Leukotriene-induced contraction is mediated by cysteinyl leukotriene receptor CysLT1 in guinea pig fundus but by CysLT1 and CysLT2 in antrum

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A B S T R A C T

Aims: Leukotriene D₄ (LTD₄) causes contraction of the stomach through unclear receptors. The aim of the present study is to characterize the cysteinyl leukotriene receptor (CysLT) mediating leukotriene-induced muscle contraction in the stomach.

Main methods: We measured contraction of gastric muscle strips isolated from the guinea pig fundus and antrum caused by cysteinyl leukotrienes, including LTC₄, LTD₄ and LTE₄, as well as the dihydroxy leukotriene LTB₄ in vitro.

Key findings: In both fundic and antral muscle strips, LTC₄ and LTD₄ caused moderate, concentration-dependent contractions. In contrast, LTB₄ caused only small contraction. The relative potencies for cysteinyl leukotrienes to cause contraction in both fundus and antrum were LTC₄ = LTD₄ > LTB₄. The LTD₄-induced contraction was not affected by tetrodotoxin or atropine, suggesting that the action is not neurally mediated. The LTD₄-induced contraction in the fundus was almost abolished by the CysLT₁ selective antagonist montelukast. In contrast, the LTD₄-induced contraction in the antrum was only partially inhibited by montelukast or the dual CysLT₁ antagonist BAY u9773, indicating enhancement of inhibition.

Significance: The results of the present study demonstrate that cysteinyl leukotrienes LTC₄, LTD₄ and LTE₄ cause moderate to marked whereas the dihydroxy leukotriene LTB₄ causes small muscle contraction in the stomach in vitro. The leukotriene-induced contraction is mediated by CysLT₁ in fundus but by CysLT₁ and CysLT₂ in antrum.

Keywords: Leukotriene, Stomach, Contraction, CysLT₁ receptor, CysLT₂ receptor

Introduction

Leukotrienes are inflammatory mediators derived from the arachidonic acid and divided by structure into two groups: cysteinyl leukotriene (cysLT) and dihydroxy leukotriene. Cysteinyl leukotrienes, i.e. leukotriene C₄ (LTC₄), LTD₄, and LTE₄, are produced by granulocytes (eosinophils, basophils), macrophages and mast cells, whereas the dihydroxy leukotriene LTB₄ is produced by granulocytes (eosinophils, neutrophils) and macrophages (Brink et al., 2003; Kanaoka and Boyce, 2004; Capra et al., 2007). Cysteinyl leukotrienes are implicated in respiratory inflammatory diseases, such as asthma and allergic rhinitis, as well as cardiovascular, gastrointestinal and skin inflammatory disorders, including atherosclerotic cardiovascular disease, eosinophilic gastroenteritis and atopic eczema (Brink et al., 2003; Kanaoka and Boyce, 2004; Capra et al., 2007; Riccioni et al., 2010). Two subtypes of cysteinyl leukotriene receptors (CysLT), i.e. CysLT₁ and CysLT₂, have been cloned and characterized (Lynch et al., 1999; Sarau et al., 1999; Nothacker et al., 2000). The rank order of agonist potency at the CysLT₁ receptor is LTC₄ > LTD₄ > LTE₄, whereas the rank order of agonist potency at the CysLT₂ receptor is LTC₄ = LTD₄ > LTE₄ (Bäck, 2002). Many potent and selective CysLT₁ receptor antagonists, including montelukast, zafirlukast, pranlukast and pobilukast, have been developed. They block leukotriene-induced calcium mobilization in CysLT₁ but not CysLT₂ transfected cells (Takasaki et al., 2000; Snyder and Krell, 1986). On the other hand, only two selective CysLT₂ receptor antagonists, Bay cysLT2 and HAMI379, have been reported to date (Huang et al., 2008; Wunder et al., 2010). Another CysLT₂ antagonist, BAY u9773, is a dual CysLT₁ and CysLT₂ antagonist (Bäck, 2002).

Both CysLT₁ and CysLT₂ receptors are expressed in the gastrointestinal system. CysLT₁ receptors are expressed in the small intestine, colon and liver, whereas CysLT₂ receptors are found in the stomach, small intestine, colon, liver and pancreas (Brink et al., 2003; Kanaoka and Boyce, 2004; Capra et al., 2007; Bäck, 2002; Heise et al., 2000). Previous studies demonstrated that cysteinyl leukotrienes cause contraction of the esophagus (Chang et al., 2008; Kim et al., 1998), lower esophageal sphincter (Huang, 2009; Kim et al., 1998), stomach (Goldenberg and Subers, 1983; Miura et al., 1992; Miyata et al., 1995), ileum (Bäck et al., 1996), colon (Ieiri et al., 2001) and gallbladder (Falcone and Krell, 1992;
Freedman et al., 1993) as well as relaxation in the internal anal sphincter (de Godoy et al., 2009). The cysteiny l leukotriene-induced contractions are mediated by CysLT$_1$ receptors in the esophagus, lower esophageal sphincter and gallbladder (Falcone and Krell, 1992; Freedman et al., 1993; Chang et al., 2008; Huang, 2009). On the other hand, the cysteiny l leukotriene-induced contraction is mediated by CysLT$_1$ receptors in the ileum (Bäck et al., 1996). The subtype of the cysteiny l leukotriene receptor mediating the cysteiny l leukotriene-induced relaxation of internal anal sphincter is not clear (de Godoy et al., 2009).

LTD$_4$ and LTC$_4$ cause contraction in the rat stomach and these contractions are sensitive to CysLT$_1$ receptor antagonists (Goldenberg and Subers, 1983; Miura et al., 1992; Miyata et al., 1995). However, effects of other leukotrienes in the gastric contraction and inhibitory effects of potent, selective CysLT$_1$ antagonists in the leukotriene-induced gastric contraction were not clearly known. The subtype of the cysteiny l leukotriene receptor mediating the leukotriene-induced contraction of stomach was not clear. The purpose of the present study is to characterize cysteiny l leukotriene receptors in the stomach mediating leukotriene-induced muscle contraction.

Materials and methods

Male Hartley guinea pigs (300–350 g) were obtained from National Laboratory Animal Center, Taiwan. LTD$_4$, LTC$_4$, LTE$_4$, montelukast and BAY u9773 were purchased from Cayman Chemical, Ann Arbor, Michigan; Carbachol, atropine, l-serine, boric acid, l-cysteine and all buffer reagents were purchased from Sigma Chemical, St. Louis, MO, USA. Tetrodotoxin was obtained from To cris Cookson, Avonmouth Bristol, UK. l-serine borate was prepared from Sigma Chemical, St. Louis, MO, USA. The incubation solution into the submucosa space to separate the mucosa from the muscle layers was gassed with 95% O$_2$ • 5% CO$_2$. The final pH at 37 °C was 7.40 ± 0.05. The stomach was cut open in the longitudinal direction along the lesser curvature. The mucosa was gently removed with micro-scissors after injecting the standard incubation solution into the submucosa space to separate the mucosa from the muscle layers. The fundus was obtained proximally to the gastro-esophageal junction. Two muscle strips (1.0 × 0.3 cm, with comparable cross sectional area) from the mid-fundus or distal antrum were cut parallel to the circular muscle layer, as seen by the muscle fiber direction of the upper most muscle layer (James et al., 2005). In preliminary experiments, proper muscle layer orientation was confirmed by histological evaluation of the tissue with hematoxylin and eosin staining.

Measurements of contraction of isolated gastric muscle strips

All procedures were performed in compliance with relevant laws and institutional guidelines. The Institutional Animal Care and Use Committee of E-Da Hospital approved the protocol for this work. Guinea pigs were sacrificed with CO$_2$. The stomach was quickly removed and placed in oxygenated standard incubation solution, containing 118 mM NaCl, 25 mM NaHCO$_3$, 4.7 mM KCl, 14 mM glucose, 1.2 mM NaH$_2$PO$_4$, 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 stomach was cut open in the longitudinal direction along the lesser curvature. The mucosa was gently removed with micro-scissors after injecting the standard incubation solution into the submucosa space to separate the mucosa from the muscle layers. The fundus was obtained proximally to the gastro-esophageal junction. Two muscle strips (1.0 × 0.3 cm, with comparable cross sectional area) from the mid-fundus or distal antrum were cut parallel to the circular muscle layer, as seen by the muscle fiber direction of the upper most muscle layer (James et al., 2005). In preliminary experiments, proper muscle layer orientation was confirmed by histological evaluation of the tissue with hematoxylin and eosin staining.

Measurements of contraction of isolated fundic and antral strips were performed according to the procedure published previously with minor modifications (Chang et al., 2008; Huang, 2009; James et al., 2005; Dick et al., 2000; von Schrenck et al., 1989). In brief, the isolated fundic and antral 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 (FT03; Grass Technologies, West Warwick, RI, USA), which were connected to an amplifier (Gould Instrument Systems, Valley View, Ohio, USA) and a computer recording system (BIOPAC systems, Goleta, CA, USA). The basal tension of the fundic and antral muscle strips was adjusted to 1.0 g (Chang et al., 2008; Huang, 2009; Dick et al., 2000; von Schrenck et al., 1989). Experiments were started after a 45 min equilibration period. All contraction experiments with LTC$_4$, LTD$_4$ and LTE$_4$ were performed in a cumulative manner because of lack of immediate desensitization of the gastric muscle to the cumulative administration of these agents (Chang et al., 2008; Huang, 2009). All contraction experiments with LTE$_4$ were performed in a non-cumulative manner because cumulative administration may mask LTE$_4$ contractile responses (Sakata et al., 2004). Carbachol (1 μM)-induced contraction was used as a reference to express contractile response to agonists. Tissues were incubated with 5 mM l-cysteine, an inhibitor of glycine nase, 7 min before beginning the experiments to reduce peptide degradation (Kim et al., 1998; Chang et al., 2008; Huang, 2009). l-cysteine caused a transient, small contraction in fundic and antral strips (Fig. 1). In some contraction experiments with LTC$_4$, the preparations were incubated for 30 min with l-serine borate, 45 mM, before beginning the experiments to inhibit the metabolism of LTC$_4$ into LTD$_4$ (Bäck et al., 2001). For studies using atropine and tetrodotoxin, the muscle strips were exposed to the indicated concentrations of these agents for 6 min and 15 min, respectively, and then to the various concentrations of leukotrienes (Chang et al., 2008; Goldenberg and Subers, 1983; Huang, 2009; von Schrenck et al., 1989). Only one cumulative concentration–contraction response curve, with or without the cysteiny l leukotriene receptor antagonist, tetrodotoxin or atropine, was constructed with each preparation in the experiments (Chang et al., 2008; Huang, 2009).

Analysis of data

Results

Effects of cysteiny l and dihydroxy leukotrienes on gastric fundus and antrum contraction

In muscle strips isolated from the gastric fundus, LTD$_4$ and LTC$_4$ induced a marked and long-duration muscle contraction (Fig. 1). In terms of the maximal tension of contraction, LTD$_4$ and LTC$_4$ were equal in efficacy (Fig. 2). In the fundus, LTD$_4$ caused detectable contraction of muscle strips at 0.1 nM, half-maximal contraction at 0.0 ± 0.5 nM and maximal contraction at 1.0 μM. The maximal tension
carbachol (1 μM)-induced tone. Results given are from at least three experiments. Vertical bars represent ± SEM.

In both fundic and antral muscle strips, the LTC₄-induced contraction was not altered by l-serine borate (Fig. 3). Specifically, in the presence of l-serine borate (45 mM), LTC₄ caused detectable contraction of muscle strips at 0.1 nM, half-maximal contraction at 1.2 ± 0.8 nM (p < 0.05, compared with half-maximal contraction in the absence of l-serine borate) and maximal contraction at 0.1 μM in the fundus. The maximal tension caused by LTC₄ (0.1 μM) was 95 ± 5% of the tension caused by 1 μM carbachol (p < 0.05, compared with maximal tension in the absence of l-serine borate). Similarly, in the presence of l-serine borate (45 mM), LTC₄ caused detectable contraction of muscle strips at 0.1 nM, half-maximal contraction at 1.5 ± 0.5 nM (p < 0.05, compared with half-maximal contraction in the absence of l-serine borate) and maximal contraction at 1 μM in the antrum. The maximal tension caused by LTC₄ (1 μM) was 107 ± 12% of the tension caused by 1 μM carbachol (p < 0.05, compared with maximal contraction in the absence of l-serine borate; Fig. 3).

**Effects of CysLT antagonists, tetrodotoxin and atropine on LTD₄-induced gastric fundus and antrum contraction**

In fundic and antral muscle strips, selective CysLT₁ receptor antagonists, montelukast (1 μM) and the dual CysLT₁ and CysLT₂ antagonist BAY u9773 (1 μM) alone did not cause contraction (data not shown). In the fundus, 1 μM montelukast almost abolished the LTD₄ response (Fig. 4). Specifically, the tension caused by 1 μM LTD₄ alone was 102 ± 9% of the tension caused by 1 μM carbachol, whereas the tension caused by 1 μM LTD₄ plus 1 μM montelukast was 6 ± 3% of the tension caused by 1 μM carbachol (Fig. 4). BAY u9773 (3 μM) markedly inhibited LTD₄-induced contraction. The tension caused by 1 μM LTD₄ plus 3 μM BAY u9773 were 22 ± 6% of the tension caused by 1 μM carbachol (Fig. 4). The combination of both BAY u9773 (3 μM) and montelukast (1 μM) almost abolished the LTD₄ response (Fig. 4). The tension caused by 1 μM LTD₄ plus the combination of montelukast (1 μM) and BAY u9773 (3 μM) was 8 ± 5% of the tension caused by 1 μM carbachol.

In the antrum, 1 μM montelukast or 3 μM BAY u9773 partially inhibited the LTD₄ response (Fig. 4). Specifically, the tension caused by 1 μM LTD₄ alone was 78 ± 5% of the tension caused by 1 μM carbachol, whereas the tension caused by 1 μM LTD₄ plus 1 μM montelukast, and 3 μM BAY u9773 were 38 ± 9%, and 40 ± 9% of the tension caused by 1 μM carbachol, respectively (p < 0.05, compared with LTD₄ alone; Fig. 4). The combination of both BAY u9773 (3 μM) and montelukast (1 μM) almost abolished the LTD₄ response (Fig. 4). The tension caused by 1 μM LTD₄ plus the combination of montelukast (1 μM) and BAY u9773 (3 μM) was 7 ± 7% of the tension caused by 1 μM carbachol (p < 0.05, compared with LTD₄ plus montelukast 1 μM or BAY u9773 3 μM; Fig. 4).

**Fig. 2.** Ability of leukotrienes, LTC₄, LTD₄, LTE₄ and LTB₄, to cause contraction of the guinea pig gastric fundus (left) and antrum (right). Values are expressed as percent of carbachol (1 μM)-induced tone. Results given are from at least three experiments. Vertical bars represent ± SEM.

**Fig. 3.** Ability of leukotriene LTC₄ in the absence or presence of l-serine borate, 45 mM, to cause contraction of the guinea pig gastric fundus (left) and antrum (right). Values are expressed as percent of carbachol (1 μM)-induced tone. Results given are from at least three experiments. Vertical bars represent ± SEM.
In both fundic and antral muscle strips, the LTD4-induced contraction was not affected by 1 μM tetrodotoxin or 1 μM atropine (Fig. 5).

**Effects of CysLT1 antagonist montelukast on LTC4-induced gastric fundus and antrum contraction in the presence of L-serine borate**

In the presence of the inhibitor of LTC4 metabolism L-serine borate, the LTC4-induced contraction was not and only partially inhibited by montelukast in the fundus and antrum, respectively (Fig. 6). Specifically, in the presence of L-serine borate (45 mM) the maximal tension caused by LTC4 (0.1 μM) plus 1 μM montelukast in the fundus was 62 ± 12% of the tension caused by 1 μM carbachol (p < 0.05, compared with LTC4 alone; Fig. 6). In contrast, in the presence of L-serine borate (45 mM) the maximal tension caused by LTC4 (0.1 μM) plus 1 μM montelukast in the antrum was 51 ± 5% of the tension caused by 1 μM carbachol (p < 0.05, compared with LTC4 alone; Fig. 6).

**Discussion**

In the present study we demonstrated for the first time that cysteinyl leukotrienes LTC4, LTD4 and LTE4 cause moderate to marked whereas the dihydroxy leukotriene LTE4 causes only small muscle contraction in the stomach in vitro. Furthermore, we found that the leukotriene-induced contraction is mediated by CysLT1 in the fundus but by both CysLT1 and CysLT2 in the antrum.

Previous studies showed LTD4 and LTC4 caused contraction of the rat stomach and these contractions were inhibited by CysLT1 receptor antagonists (Goldenberg and Subers, 1983; Miura et al., 1992; Miyata et al., 1995). In the present study we found that, in addition to LTD4 and LTC4, LTE4 caused contraction of the stomach and the relative potencies for cysteinyl leukotrienes to cause contraction were LTD4 = LTC4 > LTE4 in both fundus and antrum. The selective CysLT1 receptor antagonist montelukast almost abolished but only partially inhibited LTD4-induced contraction in fundus and antrum, respectively. The dual CysLT1 and CysLT2 receptor antagonist BAY u9773 partially inhibited LTD4-induced contraction in the antrum as well. We did not test the potent, selective CysLT2 antagonist HAMI3379 (Wunder et al., 2010). Interestingly, the combination of montelukast and BAY u9773 almost abolished LTD4-induced contraction in the antrum, indicating enhancement of inhibition. These indicate that leukotriene-induced contraction is mediated by CysLT1 in fundus and by both CysLT1 and CysLT2 in antrum. More studies using potent, selective CysLT2 receptor antagonists are needed to clarify the CysLT subtype mediating antral contraction. If confirmed, the leukotriene-induced contraction in the antrum mediated by both CysLT1 and CysLT2 is different to the esophagus, lower esophageal sphincter and gallbladder, in which CysLT1 mediating leukotriene-induced contraction (Falcone and Krell, 1992; Freedman et al., 1993; Chang et al., 2008; Huang, 2009), and different from ileum, in which CysLT2 mediating contraction (Bäck et al., 1996; Bäck, 2002). The effects of LTD4 were not affected by atropine or tetrodotoxin. This indicates that the cysteinyl leukotriene action is not neurally mediated and suggests a direct effect of cysteinyl leukotrienes on the gastric muscle. Direct effects have also been described on the cysteinyl leukotriene-induced contraction in the gallbladder, esophagus and lower esophageal sphincter (Freedman et al., 1993; Chang et al., 2008; Huang, 2009).

In the presence of L-serine borate, the CysLT1 receptor antagonist montelukast did not inhibit and only partially inhibited the LTC4-induced contractile responses in the fundus and antrum, respectively. This is in agreement with a previous study showing a difference in the antagonist sensitivity between LTC4 and LTD4 in gastric smooth muscle cells and suggests that both CysLT1 and CysLT2 might mediate leukotriene-induced contraction not only in the antrum but also in the fundus (DeLegge et al., 1993; Bäck et al., 2001).

Our results in the present study demonstrate that CysLT1 mediates leukotriene-induced contraction in the fundus and suggest that both CysLT1 and CysLT2 mediate leukotriene-induced contraction in the antrum in vitro. Further studies on the effects of leukotrienes in vivo are required to elucidate the leukotriene influence on the gastric
motility. In the gastrointestinal system, cysteinyl leukotrienes have been implicated in eosinophilic esophagitis and eosinophilic gastroenteritis. CysLT₁ receptor antagonists are used clinically as therapeutic agents in these diseases (Brink et al., 2003; Kanaoka and Boyce, 2004; Capra et al., 2007; Khan, 2005; Attwood et al., 2003). Recently, fundic relaxants are a new approach to treatment of impaired gastric accommodation in functional dyspepsia. Emerging therapeutic agents include the 5-HT₁A agonist (Tack, 2009). CysLT₁ receptor antagonists inhibit cysteinyl leukotriene-induced contraction in the fundus. Therefore they might be potential fundic relaxants. On the other hand, gastric distension, especially the antral distension, by food is important in regulation of food intake and appetite (Delzenne et al., 2010). CysLT₂ and CysLT₃ related agents, influencing antral and gastric contraction, respectively, might have therapeutic potential in appetite modulation and obesity.

Conclusion

The results of the present study demonstrate that cysteinyl leukotrienes CysLT₄, LTD₄ and LTE₄ cause moderate to marked whereas the dihydroxy leukotriene LTB₄ causes small muscle contraction in the stomach in vitro. The LTD₄-induced contraction is mediated by CysLT₁ in fundus but by CysLT₁ and CysLT₂ in antrum.

Conflict of interest statement

The author declares that there are no conflicts of interest.

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References


Fig. 6. Ability of CysLT₁ receptor antagonist montelukast, in the presence of (-)-serine borate, to inhibit leukotriene LTC₄-induced contraction in the guinea pig gastric fundus (left) and antrum (right). Values are expressed as percent of carbachol (1 μM)-induced tone. Results given are from at least three experiments. Vertical bars represent ± SEM. * p < 0.05, compared with LTC₄ alone.


