Full Paper

Action of Cyclooxygenase Inhibitors and a Leukotriene Biosynthesis Inhibitor on Cisplatin-Induced Acute and Delayed Emesis in the Ferret

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Abstract. Cisplatin at 5 mg/kg, i.p. induced an acute (day 1) and delayed (days 2 and 3) emetic response in the ferret that was used to investigate the anti-emetic activity of the non-selective cyclooxygenase inhibitor indomethacin (3 – 30 mg/kg, i.p., three times per day) and two cyclooxygenase-2 inhibitors, DFU [5,5-dimethyl-3-(3-fluorophenyl)-4-(4-methylsulphonyl)phenyl-2(5*H*)-furanone; 1 – 10 mg/kg, i.p. administered at 40 and 48 h] and L-745,337 [5-methane-sulphonamido-6-(2,4-diflurothiophenyl)-1-indanone; 10 mg/kg, i.p., administered at 40 and 48 h]. Only indomethacin potentiated significantly cisplatin-induced retching + vomiting ($P<0.05$); DFU antagonized delayed emesis ($P<0.05$) but the action was not dose-related and L-745,337 was inactive ($P>0.05$). However, indomethacin alone (30 mg/kg) also induced emesis ($P<0.05$). The leukotriene biosynthesis inhibitor, MK-886 {3-[1-(*p*-chlorobenzyl)-5-(isopropyl)-3-*tert*-butylthioindol-2-yl]-2,2-dimethylpropanoic acid; 1 – 10 mg/kg, i.p., three times per day} had no action to modify cisplatin-induced emesis ($P>0.05$). The combination treatment of indomethacin (10 mg/kg, i.p., three times per day) with MK-886 (10 mg/kg, i.p., three times per day) did not antagonize cisplatin-induced acute delayed retching + vomiting and had a different profile compared to the action of dexamethasone (1 mg/kg, i.p., three times per day; $P<0.05$). Inhibition of the cyclooxygenase and lipoxygenase pathways does not account for the anti-emetic of dexamethasone.

Keywords: emesis, cisplatin, cyclooxygenase, leukotriene, dexamethasone

Introduction

Cisplatin based chemotherapy is well known to be associated with the side effects of nausea and vomiting. The emesis that occurs on the first day of treatment (i.e., acute emesis) is particularly sensitive to 5-HT$_3$-receptor antagonists such as ondansetron and granisetron and probably relates to blocking 5-HT$_3$ receptors in the brainstem and on the vagus nerves (1). Unfortunately, emesis occurring on subsequent days (i.e., delayed emesis) is not controlled satisfactorily by the 5-HT$_3$-receptor antagonists or by single agent therapy (2). Indeed, the mechanism(s) involved in acute and delayed emesis are only partly understood (3).

Glucocorticoids such as dexamethasone are extensively used in combination with the 5-HT$_3$-receptor antagonists, tachykinin NK$_1$-receptor antagonists, and other anti-emetic drugs to control the acute and delayed emesis (3 – 5). The anti-emetic mechanism of action of the glucocorticoids is essentially unknown but may involve an ability to reduce inflammation and/or the production of inflammatory mediators (6), or by interfering with adrenergic mechanisms in the nucleus tractus solitarius (7). However, glucocorticoids do not seem to exert their anti-emetic action by non-specifically suppressing the emetic reflex (8).

Inflammation involves components of the immune system and many different mediators, some of which are known to activate the emetic reflex. For example, bacterial lipopolysaccharides inducing inflammation

causes emesis that is abolished by cyclooxygenase inhibitors, implicating a role for prostanoids in inflammatory-emetic mechanisms (9, 10). Other experiments have shown that *Staphylococcal enterotoxin* B-induced emesis can be abolished by L171883, a leukotriene D_4/E_4-receptor antagonist, to implicate a role for leukotrienes in the emetic reflex (11). Glucocorticoids affect several mechanisms involved in inflammation including an action to reduce the formation of eicosanoids (12). It is not known if this mechanism is relevant to the anti-emetic mechanism of the glucocorticoids or if eicosanoids contribute to the emetic action of cisplatin. However, plasma prostaglandin $F_{2\alpha}$ and thromboxane A_2 levels can be elevated in some patients receiving chemotherapy and this has been hypothesized to be a cause of gastrointestinal toxicity and vomiting (13).

In the present studies, we used the ferret cisplatin-induced acute and delayed emesis model to investigate the anti-emetic activity of the non-selective cyclooxygenase (COX) inhibitor indomethacin (14) and a 5-lipoxygenase biosynthesis inhibitor, MK-886 {3-[1-(*p*-chlorobenzyl)-5-(isopropyl)-3-*tert*-butylthioindol-2-yl]-2,2-dimethylpropanoic acid} (15), and their combination. We also investigated the anti-emetic potential of selective COX-2 inhibitors, DFU [5,5-dimethyl-3-(3-fluorophenyl)-4-(4-methylsulphonyl)phenyl-2(5*H*)-furanone] (16) and L-745,337 [5-methanesulphonamido-6-(2,4-diflurothiophenyl)-1-indanone] (17), but drug administrations were only targeted to the delayed emetic response. This approach seemed logical since the COX-2 enzyme is induced during inflammation to produce prostanoids and can be prevented by dexamethasone (18). Dexamethasone was also included in some of the studies for comparative purposes (dose used based on its ability to reduce cisplatin-induced acute and delayed emesis in the ferret, ref. 6). The doses of the COX inhibitors and MK-886 used in the present studies were based on their in vivo activity to reduce oedema, pyrexia, or hyperalgesia and/or to inhibit prostaglandin or leukotriene synthesis in animal models of inflammation (15 – 17, 19).

Materials and Methods

Animals

Castrated male ferrets (0.8 – 1.8 kg) were obtained from Southland Ferrets (Invercargill, New Zealand) and were housed communally at $22 \pm 1°C$ under artificial lighting, with lights on between 07.00 and 21.00 h. They were fed a dry pellet diet (Laboratory Feline Diet 5003; PMI Nutrition Inc., St. Louis, MO, USA); water was available ad libitum. All experiments were conducted under licence from the Government of the Hong Kong

SAR and the Animal Research Ethics Committee, The Chinese University of Hong Kong, or by guidelines established by the Canadian Council on Animal Care and Approved by the Animal Care Committee, Merck Frosst.

Induction and measurement of emesis

Animals were transferred to individual observation cages and allowed at least 48 h to adapt to the new environment. On the day of the experiment (at 14.30 h), they were presented with 100 g of commercially available cat food (Whiskas®; Effem Foods Pty. Ltd., Woodonga, Australia). At 15.00 h, the ferrets were removed from their observation cages and injected intraperitoneally with indomethacin (3 – 30 mg/kg) and/or MK-886 (1 – 10 mg/kg), dexamethasone (1 mg/kg), or their respective vehicles, 30 s after the administration of cisplatin (5 mg/kg, i.p., at t = 0). Drug or vehicle treatment was continued at regular 8-h intervals for the duration of the experiment. After treatment, the animals were returned to individual observation cages for the assessment of retching and/or vomiting during the subsequent 72-h observation period. During this time period, food (Laboratory Feline Diet 5003, PMI Nutrition Inc.) and water was available ad libitum. In a separate experiment, animals were injected with cisplatin (5 mg/kg, i.p.) and allowed to develop an emetic response. At 40- and 48-h post cisplatin injection, the animals were administered DFU (1 – 10 mg/kg, i.p.), L-745,337 (10 mg/kg, i.p.), or their respective vehicles. A final set of experiments investigated the emetic potential of the drugs and vehicles used in the studies. The dosing protocol was identical to that described above.

Animal behavior was recorded remotely using a closed circuit video recording system and analyzed at the end of the experiments. Emesis was characterized by rhythmic abdominal contractions that were either associated with the 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). An episode of retching and/or vomiting was considered separate when the animal changed its location in the observation cage or when the interval between retches and/or vomits exceeded 5 s.

Statistical analyses

In animals receiving cisplatin, the latency to retch or vomit and/or the total number of retches, vomits, and episodes was calculated in each 1-h period and for the acute (0 – 24 h) and delayed (24 – 48 and 48 – 72 h) periods. Data were also specifically analyzed for the 0 – 40-, 40 – 48-, and 40 – 56-h period to coincide with

the administration and duration of action of DFU in the ferret (10 mg/kg, i.p. produces a plasma level of about 0.7 μg/ml that remains elevated for about 6 h; D. Riendeau and A. Robichaud, unpublished data). Latency data were analyzed using a Kruskal-Wallis test followed by a Dunn's multiple comparison test (GraphPad Prism version 4.00; GraphPad Software, San Diego, CA, USA). If 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. The significance of the difference between the retching + vomiting data were assessed by an unpaired Student's *t*-test or a one-way analysis of variance (ANOVA) followed by pre-planned contrasts of specified means (SuperANOVA version 1.11; Abacus Concepts Inc., Berkeley, CA, USA). Differences were considered significant when $P<0.05$.

Drugs used

Cisplatin was purchased as a sterile saline solution at an active concentration of 1 mg/ml (David Bull Laboratories, Victoria, Australia). Indomethacin (Sigma-Aldrich, St. Louis, MO, USA) was formulated in 10% (w/v; in saline 0.9% w/v) $NaHCO_3$ (British Drug Houses Laboratory Supplies, Dorset, UK) and administered in a volume of 1 – 2 ml/kg. MK-886 (Merck Frosst, Quebec, Canada) and L-745,337 (Merck Frosst) were formulated in 25% molecusol (2-hydroxypropyl β-cyclodextrin; Research Biochemicals, Natick, MA, USA) and administered in a volume of 2 ml/kg. Dexamethasone 21-phosphase disodium salt (Sigma-Aldrich) was formulated in 10% (w/v) $NaHCO_3$ (British Drug Houses Laboratory Supplies) and administered in a volume of 1 ml/kg. DFU (Merck Frosst) was dissolved in 60% polyethylene glycol (Riedel-DeHaën AG, Seelze, Germany) and administered in a volume of 2 ml/kg. Doses are expressed as the free base.

Results

Effect of indomethacin on cisplatin-induced emesis

In vehicle-treated animals, cisplatin induced a retching and/or vomiting response following a latency of 5.7 ± 2.5 h and comprised 78.0 ± 37.1 retches + vomits during the 0 – 24-h period, 112.8 ± 53.3 retches + vomits during the 24 – 48-h period and 140.0 ± 62.4 retches + vomits during the 48 – 72-h period (Fig. 1). Indomethacin at 30 mg/kg administered 3 times per day, potentiated significantly the retching + vomiting response during the 0 – 24-h period by 127.9% ($P<0.05$). Indomethacin at 3 mg/kg, administered 3 times per day, potentiated significantly the retching + vomiting response occurring during the 24 – 48-h period by 240.1% ($P<0.05$). Indomethacin had no action to modify the latency to onset of cisplatin-induced retching and/or vomiting ($P>0.05$, Fig. 1).

Effect of MK-886 on cisplatin-induced emesis

In vehicle-treated animals, cisplatin induced a retching and/or vomiting response following a latency of 12.0 ± 7.4 h and comprised 100.3 ± 47.7 retches + vomits during the 0 – 24-h period, 40.7 ± 40.7 retches + vomits during the 24 – 48-h period, and 150.8 ± 52.1 retches + vomits during the 48 – 72-h period (Fig. 2). The regimen of MK-886 at 3 mg/kg, administered 3 times per day, and cisplatin was toxic in 1 out of 6 animals (the ferret died on day 3) and the regimen of MK-886 at 10 mg/kg, administered 3 times per day, in combination with cisplatin was toxic in 2 out of 6 animals (1 ferret died on day 2 and 1 died on day 3); only data from the surviving animals was included in the analysis. The highest dose of MK-886 shortened the latency of onset of emesis by approximately 8.3 h ($P>0.05$) but none of the doses tested (1 – 10 mg/kg) significantly affected the retching + vomiting response ($P>0.05$).

Effect of a combination of indomethacin and MK-886 on cisplatin-induced emesis

In control animals, cisplatin induced a retching and/or vomiting response following a latency of 2.3 ± 0.6 h and comprised 190.5 ± 44.0 retches + vomits during the 0 – 24-h period, 38.7 ± 25.0 retches + vomits during the 24 – 48-h period, and 107.0 ± 38.6 retches + vomits during the 48 – 72-h period (Fig. 3). As single treatment regimens, MK-886 and dexamethasone failed to modify cisplatin-induced retching + vomiting ($P>0.05$). However, indomethacin at 10 mg/kg, administered 3 times per day, potentiated significantly cisplatin-induced retching + vomiting during the 24 – 48-h period by 365.5% ($P<0.05$). Further analysis of the data revealed that the single regimens of indomethacin, MK-886, and dexamethasone had 59.1% ($P<0.05$), 80.8% ($P<0.01$), and 83.8% ($P<0.01$), respectively, significantly fewer retches + vomits than the combination regimen of indomethacin and MK-886 during the 0 – 24-h period. Indeed, during the 24 – 48- and 48 – 72-h periods, the animals receiving the dexamethasone regimen also had 63.8% ($P<0.05$) and 83.6% ($P<0.01$) fewer retches + vomits, respectively, than the combination regimen of indomethacin and MK-886. None of the drug treatments, or combinations, significantly affected the latency to onset of cisplatin-induced emesis ($P>0.05$).

Effect of DFU on cisplatin-induced delayed emesis

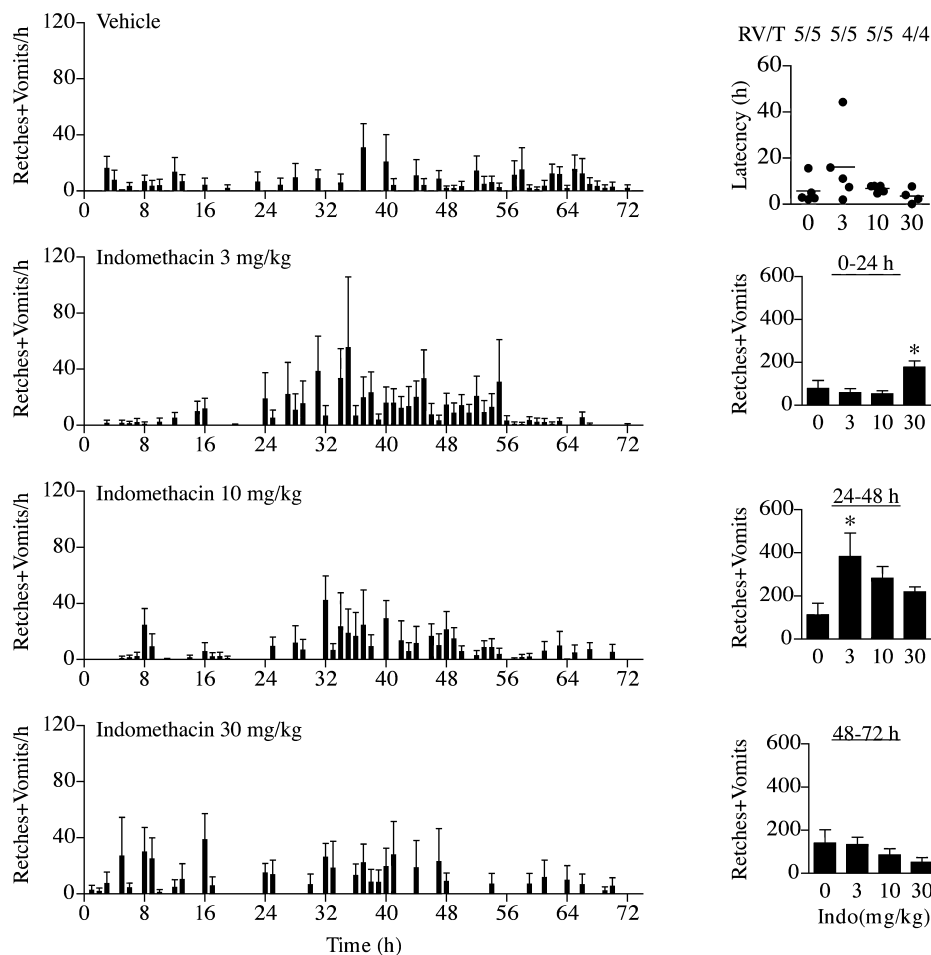All animals received cisplatin at 5 mg/kg at t = 0 and

Fig. 1. The effect of indomethacin at 3 – 30 mg/kg, i.p., administered 3 times per day, on the profile of retching + vomiting in the ferret induced by a single injection of cisplatin at 5 mg/kg, i.p. Indomethacin or vehicle (10% w/v NaHCO₃, 2 ml/kg) was administered 30 s following cisplatin injection and then at 8-h intervals for the duration of the experiment. Results represent the mean ± S.E.M. of the total numbers of retches + vomits occurring during 1-, 0 – 24-, 24 – 48-, and 48 – 72-h periods. The number of animals retching and/or vomiting out of the number of animals tested (RV/T) is also shown. Individual latencies to the first episode of retching and/or vomiting are shown as filled circles (horizontal lines on the latency plot represent the mean latencies of the respective treatment groups). Significant differences relative to the respective vehicle-treated animals are indicated as *P<0.05.

were randomized to receive DFU at 1 – 10 mg/kg, i.p. or 60% (v/v) PEG at 40 and 48 h. In control animals scheduled to receive 60% (v/v) PEG, cisplatin induced a retching and/or vomiting response following a latency of 17.4 ± 7.2 h and comprised 161.1 ± 52.5 retches + vomits during the 0 – 40-h period; animals scheduled to receive DFU had similar responses to cisplatin (*P*>0.05). However, at 40 h, the injection of DFU at 3 and 10 mg/kg produced a near abolition of emesis that lasted for approximately 2 h; this was also seen at 3 mg/kg at 48 h whilst 60% (v/v) PEG had no such action (Fig. 4). There was no significant difference between the data of the control and DFU-treated animals during the 40 – 48-h period, but DFU at 3 mg/kg reduced significantly the

retching + vomiting response occurring during the 40 – 56-h period by 55.4% (*P*<0.05).

Effect of L-745,337 on cisplatin-induced delayed emesis

All animals received cisplatin at 5 mg/kg at t = 0 and were randomized to receive L-745,337 at 10 mg/kg or vehicle at 40 and 48 h. Cisplatin induced retching and/or vomiting following a latency of 10.8 ± 8.2 h in the control vehicle-treated animals and at 15.0 ± 5.8 h in the L-745,337-treated animals (*P*>0.05). There were no significant differences in the numbers of retches + vomits between the control and L-745,337-treated animals during the periods of 0 – 40 (controls: 312.0 ± 83.5, L-745,337-treated: 214.8 ± 65.1; *P*<0.05), 40 – 48
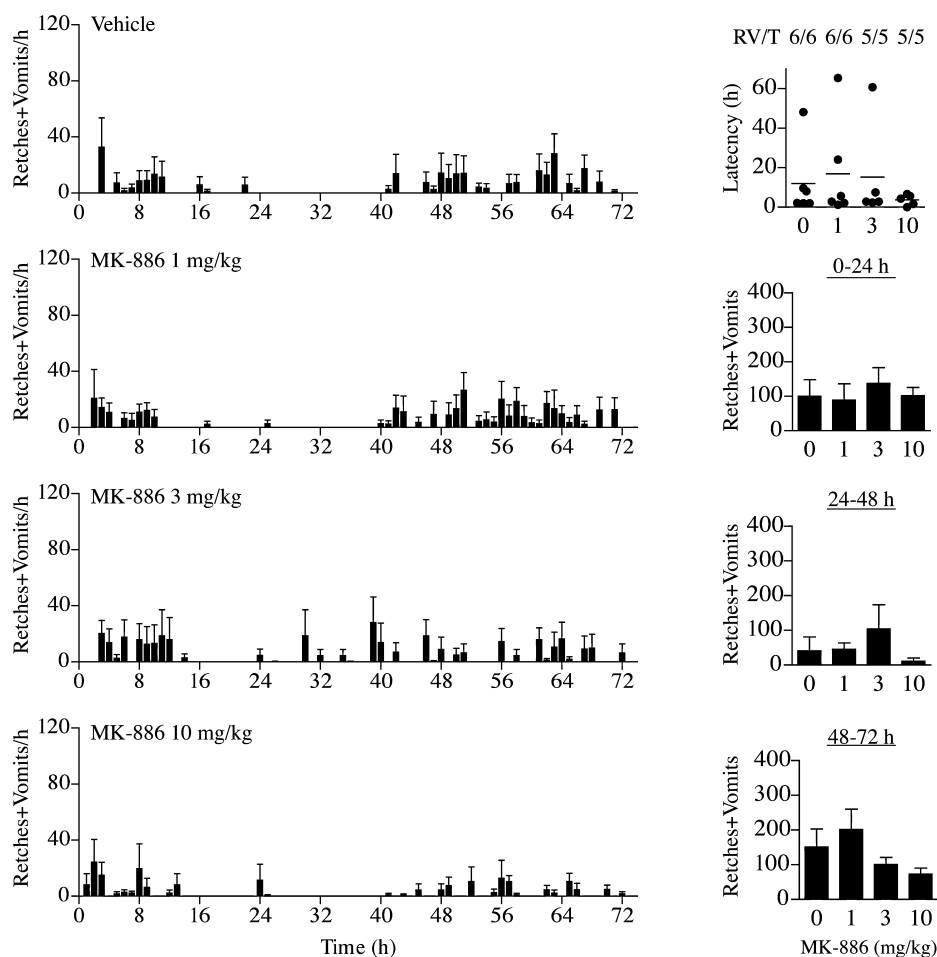
Fig. 2. The effect of MK-886 at 1 – 10 mg/kg, i.p., administered 3 times per day, on the profile of retching + vomiting in the ferret induced by a single injection of cisplatin at 5 mg/kg, i.p. MK-886 or vehicle (25% molecusol, 2 ml/kg, i.p.) was administered 30 s following cisplatin injection and then at 8-h intervals for the duration of the experiment. Results represent the mean ± S.E.M. of the total numbers of retches + vomits occurring during 1-, 0 – 24-, 24 – 48-, and 48 – 72-h periods. The number of animals retching and/or vomiting out of the number of animals tested (RV/T) is also shown. Individual latencies to the first episode of retching and/or vomiting are shown as filled circles (horizontal lines on the latency plot represent the mean latencies of the respective treatment groups). There were no significant differences relative to the respective vehicle-treated animals (P>0.05).

(controls: 174.0 ± 60.4, L-745,337-treated: 182.0 ± 58.1; P<0.05), or 40 – 56 h (controls: 238.8 ± 75.7, L-745,337-treated: 350.0 ± 93.0; P<0.05) (see Fig. 5).

Emetic potential of vehicles and biosynthesis inhibitors

During the course of the experiments, it was noted that some of the treatments (in particular, indomethacin) appeared to potentiate cisplatin-induced retching and vomiting. It was considered appropriate, therefore, to investigate any emetic potential of the drugs and vehicles used in the study. In these experiments, the three times per day administration of all drugs and vehicles, produced some level of retching and/or vomiting, with the episodes mainly coinciding with the time of administration (see Fig. 6 and Table 1). Com-

pared to the control groups, indomethacin at 30 mg/kg produced a significant number of retches + vomits over the 0 – 24- and 24 – 48-h periods (P<0.05, Table 1). Although, not statistically significant, it is notable that even the combined regimen of indomethacin (10 mg/kg, i.p.) plus MK 886 (10 mg/kg, i.p.), three times per day, also produced emesis. PEG (60%) only produced a few retches + vomits, as did DFU at 10 mg/kg (see Table 1), although this was only studied for 2 administrations spaced 8-h apart, to mimic the original protocol involving cisplatin (see Fig. 4). Unfortunately, complete dose responses for the drugs could not be performed due to a limited number of animals; and in the case of L-745,337, no studies were conducted due to a limited supply of the compound.
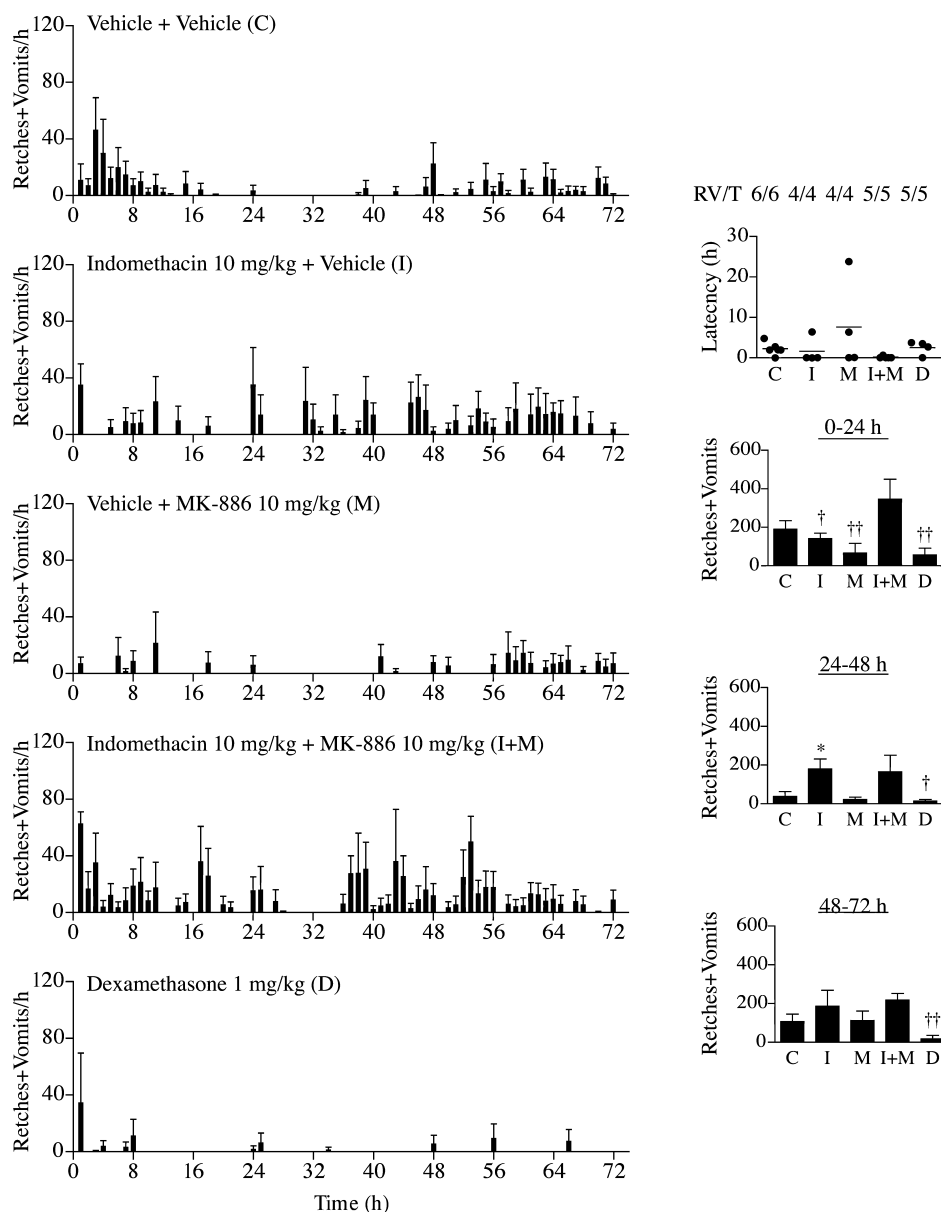
Fig. 3. The effect of indomethacin (10 mg/kg) + 25% molecusol (2 ml/kg) (I), MK-886 (10 mg/kg) + 10% w/v NaHCO₃ (1 ml/kg) (M), indomethacin (10 mg/kg) + MK-886 (10 mg/kg) (I + M), or dexamethasone (1 mg/kg) + 25% molecusol (2 ml/kg) (D) on the profile of retching + vomiting in the ferret induced by a single injection of cisplatin at 5 mg/kg. All drugs or vehicles (10% w/v NaHCO₃ (1 ml/kg) + 25% molecusol (2 ml/kg) (C) were administered intraperitoneally. The first administration of vehicles, indomethacin, MK-886, dexamethasone, or their respective combination was 30 s after injection of cisplatin and then at regular 8-h intervals. Results represent the mean ± S.E.M. of the total numbers of retches + vomits occurring during 1-, 0 − 24-, 24 − 48-, and 48 − 72-h periods. The number of animals retching and/or vomiting out of the number of animals tested (RV/T) is also shown. Individual latencies to the first episode of retching and/or vomiting are shown as filled circles (horizontal lines on the latency plot represent the mean latencies of the respective treatment groups). Significant differences relative to the NaHCO₃ + molecusol-treated animals is indicated as *$P<0.05$. Significant differences relative to the indomethacin + MK-886-treated animals are indicated as †$P<0.05$, ††$P<0.01$.

Discussion

We have previously hypothesized the anti-emetic action of glucocorticoids to antagonize cisplatin-induced emesis may involve inhibition of the production of inflammatory mediators such as prostanoids and leukotrienes (6); the action of glucocorticoids is also mimicked by tetracosactrin (20). In the present studies, the contribution of prostanoids and leukotrienes to cisplatin-induced emesis was more selectively tested
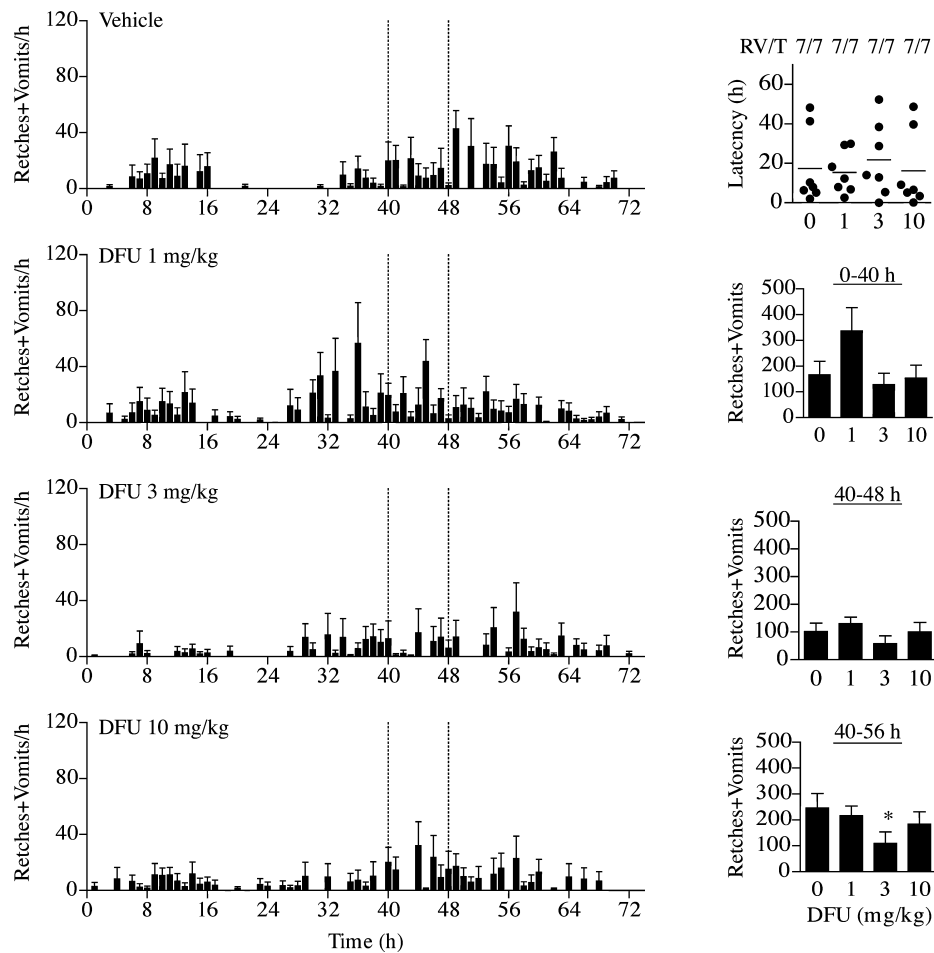
Fig. 4. The effect of DFU at 1-10 mg/kg on the profile of cisplatin (5 mg/kg, i.p.)-induced delayed retching + vomiting in the ferret. DFU or vehicle (60% PEG, 2 ml/kg) was administered intraperitoneally 40 and 48 h post cisplatin injection. Results represent the mean ± S.E.M. of the total numbers of retches + vomits occurring during 1-, 0 – 40-, 40 – 48-, and 40 – 56-h periods. The number of animals retching and/or vomiting out of the number of animals tested (RV/T) is also shown. Individual latencies to the first episode of retching and/or vomiting are shown as filled circles (horizontal lines on the latency plot represent the mean latencies of the respective treatment groups). Dotted lines on the graphs represent DFU or vehicle administration. Significant differences relative to the respective vehicle-treated animals are indicated as *$P > 0.05$.

using COX inhibitors (21) and a leukotriene bio-synthesis inhibitor (22).

We have previously demonstrated that DP, EP, TP, and IP prostanoid receptor agonists either induce or potentiate drug-induced emesis in the ferret (23); the emetic action of prostaglandin E_2 is prevented by ondansetron (24). Based on these studies, and the premise that cisplatin causes a release of inflammatory and/or pro-inflammatory mediators (see above), we expected that an inhibition of prostanoid synthesis might have provided a beneficial anti-emetic action against cisplatin. Conversely, the present studies revealed the potential of the non-selective cyclooxygenase inhibitor indomethacin to potentiate the emetic response induced by cisplatin. Certainly, the production of prostanoids has an important protective action in the gastrointestinal

tract and indomethacin may have removed this influence as a function of its known ability to cause gastric ulceration (25).

Cisplatin and other chemotherapeutic drugs are reported to increase COX-2 mRNA levels in the gastric mucosa with concurrent permeability changes and structural damage (26, 27). In our studies, the selective COX-2 inhibitor DFU had an action to transiently antagonize the delayed phase of emesis, although this was not dose-related when measured over 8 – 16-h periods, and the other COX-2 inhibitor, L-745,337, had no action. The data are therefore inconsistent but gastric ulceration is not seen with DFU (16), or L-745,337 (17), at the doses used in the present studies.

We timed the administration of the COX-2 inhibitors to coincide with the most intense period of delayed
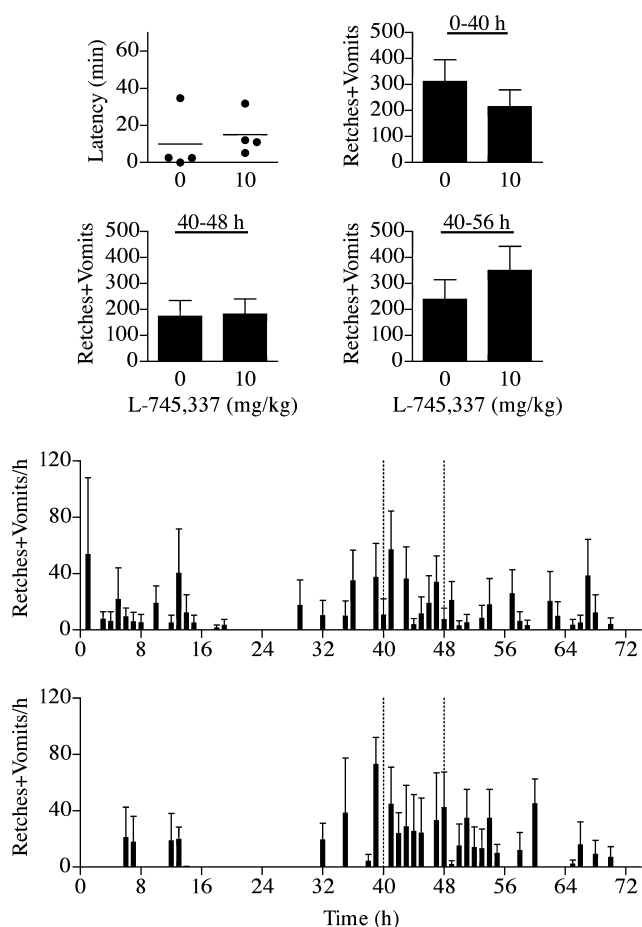
Fig. 5. The effect of L-745,337 at 10 mg/kg on the profile of cisplatin (5 mg/kg, i.p.)-induced delayed retching + vomiting in the ferret. L-745,337 or vehicle (25% molecusol, 2 ml/kg) was administered intraperitoneally 40 and 48 h post cisplatin injection. Results represent the mean ± S.E.M. of the total numbers of retches + vomits occurring during 1–, 0–40-, 40–48-, and 40–56-h periods. The number of animals retching and/or vomiting out of the number of animals tested (RV/T) is also shown. Individual latencies to the first episode of retching and/or vomiting are shown as filled circles (horizontal lines on the latency plot represent the mean latencies of the respective treatment groups). Dotted lines on the graphs represent L-745,337 or vehicle administration. There were no significant differences relative to the respective vehicle-treated animals ($P>0.05$).

emesis, where the incidence of emesis is higher. However, it should be noted that our studies with the COX-2 inhibitors are not in anyway definitive, since only 2 administrations during the delayed phase were used, and it is not known if an improved anti-emetic action could have been observed if the drugs were administered before emesis had begun (i.e., prostanoids potentially synthesized in the early course of cisplatin-induced emesis may have already triggered a cascade of other events or sensitized emetic circuits to other mediators). Certainly, the effect of DFU was not dose-related (see

above), but this may relate to higher doses possibly having an inherent emetic toxicity that may mask a useful anti-emetic effect.

There have been other studies in the dog showing that the emetic mechanism of action of sodium salicylate involves vagal afferents and the area postrema (28), suggesting the potential mechanism of action of emesis seen with COX inhibitors is complex (i.e., involving more than simple gastric irritation). It is possible, therefore, that the potentiation of cisplatin-induced emesis that we observed was only an additive phenomenon, since both indomethacin and cisplatin were both independently emetic. Yet in our studies, the vehicles also resulted in some emesis and how this may have potentially modified the cisplatin-induced emetic response is unknown.

In the pigeon, dexamethasone has a central and peripheral action to antagonize cisplatin-induced emesis, whereas indomethacin is inactive to both induce and inhibit emesis (29). However, a study in the piglet has examined a range of COX inhibitors for an ability to prevent cisplatin-induced emesis (30) and found that indomethacin, naproxen, and diclofenac, but not meloxicam, appeared to have an intrinsic emetic activity (indomethacin was the most potent) following intravenous administration (30). However, in contrast, in the ferret (present studies) and pigeon (29), indomethacin had a weak anti-emetic action to reduce 'acute' emesis, and indomethacin and meloxicam also weakly reduced the 'delayed' emesis; naproxen and diclofenac were inactive to reduce both phases of emesis. Unfortunately, the piglet study design was not balanced (i.e., 29 controls vs 5-11 for drug treatments) and there was no matching vehicle control (i.e., molecusol) for indomethacin, making the data difficult to interpret. Indeed, the anti-emetic potential of meloxicam, which has some degree of selectivity for COX-2 (31), was clearly not dose-related, and the conclusions of the study should be viewed cautiously.

MK-886 was selected as a leukotriene biosynthesis inhibitor for our studies with a mechanism of action involving direct binding to the 5-lipoxygenase-activating protein to subsequently prevent the activity of 5-lipoxygenase (22). We used MK-886 at doses up to 40 times higher than the reported oral ID$_{50}$ dose to reduce leukotriene biosynthesis (15). In man, multiple dosing of MK-886 is well tolerated with a single dose of 500 mg preventing the ex vivo synthesis of leukotriene B$_4$ by 60% (32), but in our study, MK-886 in combination with cisplatin was fatal in a few animals. For this reason, we were unable to use MK-886 at higher doses and we did not increase the numbers of animals used in the studies.
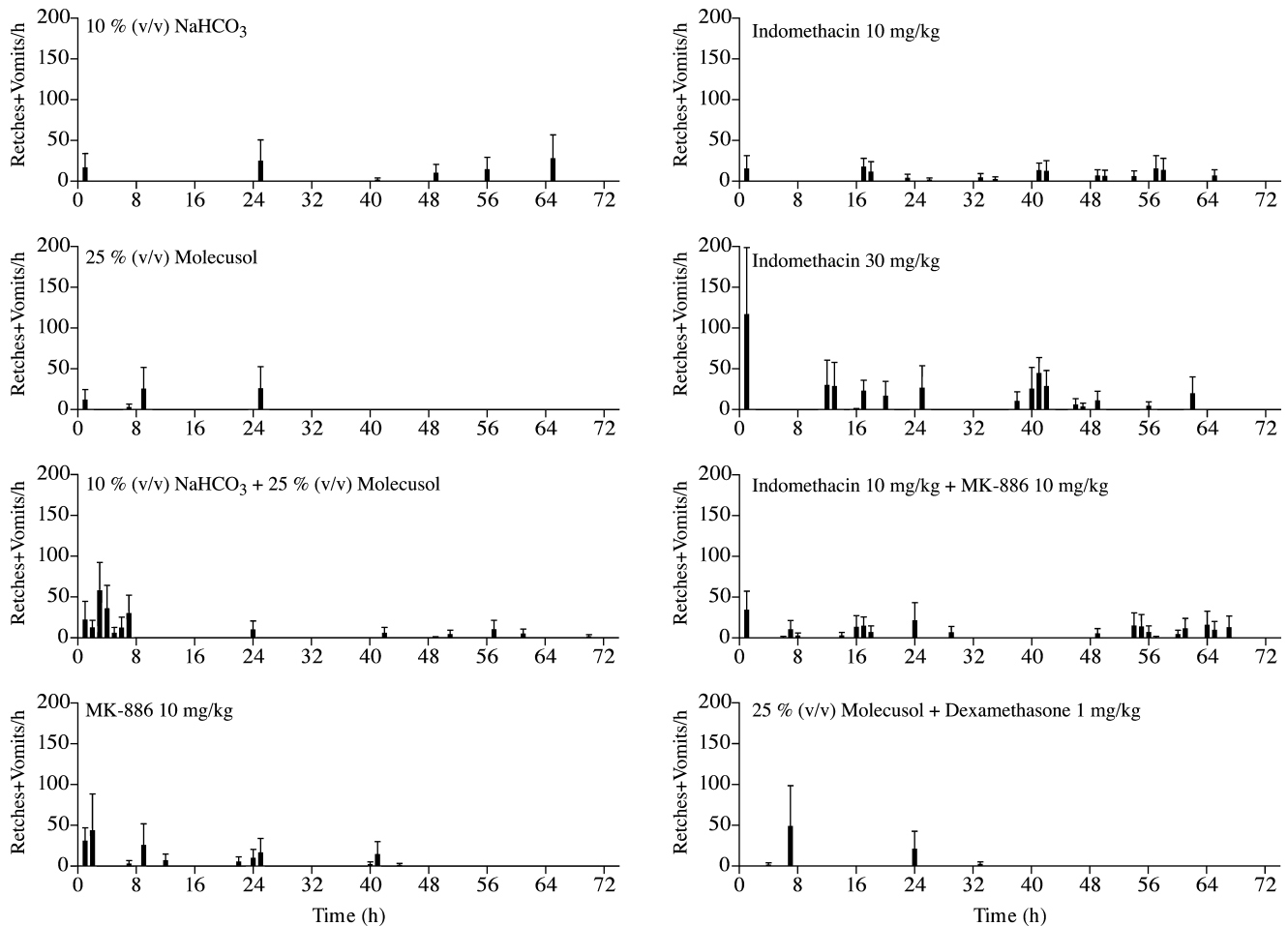
Fig. 6. Emetic profile of emetic action of eicosanoid biosynthesis inhibitors and their respective vehicles in the ferret. Results represent the mean ± S.E.M. of the total numbers of retches + vomits occurring during 1-h time intervals (n = 3). Drug and/or vehicle combinations were administered every 8 h (starting at t = 0) for 3 days.

The animals that died were not included in the final analysis, but MK-886 failed to affect cisplatin-induced acute emesis and did not significantly reduce the delayed phase of emesis (a failure to affect emesis was seen in 2 separate experiments). MK-886 formulated in molecusol was associated with some emesis, but it is possible that this action relates more to molecusol than MK-886, given that molecusol was also shown to emetic; this could have interfered with the potential anti-emetic action of MK-886. Nevertheless, the data with MK-886 suggests that leukotrienes are not involved significantly in the acute and delayed emesis induced by cisplatin in the ferret. However, to be certain of the role of leukotrienes in delayed emesis, it may be necessary to repeat the studies with other leukotriene synthesis inhibitors, or leukotriene receptor antagonists, that have negligible toxicity when combined with cisplatin and that can be delivered using inert vehicles.

In our previous studies, dexamethasone at 1 mg/kg,

i.p., administered 3 times per day, produced 60% – 85% and 58% – 90% reductions during the 0 – 24- and 24 – 72-h periods, respectively, of cisplatin-induced retching + vomiting (6, 33); and in the present studies, 70.5% and 78.5% reductions were observed for the respective periods. However, the reductions in the present studies failed to reach statistical significance and this possibly relates to interference from the vehicle used in the present studies for dexamethasone (NaHCO$_3$ and molecusol), since it had an inherent emetic potential. Whilst a lack of statistical significance of dexamethasone alone to modify emesis is a potential weakness of the present study, the data clearly showed differences in the mechanisms of dexamethasone and the regimen of indomethacin combined with MK-886 to affect emesis. Indeed, the combined regimen of indomethacin and MK-886 appeared to almost significantly potentiate the acute emetic response compared to control animals, whilst the single regimens of dexamethasone and MK-

Table 1.   Summary of the emetic potential of eicosanoid biosynthesis inhibitors and their respective vehicles in the ferret

Treatment	0 – 24 h	24 – 48 h	48 – 72 h	RV/T
10% (v/v) NaHCO$_3$	17.0 ± 17.0	27.3 ± 27.3	53.3 ± 53.3	1/3
25% (v/v) Molecusol	74.3 ± 74.3	26.3 ± 26.3	6.3 ± 6.3	1/3
10% (v/v) NaHCO$_3$ + 25% (v/v) Molecusol	179.3 ± 92.3	17.6 ± 16.7	23.3 ± 19.5	2/3
Indomethacin, 10 mg/kg	50.0 ± 25.1	35.7 ± 29.9	56.7 ± 36.7	3/3
Indomethacin, 30 mg/kg	218.0 ± 82.0*,†	148.7 ± 78.4*,†	36.3 ± 13.6	3/3
MK-886, 10 mg/kg	118.0 ± 46.6	46.7 ± 44.2	0.0 ± 0.0	3/3
Indomethacin, 10 mg/kg + MK-886, 10 mg/kg	88.7 ± 45.8	28.7 ± 19.2	100.0 ± 79.9	3/3
25% (v/v) Molecusol + Dexamethasone, 1 mg/kg	51.3 ± 51.3	24.0 ± 24.0	0.0 ± 0.0	1/3
60% (v/v) PEG	0.0 ± 0.0	0.0 ± 0.0	1.7 ± 1.7	1/3
DFU, 10 mg/kg	0.0 ± 0.0	37.5 ± 37.5	33.3 ± 33.3	1/3

All drugs/vehicles excepting PEG and DFU were administered 3 times per day for 3 days. PEG and DFU were administered at t = 40 and 48 h; see methods for dosing protocol. Significant differences relative to 10% (v/v) NaHCO$_3$-treated animals are indicated as *P<0.05; significant differences relative to 10% (v/v) NaHCO$_3$ + 25% (v/v) molecusol-treated animals are indicated as †P<0.05; there were no other significant differences between the other respective vehicle groups or drug treatments (P>0.05). The number of animals either retching and/or vomiting (RV) out of the number of animals tested (T) is also shown.

886 had no such action. Furthermore, and importantly, the single treatments of dexamethasone and MK-886, with cisplatin, produced significantly less retching and vomiting compared to the animals receiving the regimen of indomethacin combined with MK-886 and cisplatin.

In some acute models of inflammation, a dual inhibitor of leukotriene and prostanoid synthesis or the combination of a leukotriene biosynthesis inhibitor with a cyclooxygenase inhibitor provides a similar anti-inflammatory profile to dexamethasone (34 – 36). However, there are other occasions, where the immune system contributes to inflammation (e.g., in delayed hypersensitivity reactions), when the combination of leukotriene and prostanoid synthesis inhibitors fail to mimic the action of dexamethasone (37). These observations may enforce the hypothesis that glucocorticoids reduce cisplatin-induced emesis by interfering with the production of inflammatory cytokines (6). Blocking cytokine production and/or their receptors may represent a novel mechanism to antagonize cisplatin-induced acute and delayed emesis.

In conclusion, cisplatin induced emesis in the ferret is potentiated by treatment with the non-selective COX inhibitor indomethacin. This may suggest that prostanoids have a protective role against cisplatin, at least in the ferret. It is possible that protective prostanoids are produced by COX-1 during delayed emesis since the COX-2 selective inhibitors DFU and L-745,337 had no action to potentiate the emetic response (at least during the delayed phase, post 40 h) and DFU actually had short-lasting anti-emetic properties. The role of COX-2

in delayed emesis is therefore unresolved and future experiments with other selective agents on both acute and delayed emesis are required. The failure of MK-886 to affect acute and delayed emesis suggests that 5-lipoxygenase products do not contribute significantly to the emetic action of cisplatin, but the toxicity seen with MK-886 in combination with cisplatin limits the power of this statement. However, the combination of indomethacin and MK-886 clearly failed to mimic the action of dexamethasone in the model. Taken together, this may suggest that glucocorticoids exert their anti-emetic action in the ferret via mechanisms not directly related to their known action to prevent eicosanoid synthesis. The anti-emetic action of the glucocorticoids could involve a suppression of other mediators involved at other points in the inflammatory cascade (6, 20) or could be facilitated by other unknown mechanisms (3); such mechanisms may involve alterations in 5-HT function (38). Certainly, the number of genes directly activated by glucocorticoids is estimated to be between 10 and 100, with many genes being indirectly regulated through an interaction with other transcription factors and coactivators (39), meaning that future research on glucocorticoids may open new exciting possibilities for emesis control.

Acknowledgments

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