseline ciliary activity among the mucosal specimens obtained from different subjects. Ciliary activities were expressed as the percentage of change from its own individual time-zero value (each time-zero value was normalized to 100%). LTD_4 progressively inhibited ciliary activity. Significant ciliary inhibition was observed after 1-h exposure. However, LTC_4 could not induce significant ciliary inhibition for 2 h after exposure. After 4-h exposure, we could observe significant ciliary inhibition induced by LTC_4. The control showed no significant effect on ciliary activity throughout the testing period.

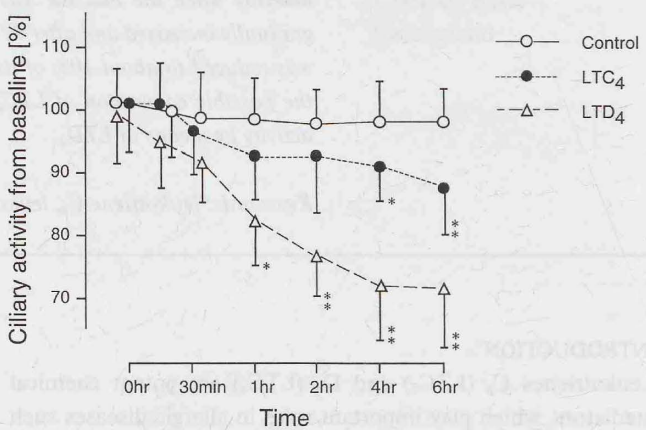

Figure 1. The time course of ciliary activity exposed to 10^{-8} M LTC_4 and LTD_4. The values are expressed as means±SD. Ten ciliated cells of each group were observed (*: $p < 0.05$; **: $p < 0.01$, compared to the control).

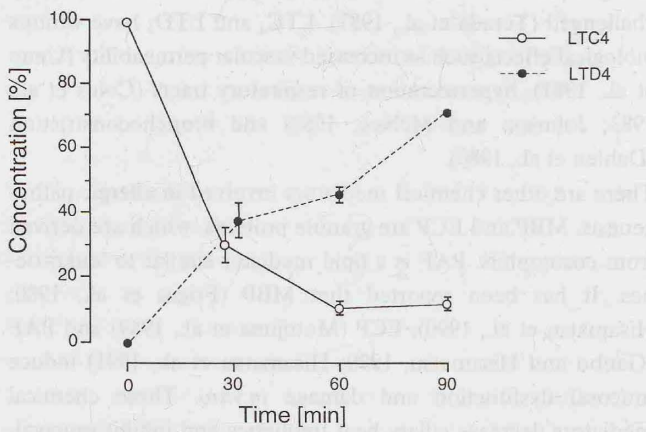

Figure 2. The time course of LTC_4 and LTD_4 concentrations in the incubation medium when the mucosa was incubated with 10^{-8} M LTC_4. The values are expressed as means±SD.

Time courses of LTC_4 and LTD_4 in the culture medium

We carried out assays of LTC_4 and LTD_4 in the incubation chamber when the mucosa was incubated with 10^{-8} M LTC_4 (Figure 2). We observed a time-dependent decrease in LTC_4 concentration in the tissue culture medium. LTC_4 concentration was reduced to 30.2% within 30 min, and within 60 min its concentration was only 10.8% of the initial concentration. The concentration of LTD_4 gradually increased to 37.9% within 30 min, and after 90 min the mean of LTD_4 concentration reached the maximum of 0.71×10^{-8} M.

DISCUSSION

In the present study, LTD_4 showed a more potent effect on ciliary activity compared to LTC_4. LTD_4 induced significant ciliary inhibition after 1-h exposure, whereas there was no significant difference in ciliary activity between LTC_4 and the control until 4 h after challenge. Particularly, ciliary inhibition could not be observed primarily in the first 30 min.

It is well known that LTC_4 can be metabolized to LTD_4 by γ-glutamyl transpeptidase (γ-GTP; Samuelsson, 1983). We detected LTD_4 in the incubation medium of LTC_4 by radioimmunoassay. It indicates the conversion of LTC_4 to LTD_4 by γ-GTP. In the preliminary study, we measured the concentration of γ-GTP in the incubation medium; however, we could not detect γ-GTP. There is a possibility that γ-GTP does not exist in the incubation medium, because it is a membrane-bound enzyme (Kuo et al., 1984). In this study, LTD_4 progressively induced ciliary inhibition. Bisgaard et al. (1987) reported that LTD_4 inhibited ciliary activity *in vitro*, using human nasal cells that were scraped from the inferior turbinate. It is also reported that LTC_4 inhibited ciliary activity in human scraped nasal cells (Bisgaard et al., 1987) and chicken tracheal epithelium (Weisman et al., 1990). In the present study, a rapid decrease in LTC_4 concentration was observed; it was possibly due to its conversion to LTD_4. Therefore, it was suggested that we observed the effects of both LTC_4 and LTD_4 on ciliary activity when the mucosal specimen was incubated with LTC_4. Ciliary inhibition after 30-min exposure might be induced by LTD_4, to which LTC_4 was converted. However, it is certain that LTC_4 subsequently induced ciliary dysfunction.

In contrast, there have been some reports concerning ciliary stimulation induced by LTC_4 and LTD_4. Wanner et al. (1983, 1986) and Tamaoki et al. (1991) reported that both LTC_4 and LTD_4 promoted ciliary activity during short 20- to 30-min observation periods in studies using sheep single free ciliated cells that were scratched off from the bronchus (Wanner et al., 1983, 1986), and cultured canine tracheal epithelium (Tamaoki et al., 1991). These discrepancies in the results might be attributable to the differences between the experimental conditions such as observation time, culture forms of ciliated cells and variance of animals. However, tissue culture is considered a more physiological form than single cell culture, because it could preserve interaction between ciliated cells, which is an important component of ciliary activity. Consequently, it needs adequately long periods of observation to determine the effect of LTC_4 and LTD_4 on ciliary activity considering the metabolic series of leukotrienes. Moreover, it is important to use human material to investigate the pathogenesis of human diseases.

LTC_4 and LTD_4 increase vascular permeability (Ueno et al., 1981) and mucus secretion (Marom et al., 1982). These biological responses might change the mucus blanket. It is also clear in the present study that LTC_4 and LTD_4 inhibit ciliary activity. These pathological effects of LTC_4 and LTD_4 can cause mucosal dysfunction and inhibit mucociliary clearance in the respiratory tract, similar to other mediators such as MBP, ECP and PAF. For a better understanding of the action of leukotrienes upon ciliated cells, it may be necessary to investigate the more detailed mechanisms of their metabolism on the mucosa.

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