Parapheromones Suppress Chemotherapy Side Effects

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ABSTRACT

The cytotoxic drugs used in chemotherapy are often accompanied by nausea and vomiting. Despite the use of antiemetic drugs, chemotherapy-induced nausea and vomiting (CINV) remain significant side effects for cancer patients and are associated with serotonin type 3 receptor (5-HT3R) activation in the brainstem. Farnesol and nerolidol are sesquiterpene alcohols found in essential oils of plants such as roses, citronella, and lemon grass and are used as antiemetic parapheromones. Medicinal plants often are effective in treating gastrointestinal disorders, including CINV, although the mechanism of action remains unclear. In the current work, the antiemetic efficacy of the naturally occurring racemic mixture of farnesol (m-farnesol) and nerolidol (m-nerolidol) against cisplatin CINV was tested using the pica behavior (consumption of nonnutritive substances) of rats. Animals treated with m-farnesol or m-nerolidol consumed a smaller amount of kaolin than of saline-treated control animals. This result is consistent with the antiemetic efficacy of farnesol and nerolidol. Compared with controls, m-farnesol– but not m-nerolidol–treated animals consumed more food and lost less body weight. Thus, farnesol effectively reduced appetite suppression and weight loss induced by cisplatin. In separate experiments, isomers of farnesol and nerolidol were tested on 5-HT–mediated responses of acutely isolated nodose neurons using patch-clamp methods. All the tested constituents inhibited 5-HT3R–mediated current in a noncompetitive manner. Thus, both farnesol and nerolidol may exert antiemetic efficacy by inhibiting 5-HT signaling in cranial visceral afferents, resulting in interruption of emetogenic signaling; however, nerolidol failed to suppress cisplatin–induced anorexia and weight loss, suggesting that additional mechanisms may contribute.

Introduction

The cytotoxic drugs used in chemotherapy cause diverse adverse effects that include nausea and vomiting (NV), diarrhea, anorexia, and weight loss (Hofman et al., 2004; Devlin et al., 2017). These chemotherapy-induced side effects often interrupt drug treatment and negatively impact outcome of the therapy (Andreyev et al., 1998; Hess et al., 2007). NV, the most common and debilitating of side effects, is commonly treated with antiemetic drugs, including serotonin type 3 receptor (5-HT3R) antagonists, corticosteroids, and substance P antagonists. Activation 5-HT3R in the brainstem is associated with triggering nausea pathways, and vagal afferents appear critical in these responses (Babic and Browning, 2014). Nevertheless, many cancer patients still suffer chemotherapy-induced nausea and vomiting (CINV) (Haiderali et al., 2011; Hernandez Torres et al., 2015). To suppress CINV further, medicinal plants (i.e., ginger and bitter orange) are often recommended (Pillai et al., 2011; Suryawanshi, 2011; Ryan et al., 2012). Sesquiterpene alcohols farnesol and nerolidol (Fig. 1) are common constituents of those medicinal plants and widely exist in fruits and herbs.

These compounds are used widely in perfumes as well as in food additives to enhance flavors (Suryawanshi, 2011; Nampoothiri et al., 2012; Chan et al., 2016). These constituents have antimicrobial and antineoplastic activity; however, their actions in CINV are not understood (Jabra-Rizk et al., 2006; Joo and Jetten, 2010). Plant-derived monoterpenoids with 5-HT3R–inhibiting potency have potential antiemetic efficacy to inhibit diverse gastrointestinal symptoms, including NV (Tyers and Freeman, 1992; Ashoor et al., 2013; Jarvis et al., 2016). Such evidence suggests that farnesol and nerolidol may have potential antiemetic efficacy against CINV.

To evaluate this assumption, we tested the functional efficacy of farnesol and nerolidol on CINV in a rat-pica model. We also assessed their potential mechanism of action by assaying effects on 5-HT3R–mediated currents in acutely isolated visceral afferent nodose neurons.

Materials and Methods

Ethics Statement. All animal procedures were conducted with the approval of the institutional Animal Care and Use Committee of the National Research Foundation of Korea (NRF-2017R1D1A1B03033436). ABBREVIATIONS: 5-HT3R, serotonin type 3 receptor; ACSF, artificial cerebrospinal fluid; CINV, chemotherapy-induced nausea and vomiting; MA, megestrol acetate; m-farnesol, racemic mixture of farnesol; m-nerolidol, racemic mixture of nerolidol; NV, nausea and vomiting; RM ANOVA, repeat measures analysis of variance.
Nerolidol and Farnesol Reduce Cisplatin-Induced Kaolin Consumption. Antiemetic effects of farnesol and nerolidol, racemic mixture of farnesol (m-farnesol), trans-nerolidol, cis-nerolidol, racemic mixture of cis and trans nerolidol (m-nerolidol), and linalool were purchased from Sigma-Aldrich. The 5-HT3R selective antagonist ondansetron was from Tocris Cookson (Ballwin, MO). Farnesol and nerolidol were first dissolved in ethanol, and then the stock solution was diluted with the external solution just before use. The final concentrations of ethanol were always <0.01%. At these concentrations, ethanol alone had no effect on membrane potential or on electrical activity. All drugs were applied via a rapid application Y-tube microperfusion system that provided complete changes in the solution surrounding the recorded neurons within 0.1 second (Murase et al., 1989).

Statistical Analysis. Values were expressed as the mean ± S.E.M. Statistical comparisons between two groups were analyzed by the Student’s t test. For multiple comparisons, one-way or two-way analysis of variance (ANOVA) was followed by post hoc testing for multiple comparisons (Bonferroni/Dunn’s correction, StatView; SAS Institute Inc., Cary, NC). Differences were considered statistically significant for P values <0.05.

Results

Nerolidol and Farnesol Reduce Cisplatin-Induced Kaolin Consumption. Antiemetic effects of farnesol and nerolidol were tested on cisplatin-induced emesis in a rat-pica model with their naturally occurring racemic mixtures m-farnesol and m-nerolidol. Animals were conditioned for 3 days before the
experiments by intraperitoneal cisplatin injections (6 mg/kg per day). In a control experiment, cisplatin administration significantly increased kaolin consumption \(P < 0.01\), repeat measures analysis of variance (RM ANOVA) compared with days before injection (Fig. 2). On days 1 and 2 of cisplatin administration, kaolin intake was significantly greater than the saline-treated group (\(n = 6\), \(P < 0.01\), RM ANOVA). Treatment with the 5-HT3R antagonist, ondansetron (3 mg/kg, p.o.) prevented cisplatin-mediated increases in kaolin intake. Kaolin intake was not significantly different in the saline-saline and cisplatin-ondansetron group animals. In the same conditions, \(m\)-nerolidol and \(m\)-farnesol (100 mg/kg) significantly inhibited the cisplatin-induced increase in kaolin intake at days 2 compared with the cisplatin-saline group (\(m\)-nerolidol \(P < 0.05\), \(m\)-farnesol \(P < 0.01\); Fig. 3). When their concentrations were increased to 500 mg/kg, both compounds reduced the cisplatin-induced kaolin intake at days 1 and 2 (\(P < 0.01\)). Neither \(m\)-farnesol nor \(m\)-nerolidol alone had a significant effect on the kaolin intake (Fig. 3B).

Farnesol and Nerolidol Effects on Cisplatin-Induced Changes in Body Weight, Food and Water Intake. In addition to NV, cytotoxic chemotherapy also suppresses appetite for food and reduces body weight (for review, see Suzuki et al., 2013). In our experiments, cisplatin reduced body weight and food and water intake (Fig. 4). Unexpectedly, animals treated with \(m\)-farnesol were less affected by cisplatin-induced side effects. Cisplatin caused weight loss in all tested groups but was significantly reduced by 100 mg/kg (\(P < 0.05\), RM ANOVA) and 500 mg/kg (\(P < 0.01\), RM ANOVA) \(m\)-farnesol treatment (Fig. 4A). After 4 days of cisplatin administration, rats treated with \(m\)-farnesol, 100 and 500 mg/kg, retained 86% ± 2.4% and 92% ± 4.8% of their original body weight, respectively, and that was significantly higher than the control group weight (83% ± 2.2%, \(P < 0.05\)). In contrast, neither \(m\)-nerolidol nor ondansetron affected cisplatin-induced weight loss. Cisplatin-treated animals consumed progressively smaller amounts of food, whereas animals treated with \(m\)-farnesol (500 mg/kg) consumed significantly more food than did the control groups on days 1 and 4 (\(P < 0.05\), RM ANOVA) (Fig. 4B). Moreover, \(m\)-farnesol (100 and 500 mg/kg) and ondansetron significantly inhibited the cisplatin-induced decreases in water intake at day 4 (\(P < 0.05\) or 0.01). Meanwhile, \(m\)-nerolidol had no effect on cisplatin-induced decrease in food and water intake (Fig. 4C). \(M\)-farnesol (500 mg) alone did not affect body weight or food and water intake compared with saline-treated controls (\(P > 0.05\)).

Farnesol and Nerolidol Inhibit 5-HT Currents in Sensory Neurons. Our work has revealed a novel antiemetic efficacy of farnesol and nerolidol against CINV. The mechanisms or sites of action for these antiemetic compounds are not known. Recent studies of the natural monoterpene (i.e., menthol, citral, and linalool) indicate efficacy in 5-HT3R inhibition (Ashoor et al., 2013; Jarvis et al., 2016). These results, coupled with the finding that 5-HT3R selective antagonists suppress CINV (Tyers and Freeman, 1992), suggest that this receptor may be targeted. As 5-HT3Rs are prominently

![Fig. 2. Effects of ondansetron (Ondan) on cisplatin-induced increase in kaolin intake. Cisplatin or vehicle (saline) was administered on day 1. Ondansetron was administered 60 minutes before cisplatin. Data are expressed as mean ± S.E.M. \(n = 6\). *\(P < 0.05\); **\(P < 0.01\) vs. saline-treated group.](image)

![Fig. 3. Effects of \(m\)-nerolidol (Nel) and \(m\)-farnesol (Far) on cisplatin-induced kaolin intake. (A) Nerolidol (100 and 500 mg/kg, p.o.) and farnesol (100 and 500 mg/kg, p.o.) were administered 60 minutes before cisplatin. *\(P < 0.05\); **\(P < 0.01\) vs. cisplatin-saline-treated group. (B) Nerolidol and farnesol were administered 60 minutes before saline. All data are expressed as mean ± S.E.M.](image)
expressed in vagal afferents, which participate in emetic pathways (Babic and Browning, 2014), we decided to test farnesol and nerolidol on visceral afferent nodose neurons.

5-HT induced inward currents in 38% (n = 70/184 neurons) of the tested neurons, as previously reported (Kim et al., 2015). This response was completely inhibited by the 5-HT3R-selective antagonist ondansetron (n = 8). The 5-HT-evoked inward currents increased in a concentration-dependent manner (Fig. 5). The 5-HT current amplitude was well fit by the Michaelis-Menten equation, in which the EC50 and the Hill coefficient were 9.1 and 1, respectively. Thus, fast application of 1 mM 5-HT evoked inward current responses in nodose neurons by activating 5-HT3Rs.

We tested the effect of farnesol and nerolidol on the 5-HT3R currents, including their stereoisomers and racemic mixtures independently. In addition, we compared linalool, a monoterpene alcohol, to compare with farnesol and three forms of nerolidol (cis- and trans-isomers and their racemic mixture) (Fig. 6A). The order of inhibitory potency was cis-nerolidol > m-nerolidol > trans-nerolidol. The IC50 of m-nerolidol was 2-fold lower than trans-nerolidol and 1.3 times higher than cis-nerolidol (Table 1). Farnesol has four different isomers; among them, trans, trans from isomer is the most common in nature. Trans, trans-farnesol, and a racemic mixture of farnesol (m-farnesol) inhibited the 5-HT response in a concentration-dependent manner (Fig. 6B) with an estimated IC50s of 2.7 and 3.9 μM for trans, trans-farnesol, and m-farnesol, respectively (Table 1); however, linalool failed to inhibit 5-HT response, even at 100 μM. In summary, cis-nerolidol and trans, trans-farnesol have the highest inhibitory efficacies against 5-HT currents in nodose neurons, respectively.

Farnesol and Nerolidol Act as Noncompetitive Antagonists. To characterize the inhibitory mechanism of farnesol and nerolidol on the 5-HT3Rs, 5-HT concentration-response curves were examined in the presence of cis-nerolidol or trans, trans-farnesol. The maximum responses to 1 mM 5-HT were significantly decreased by cis-nerolidol (P < 0.05, n = 5) to 82% ± 5.9%, 45% ± 3.5%, and 19% ± 2.0% of control values for
2, 5, and 10 μM, respectively (Fig. 7); however, the EC_{50} value for 5-HT was not affected by cis-nerolidol (P > 0.05). Analysis with Lineweaver-Burk double reciprocal plot indicates that cis-nerolidol inhibits the 5-HT response noncompetitively. Similarly, trans, trans-farnesol significantly decreased 1 mM 5-HT responses (P < 0.05, n = 5) to 98% ± 0.1%, 67% ± 11.2%, and 48% ± 12.4% of control values, for 1, 3, and 5 μM, respectively, without altering the 5-HT EC_{50} (P > 0.05).

Lineweaver-Burk analysis shows that trans, trans-farnesol also inhibits the 5-HT response noncompetitively.

**Discussion**

Our experiments indicate that naturally occurring racemic mixtures of m-farnesol and m-nerolidol acting at 5-HT_{3}Rs exert antiemetic efficacy in a cisplatin-induced pica behavior assay. M-farnesol also reduced the cisplatin-induced decreased weight and food and water intake. A selective 5-HT_{3}R antagonist, ondansetron, and m-nerolidol had no effect on the cisplatin-induced decrease in weight and food and water intake. Our electrophysiologic recordings indicate that sesquiterpene alcohol isomers of farnesol and nerolidol inhibited 5-HT–evoked responses in a concentration-dependent manner at vagal afferent neurons, a potential site of action in the behavioral assays. The order of inhibitory potency was trans, trans-farnesol > racemic mixture-farnesol > cis-nerolidol > racemic mixture-nerolidol > trans-nerolidol. The highest potency inhibitors, trans, trans-farnesol, and cis-nerolidol, noncompetitively inhibited 5-HT nodose responses.

**Antiemetic Action of Farnesol and Nerolidol against Cytotoxic CINV.** Pica behavior in rats closely parallels the pharmacologic mechanisms of vomiting in humans (Takeda et al., 1993). Here, we found that naturally occurring racemic mixtures of farnesol suppressed cisplatin-induced kaolin consumption. Thus, both farnesol and nerolidol exerted antiemetic efficacy against cytotoxic CINV.

Isomers of the farnesol and nerolidol inhibited 5-HT_{3}R–mediated 5-HT responses on visceral afferent neurons. Emetogenic chemotherapy increases 5-HT concentrations in the plasma and intestine (Schwörer et al., 1991; Castejon et al., 1999), and this elevated the activity of the gastrointestinal visceral afferents through 5-HT_{3}R activation (Horn et al., 2004). Hence, selective 5-HT_{3}R competitive antagonists are prescribed to alleviate CINV. Despite the appropriate use of diverse kinds of antiemetic drugs, many cancer patients still suffer from CINV (Pillai et al., 2011; Hernandez Torres et al., 2015) suggesting the need for new, alternative classes of antiemetic agents. Recent clinical trials found that add-on use of ginger augmented conventional
antiemetic medications for resistant NV (Ryan et al., 2009; Pillai et al., 2011). These ginger-mediated antiemetic actions have been attributed to the noncompetitive inhibition of 5-HT3Rs by its major constituents, gingerol and shogaol (Jin et al., 2014). Until now, several 5-HT3R noncompetitive antagonists have been derived from plant constituents (i.e., citral, linalool, eucalyptol, and menthol) (Ashoor et al., 2013; Jarvis et al., 2016); however, none of these has greater inhibitory efficacy at 5-HT3Rs than ginger constituents. Besides their antiemetic efficacy against CINV, their characterizations are quite incomplete. In contrast, isomers of the farnesol and nerolidol have lower IC50 values than any currently known compounds. Taken together, our current results indicate that isomers of the farnesol and nerolidol have promising potency in therapy-resistant CINV.

**Farnesol Effects on Cisplatin-Induced Reduced Food Intake and Weight Loss.** In addition to NV, cytotoxic chemotherapy causes appetite loss (anorexia) and weight loss (Kokal, 1985; Holmes, 1993; Andreyev et al., 1998; De Jonghe et al., 2009). Patient weight loss is associated with poor outcomes (Andreyev et al., 1998; Hess et al., 2007). The progesterone derivative megestrol acetate (MA) and cannabis-based medicines stimulate appetite and reduce weight loss in cancer patients (Jatoi et al., 2002; Walsh et al., 2005; Brisbois et al., 2011; Cuvelier et al., 2014). Despite their positive efficacy, significant side effects can curtail their use. For example, MA treatment can cause adrenal insufficiency in cancer patients with weight loss (Meacham et al., 2003; Orme et al., 2003). Meanwhile, the cannabis-based medicine induces psychotropic side effects through activating cannabinoid type 1 receptor in the central nervous system (Parmar et al., 2016). Despite their positive efficacy, significant side effects can curtail their use. For example, MA treatment can cause adrenal insufficiency in cancer patients with weight loss (Meacham et al., 2003; Orme et al., 2003). Meanwhile, the cannabis-based medicine induces psychotropic side effects through activating cannabinoid type 1 receptor in the central nervous system (Parmar et al., 2016). In contrast, farnesol is a common plant constituent long used in food, medicine, and perfume (Suryawanshi, 2011; Scheman et al., 2014). In many countries, farnesol flavors food so that there are no specific health concerns related to its ingestion (Lewis, 1989). Farnesol has antimicrobial and antineoplastic use (Jabra-Rizk et al., 2006; Joo et al., 2010). Our report now shows promising use in chemotherapy-associated appetite and

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<tr>
<th>Compound</th>
<th>IC50 (M)</th>
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<tr>
<td>Cis-nerolidol</td>
<td>4.89 x 10^-6</td>
</tr>
<tr>
<td>Trans-nerolidol</td>
<td>1.32 x 10^-5</td>
</tr>
<tr>
<td>Racemic mixture-nerolidol</td>
<td>6.58 x 10^-6</td>
</tr>
<tr>
<td>Trans, trans-farnesol</td>
<td>2.66 x 10^-6</td>
</tr>
<tr>
<td>Racemic mixture-farnesol</td>
<td>3.96 x 10^-6</td>
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**Fig. 7.** Characterization of the inhibition by cis-nerolidol and trans, trans-farnesol. (A) Lineweaver-Burk plot showing the mode of inhibition of cis-nerolidol. The concentration-response curves for 5-HT–induced responses were obtained in the presence of 2 (□), 5 (○), and 10 μM (△) of cis-nerolidol. (B) Lineweaver-Burk plot showing the mode of inhibition of t,t-farnesol. The concentration-response curves for 5-HT–induced responses were obtained in the presence of 1 (□), 3 (○), and 5 μM (△) of t,t-farnesol. All responses were normalized for the peak current induced by 1 mM 5-HT alone. The IC50 value was calculated by fitting the data using nonlinear regression (Origin 7). Each point is the average of five experiments. Vertical bars show ±S.E.M.
weight loss. Farnesol alone did not affect food intake in our saline-treated control animals (Fig. 3). Hence, its appetite-stimulating efficacy is specific for anorexia of chemotherapy. The mechanism of action of farnesol represents a quite different approach compared with MA or cannabis-based therapies. Further studies are needed to elucidate these mechanisms.

In conclusion, we have found a novel antiemetic efficacy of the farnesol and nerolidol. These edible sesquiterpene alcohol constituents non-competitively inhibited 5-HT3R-induced responses. This antiemetic mechanism of action may help inhibit conventional antiemetics (5-HT3R competitive antagonists) by overlapping the inhibition of serotonergic emetogenic signaling. Therefore, farnesol has promising efficacy for suppress antiemetic drug-resistant CINV and improving appetite and weight loss without serious side effects.

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Authorship Contributions

Performed data analysis: Lee, Yang, Kim.

References


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