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Original Article

Cysteinyl Leukotriene Receptor Type 1 ($CysLT_1$) Mediates Contraction of the Guinea Pig Lower Esophageal Sphincter

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Abstract

Objective: Leukotriene D_4 (LTD_4) causes contraction of the cat lower esophageal sphincter. The effects of leukotrienes in the guinea pig lower esophageal sphincter and the cysteinyl leukotriene receptor (CysLT) subtype that mediate this contraction are not known. The purpose of the present study was to characterize the CysLT receptors in the guinea pig lower esophageal sphincter.

Materials and Methods: We measured the contractions of transverse strips from the guinea pig lower esophageal sphincter caused by cysteinyl leukotrienes, LTC_4, LTD_4 and LTE_4, and the dihydroxy leukotriene, LTB_4. We also measured LTD_4-induced contraction inhibited by CysLT receptor antagonists, tetrodotoxin and atropine.

Results: In the guinea pig lower esophageal sphincter strips, LTC_4, LTD_4 and LTE_4, but not LTB_4, caused concentration-dependent contractions. The relative potencies for cysteinyl leukotrienes to cause contraction were $LTD_4 = LTC_4 > LTE_4$. LTE_4 was a partial agonist. The LTD_4-induced contraction was inhibited by two selective $CysLT_1$ receptor antagonists, montelukast and zafirlukast, and by the dual $CysLT_1$ and $CysLT_2$ receptor antagonist BAY u9773. The combination of both montelukast and BAY u9773 did not potentiate the inhibition caused by montelukast alone. These findings indicate that $CysLT_1$ mediates the contraction in the lower esophageal sphincter. Furthermore, LTD_4-induced contraction was not affected by tetrodotoxin or atropine, suggesting a direct effect.

Conclusion: These results demonstrate that cysteinyl leukotrienes, but not the dihydroxy leukotriene LTB_4, cause contraction of the guinea pig lower esophageal sphincter. The $CysLT_1$ receptor mediates this contraction. [*Tzu Chi Med J* 2009;21(1):28–33]

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1. Introduction

Cysteinyl leukotrienes (Cys-LTs), such as LTC_4, LTD_4, and LTE_4, and the dihydroxy leukotriene, LTB_4, are inflammatory mediators that are derived from the lipoxygenase pathway of arachidonic acid metabolism. Cysteinyl leukotrienes are peptide-conjugated lipids and are produced by eosinophils, basophils, mast cells, and macrophages [1–3]. They were identified on the basis of their contractile properties for intestinal and bronchial smooth muscle, and they are potent inflammatory mediators. These cysteinyl leukotrienes play an important role in respiratory diseases such as asthma and allergic rhinitis, and have been implicated in other inflammatory conditions including cardiovascular, gastrointestinal, skin and immune disorders [1–3]. Two receptor subtypes for the cys-LTs, the type 1 and type 2 cys-LT receptors ($CysLT_1$ and $CysLT_2$, respectively), have been cloned and characterized. $CysLT_1$ receptors have been found in the spleen, lung, placenta and small intestine, whereas $CysLT_2$ receptors have been found in the lung, spleen, heart, lymph nodes and brain [1–10]. The rank order of agonist potency at the $CysLT_1$ receptor is $LTC_4 = LTD_4 > LTE_4$, at which LTE_4 is a partial agonist, or $LTC_4 = LTD_4 = LTE_4$. The rank order of agonist potency at the $CysLT_2$ receptor is $LTC_4 = LTD_4 >> LTE_4$ (LTE_4 is inactive) [4–10]. Many selective antagonists for $CysLT_1$ have been developed. These antagonists, including montelukast, zafirlukast, pranlukast, pobilukast and MK571, block CysLT-induced calcium mobilization in $CysLT_1$ receptor-transfected cells but not in cells transfected with the $CysLT_2$ receptor. On the other hand, selective $CysLT_2$ receptor antagonists have not been reported to date. BAY u9773 is an antagonist at both the $CysLT_1$ and $CysLT_2$ receptors [1–4,11,12].

In the gastrointestinal system, $CysLT_1$ receptors are expressed in the small intestine, colon, liver, and in cancers such as colorectal cancer, whereas $CysLT_2$ receptors are expressed in the stomach, small intestine, colon, liver and pancreas [1–4,6–9,13,14]. Cysteinyl leukotrienes have been implicated in inflammatory conditions including eosinophilic esophagitis and eosinophilic gastroenteritis [1–3,15–18]. Previous studies have demonstrated that cysteinyl leukotrienes cause contraction of the cat esophagus and lower esophageal sphincter, and the rat stomach and colon, as well as the guinea pig ileum, colon and gallbladder [18–22]. In the guinea pig gallbladder, cysteinyl leukotrienes cause contractions through interaction with $CysLT_1$ receptors whereas in the guinea pig ileum, they cause contraction mainly through interaction with $CysLT_2$ receptors [1,4,12,18,19].

LTD_4 has been demonstrated to cause contraction of the cat lower esophageal sphincter [20]. At the present time, no data are available on the effects caused by leukotrienes in the guinea pig lower esophageal sphincter. Furthermore, the subtype of the cysteinyl leukotriene receptor mediating the contraction of the lower esophageal sphincter is unknown. We have demonstrated that the endothelin ET_A receptor, ET_B receptor, protease-activated receptor-1 (PAR_1) and PAR_2 mediate contraction or relaxation in the guinea pig lower esophageal sphincter [23,24]. The purpose of the present study was to characterize the CysLT receptors in the guinea pig lower esophageal sphincter, a ring of thickened circular muscle at the gastroesophageal junction [23].

2. Materials and methods

Male Hartley guinea pigs were obtained from the Animal Center, National Science Council, Taiwan. The leukotrienes LTB_4, LTC_4, LTD_4 and LTE_4, and the cysteinyl leukotriene antagonists montelukast, zafirlukast and BAY u9773, were purchased from Cayman Chemical (Ann Arbor, MI, USA). Carbachol, atropine and all buffer reagents were purchased from Sigma Chemical (St. Louis, MO, USA). Tetrodotoxin was obtained from Tocris Cookson (Avonmouth, Bristol, UK).

2.1. Measurement of contraction or relaxation of isolated lower esophageal sphincter strips

The Institutional Animal Care and Use Committee of Buddhist Tzu Chi General Hospital, Hualien, approved the protocol for this study. Male guinea pigs, weighing 350–400 g, were sacrificed with CO_2. The stomach, including a portion of the esophagus, was quickly removed and placed in oxygenated standard incubation solution (see below). The esophagus and stomach were cut open in the longitudinal direction along the greater curvature and pinned flat with the mucosal side up. The mucosa was removed with micro-scissors. Transverse strips (2 mm wide and 10 mm long) were cut from the area of the lower esophageal sphincter, which was easily identified as a thickened region of muscle between the esophagus and the stomach. In preliminary experiments, the thickening of the circular smooth muscle was confirmed by observing hematoxylin and eosin-stained tissue sections under a microscope (data not shown) [23].

Measurements of contraction and relaxation of isolated lower esophageal sphincter strips from the guinea pig esophagus were performed as previously described [23,24]. In brief, the strips were placed in a standard incubation solution containing 118 mM NaCl, 25 mM $NaHCO_3$, 4.7 mM KCl, 14 mM glucose, 1.2 mM NaH_2PO_4, 1.8 mM $CaCl_2$, gassed with 95% O_2 • 5% CO_2. The final pH at 37°C was 7.40±0.05. The sphincter strips were attached to organ baths using surgical silk sutures and incubated at 37°C in the standard incubation solution

continuously gassed with 95% O_2 • 5% CO_2. The strips were connected to isometric transducers (FT.03; Grass Technologies, West Warwick, RI, USA), which were connected to an amplifier (Gould Instrument Systems, Valley View, OH, USA) and a computer recording system (BIOPAC Systems, Goleta, CA, USA). The basal tension of the muscle strips was adjusted to 1.0 g [23,24]. Experiments were started after a 45-minute equilibration period. All contraction experiments with agonists were performed in a cumulative manner because of the absence of immediate desensitization of the lower esophageal sphincter muscle to the cumulative administration of these agents, similar to the guinea pig gallbladder [19]. Carbachol (1 μM)-induced contraction was used as a reference to express the contractile response to agonists. In the relaxation experiments, leukotrienes were added to the carbachol-contracted muscle strips. Carbachol-induced tone before the addition of leukotrienes was used as a reference to express relaxation to these agents. Carbachol (1 μM) induced a fast and long-duration contraction [23,24]. The leukotrienes were added in a single administration 15 minutes after the addition of carbachol during the relaxation experiments. Tissues were incubated with 5 mM L-cysteine, an inhibitor of glycinase, 7 minutes before starting the experiments to reduce peptide degradation [20,25]. In preliminary experiments, L-cysteine caused a transient, negligible contraction (Fig. 1), or relaxation, or neither contraction nor relaxation (data not shown). For studies using atropine and tetrodotoxin, the muscle strips were exposed to the indicated concentrations of these agents for 6 minutes and 15 minutes, respectively, and then to the various concentrations of leukotrienes [23,24]. Only one cumulative concentration-contraction response curve, with or without the cysteinyl leukotriene antagonist, tetrodotoxin or atropine, was constructed with each preparation in the experiments.

2.2. *Data analysis*

Results are expressed as mean±standard error of the mean. Statistical evaluation was performed using non-paired Student's *t* test. A value of $p<0.05$ was considered statistically significant.

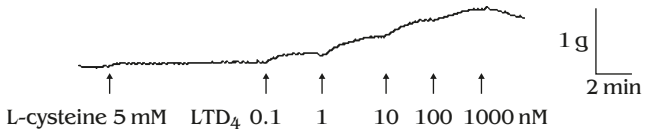

Fig. 1 — A typical tracing showing the contraction of the guinea pig lower esophageal sphincter strip with cumulative addition of leukotriene LTD_4.

3. Results

3.1. *Effects of leukotrienes on contraction of the guinea pig lower esophageal sphincter*

To test the ability of leukotrienes to cause muscle contraction, transverse strips from isolated guinea pig lower esophageal sphincter were prepared and responses to cysteinyl leukotrienes, including LTC_4, LTD_4, LTE_4, and the dihydroxy leukotriene, LTB_4, were studied. In the lower esophageal sphincter strips, both LTD_4 and LTC_4 induced a slow- and long-duration muscle contraction (Fig. 1). In terms of the maximal tension of contraction, LTD_4 and LTC_4 had equal efficacy (Fig. 2). LTD_4 caused a detectable contraction of the sphincter strips at 0.1 nM, half-maximal contraction at 4±2 nM and maximal contraction at 1 μM. The maximal tension caused by 1 μM LTD_4 was 29±5% of the tension caused by 1 μM carbachol. LTC_4 was as potent as LTD_4, causing half-maximal contraction at 10±5 nM and maximal contraction at 1 μM. LTE_4 was less efficacious and less potent than LTD_4. LTE_4 caused a detectable contraction at 10 nM, half-maximal contraction of the sphincter strips at 160±50 nM ($p<0.05$, compared with LTD_4; Fig. 2) and maximal contraction at 1 μM. LTE_4, 1 μM, caused a contractile response of 14±3% of the tension caused by 1 μM carbachol ($p<0.05$, compared with LTD_4; Fig. 2). In contrast, the dihydroxy leukotriene LTB_4 did not cause contraction at concentrations up to 1 μM (Fig. 2).

In the carbachol (1 μM)-contracted lower esophageal sphincter strips, LTD_4, up to 1 μM, did not cause relaxation (data not shown).

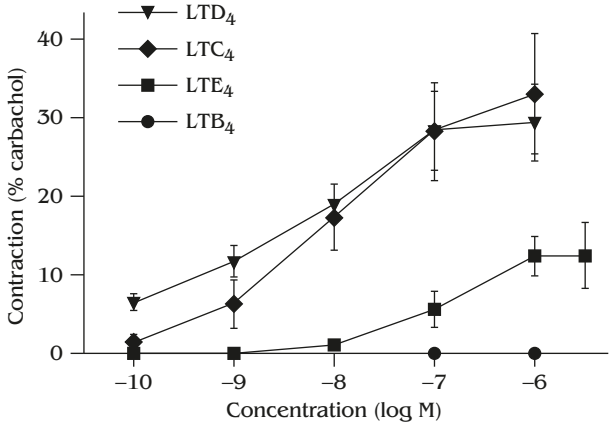

Fig. 2 — Ability of leukotrienes, LTC_4, LTD_4, and LTE_4 to cause contraction of the guinea pig lower esophageal sphincter. LTB_4 did not cause contraction up to 1 μM. Values are expressed as the percent of carbachol (1 μM)-induced tone. Results given are from at least three experiments. Vertical bars represent ± standard error of the mean.

3.2. *Effects of CysLT receptor antagonists on LTD_4-induced lower esophageal sphincter contraction*

To further characterize the CysLT receptors mediating the lower esophageal sphincter contraction, the abilities of two selective $CysLT_1$ receptor antagonists, montelukast and zafirlukast, and the dual $CysLT_1$ and $CysLT_2$ receptor antagonist BAY u9773, to inhibit LTD_4-induced contraction were determined.

Montelukast (1 μM), zafirlukast (1 μM) and BAY u9773 (1 μM) alone did not cause contraction (data not shown) in the strips. As shown in Fig. 3, montelukast (1 μM) and zafirlukast (1 μM) shifted the LTD_4 concentration-response curve to the right. The tensions caused by 1 μM LTD_4 plus montelukast (1 μM) and zafirlukast (1 μM) were 12±4% and 12±2%, respectively, of the tension caused by 1 μM carbachol ($p<0.05$, compared with 1 μM LTD_4 alone). Similarly, the dual $CysLT_1$ and $CysLT_2$ receptor antagonist BAY u9773 (1 μM) also shifted the LTD_4 concentration-response curve to the right. The tension caused by 1 μM LTD_4 plus BAY u9773 (1 μM) was 8.3±2.3% of the tension caused by 1 μM carbachol ($p<0.05$, compared with 1 μM LTD_4 alone). The combination of both montelukast and BAY u9773 shifted the LTD_4 concentration-response curve to the right but did not potentiate the shift caused by montelukast alone. The tension caused by 1 μM LTD_4 plus both montelukast (1 μM) and BAY u9773 (1 μM) was 12±1% of the tension caused by 1 μM carbachol ($p>0.05$, compared with 1 μM LTD_4 plus montelukast, 1 μM; Fig. 3).

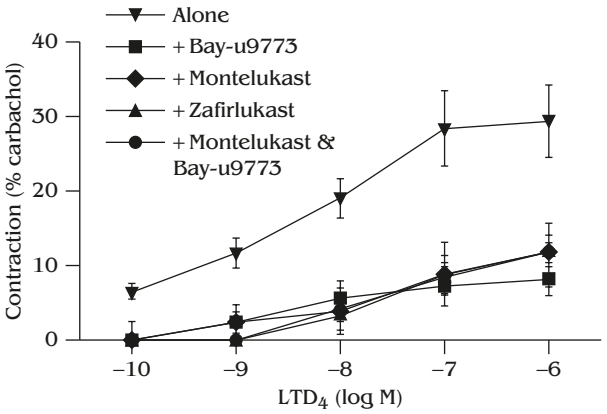

Fig. 3 — Ability of cysteinyl leukotriene receptor antagonists to inhibit leukotriene LTD_4-induced contraction in the guinea pig lower esophageal sphincter. Values are expressed as the percent of carbachol (1 μM)-induced tone. Results given are from at least three experiments. Vertical bars represent ± standard error of the mean.

3.3. *Effects of tetrodotoxin and atropine on LTD_4-induced lower esophageal sphincter contraction*

In the lower esophageal sphincter strips, the LTD_4-induced contraction was not affected by tetrodotoxin or atropine, at concentrations of 1 μM each (Fig. 4).

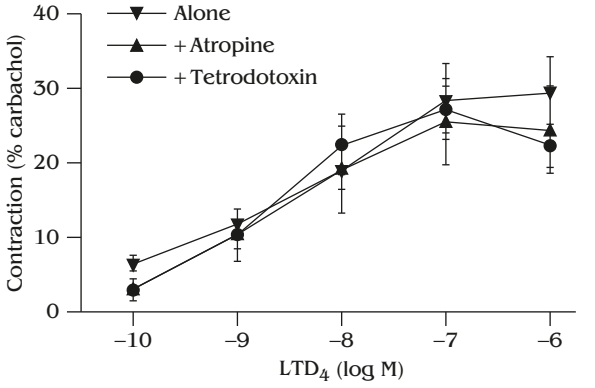

Fig. 4 — Ability of cysteinyl leukotriene LTD_4 in the absence or presence of tetrodotoxin or atropine, 1 μM, to cause contraction of the guinea pig lower esophageal sphincter. Values are expressed as the percent of carbachol (1 μM)-induced tone. Results given are from at least three experiments. Vertical bars represent ± standard error of the mean.

4. Discussion

In the present study, we have shown for the first time that cysteinyl leukotrienes elicit contraction in the guinea pig lower esophageal sphincter and $CysLT_1$ mediates this contraction. In addition, this study provides evidence that the dihydroxy leukotriene LTB_4 does not cause contraction of the lower esophageal sphincter.

Similar to a previous study on the contractile effect of LTD_4 in cat lower esophageal sphincter muscle cells (20), this study demonstrated that LTD_4 causes contractions in guinea pig lower esophageal sphincter strips. In addition, this study showed that LTC_4 and LTE_4 cause contractions in the guinea pig lower esophageal sphincter. LTC_4 and LTD_4 were of the same potency and efficacy whereas LTE_4 was a partial agonist. Similar to that in the guinea pig gallbladder, the relative potencies for cysteinyl leukotrienes to cause contraction of the lower esophageal sphincter were $LTD_4=LTC_4>LTE_4$ (4,18). In addition, the LTD_4-induced contraction was inhibited by two selective $CysLT_1$ receptor antagonists, montelukast and zafirlukast, and by the dual $CysLT_1$ and $CysLT_2$ receptor antagonist BAY u9773. Furthermore, the combination of both montelukast and BAY u9773 did not potentiate the inhibition caused by montelukast alone. These findings indicate that $CysLT_1$ mediates this contraction in the lower esophageal sphincter, similar

to the guinea pig gallbladder [18,19]. In contrast, the dihydroxy leukotriene LTB_4 did not cause contraction in the lower esophageal sphincter. This suggests that leukotriene LTB_4 receptors are not involved in the contraction of the lower esophageal sphincter.

We demonstrated that the contractile response of the guinea pig lower esophageal sphincter to LTD_4 was insensitive to atropine and tetrodotoxin, similar to studies showing the same responses in guinea pig gallbladder [18,19]. These findings indicate that indirect neural mechanisms are probably not involved and suggest a direct effect of cysteinyl leukotrienes on the lower esophageal sphincter muscle.

In the lower esophageal sphincter, LTC_4, LTD_4 and LTE_4 induce contractions and $CysLT_1$ mediates contractions. Thus cysteinyl leukotrienes might play an important role in the control of lower esophageal sphincter motility. Studies on the endogenous release of these cysteinyl leukotrienes in the esophagus and on the role of cysteinyl leukotrienes in the human lower esophageal sphincter are warranted. Cysteinyl leukotrienes have been implicated in inflammatory conditions, including eosinophilic esophagitis and eosinophilic gastroenteritis [1–3,15,16]. The high pressure of the lower esophageal sphincter in patients with eosinophilic esophagitis might be caused by cysteinyl leukotrienes released by eosinophils [26]. On the other hand, because $CysLT_1$ antagonists influence lower esophageal sphincter motility, the use of $CysLT_1$ receptor antagonists for treating asthma or other diseases might decrease lower esophageal sphincter contraction and worsen gastroesophageal reflux.

In conclusion, these results demonstrate that cysteinyl leukotrienes, but not the dihydroxy leukotriene LTB_4, elicit contraction of the guinea pig lower esophageal sphincter. $CysLT_1$ mediates this contraction.

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