European Journal of Pharmacology 628 (2010) 195–201

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Pulmonary, Gastrointestinal and Urogenital Pharmacology

Pranlukast prevents cysteinyl leukotriene-induced emesis in the least shrew (*Cryptotis parva*)

Seetha Chebolu, Yaozhi Wang [1], Andrew P. Ray, Nissar A. Darmani *

Department of Basic Medical Science, College of Osteopathic Medicine of the Pacific, Western University of Health Sciences, 309 East Second Street, Pomona, CA 91766, USA

ARTICLE INFO

Article history:
Received 20 May 2009
Received in revised form 2 November 2009
Accepted 16 November 2009
Available online 24 November 2009

Keywords:
Leukotriene A_4
Leukotriene B_4
Fos
Dorsal vagal complex
Enteric nervous system

ABSTRACT

Many chemotherapeutic agents activate multiple signaling systems, including potentially emetogenic arachidonic acid metabolites. Of these messengers, the emetic role of the leukotriene family has been neglected. The aims of this study were to test the emetic potential of key leukotrienes (LTA_4, LTB_4, LTF_4, and the cysteinyl leukotrienes LTC_4, LTD_4 and LTE_4), and to investigate whether the leukotriene $CysLT_1$ receptor antagonist pranlukast or mixed leukotriene $CysLT_{1/2}$ receptor antagonist Bay u9773 can prevent the LTC_4-induced emesis. Least shrews were injected with varying doses of one of the six tested leukotrienes and vomiting parameters were measured for 30 min. LTC_4 and LTD_4 were most efficacious, and significantly increased both the frequency and percentage of animals vomiting at doses from 0.1 and 0.05 mg/kg, respectively. The other tested leukotrienes were either weakly emetic or ineffective at doses up to 4 mg/kg. The relative emetogenic activities of the cysteinyl leukotrienes ($LTC_4 = LTD_4 > LTE_4$) suggest that leukotriene $CysLT_2$ receptors have a key role in emesis. However, pranlukast dose-dependently, and at 10 mg/kg completely, blocked LTC_4-induced vomiting, implicating a leukotriene $CysLT_1$ receptor-mediated emetic effect. Bay u9773 dose-dependently reduced the percentage of animals vomiting, but did not significantly reduce vomiting frequency. Fos immunoreactivity, measured subsequent to LTC_4-induced vomiting to define its putative anatomical substrates, was significantly increased in the enteric nervous system and medullary dorsal vagal complex following LTC_4 ($P<0.05$) versus vehicle injections. This study is the first to show that some leukotrienes induce emesis, possibly involving both central and peripheral leukotriene $CysLT_1$ and/or leukotriene $CysLT_2$ receptors.

1. Introduction

One of the most distressing side effects of antitumor treatment is chemotherapy-induced vomiting (Slatkin, 2007). Although its full mechanism has continued to elude discovery, chemotherapy-induced vomiting appears to be a multifactorial process with dopamine, serotonin, substance P, and eicosanoids all contributing to its genesis (Andrews and Rudd, 2004; Minami et al., 2003; Darmani et al., 2009). One key eicosanoid, arachidonic acid, is released from cell membrane phospholipids by phospholipase A2. Depending on the cell and tissue type, arachidonic acid is then metabolized into different compounds by two key enzymes: 1) cyclooxygenases, which lead to formation of prostaglandins; and 2) lipoxygenases, which catalyze its conversion to hydroperoxyeicosatetraenoic acid (5-HPETE) and then to the rapidly metabolized intermediate leukotriene A_4 (LTA_4). LTA_4 is then converted to leukotriene B_4 via LTA_4 hydrolase or conjugated with glutathione to form the cysteinyl (or peptidyl) leukotriene, leukotriene C_4 (LTC_4). Enzymatic cleavage of specific portions of the glutathione moiety produces the other cysteinyl leukotrienes, leukotrienes D_4 (LTD_4), and E_4 (LTE_4) (Barnes, 1997; Capra et al., 2006), and also leukotriene F_4 (LTF_4), which is related to LTC_4 but like LTB_4 does not bind the cysteinyl leukotriene receptors. Two G-protein coupled receptors (GPCR) specific for cysteinyl leukotrienes (leukotriene $CysLT_1$ and leukotriene $CysLT_2$) have been cloned and characterized (Kanaoka and Boyce, 2004; Figueroa et al., 2001; Heise et al., 2000). Leukotriene $CysLT_1$ is expressed mostly in respiratory and gastrointestinal tissues and leukotriene $CysLT_2$ in cardiovascular and brain tissues (Capra et al., 2006).

Despite the knowledge that arachidonic acid and its related metabolites (prostaglandins E_2 and $F_{2\alpha}$ and their analogs) are potent emetogens (Darmani, 2002; Kan et al., 2003; Kan et al., 2006), little attention has been paid to the emetic potential of its other metabolites such as the leukotrienes. The only study that has indirectly attempted to determine the emetogenicity of the leukotrienes is the unsuccessful use of the leukotriene biosynthesis inhibitor MK-886 to block chemotherapy-induced vomiting in ferrets (Sam et al., 2007). Rather, extensive research on cysteinyl leukotrienes has examined their key role in asthma. Therefore, the aims of this study were 1) to test key leukotrienes

* Corresponding author. Tel.: +1 909 469 5654; fax: +1 909 469 5577.
E-mail addresses: schebolu@westernu.edu (S. Chebolu), yaw007@ucsd.edu (Y. Wang), aray@westernu.edu (A.P. Ray), ndarmani@westernu.edu (N.A. Darmani).
[1] Present address: Department of Neuroscience, University of California at San Diego, 9500 Gilman Drive # 0662, La Jolla, CA 92093, USA.

0014-2999/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2009.11.030

as putative emetogens; and 2) to investigate if the currently available leukotriene $CysLT_1$ receptor antagonists could prevent leukotriene-induced vomiting. For this, we used the previously characterized (Barnes, 1997; Evans, 2003) human leukotriene $CysLT_1$ receptor selective antagonist pranlukast, and the mouse leukotriene $CysLT_{1/2}$ dual antagonist Bay u9773 (4-[[(1R,2E,4E,6Z,9Z)-1-[(1S)-4-carboxy-1-hydroxybutyl]-2,4,6,9-pentadecatetraen-1-yl]thio]benzoic acid). Additionally, immunohistochemistry for Fos protein was used to indicate neuronal activation related to leukotriene-induced vomiting and provide information on its anatomical substrates. In this study we used the least shrew (*Cryptotis parva*), an emesis-competent mammal whose responses to common emetogens are well defined and correlate well with human responses (Darmani, 1998; Darmani et al., 2008).

2. Materials and methods

2.1. Animals

Adult least shrews (*C. parva*) were bred in the animal facility at Western University. Previous studies had demonstrated no gender differences, so both males and females were used. They were housed in groups on a 14:10 light:dark cycle, fed with food and water ad libitum. All the shrews used were 45–60 days old and weighed between 4 and 5 g. All experiments were conducted between 9:00 and 16:00 h and in accordance with Western University IACUC standards.

2.2. Drugs

Pranlukast, Bay u9773, LTA_4, LTB_4, LTC_4, LTD_4, LTE_4 and LTF_4 were purchased from Cayman Chemicals. All the leukotrienes were supplied in ethanol. The ethanol was evaporated under nitrogen and the drug was dissolved to twice the stated concentrations in a 1:1:18 solution of ethanol: emulphor™: 0.9% saline. This solution was then diluted with an equal volume of saline to a final ethanol concentration of 2.5%. Pranlukast and Bay u9773 were dissolved to twice the stated concentrations in DMSO and this was then diluted with an equal volume of distilled water to a final DMSO concentration of 50%.

2.3. Experimental protocols

On the day of the experiment, shrews were brought from the animal facility, separated into individual cages and allowed to adapt for at least two hours. One hour before the experiment food was withheld. The experiment was initiated when the shrews were injected intraperitoneally (i.p.) with either the vehicle for control animals, or one of the different leukotrienes at varying doses (0.025, 0.0375, 0.05, 0.1, 0.25, 0.5, 1, 2 or 4 mg/kg, $n=5$–18 per dose). Each individual shrew received one dose of one leukotriene only. Shrews were given 4 mealworms each prior to the injections, to aid in identifying wet vomits as described previously (Darmani, 1998). The shrews were observed for 30 min for vomiting behavior (number of animals vomiting within groups and frequency of vomits). Animals were used once and then euthanized with an overdose of pentobarbital (100 mg/kg i.p.) following the behavioral studies. Some were transcardially perfused with paraformaldehyde solution following anesthesia for use in subsequent immunohistochemical (Fos immunoreactivity) studies (see below).

For the agonist–antagonist studies, pranlukast (0, 2.5, 5 or 10 mg/kg, $n=7$–11 per dose) or Bay u9773 (1 or 5 mg/kg, $n=6$–8 per dose) was injected at 0 min. Immediately after this injection, shrews were given 4 mealworms each, and 30 min later the leukotriene agonist (1 mg/kg LTC_4) was injected. The agonist was chosen on the basis at which it caused maximum emesis in all tested animals at the lowest dosage. Each treated shrew was observed for 30 min post agonist injection and the number of animals vomiting within groups and frequency of vomits were recorded. Animals were euthanized with an overdose of pentobarbital (100 mg/kg i.p.) following the behavioral studies.

Immunohistochemical procedures were used to support the behavioral data and identify potential anatomical sites of action of leukotriene-mediated vomiting, and were performed as described elsewhere (Ray et al., 2009b). The number of Fos-immunoreactive (Fos-positive) nuclei resulting from LTC_4-induced vomiting versus non-vomiting, vehicle-injected shrews was compared in brain and gut regions previously found to be associated with emesis (i.e., the dorsal vagal complex and enteric nervous system of the small intestine). The brains, as well as intestinal sections 2 cm long, beginning 1 cm from the stomach, were taken from transcardially perfused shrews used in either control or LTC_4-treated behavioral groups. All tissue was processed immunohistochemically using the same methods (Ray et al., 2009b) regardless of its source.

2.4. Statistical analysis

The vomiting frequency data and dose-dependence were analyzed using the Kruskal–Wallis non-parametric analysis of variance (ANOVA). Animals within a group which did not vomit were still counted (as a zero frequency) for the purpose of statistical analysis. The percentage of animals vomiting across groups at different doses was compared using the Mann–Whitney *U* test. The mean numbers of Fos-positive nuclei across vomiting and non-vomiting conditions were counted by an observer blind to the drug condition and analyzed via Student's *t*-test. Nuclei were counted on a per section basis in all tissues. In the ENS, sections were also subdivided into 20 µm lengths for counting because the oblique sectioning made identifying individual enteric ganglia difficult in some sections. In all cases, a P-value<0.05 was necessary for statistical significance.

3. Results

3.1. Leukotriene dose–response emesis studies

The different leukotrienes tested were LTA_4, LTB_4, LTC_4, LTD_4, LTE_4 and LTF_4. As mentioned, LTC_4, LTD_4, and LTE_4 are the cysteinyl leukotrienes.

3.1.1. Cysteinyl leukotrienes

Dose–response vomiting behavior data for the cysteinyl leukotrienes are presented in Fig. 1. Both LTC_4 (Fig. 1A, B) and LTD_4 (Fig. 1C, D) showed dose-dependent increases in vomiting behaviors. Compared to vehicle injection, LTC_4 demonstrated significantly higher frequencies of vomiting ($P<0.05$) at doses greater than 0.05 mg/kg, and significant percentage of animals vomited ($P<0.05$) at doses greater than 0.1 mg/kg. LTD_4 had a significantly greater frequency of vomiting ($P<0.05$) and percentage of animals vomiting ($P<0.05$) at all tested doses from 0.05 to 2 mg/kg. Only LTC_4 produced 100% vomiting, at the 1 mg/kg dosage ($U=84$, 0; $P<0.0005$), while LTD_4 produced 91% vomiting at the same dosage ($U=126$, 6; $P<0.0005$). LTE_4 demonstrated significant increases in the vomiting frequency ($P<0.05$) and the percentage of animals vomiting ($P<0.05$) at doses greater than 1 and 2 mg/kg, respectively (Fig. 1E, F).

3.1.2. Non-cysteinyl leukotrienes

As shown in Fig. 2A/B, LTA_4 administration caused significant emesis only at its highest tested (4 mg/kg) dose (frequency, $P<0.01$; percentage vomiting, $U=114$, 42; $P<0.02$) when compared to the vehicle treated group. LTB_4 failed to induce significant vomiting at doses up to 4 mg/kg (Fig. 2C, D). On the other hand, LTF_4 injection caused emesis in 36–40% of shrews ($P<0.05$, $U=84$, 36; $P<0.05$, $U=42$, 18, respectively) at 1 and 4 mg/kg doses, statistically significant when compared against vehicle treated controls. However, LTF_4 showed a significant increase in emesis frequency at 1 mg/kg ($P<0.05$) but not at the 4 mg/kg dose ($P>0.05$) (Fig. 2E, F).