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The anti-asthmatic drug pranlukast suppresses the delayed-phase vomiting and reverses intracellular indices of emesis evoked by cisplatin in the least shrew (Cryptotis parva)

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ABSTRACT

The introduction of second generation serotonin 5-HT_3 receptor (5-HT_3) antagonist palonosetron combined with long-acting substance P neurokinin NK_1 receptor (NK_1) antagonists (e.g. netupitant) has substantially improved antiemetic therapy against early- and delayed-phases of emesis caused by highly emetogenic chemotherapeutics such as cisplatin. However, the improved efficacy comes at a cost that many patients cannot afford. We introduce a new class of antiemetic, the antiasthmatic leukotriene CysLT1 receptor antagonist pranlukast for the suppression of cisplatin-evoked vomiting. Pranlukast (10 mg/kg) by itself significantly reduced the mean frequency of vomits (70%) and fully protected least shrews from vomiting (46%) during the delayed-phase of cisplatin (10 mg/kg)-evoked vomiting. Although, pranlukast tended to substantially reduce both the mean frequency of vomits and the number of shrews vomiting during the early-phase, these reductions failed to attain significance. When combined with a first (tropisetron)- or a second (palonosetron)-generation 5-HT_3 receptor antagonist, pranlukast potentiated their antiemetic efficacy during both phases of vomiting. In addition, pranlukast by itself prevented several intracellular signal markers of cisplatin-evoked delayed-vomiting such as phosphorylation of ERK1/2 and PKA. When pranlukast was combined with either palonosetron or tropisetron, these combinations suppressed the evoked phosphorylation of: i) ERK1/2 during both acute- and delayed-phase, ii) PKCα/β at the peak acute-phase, and iii) PKA at the peak delayed-phase. The current and our published findings suggest that overall behavioral and intracellular signaling effects of pranlukast via blockade of CysLT1 receptors generally appear to be similar to the NK_1 receptor antagonist netupitant with some differences.

1. Introduction

Chemotherapy-induced nausea and vomiting (CINV) caused by cisplatin-type cytotoxic chemotherapeutics occurs in two phases in both patients (Hesketh et al., 2003b, 2015) and vomit-competent animals (Darmani et al., 2009; Rudd et al., 2016). Cisplatin-evoked vomiting observed during the first 24 h has been referred to as the acute (immediate)-phase CINV, whereas its delayed-phase is known to persist from 2 to 7 days depending upon the dose used and species examined (Darmani and Ray, 2009). The first-generation serotonin 5-HT_3 receptor antagonists such as tropisetron, ondansetron, or granisetron have been extensively used to reduce the acute-phase (Hesketh, 2008), but are relatively less effective in the control of delayed-phase CINV (Andrews and Rudd, 2004). To improve the overall control of both acute and delayed CINV, 5-HT_3 receptor antagonists were initially combined with a glucocorticoid such as dexamethasone (Hesketh et al., 1994; Ioannidis et al., 2000). Later, based upon animal studies (Tattersall et al., 1993; Andrews and Rudd, 2004), a neurokinin NK1 receptor antagonist (aprepitant) was also added in the antiemetic regimen (Hesketh et al., 2003a; Jordan et al., 2005). Currently this constitutes the standard triple prophylactic antiemetic therapy against highly emetogenic chemotherapeutics in cancer patients.

The triple regimen was founded upon early neurotransmitter studies since during the acute-phase cisplatin was shown to release serotonin from the enterochromaffin cells of the gastrointestinal tract (GIT) (Cubeddu et al., 1992; Janes et al., 1998; Wilder-Smith et al., 1993), which then stimulates 5-HT_3 receptors present on the vagal afferents to initiate the vomiting reflex (Naylor and Rudd, 1996;

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Minami et al., 2003). Furthermore, the delayed vomiting was thought to be primarily due to activation of NK_1 receptors subsequent to release of substance P (SP) in the brainstem (Tattersall et al., 1996; Andrews and Rudd, 2004). This elegant but simplistic view of the transmitter basis of CINV has been recently revised since cisplatin not only simultaneously increases 5-HT and SP turnover during both acute- and delayed-phases of CINV in the brainstem, GIT and urine (Darmani et al., 2009; Higa et al., 2006), but it can concomitantly enhance dopamine and eicosanoids turnover as well (Darmani and Ray, 2009; Darmani et al., 2009). Further support for this revision comes from the antiemetic potential of second generation 5-HT_3 receptor (palonosetron)- and long-acting NK_1 receptor (netupitant or rolapitant)- antagonists since these agents significantly attenuate both phases of CINV either alone or in combination, in both animals (Darmani et al., 2015; Duffy et al., 2012; Rudd et al., 2016) and cancer patients (Eisenberg et al., 2004; Hesketh et al., 2015; Rapoport et al., 2015; Rojas and Slusher, 2015). Dopaminergic antagonists are less effective in the clinic and were used against CINV prior to the advent of 5-HT_3 receptor antagonists (Darmani and Ray, 2009). Arachidonic acid can be transformed by the cyclooxygenase and lipoxygenase pathways to prostaglandins, thromboxane and leukotrienes which are collectively called eicosanoids (Darmani and Ray, 2009; Darmani and Chebolu, 2013). Indeed, arachidonic acid itself and several prostaglandins (e.g. PGE2, PGF2α) and cysteinyl leukotrienes (LTC_4=LTD_4 > LTE_4) behave as potent emetogens (Chebolu et al., 2010; Darmani and Chebolu, 2013; Kan et al., 2002, 2003). In fact in the least shrew cysteinyl leukotrienes exhibit structure-emetic-activity relationship with LTC_4 being the most potent emetogen. Moreover, the LTC_4-evoked vomiting was potently blocked by the human leukotriene CysLT1 receptor antagonist pranlukast (Chebolu et al., 2010), which is currently used as an antiasthmatic agent in the clinic (Matsuse and Kohno, 2014). Based on these findings, we envisaged pranlukast may be an effective antiemetic against CINV and introduction of such new classes of antiemetics may help to reduce the cost of CINV prophylaxis (Broder et al., 2014; Schwartzberg et al., 2015).

Thus, the aim of this study was to determine whether pranlukast: 1) by itself has efficacy against the acute, delayed or both phases of cisplatin-evoked vomiting in the least shrew model of emesis; 2) can potentiate the antiemetic efficacy of the very potent second generation 5-HT_3 receptor antagonist palonosetron and of the first generation 5-HT_3 receptor antagonist tropisetron, and 3) either alone or in combination with palonosetron or tropisetron, can prevent some of the phosphorylated intracellular markers of cisplatin-evoked vomiting [phosphorylated extracellular signal-regulated protein kinases 1 and 2 (pERK1/2); phosphorylated protein kinase C alpha/beta (p-PKCα/β) and phosphorylated protein kinase A (p-PKA)] at peak immediate (2 h) and delayed emetic phases (33 h) (Darmani et al., 2013, 2015).

2. Materials and methods

2.1. Animals and treatment protocols

Male and female shrews were bred and housed in our animal facilities. Shrews weighing 4–6 g (45–70 days old) were used throughout the study. The animals were housed in groups of 5–10 and kept on a 14/10-h light/dark cycle at a room temperature of 22 ± 1 °C in a humidity-controlled environment with ad lib supply of food and water as described previously (Darmani, 1998). All animals received care according to the Guide for the Care and Use of Laboratory Animals (Department of Health and Human Services Publication, revised, 2011). All experimental procedures were conducted between 9:00 and 16:00 h and were approved by the Western University Institutional Animal Care and Use Committee standards.

2.2. Drugs

Palonosetron was a generous gift from the Helsinn Health Care (Lugano, Switzerland) and was dissolved in water. Tropisetron (Tocris, Minneapolis, MN) and Pranlukast (Cayman Chemicals, Ann Arbor, MI) were dissolved in water and 25% DMSO respectively. Cisplatin [cis-platinum (II) diamine dichloride (Pt (NH3)2cl2] (Sigma, St. Louis, MO) was dissolved in saline by sonication. All drugs were administered at a volume of 0.1 ml/10 g body weight.

2.3. Behavioral studies

Based upon our previously published and preliminary studies, a subcutaneous 0.1 mg/kg dose of palonosetron, a 10 mg/kg (s.c.) dose of tropisetron, and a 10 mg/kg (i.p.) dose of pranlukast were chosen to study their antiemetic potential against both phases of cisplatin-induced vomiting (Chebolu et al., 2010; Darmani, 1998; Darmani et al., 2014). On the day of the experiment, shrews were brought into the laboratory from the animal facility, separated into individual cages and allowed to adapt for at least two h. Two h before the experiment daily food was withheld but shrews were given 4 mealworms (Tenebrio sp.) each prior to cisplatin injection to aid in identifying wet vomits as described previously (Darmani, 1998).

We have previously demonstrated that cisplatin (5–20 mg/kg, i.p.) administration causes dose-dependent acute and delayed vomiting across a 40 h post-injection observation period in the least shrew (Darmani et al., 2009). The 20 mg/kg cisplatin dose was too toxic, the 5 mg/kg did not produce sufficient frequency of emesis per h, while the 10 mg/kg dose produced robust frequencies of vomits during immediate (1–2 h post-injection)- and delayed (31–34 h post-injection)-phases over the 40 h observation period. Thus, different groups of shrews were pretreated with either vehicle (vehicle+vehicle, n=12–16), palonosetron (0.1 mg/kg+pranlukast vehicle, n=12), tropisetron (10 mg/kg+pranlukast vehicle, n=9), pranlukast (10 mg/kg+palonosetron/tropisetron vehicle, n=11–15), a combination of palonosetron (0.1 mg/kg) and pranlukast (10 mg/kg) (n=15), or a combination of tropisetron (10 mg/kg) and pranlukast (10 mg/kg) (n=8), 30 min prior to the administration of cisplatin (10 mg/kg). Following administration of cisplatin each animal was placed in an individual cage with free access to food (mealworms) and water and the mean frequency of emetic behaviors were videotaped and scored per h (mean ± S.E.M.) for 40 h in accord with our published methods (Darmani et al., 2009).

2.4. Western blot

Shrews were decapitated at the indicated time points for different experimental treatment groups (n=3 animals per group). Brainstem samples were collected on ice and were kept at −80 °C before use. Brainstem tissue samples were homogenized in cold lysis buffer (50 mM Tris-HCl, pH 8, 150 mM NaCl and 1% NP-40) containing protease- and phosphatase-inhibitors cocktail (Pierce, Rockford, IL), and centrifuged at 10,000×*g* for 20 min at 4 °C. Total protein concentrations in supernatants were confirmed using the BCA protein assay kit (Pierce, Rockford, IL). All samples were subjected to 12% SDS-polyacrylamide gel electrophoresis. Proteins were transferred to a polyvinylidene difluoride membrane for 90 min at 90 V. After blocking with TBST solution (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, and 0.1% Tween 20) containing 5% BSA for 1 h at room temperature, membranes were incubated overnight at 4 °C with the following antibodies: ERK1/2 mouse antibody (#9107, 1:2000, Cell signaling), phospho-ERK1/2 (Thr202/Tyr204) rabbit antibody (#9101, 1:2000, Cell signaling), phospho-PKA C (Thr197) rabbit antibody (#5661, 1:1000, Cell signaling)), phospho-PKCα/β II (Thr638/641) rabbit antibody (#9375, 1:1000, Cell signaling), or glyceraldehyde-3-phosphate dehydrogenase mouse antibody (GAPDH) (#MAB374, 1:10,000, Millipore). Infrared fluorescent-labeled anti-goat and anti-mouse secondary antibodies