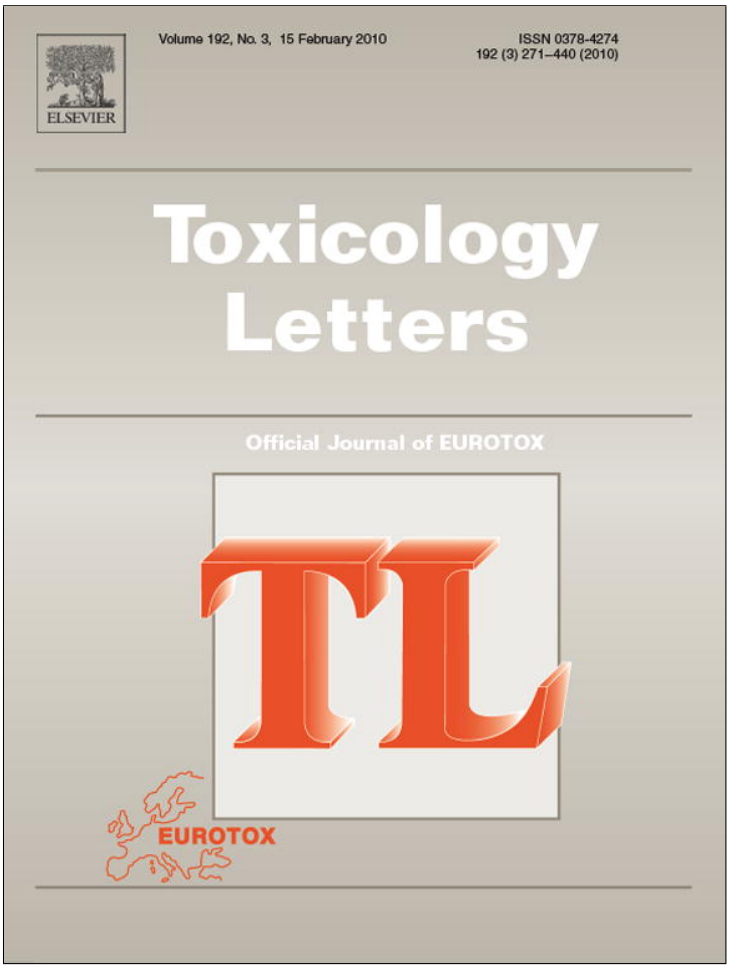

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Olvanil, a non-pungent vanilloid enhances the gastrointestinal toxicity of cisplatin in the ferret

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ABSTRACT

Pungent transient receptor potential vanilloid (TRPV1) channel activators have been shown to have broad inhibitory anti-emetic activity against centrally- and peripherally acting challenges but only at doses that have adverse effects on the cardiovascular system and on temperature homeostasis. In the present studies, we investigated the anti-emetic potential of the non-pungent TRPV1 activator, olvanil (0.05–5 mg/kg, s.c., 3 times per day, for 3 days) to antagonise the acute and delayed emesis induced by cisplatin (5 mg/kg, i.p.) in ferrets that had been implanted with radiotelemetry devices to enable an analysis of heart rate and temperature. Cisplatin induced an acute (day 1: 48.0 ± 18.3 retches + vomits) and delayed (day 2: 111.7 ± 35.5; day 3: 147.5 ± 20.2 retches + vomits) emetic response that was associated with reduced food (−98.7% at day 3, $P < 0.001$) and water consumption (−70.2% at day 3, $P < 0.001$) and progressive weight loss (−12.0% at day 3, $P < 0.001$). Olvanil did not prevent either emesis or the weight loss and negative effects on food and water consumption ($P > 0.05$); the effect on food consumption appeared potentiated by a further 21.2% at 0.05 mg/kg ($P < 0.05$) and 19.9% at 0.5 mg/kg ($P < 0.05$). Cisplatin did not alter body temperature (basal: 37.7 ± 0.1 °C) or heart rate (basal: 233.7 ± 5.5 beats per min (BPM); $P > 0.05$), but hypothermia (−1.6 °C) and increases in locomotor activity (50–90%) were recorded in animals concomitantly treated with olvanil ($P < 0.05$). These data indicate that non-pungent activators as exemplified by olvanil are unlikely to be useful clinically for the control of the gastrointestinal side effects induced by cisplatin.

1. Introduction

Tachykinin NK_1 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 NK_1 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- (apomorphine, loperamide), peripherally- (copper sulphate, radiation), and mixed centrally- and peripherally acting ('high-dose' cisplatin) stimuli in ferrets (Andrews and Bhandari, 1993).

Unfortunately, pungent/irritant TRPV1 activators such as resiniferatoxin and capsaicin have undesirable actions on cardiovascular and pulmonary systems, as well as a capacity to cause hypothermia (Szallasi and Blumberg, 1999). The mechanism of pungency probably relates to a rapid release of substance P in the brainstem, which may explain emesis or fictive vomiting seen in shrews and dogs (Andrews et al., 2000; Cheng et al., 2005; Rudd and Wai, 2001; Shiroshita et al., 1997; Smith et al., 2002).

Olvanil is a non-pungent TRPV1 activator that does not affect cardiovascular performance or cause hypothermia in rodents (Appendino et al., 2005; Dray et al., 1990; Iida et al., 2003). In ferrets, olvanil antagonises apomorphine-induced emesis and the emesis induced by 'high-dose' cisplatin (10 mg/kg, i.p.) without affecting blood pressure, temperature, or generalised behaviour including changes in feeding and drinking (Chu et al., 2009).

The 'high-dose' cisplatin-induced emesis ferret model that we used previously is considered predictive of drugs to prevent acute

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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 vanilloids (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 aseptic 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 tunnelled 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 (Bublot 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 × 49 cm × 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-CP460/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

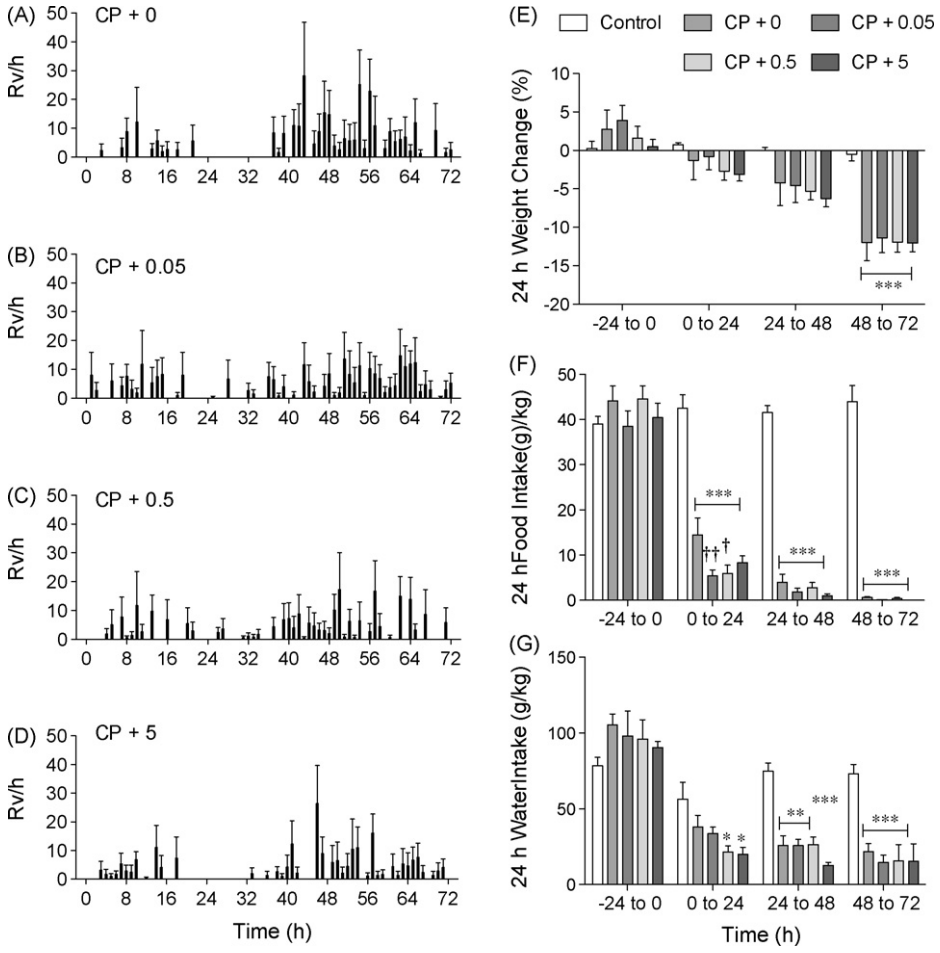

Fig. 1. The effect of olvanil (+0.05 = 0.05 mg/kg, s.c.; +0.5 = 0.5 mg/kg, s.c.; +5 = 5 mg/kg, s.c.) or vehicle (+0) on cisplatin-induced (CP; 5 mg/kg, i.p.) retching + vomiting (A–D) and on bodyweight (E), food (F), and water consumption (G). Data represent the mean ± S.E.M. of 7–8 determinations. Significant 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 $\dagger P < 0.05$, $\dagger\dagger P < 0.01$ (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 DQA.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 Log_{10} 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 28.6 ± 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), and 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).

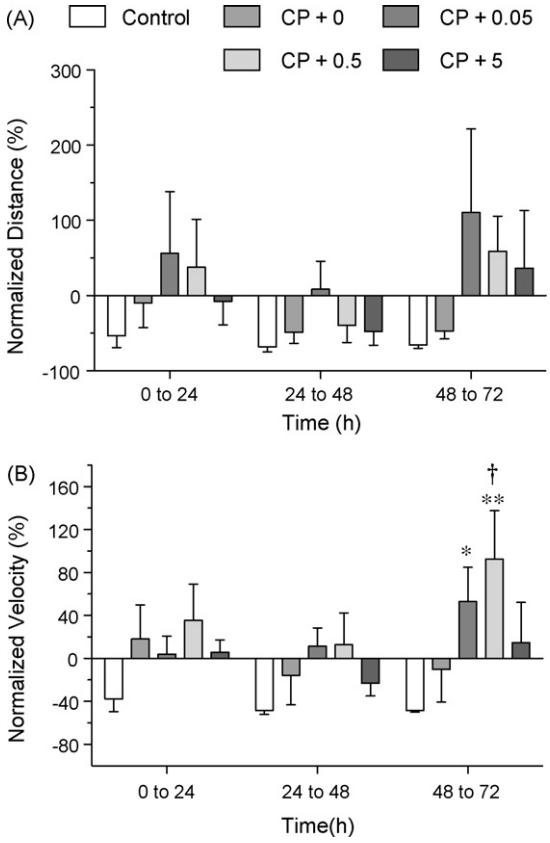

Fig. 2. The effect of cisplatin (CP; 5 mg/kg, i.p.) in combination with olvanil (+0.05 = 0.05 mg/kg, s.c.; +0.5 = 0.5 mg/kg, s.c.; +5 = 5 mg/kg, s.c.) or vehicle (+0) on spontaneous locomotor activity. Data represent the mean ± S.E.M. of 7–8 determinations. Significant differences relative to the cisplatin vehicle + olvanil vehicle treated (Control) animals are indicated as $^{*}P < 0.05$, $^{**}P < 0.01$; significant differences relative to cisplatin treated + the vehicle for olvanil treated animals (CP + 0) are indicated as $\dagger P < 0.05$ (two-way ANOVA with Bonferroni post tests). Changes in distance travelled (A) and velocity (B) normalised on spontaneous locomotor activity measures made over the −24 to 0 h period prior to cisplatin or vehicle administration at $t = 0$.

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

Table 1
The effect of olvanil or vehicle on cisplatin-induced (CP; 5 mg/kg, i.p.) acute and delayed retching + vomiting.

Treatment	Latency (h)	Retches + vomits		
		0–24 h	24–48 h	48–72 h
CP + vehicle	9.1 [6.4–13.6]	48.0 ± 18.3	111.7 ± 35.5	145.7 ± 20.2
CP + olvanil 0.05 mg/kg, s.c.	10.1 [2.2–31.3]	75.6 ± 29.7	63.3 ± 21.6	146.6 ± 41.7
CP + olvanil 0.5 mg/kg, s.c.	11.5 [7.3–36.7]	56.3 ± 34.9	60.9 ± 16.8	113.1 ± 36.1
CP + olvanil 5 mg/kg, s.c.	6.8 [4.6–40.4]	48.3 ± 16.3	61.0 ± 21.6	101.5 ± 29.1

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).

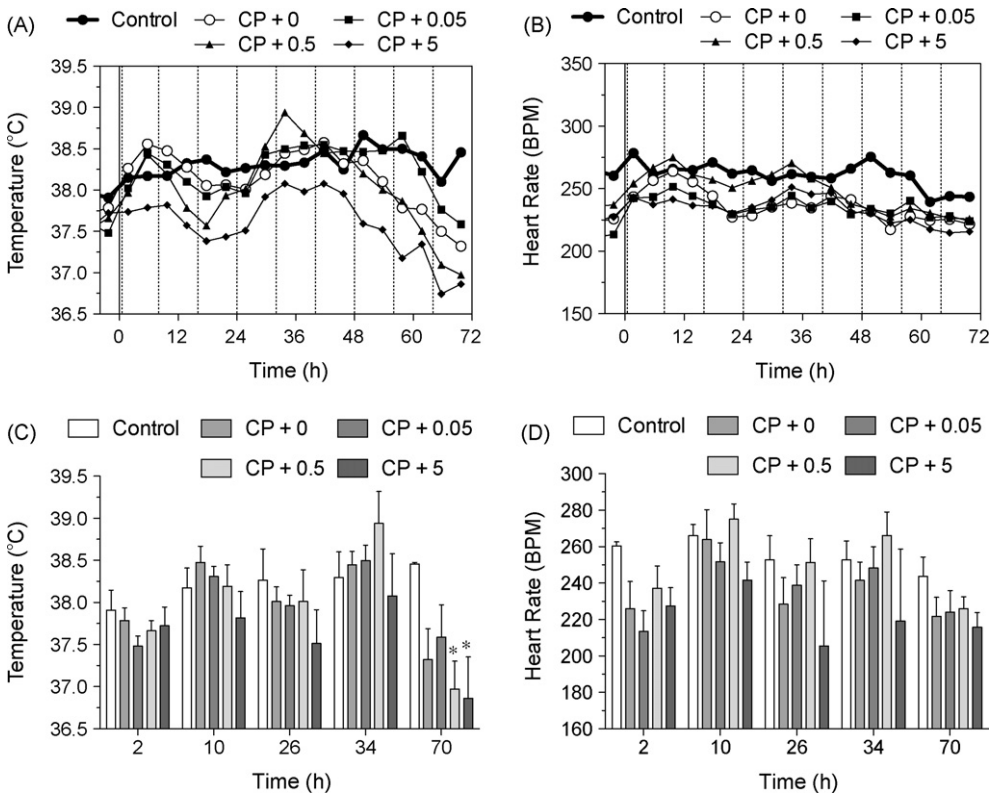

Fig. 3. The effect of cisplatin (CP; 5 mg/kg, i.p.) in combination with olvanil (+0.05 = 0.05 mg/kg, s.c.; +0.5 = 0.5 mg/kg, s.c.; +5 = 5 mg/kg, s.c.) or vehicle (+0) on body temperature and heart rate. Measurements were made over 4 h periods (A and C) and data points represent the mean ± S.E.M. of 7–8 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).

70.2% ($P < 0.001$), respectively, recorded at 48 and 72 h (Fig. 1G); a 12% reduction in bodyweight was also recorded at 72 h ($P < 0.001$; Fig. 1E).

Treatment of ferrets with olvanil (0.05–5 mg/kg) did not modify the latency to onset of cisplatin-induced retching + vomiting, or the number of retches + vomits recorded during the 72 h observation time ($P > 0.05$; Table 1 and Fig. 1A–D). Similarly, olvanil also failed to reverse the cisplatin-induced decreases in food and water consumption, and the cisplatin-induced decrease in bodyweight ($P > 0.05$; Fig. 1E–G). Indeed, at 24 h the combination of olvanil at 0.05 and 0.5 mg/kg enhanced significantly the cisplatin-induced decrease in food consumption by a further 21.2% and 19.9%, respectively ($P < 0.05$; Fig. 1F).

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-HT_3 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 an 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; Yox 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 channels are reported to not be involved in the modulation of thirst (Taylor et al., 2008), although TRPV1 channels are on sensory nerves in the renal pelvis. (Zhu and Wang, 2008). Indeed, in our previous studies, the administration of olvanil (0.05-5 mg/kg, s.c.) did not affect bodyweight, feeding, or drinking when measured over 24 h (Chu et al., 2009). Further, in acute experiments, olvanil also failed to potentiate the inhibition of food and water intake caused by apomorphine, copper sulphate, and cisplatin (10 mg/kg, i.p.) (Chu et al., 2009). It seems likely that the action of olvanil to potentiate the anorexic action of cisplatin 5 mg/kg, i.p. in the present studies resulted from the repeated dosing schedule that we used. Alternatively, the differential action of olvanil in the high-dose and low-dose cisplatin-induced emesis models may relate to different mechanisms driving the emetic responses. For example, the high-dose model is comparatively more sensitive to the anti-emetic action of 5-HT_3 receptor antagonists, and the low-dose model is more sensitive to the action of glucocorticoids (Rudd and Andrews, 2004).

As an additional approach to measure toxicity, and indirectly assessing the pungency of olvanil, the ferrets had been implanted with radiotelemetry devices capable of transmitting ECG and temperature data, and locomotor activity was also recorded. We did not expect that cisplatin alone would impact on heart rate since cardiovascular toxicity is not reported frequently in man (Von Hoff et al., 1979). Indeed, cisplatin alone, or in combination with olvanil, had no significant action to modify the heart rate during the acute or delayed phase of cisplatin-induced emesis, but we did not perform a focused analysis of data around individual episodes of retching and/or vomiting which would be required to detect subtle changes in autonomic control of the heart (Bellg et al., 1995; Fredrikson et al., 1993). Olvanil combined with cisplatin affected locomotor activity, with increases in velocity but not distance travelled being observed at the end of the experiment. The significance of this observation is not clear as pungent vanilloids can reduce locomotor activity (Di Marzo et al., 2001).

Cisplatin itself did not seem to impact significantly on the body temperature of the ferrets. However, towards the end of the experiment, animals receiving olvanil and cisplatin had lower temperatures than the vehicle plus vehicle controls; this was particularly evident for animals treated with cisplatin and olvanil at 0.5 and 5 mg/kg, s.c. The physiological mechanisms for temperature homeostasis are complex. The hypothalamus, particularly the preoptic region that expresses TRPV1 channels, is well known to be responsible for thermoregulation (Boulant, 2000; Szabo et al., 2002). The preoptic region is integrated with the periaquaductal grey and reticular formation to effect cutaneous vasodilatation (Nagashima et al., 2000), and the rostral hypothalamus to produce sweating (Kanosue et al., 1994). A single administration of olvanil does not cause hypothermia in ferrets, which is in contrast to the action of the pungent vanilloid, resiniferatoxin (Andrews and Bhandari, 1993; Chu et al., 2009). The fall in temperature seen at 70 h in animals treated with cisplatin and olvanil was therefore unexpected. However, as the health of the animals is deteriorating, homeostatic mechanisms may be less responsive, and it is possible that subtle effects of drug treatment could be magnified. Nevertheless, a fall in body temperature concurrent with hyperactivity has been seen with other drug treatments including morphine (opioid; Ganesan, 1993), apomorphine (dopamine receptor agonist; Maj et al., 1990), and neurotensin (neuropeptide; Kaliva, 1984), indicating that the temperature homeostasis and locomotor activity may have complex control mechanisms.

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

Andrews, P.L.R., Bhandari, P., 1993. Resinferatoxin, an ultrapotent capsaicin analogue, has anti-emetic properties in the ferret. Neuropharmacology 32, 799–806.

Andrews, P.L.R., Okada, F., Woods, A.J., Hagiwara, H., Kakaimoto, S., Toyoda, M., Matsuki, N., 2000. The emetic and anti-emetic effects of the capsaicin analogue resiniferatoxin in *Suncus murinus*, the house musk shrew. Br. J. Pharmacol. 130, 1247–1254.

Andrews, P.L.R., Rudd, J.A., 2004. The role of tachykinins and the tachykinin receptor in nausea and emesis. In: Holzer, P. (Ed.), Handbook of Experimental Pharmacology, vol. 164. Springer-Verlag, Berlin/Heidelberg, Germany, pp. 359–440.

Appendino, G., De Petrocellis, L., Trevisani, M., Minassi, A., Daddario, N., Moriello, A.S., Gazzieri, D., Ligresti, A., Campi, B., Fontana, G., Pinna, C., Geppetti, P., Di Marzo, V., 2005. Development of the first ultra-potent "capsaicinoid" agonist at transient receptor potential vanilloid type 1 (TRPV1) channels and its therapeutic potential. J. Pharmacol. Exp. Ther. 312, 561–570.

Bellg, A.J., Morrow, G.R., Barry, M., Angel, C., DuBeshter, B., 1995. Autonomic measures associated with chemotherapy-related nausea: techniques and issues. Cancer Invest. 13, 313–323.

Boulant, J.A., 2000. Role of the preoptic-anterior hypothalamus in thermoregulation and fever. Clin. Infect. Dis. 31 (Suppl. 5), S157–161.

Bublot, I., Wayne Randolph, R., Chalvet-Monfray, K., Joel Edwards, N., 2006. The surface electrocardiogram in domestic ferrets. J. Vet. Cardiol. 8, 87–93.

Cheng, F.H.M., Andrews, P.L.R., Moreaux, B., Ngan, M.P., Rudd, J.A., Sam, T.S.W., Wai, M.K., Wan, C., 2005. Evaluation of the anti-emetic potential of anti-migraine drugs to prevent resiniferatoxin-induced emesis in *Suncus murinus* (house musk shrew). Eur. J. Pharmacol. 508, 231–238.

Chu, K.M., Ngan, M.P., Wai, M.K., Yeung, C.K., Andrews, P.L.R., Percie du Sert, N., Rudd, J.A., 2009. Olvanil: a non-pungent TRPV1 activator has anti-emetic properties in the ferret. Neuropharmacology, doi:10.1016/j.neuropharm.2009.10.002.

Cortright, D.N., Szallasi, A., 2004. Biochemical pharmacology of the vanilloid receptor TRPV1. An update. Eur. J. Biochem. 271, 1814–1819.

De Jonghe, B.C., Horn, C.C., 2008. Chemotherapy-induced pica and anorexia are reduced by common hepatic branch vagotomy in the rat. Am. J. Physiol. Regul. Integr. Comp. Physiol. 294, R756–765.

Di Marzo, V., Lastres-Becker, I., Bisogno, T., De Petrocellis, L., Milone, A., Davis, J.B., Fernandez-Ruiz, J.J., 2001. Hypolocomotor effects in rats of capsaicin and two long chain capsaicin homologues. Eur. J. Pharmacol. 420, 123–131.

Dray, A., Bettaney, J., Rueff, A., Walpole, C., Wrigglesworth, R., 1990. NE-19550 and NE-21610, antinociceptive capsaicin analogues: studies on nociceptive fibres of the neonatal rat tail *in vitro*. Eur. J. Pharmacol. 181, 289–293.

Fredrikson, M., Hursti, T., Salmi, P., Borjeson, S., Furst, C.J., Peterson, C., Steineck, G., 1993. Conditioned nausea after cancer chemotherapy and autonomic nervous system conditionability. Scand. J. Psychol. 34, 318–327.

Fukuda, H., Nakamura, E., Koga, T., Furukawa, N., Shiroshita, Y., 1999. The site of the anti-emetic action of tachykinin NK_1 receptor antagonists may exist in the medullary area adjacent to the semicompact part of the nucleus ambiguus. Brain Res. 818, 439–449.

Fukunaka, N., Sagae, S., Kudo, R., Endo, T., Hirafuji, M., Minami, M., 1998. Effects of granisetron and its combination with dexamethasone on cisplatin-induced delayed emesis in the ferret. Gen. Pharmacol. 31, 775–781.

Gan, T.J., Apfel, C.C., Kovac, A., Philip, B.K., Singla, N., Minkowitz, H., Habib, A.S., Knighton, J., Carides, A.D., Zhang, H., Horgan, K.J., Evans, J.K., Lawson, F.C., 2007. A randomized, double-blind comparison of the NK_1 antagonist, aprepitant, versus ondansetron for the prevention of postoperative nausea and vomiting. Anesth. Analg. 104, 1082–1089.

Ganesan, R., 1993. Effects of morphine during pregnancy and lactation in mice. Pharmacol. Biochem. Behav. 45, 393–398.

Gunthorpe, M.J., Benham, C.D., Randall, A., Davis, J.B., 2002. The diversity in the vanilloid (TRPV) receptor family of ion channels. Trends Pharmacol. Sci. 23, 183–191.

Harrison, A.P., Erlwanger, K.H., Elbrond, V.S., Andersen, N.K., Unmack, M.A., 2004. Gastrointestinal-tract models and techniques for use in safety pharmacology. J. Pharmacol. Toxicol. Methods 49, 187–199.

Iida, T., Moriyama, T., Kobata, K., Morita, A., Murayama, N., Hashizume, S., Fushiki, T., Yazawa, S., Watanabe, T., Tominaga, M., 2003. TRPV1 activation and induction of nociceptive response by a non-pungent capsaicin-like compound, capsiate. Neuropharmacology 44, 958–967.

Jordan, K., Kasper, C., Schmoll, H.J., 2005. Chemotherapy-induced nausea and vomiting: current and new standards in the antiemetic prophylaxis and treatment. Eur. J. Cancer 41, 199–205.

Kaliva, P.W., 1984. Neurotensin in the ventromedial mesencephalon of the rat: anatomical and functional considerations. J. Comp. Neurol. 226, 495–507.

Kanosue, K., Zhang, Y.H., Yanase-Fujiwara, M., Hosono, T., 1994. Hypothalamic network for thermoregulatory shivering. Am. J. Physiol. 267, R275–R282.

Kramer, K., Kinter, L.B., 2003. Evaluation and applications of radiotelemetry in small laboratory animals. Physiol. Genom. 13, 197–205.

Lee, S., Kim, W., Moon, S.O., Sung, M.J., Kim, D.H., Kang, K.P., Jang, Y.B., Lee, J.E., Jang, K.Y., Park, S.K., 2006. Rosiglitazone ameliorates cisplatin-induced renal injury in mice. Nephrol. Dial. Transplant. 21, 2096–2105.

Liu, Y.L., Malik, N.M., Sanger, G.J., Andrews, P.L.R., 2006. Ghrelin alleviates cancer chemotherapy-associated dyspepsia in rodents. Cancer Chemother. Pharmacol. 58, 326–333.

Maj, J., Rogoz, Z., Skuka, G., Sowinska, H., 1990. Some central pharmacological effects of (+)- and (−)-oxaprotiline. J. Neural Transm. 80, 129–143.

Malik, N.M., Moore, G.B., Kaur, R., Liu, Y.L., Wood, S.L., Morrow, R.W., Sanger, G.J., Andrews, P.L.R., 2008. Adaptive upregulation of gastric and hypothalamic ghrelin receptors and increased plasma ghrelin in a model of cancer chemotherapy-induced dyspepsia. Regul. Pept. 148, 33–38.

Nagashima, K., Nakai, S., Tanaka, M., Kanosue, K., 2000. Neuronal circuitries involved in thermoregulation. Auton. Neurosci. 85, 18–25.

Naylor, R.J., Rudd, J.A., 1996. Mechanisms of chemotherapy/radiotherapy-induced emesis in animal models. Oncology 53 (Suppl. 1), 8–17.

Ramesh, G., Reeves, W.B., 2002. TNF-alpha mediates chemokine and cytokine expression and renal injury in cisplatin nephrotoxicity. J. Clin. Invest. 110, 835–842.

Reinhart, G.A., Fryer, R.M., Osinski, M.A., Polakowski, J.S., Cox, B.F., Gintant, G.A., 2005. Predictive, non-GLP models of secondary pharmacodynamics: putting the best compounds forward. Curr. Opin. Chem. Biol. 9, 392–399.

Rudd, J.A., Andrews, P.L.R., 2004. Mechanisms of acute, delayed and anticipatory vomiting in cancer and cancer treatment. In: Hesketh, P. (Ed.), Management of Nausea and Vomiting in Cancer and Cancer Treatment. Jones and Barlett Publishers Inc., New York, pp. 15–66.

Rudd, J.A., Wai, M.K., 2001. Genital grooming and emesis induced by vanilloids in *Suncus murinus*, the house musk shrew. Eur. J. Pharmacol. 422, 185–195.

Sharkey, K.A., Cristino, L., Oland, L.D., Van Sickle, M.D., Starowicz, K., Pittman, Q.J., Guglielmotti, V., Davison, J.S., Di Marzo, V., 2007. Arvanil, anandamide and N-arachidonoyl-dopamine (NADA) inhibit emesis through cannabinoid CB1 and vanilloid TRPV1 receptors in the ferret. Eur. J. Neurosci. 25, 2773–2782.

Shiroshita, Y., Koga, T., Fukuda, H., 1997. Capsaicin in the 4th ventricle abolishes retching and transmission of emetic vagal afferents to solitary nucleus neurons. Eur. J. Pharmacol. 339, 183–192.

Smith, J.E., Paton, J.F., Andrews, P.L.R., 2002. An arterially perfused decerebrate preparation of *Suncus murinus* (house musk shrew) for the study of emesis and swallowing. Exp. Physiol. 87, 563–574.

South, E.H., Ritter, R.C., 1983. Overconsumption of preferred foods following capsaicin pretreatment of the area postrema and adjacent nucleus of the solitary tract. Brain Res. 288, 243–251.

South, E.H., Ritter, R.C., 1988. Capsaicin application to central or peripheral vagal fibers attenuates CCK satiety. Peptides 9, 601–612.

Szabo, T., Biro, T., Gonzalez, A.F., Palkovits, M., Blumberg, P.M., 2002. Pharmacological characterization of vanilloid receptor located in the brain. Brain Res. Mol. Brain Res. 98, 51–57.

Szallasi, A., Blumberg, P.M., 1999. Vanilloid (capsaicin) receptors and mechanisms. Pharmacol. Rev. 51, 159–212.

Tattersall, F.D., Rycroft, W., Francis, B., Pearce, D., Merchant, K., MacLeod, A.M., Ladduwahetty, T., Keown, L., Swain, C., Baker, R., Cascieri, M., Ber, E., Metzger, J., MacIntyre, D.E., Hill, R.G., Hargreaves, R.J., 1996. Tachykinin NK_1 receptor antagonists act centrally to inhibit emesis induced by the chemotherapeutic agent cisplatin in ferrets. Neuropharmacology 35, 1121–1129.

Taylor, A.C., McCarthy, J.J., Stocker, S.D., 2008. Mice lacking the transient receptor vanilloid potential 1 channel display normal thirst responses and central Fos activation to hypernatremia. Am. J. Physiol. Regul. Integr. Comp. Physiol. 294, R1285–R1293.

Von Hoff, D.D., Schilsky, R., Reichert, C.M., Reddick, R.L., Rozencweig, M., Young, R.C., Muggia, F.M., 1979. Toxic effects of cis-dichlorodiammineplatinum(II) in man. Cancer Treat. Rep. 63, 1527–1531.

Wang, X., Miyares, R.L., Ahern, G.P., 2005. Oleoylethanolamide excites vagal sensory neurones, induces visceral pain and reduces short-term food intake in mice via capsaicin receptor TRPV1. J. Physiol. 564, 541–547.

Yamakuni, H., Sawai-Nakayama, H., Imazumi, K., Maeda, Y., Matsuo, M., Manda, T., Mutoh, S., 2002. Resiniferatoxin antagonizes cisplatin-induced emesis in dogs and ferrets. Eur. J. Pharmacol. 442, 273–278.

Yox, D.P., Stokesberry, H., Ritter, R.C., 1991. Fourth ventricular capsaicin attenuates suppression of sham feeding induced by intestinal nutrients. Am. J. Physiol. 260, R681–R687.

Zhong, F., Christianson, J.A., Davis, B.M., Bielefeldt, K., 2008. Dichotomizing axons in spinal and vagal afferents of the mouse stomach. Dig. Dis. Sci. 53, 194–203.

Zhu, Y., Wang, D.H., 2008. Segmental regulation of sodium and water excretion by TRPV1 activation in the kidney. J. Cardiovasc. Pharmacol. 51, 437–442.