Olvanil, a non-pungent vanilloid enhances the gastrointestinal toxicity of cisplatin in the ferret

Kit-Man Chu a, Man-Piu Ngan b, Man-Keung Waia, Chi-Kong Yeunga, Paul L.R. Andrews b, Nathalie Percie du Sert b, Ge Lin a, John A. Rudd a,∗

a Emesis Research Group, Brain-Gut Laboratory, Department of Pharmacology, Faculty of Medicine, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong, China
b Division of Basic Medical Sciences, St. George’s University of London, Cranmer Terrace, London SW17 0RE, UK

1. Introduction

Tachykinin NK1 receptor antagonists represent a relative new class of anti-emetic for the treatment of chemotherapy-induced acute and delayed emesis (Andrews and Rudd, 2004; Jordan et al., 2005) and the emesis induced by surgery and anaesthesia (Gan et al., 2007). The anti-emetic mechanism probably involves a block of the action of substance P at NK1 receptors in the nucleus tractus solitarius (NTS) and/or closely associated brainstem structures (Fukuda et al., 1999; Tattersall et al., 1996). Activators of transient receptor potential vanilloid (TRPV1) channels, which regulate neuropeptide release (including substance P) from sensory nerves (Cortright and Szallasi, 2004; Gunthorpe et al., 2002; Szallasi and Blumberg, 1999), have also been examined for their potential to inhibit emesis. In particular, the ultrapotent capsaicin analog, resiniferatoxin, has been shown to antagonise emesis induced by centrally-(apomor-
emesis in man (Rudd and Andrews, 2004). The aim of the present studies was to take our investigations forward to examine if olvanil also had the capacity to antagonise cisplatin-induced delayed emesis. This was done utilising the ferret lower-dose cisplatin (5 mg/kg, i.p.) model, with extended observation times up to 72 h (Naylor and Rudd, 1996; Rudd and Andrews, 2004). The experiments were performed using animals surgically implanted with radiotelemetry devices to transmit biopotentials for determining heart rate and temperature. This provided a means of ascertaining if a repeated dosing of olvanil in combination with cisplatin would produce an inhibition of emesis in the absence of an adverse effect on homeostatic mechanisms known to be affected by pungent vaniloids (Reinhart et al., 2005). Feeding and drinking and spontaneous locomotor activity were also assessed as an additional index of toxicity during the experiments (Harrison et al., 2004; Reinhart et al., 2005).

2. Materials and methods

2.1. Animals

Castrated male ferrets weighing between 1 and 2 kg were used. They were obtained from Southland Ferrets (Invercargill, New Zealand) and housed at 24 ± 1°C. Artificial lighting was provided between 0600 and 1800 h, and water and dry pelleted cat chow (Feline Diet 5003, PMI Feeds, St. Louis, U.S.A.) were available ad libitum. All experiments were conducted under a licence provided by the Government of the Hong Kong SAR, and the Animal Experimentation Ethics Committee, The Chinese University of Hong Kong.

2.2. Implantation of radiotelemetric devices to record heart rate and temperature

Animals were injected with buprenorphine (0.05 mg/kg, s.c.) as a preoperative analgesic and were anaesthetised with xylazine (2 mg/kg, i.m.) and ketamine (35 mg/kg, i.m.), using aspetic techniques, the bodies of the transmitters (TL11M2-C50-PXT, Data Sciences International, U.S.A.) were implanted subcutaneously on the dorsal aspect of the animal, and its two biopotential leads were tunneled subcutaneously in an approximate lead II configuration for recording the electrocardiogram (ECG); one lead was just caudal to the right forelimb at approximately the first or second rib space and the other lead was at the apex beat of the heart which is found around the eighth to ninth intercostal space on the right side (Rubliot et al., 2006; Kramer and Kinter, 2003). All skin incisions were closed with interrupted suture and sprayed with antibiotic aerosol (Tribiotic Spray®, Riker Laboratories, UK) and silicone wound dressing (Opsite®, Smith and Nephew, UK). Animals then received a second buprenorphine (0.05 mg/kg, s.c.) injection 12 h post surgery and were allowed 7 days recovery prior to experimentation.

2.3. Behavioural observation and recording of radiotelemetric data

Ferrets were transferred to observation chambers (49 cm x 49 cm x 60 cm) illuminated to 15 ± 1 lx. Recordings commenced 24 h after introduction of the animals to the observation cage. The image of each animal was captured by a camera (Panasonic WV-CT460/P; Panasonic, Yokahoma, Japan) and the analog-video signal was converted to digital by a frame grabber. Calculations of movement (sensitivity, 2 cm) were made using EthoVision Color Pro software (Version 2.3; Noldus Information Technology, Costerweg, Netherlands) running on a personal computer. Emesis was characterized by rhythmic abdominal contractions that were either associated with the forceful oral expulsion of solid or liquid material from the gastrointestinal tract (i.e. vomiting) or not associated with the passage of material (i.e. retching movements). Consecutive episodes of retching and/or vomiting were considered separate

Fig. 1. The effect of olvanil (+0.05 = 0.05 mg/kg, s.c.; +0.5 = 0.5 mg/kg, s.c.; +5 = 5 mg/kg, s.c.) or vehicle (+0) on cisplatin-induced (CP; 5 mg/kg, i.p.) retching + vomiting (A–D) and on bodyweight (E), food (F), and water consumption (G). Data represent the mean ± S.E.M. of 7–8 determinations. Significant differences relative to the cisplatin vehicle + olvanil vehicle treated (Control) animals are indicated as *P < 0.05, **P < 0.01, ***P < 0.001; significant differences relative to cisplatin treated + the vehicle for olvanil treated animals (CP + 0) are indicated as †P < 0.05, ††P < 0.01 (two-way ANOVA with Bonferroni post tests). Changes in body weight and food and water consumption were calculated based on measurements taken over the −24 to 0 h period prior to cisplatin or vehicle administration at t = 0.
when the animal changed its location in the observation cage, or when the interval between episodes exceeded 5 s. Food and water consumption was assessed in 24 h periods based on the administration of cisplatin at t = 0. ECG and temperature were recorded wirelessly via RMC1 receivers (Dataquest Acquisition and Analysis, Data Sciences International, U.S.A.) placed in the vicinity of the cages. After 4 h of recording basal activity, the animals were injected with cisplatin (5 mg/kg, i.p.) or vehicle (1% mannitol in saline 0.9% (w/v), 5 ml/kg). Thirty minutes later the animals were removed from their observation cages and injected with olvanil (0.05–5 mg/kg), or vehicle (0.5 ml/kg. Tween 80/ethanol and saline 0.9% (w/v) in the ratio of 1:1:8). The administration of olvanil or its vehicle was then repeated 8 h post cisplatin administration, and then at regular 8 h intervals for the duration of the 72 h observation time. Treatments were randomised and administered following a Latin square design, and behavioural measurements were made by investigators who were blinded to the treatments.

2.4. Drug used

D-Mannitol was from Sigma–Aldrich, St. Louis, U.S.A. Olvanil (N-vanillyloleoylamide) was from Tocris, Bristol, UK. Cisplatin (1 mg/ml in 0.1% mannitol in saline) was from David Bull Laboratories, Victoria, Australia. Doses are expressed as the free base.

2.5. Data analysis

The radiotelemetry data were analysed using DQ A.R.T. Gold software (Dataquest Acquisition and Analysis, Data Sciences International, U.S.A.) or Spike 2 (Cambridge Electronic Design, Ltd., Cambridge, England). Differences between behavioural and physiological data of control and drug treated animals were analysed by various techniques using GraphPad Prism 5.0a (GraphPad Software, CA, U.S.A.). The Log10 transformed locomotor activity data, and the retching and vomiting and food and water consumption data were analysed using a one-way analysis of variance (ANOVA) followed by Bonferroni multiple comparison tests. Heart rate (beats per min; BPM) and temperature data, which were sampled every 30 min, were analysed by a repeated measures 2-way ANOVA followed by Bonferroni multiple comparison tests. Latency data and all other behaviours were analysed using a Kruskall–Wallis test followed by Dunn’s multiple comparison tests. When an animal failed to retch or vomit, a latency value equal to the test period observation time (i.e. 72 h) was used to perform the statistical analysis. Results are expressed as the mean ± S.E.M., unless otherwise stated. In all cases, difference between treatment groups were considered significant when P < 0.05.

3. Results

3.1. Effect of olvanil on cisplatin-induced changes in food and water consumption, emesis, and locomotor activity

During the 24 h period prior to drug or vehicle treatment the animals consumed 41.5 ± 1.4 g of food and 28.0 ± 3.6 g of water (pooled data, n = 35, t = 0); there were no significant differences between the consumption observed of the respective treatment groups (P > 0.05; Fig. 1). Ferrets also travelled 215.05 ± 29.41 m at 0.06 ± 0.01 m/s. Subsequently, animals treated with the vehicle for cisplatin (1% mannitol in saline 0.9% (w/v), 5 ml/kg) in combination with the vehicle for olvanil (9 administrations; 0.5 ml/kg. Tween 80/ethanol and saline 0.9% (w/v) in the ratio of 1:1:8) did not exhibit emesis (data not shown). Food and water consumption measured over the next 3 successive 24 h periods did not deviate significantly from these starting values (P > 0.05; Fig 1F and G). However, body weight also remained relatively constant when normalised to data recorded 24 h prior to vehicle administration (P > 0.05; Fig 1F). Locomotor activity appeared variable during the recording period, but significant 52.9% (P < 0.05) and 92.3% (P < 0.01) increases in velocity, respectively, were recorded during the 48–72 h period in animals treated with olvanil 0.05 and 0.5 mg/kg plus cisplatin, when compared with the vehicle plus vehicle controls (Fig. 2).

In the animals treated with cisplatin (5 mg/kg, i.p.) plus the vehicle for olvanil there were 48, 112, and 146 retches + vomits during the 0–24, 24–48 and 48–72 h time periods, respectively (see Table 1 and Fig. 1A–D). Compared with the vehicle for cisplatin plus the vehicle for olvanil treated animals, there were significant 66.1% (P < 0.001), 90.1% (P < 0.001), and 98.7% (P < 0.001) reductions in the amount of food consumed measured 24, 48 and 72 h post cisplatin, respectively. There was also a reduction in the amount of water consumed, with significant reductions of 65.6% (P < 0.01) and 93.7% (P < 0.01) when compared with the controls (Fig. 1A and B). Retching + vomiting data was obtained during 24 h observation periods and are shown as the mean ± S.E.M. Latency data are shown as medians with 25% and 75% percentiles indicated in square brackets. There were no significant differences between the data obtained from the cisplatin + vehicle treated animals and the other treatment groups (P > 0.05; one-way ANOVA or Kruskall–Wallis tests, as appropriate).

Table 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Latency (h)</th>
<th>Retches + vomits</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>0–24 h</td>
<td>24–48 h</td>
</tr>
<tr>
<td>CP + vehicle</td>
<td>9.1 ± 1.2</td>
<td>48.9 ± 18.3</td>
</tr>
<tr>
<td>CP + olvanil 0.05 mg/kg, s.c.</td>
<td>10.1 ± 3.3</td>
<td>75.6 ± 29.7</td>
</tr>
<tr>
<td>CP + olvanil 0.5 mg/kg, s.c.</td>
<td>11.5 ± 3.6</td>
<td>56.3 ± 34.9</td>
</tr>
<tr>
<td>CP + olvanil 5 mg/kg, s.c.</td>
<td>6.8 ± 4.6</td>
<td>48.3 ± 16.3</td>
</tr>
</tbody>
</table>

Retching + vomiting data was obtained during 24 h observation periods and are shown as the mean ± S.E.M. Latency data are shown as medians with 25% and 75% percentiles indicated in square brackets. There were no significant differences between the data obtained from the cisplatin + vehicle treated animals and the other treatment groups (P > 0.05; one-way ANOVA or Kruskall–Wallis tests, as appropriate).
Fig. 3. The effect of cisplatin (CP; 5 mg/kg, i.p.) in combination with olvanil (+0.05 = 0.05 mg/kg, s.c.; +0.5 = 0.5 mg/kg, s.c.; +5 = 5 mg/kg, s.c.) or vehicle (+0) on body temperature and heart rate. Measurements were made over 4 h periods (A and C) and data points represent the mean ± S.E.M. of 7–8 determinations. A focused analysis was also made on data recorded at every 2 h point post administration of olvanil (dotted line) or its vehicle (B and D). Significant differences relative to the cisplatin vehicle + olvanil vehicle treated (Control) animals are indicated as *P < 0.05 (two-way ANOVA with Bonferroni post tests).

3.2. Effect of olvanil on temperature and heart rate in cisplatin treated animals

The body temperature of the ferrets was 37.7 ± 0.1 °C and heart rate was 233.7 ± 5.5 BPM in the 4 h period prior to the start of drug or vehicle administration (data pooled, n = 31); these values were relatively constant in animals treated with the vehicle for cisplatin in combination with the vehicle for olvanil ([Fig. 3A and B]). However, animals treated with cisplatin plus the vehicle for olvanil, or cisplatin in combination with olvanil appeared to have a more variable temperature control ([Fig. 3C]). A more focused analysis of temperature and heart rate was then conducted at time points 1.5–2 h post vehicle or olvanil administration; this decision was based on data we had obtained previously with another vanilloid, resiniferatoxin, which showed maximal effects on temperature at these time intervals ([Chu et al., 2009]). However, cisplatin alone did not show significant effects on temperature or heart rate. Interestingly, the combination of cisplatin with olvanil at 0.5 and 5 mg/kg resulted in an approximate 1.6 °C fall in temperature at the end of the experiment at 70 h compared with the control vehicle plus vehicle treated animals whilst the heart rate was unaffected (P < 0.05; [Fig. 3C]).

4. Discussion

Our previous studies using a single administration of olvanil have shown an antagonism of apomorphine-induced (0.25 mg/kg, s.c.) and acute cisplatin-induced (10 mg/kg, i.p.) emesis over the 0.5–5 mg/kg, s.c. dose range. The reduction of apomorphine-induced emesis was almost complete (∼90%), and the reduction of cisplatin-induced emesis during the first 6 h period was approximately 40% ([Chu et al., 2009]). In the present studies, we used a lower dose of cisplatin (5 mg/kg, i.p.) in order to induce both an acute and delayed emetic response, and an 8 h dosing interval for olvanil. The model has been used previously to demonstrate the anti-emetic potential of resiniferatoxin to inhibit emesis ([Yamakuni et al., 2002]). Olvanil, however, was inactive in the present “low dose” cisplatin study with no evidence for a reduction in emesis or a delay in the onset of the first episode of retching emesis. Not only did olvanil fail to reduce emesis, it appeared to enhance the anorexic action of cisplatin, as exemplified by a significant reduction of food intake. The potentiating action was apparent at 24 h, and olvanil also contributed towards causing hypothermia and changes in locomotor activity that were seen towards the end of the experiment.

Whilst we hypothesised that if olvanil had anti-emetic properties, it would also be able to prevent the cisplatin-induced weight loss, which probably results from physical exertion and metabolism, a loss of gastric contents, and/or reduced food intake. Consistent with this reasoning, Fukunaka et al. (1998) have reported that the 5-HT₃ receptor antagonist, granisetron, alone or in combination with dexamethasone, antagonised acute and...
delayed emesis induced by cisplatin (5 mg/kg, i.p.). There was also a significant increase in drinking frequency in animals when assessed over 72 h; only granisetron combined with dexamethasone significantly increased feeding frequency. Unfortunately, there was no control group for the cisplatin and vehicle treated animals, nor reports of the amount of food and water consumed during the respective acute and delayed phases, so it was not possible to detect the effect of cisplatin alone to modify any daily parameters, or to observe the effect alone to cause weight loss (Fukunaka et al., 1998). Our studies, therefore, are the first to report detailed food and water consumption during the acute and delayed emesis experiments and to show associated changes in cisplatin-induced weight loss.

The ferret is not a common laboratory animal, so most studies addressing the mechanism of anorexia induced by cisplatin have used rodents incapable of emesis. Experiments in the rat utilising cisplatin at 6 mg/kg, i.p. show a similar reduction in feeding, which becomes particularly pronounced at 2 days, with a mechanism involving gastric stasis, increased levels of plasma ghrelin, and a up-regulation of ghrelin receptors in the stomach and hypothalamus (Malik et al., 2008); these effects can be ameliorated to some degree by treatment with ghrelin (Liu et al., 2006). Other studies also indicate that the mechanism may also involve an activation of the common hepatic branch of the vagus, with similar pathways and mechanisms also affecting cisplatin-induced pica in these animals (De Jonghe and Horn, 2008). Certainly, there are TRPV1 channels on the vagi innervating the gastrointestinal tract (e.g. human, mouse) and their activation may reduce feeding (Wang et al., 2005; Zhong et al., 2008). The control of food intake is more complex, with TRPV1 channels located in the nucleus tractus solitarius, dorsal motor nucleus of the vagus, with area postrema also playing a role (Sharkey et al., 2007; South and Ritter, 1983, 1988; Yov et al., 1991).

Cisplatin clearly reduced water intake during our studies, and it is known to cause nephrotoxicity, with effects being manifested after 2 days (Fukunaka et al., 1998; Lee et al., 2006; Ramesh and Reeves, 2002). We did not expect olvanil when combined with cisplatin to impact significantly on water consumption since TRPV1 playing a role (Sharkey et al., 2007; South and Ritter, 1983, 1988; Yov et al., 1991).

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In conclusion, cisplatin (5 mg/kg, i.p.) induced acute and delayed emesis and inhibited food and water consumption in the ferret that probably contributed to an overall decrease in body weight. Olvanil did not antagonise emesis or the cisplatin-induced changes in food and water intake. Indeed, olvanil appeared to enhance the negative effect of cisplatin on food intake and it also contributed to causing hypothermia and altered locomotor activity profile. These data suggest that non-pungent vanilloids as exemplified by olvanil may not be useful for the treatment of gastrointestinal toxicity associated with cisplatin-based chemotherapy in man. The differences between the effects of olvanil on the emetic response to “low” (this study) and “high” doses of cisplatin and apomorphine (Chu et al., 2009) need to be reconciled before discounting this approach to anti-emesis.

Conflict of interest statement

The authors declare that there are no conflicts of interests.

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References


