The Antiemetic 5-HT₃ Receptor Antagonist Palonosetron Inhibits Substance P-Mediated Responses In Vitro and In Vivo

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ABSTRACT

Palonosetron is the only 5-HT₃ receptor antagonist approved for the treatment of delayed chemotherapy-induced nausea and vomiting (CINV) in moderately emetogenic chemotherapy. Accumulating evidence suggests that substance P (SP), the endogenous ligand acting preferentially on neurokinin-1 (NK-1) receptors, not serotonin (5-HT), is the dominant mediator of delayed emesis. However, palonosetron does not bind to the NK-1 receptor. Recent data have revealed cross-talk between the NK-1 and 5HT₃ receptor signaling pathways; we postulated that if palonosetron differentially inhibited NK-1/5-HT₃ cross-talk, it could help explain its efficacy profile in delayed emesis. Consequently, we evaluated the effect of palonosetron, granisetron, and ondansetron on SP-induced responses in vitro and in vivo. NG108-15 cells were preincubated with palonosetron, granisetron, or ondansetron; antagonists were removed and the effect on serotonin enhancement of SP-induced calcium release was measured. In the absence of antagonist, serotonin enhanced SP-induced calcium-ion release. After preincubation with palonosetron, but not ondansetron or granisetron, the serotonin enhancement of the SP response was inhibited. Rats were treated with cisplatin and either palonosetron, granisetron, or ondansetron. At various times after dosing, single neuronal recordings from nodose ganglia were collected after stimulation with SP; nodose ganglia neuronal responses to SP were enhanced when the animals were pretreated with cisplatin. Palonosetron, but not ondansetron or granisetron, dose-dependently inhibited the cisplatin-induced SP enhancement. The results are consistent with previous data showing that palonosetron exhibits distinct pharmacology versus the older 5-HT₃ receptor antagonists and provide a rationale for the efficacy observed with palonosetron in delayed CINV in the clinic.

Introduction

Nausea and vomiting are common, severe, and feared side effects of many chemotherapeutics. Inadequate control of chemotherapy-induced nausea and vomiting (CINV) impairs functional activity and may compromise adherence to treatment. 5-HT₃ receptor antagonists are most efficacious against acute emesis (0–24 h after chemotherapy administration), whereas neurokinin-1 (NK-1) receptor antagonists have been associated with the prevention of delayed emesis (24–120 h after chemotherapy administration) (Hesketh et al., 2003). Even though the terms acute and delayed are approximations with no clear distinction of when acute emesis ends and delayed emesis begins, they point to the idea that different mechanisms are at play. Palonosetron is unique among 5-HT₃ receptor antagonists in that, in addition to being effective against acute emesis, it has shown efficacy against delayed emesis (Eisenberg et al., 2003; Aapro et al., 2006; Saito et al., 2009). The reason for this unique efficacy in delayed emesis is not clear, because palonosetron does not bind to NK-1 receptors (Wong et al., 1995).

Receptor cross-talk, defined as activation of one receptor by its ligand affecting cellular responses to another receptor signaling pathways. For example, substance

ABBREVIATIONS: CINV, chemotherapy-induced nausea and vomiting; 5-HT, serotonin; NK-1, neurokinin-1; SP, substance P; AM, ace- toxymethyl.
Palonosetron, but Not Ondansetron or Granisetron, Inhibited Serotonin Enhancement of SP-Induced Calcium-Ion Release in NG-108-15 Cells. Calcium-ion release in NG-108-15 cells depended on SP and 5-HT at the EC50. Results shown in Figs. 2 to 5 are the average from 12 to 42 measurements made on isolated neurons obtained from 7 to 26 rats. Statistical Analysis. Prism (GraphPad Software Inc., San Diego, CA) was used to obtain EC50 values in vitro experiments (sigmoidal dose-response variable slope). Errors correspond to S.E.M. of at least eight independent determinations. Student’s t test (two-tailed distribution) was used to determine p values. Experimental groups in the in vivo experiments included at least 12 independent neuronal measurements from at least seven rats to obtain average values. Error bars correspond to S.E.M.

Results
TABLE 1
Effect of 5-HT3 receptor antagonists on serotonin activation of SP response in NG108-15 cells

<table>
<thead>
<tr>
<th>Treatment</th>
<th>EC50 ± S.E.M.</th>
<th>µM</th>
</tr>
</thead>
<tbody>
<tr>
<td>SP</td>
<td>67.7 ± 0.97</td>
<td>6.7</td>
</tr>
<tr>
<td>SP + 5-HT</td>
<td>0.62 ± 0.09</td>
<td>0.025</td>
</tr>
<tr>
<td>SP + 5-HT + ondansetron</td>
<td>0.66 ± 0.17</td>
<td>0.3</td>
</tr>
<tr>
<td>SP + 5-HT + granisetron</td>
<td>0.57 ± 0.10</td>
<td>0.005</td>
</tr>
<tr>
<td>SP + 5-HT + palonosetron</td>
<td>3.7 ± 0.84</td>
<td>3.7</td>
</tr>
</tbody>
</table>

10 µg/kg via intra-arterial injection neuronal activity was increased, although variable, to 4 ± 4 impulses/10 s. When measurement of the SP response was made 10 h after intraperitoneal administration of 5 mg/kg i.p. cisplatin (Fig. 2A), SP responses increased to 23 ± 3 impulses/10 s. When the dose of cisplatin was increased to 10 mg/kg, both basal and SP responses were similar within experimental error to when 5 mg/kg cisplatin was used (Fig. 2B). In short, there was an approximately 6-fold increase in the neuronal response to SP in the presence of 5 to 10 mg/kg cisplatin.

Palonosetron Dose-Dependently Inhibited Cisplatin’s Potentiation of the SP Response in Nodose Ganglia. Preliminary studies with the three antagonists suggested that only palonosetron had an effect on SP responses. Consequently, we conducted a dose response with palonosetron. Cisplatin (5 mg/kg i.p.) was administered to rats; 10 h later, single nodose ganglia neurons were isolated and four electrophysiological recordings were made at 10-min intervals (Fig. 3A). First, a basal reading was taken. Second, the neuronal response to SP (10 µg/kg intra-arterial injection) was measured. Third, the neuronal response to palonosetron (intravenous infusion of 30, 100, and 300 µg/kg) was measured. The fourth and final recording measured neuronal responses after the administration of SP (10 µg/kg intra-arterial injection). The first three responses were similar throughout the experiment: basal readings were 7.5 ± 3

Fig. 1. The effect of 5-HT3 receptor antagonists on serotonin activation of SP-induced intracellular calcium-ion release. A, serotonin activation of the SP response. NG108-15 cells were incubated with SP at various concentrations, and after 1 h internal calcium release was measured upon the addition of 10-7 M B, ondansetron (Ondan; 30 nM) was preincubated with cells for 2 h; antagonist was subsequently removed, and the effect on serotonin activation of the SP response was measured. Responses to SP and SP + 5HT in A are shown for reference. C and D, same as B except granisetron (Gran; 5 nM; C) and palonosetron (Palo; 1 nM; D) were used. Error bars correspond to ± S.E.M.; error bars in B–D for the SP and SP + 5HT traces are the same as in A. Each EC50 curve corresponds to the average of at least eight independent determinations.

Fig. 2. The effect of cisplatin on neuronal response to SP stimulation. A, experimental protocol. Cisplatin was given at 0 h; 10 h later, basal and SP responses were measured. B, animals were anesthetized after cisplatin administration, vagal nerve was isolated, and single neuronal responses were examined. Before SP stimulation basal discharge frequency (impulses/10 s) was assessed for 1 min. The discharge frequency after SP administration was subsequently measured for 3 min. Results are the average of at least 12 independent neuronal measurements from at least seven rats (***, p < 0.001; compared with no cisplatin). Error bars correspond to ± S.E.M.
The doses used for ondansetron and granisetron in these studies turned out to be in excess of their active doses reported in previous efficacy studies in animals (Eglen et al., 1995; Rudd and Naylor, 1996; Endo et al., 1999; Rudd et al., 2002). Vagal nerve activity was measured in animals 10 h after cisplatin administration (5 mg/kg i.p.). The same measurements as in the palonosetron dose-dependence experiment were made: basal response to SP, response to each antagonist alone, and response to administration of SP (Fig. 4A). Basal and SP responses were 8 ± 3 and 33 ± 6 impulses/10 s, respectively. Neither ondansetron (38 mg/kg i.v.) nor granisetron (840 µg/kg i.v.) had an effect on the basal response. When SP was administered after ondansetron or granisetron, the SP response was not affected. This lack of effect was in stark contrast to more than 70% inhibition of the SP response after palonosetron administration (Fig. 4B).

**Palonosetron’s Inhibition of the Cisplatin-Induced SP Response Was Time-Dependent.** One major consideration in these experiments and in the clinic is the time of administration of 5-HT<sub>3</sub> receptor antagonist with respect to the time of administration of the chemotherapeutic agent. In the experiments described thus far, the antagonists were administered 10 h after cisplatin. We wanted to explore whether inhibition of the SP response could be observed at earlier time points. To this end, we explored the inhibition of the neuronal response to SP when antagonists were administered 30 min before and 5 h after cisplatin. As above, the measurement of the neuronal response to SP was made 10 h after cisplatin (Fig. 5A). The neuronal response to SP administration in the presence of cisplatin was 32 ± 4 impulses/10 s. When ondansetron (38 mg/kg i.v.) or granisetron (840 µg/kg i.v.) was given at either 30 min before or 5 and 10 h after cisplatin, there was no effect on the SP response. In contrast, when palonosetron (300 µg/kg i.v.) was administered at 30 min before or 5 and 10 h after cisplatin, inhibition of the SP response was observed at every time point. There was 33 ± 7% inhibition when palonosetron was administered 30 min before cisplatin, 70 ± 8% inhibition when palonosetron was given 5 h after cisplatin, and 78 ± 10% inhibition when palonosetron was given 10 h after cisplatin (Fig. 5B).

**Discussion**

5-HT<sub>3</sub> and NK-1 receptor antagonists are common therapy to help prevent CINV. In general, 5-HT<sub>3</sub> receptor antagonists are efficacious in the acute (0–24 h) phase of CINV, whereas NK-1 receptor antagonists are most efficacious during the delayed (24–120 h) phase (Hesketh et al., 2003). Palonosetron is the only 5-HT<sub>3</sub> receptor antagonist that is labeled for the treatment of delayed emesis in moderately emetogenic chemotherapy. The mechanisms by which palonosetron helps prevent delayed emesis are not known; this is tantalizing given that palonosetron does not bind to the NK-1 receptor (Wong et al., 1995). On the other hand, palonosetron has been shown to exhibit unique molecular interactions with the 5-HT<sub>3</sub> receptor. Palonosetron exhibits allosteric binding and positive cooperativity (Rojas et al., 2008) and triggers receptor internalization and long-term inhibition of receptor function (Rojas et al., 2010), attributes not shared by other 5HT<sub>3</sub> receptor antagonists.

Various reports in the literature have shown that there is cross-talk between NK-1 and 5-HT<sub>3</sub> receptor signaling path-
ways (Hu et al., 2004). SP, a known agonist at the NK-1 receptor, was shown to potentiate 5-HT$_3$ receptor-mediated inward current in rat trigeminal ganglion neurons. Potentiation of 5-HT$_3$ receptor current through SP is thought to involve second-messenger signaling that culminates in protein kinase C activation (Hu et al., 2004). In addition, 5-HT$_3$ receptor antagonists have been shown to block SP-mediated vagal afferent activation, and NK-1 antagonists were shown to block serotonin-induced vagal afferent activation (Minami et al., 2001). Evidence of receptor signaling interaction raises the interesting possibility that palonosetron’s unique efficacy for receptor saturation, we chose to use antagonists’ internalization, whereas ondansetron and granisetron exhibit simple receptor blockade (Rojas et al., 2010). The purpose of these in vitro experiments was to determine whether preincubation with the 5-HT$_3$ antagonists, followed by their complete removal from the media, could have a persistent downstream effect on SP function. In other words, could 5-HT$_3$ receptor internalization affect serotonin’s activation of SP-induced calcium-ion release? To ensure excess drug availability for receptor saturation, we chose to use antagonists’ concentrations >15-fold their respective IC$_{50}$ values needed to inhibit serotonin-induced calcium-ion influx (Supplemental Methods and Supplemental Fig. 1, A–C; IC$_{50}$s were 0.7, 2, and 3 nM for palonosetron, granisetron, and ondansetron, respectively). These concentrations were also approximately 50 times their previously determined Kd values (Wong et al., 1995; Rojas et al., 2010). When cells were preincubated with excess palonosetron (10 nM) followed by the drug’s removal...
through extensive cell washing, the serotonin effect on the response to SP was inhibited 6-fold (rightward shift of the EC50 curve; Fig. 1B; Table 1). In contrast, when cells were preincubated with excess ondansetron (300 nM) or granisetron (50 nM) followed by their removal there was no inhibition of the serotonin-induced SP response (Fig. 1, C and D; Table 1). Cell washing to remove the antagonists from the media took into account their respective half-lives of dissociation from 5-HT3 receptors to insure that all antagonist bound to the cell surface was dissociated before calcium-ion flux measurements were initiated (Rojas et al., 2008). The results showed that exposure to palonosetron uniquely inhibited the serotonin-induced activation of the SP response in vitro even after the drug was removed from the media.

These in vitro results probably are caused by palonosetron’s distinctive ability to induce 5-HT3 receptor internalization and cause long-term reduction in 5-HT3 receptor density on the cell surface. Previously, we have shown palonosetron rapidly partitions inside cells along with the 5-HT3 receptor during antagonist preincubation; once inside the cell, palonosetron does not reappear in the extracellular milieu for at least 2 h. It is noteworthy that palonosetron’s internalization depends on the presence of the 5-HT3 receptor, because palonosetron was shown not to partition into cells that do not express the 5-HT3 receptor (Rojas et al., 2010). These data suggest that palonosetron’s unique effect on inhibition of serotonin-induced SP activation is a 5-HT3 receptor-mediated difference, not a cell permeability difference.

To determine whether the in vitro results could also be demonstrated in vivo, we examined neuronal responses in nodose ganglia to SP after cisplatin administration in rats. We used rat nodose ganglia because they have been shown to express both 5-HT3 and NK-1 receptors (Hu et al., 2004) and palonosetron because it is known to activate the mechanisms of both acute and delayed emesis (Hesketh, 2008). Cisplatin triggers the release of serotonin from enterochromafin cells, which in turn activates 5-HT3 receptors located on the surface of vagal afferents. Consistent with previous data showing that 5-HT3 receptor activity can influence NK-1 signaling (Minami et al., 2001; Hu et al., 2004), we found that pretreatment of rats with cisplatin induced a 3- to 6-fold increase of the neuronal response in nodose ganglia to SP (Fig. 2) in agreement with a previous report (Wu et al., 2009).

Because cisplatin potentiated the SP response in nodose ganglia, we explored the possibility that 5-HT3 receptor antagonists could inhibit the cisplatin-induced activation of the SP response. We found that palonosetron uniquely inhibited the cisplatin-induced neuronal response to SP in a dose-dependent manner 10 h after cisplatin administration (Figs. 3 and 4). Neither ondansetron nor granisetron had an effect. It is noteworthy that the doses of ondansetron and granisetron in these studies took into account differences in clinical dose and were higher than those used in animal models where efficacy with these antagonists was observed (Eglen et al., 1995; Rudd and Naylor, 1996; Endo et al., 1999; Rudd et al., 2002). Consequently, the lack of inhibition of SP response with the high doses of granisetron and ondansetron used obviated the need for evaluating the efficacy of lower doses. One limitation, however, is that even though these antagonists are thought to be selective for the 5-HT3 receptor the compounds could be acting on off-target sites that could affect neuronal activity in the nodose ganglia at the relatively high doses used. Minami et al. (2001) reported that granisetron inhibits the SP response under acute conditions in vivo. However, our in vivo study was different in that it was designed to explore the effect of 5-HT3 receptor antagonists on the delayed cisplatin-induced plasticity changes characterized by an enhanced SP response (Wu et al., 2009). Correspondingly, the experiment outlined in Fig. 4 involved measurements of the SP response 10 h after cisplatin administration and the potential effects of 5-HT3 receptor antagonists on this enhancement. Under these conditions only palonosetron inhibited the enhanced interaction of SP and serotonin in vagal afferent neurons. These differences probably are caused by palonosetron-triggered 5HT3 receptor internalization that is unique and would be predicted to provide more persistent 5HT3 receptor functional inhibition compared with simple...
binding followed by dissociation at the receptor on the cell surface as occurs with ondansetron and granisetron.

Because the time of antiemetic administration could have different effects on cisplatin-induced toxicity in the clinic, we also explored the time dependence of palonosetron inhibition of the cisplatin-induced neuronal response to SP and the potential that ondansetron and/or granisetron could also have an effect if administered at times other than 10 h after cisplatin administration. Additional studies were performed when antagonists were given 30 min before cisplatin and 5 h after cisplatin. When ondansetron or granisetron was used, no inhibition of the cisplatin effect was observed at either time point. In contrast, when palonosetron was administered 30 min before cisplatin or 5 h after cisplatin and the effect on SP activity was measured 10 h later, there was 33 ± 7 and 70 ± 8% inhibition was observed, respectively (Fig. 5). In short, palonosetron inhibition of the SP response could be seen when palonosetron was administered 10 h, 5 h, or 10 min before measurement of the SP response.

The half-life of palonosetron in the rat is 90 min after intravenous administration (Supplemental Data, Palonosetron Rat PK Report), which is in the same order of magnitude as the half-lives previously reported for ondansetron (20–40 min) (Yang and Lee, 2008) and granisetron (50 min) (Huang et al., 1999) in rats. Therefore, the differences in pharmacokinetics provide a rationale for the unique efficacy observed with palonosetron in delayed CINV in the clinic.

In summary, palonosetron uniquely inhibits cross-talk between the 5-HT$_3$ and NK-1 receptor pathways in a dose- and time-dependent fashion. Our results are consistent with previous data showing that palonosetron exhibits distinct pharmacology versus the older 5-HT$_3$ receptor antagonists and provide a rationale for the unique efficacy observed with palonosetron in delayed CINV in the clinic.

References


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