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# Differential involvement of neurotransmitters through the time course of cisplatin-induced emesis as revealed by therapy with specific receptor antagonists

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**Abstract**

Advances in antiemetic therapy for chemotherapy-induced emesis have resulted in improved protection against symptoms occurring within 24 h of chemotherapy. However, the vomiting which tends to occur beyond 24 h after chemotherapy (delayed-phase vomiting) is still relatively poorly controlled by the currently available drugs, suggesting that more than one mechanism may mediate these symptoms. The standard antiemetic regimen currently recommended for prevention of chemotherapy-induced emesis includes a serotonin (5-$HT_3$) antagonist and a corticosteroid. The neurokinin-1 ($NK_1$) antagonist aprepitant represents a new class of antiemetic currently in clinical development. Using data obtained in 2 Phase II clinical trials of aprepitant in patients receiving chemotherapy based on the highly emetogenic chemotherapeutic agent cisplatin, we compared the time course of antiemetic effect of aprepitant, a 5-$HT_3$ antagonist, or a combination of both. Over the entire observation period (up to 7 days post-cisplatin), patients who received the $NK_1$ antagonist had a superior prevention of emesis. However, in the first 24 h after cisplatin, emesis occurred in fewer patients who received the 5-$HT_3$ antagonist than in patients who did not receive this class of drug. Furthermore, the majority of treatment failures in patients who received the $NK_1$ antagonist occurred within the first 8–12 h of chemotherapy, whereas the treatment failures in patients who received a 5-$HT_3$ antagonist were more evenly distributed over time. Patients who received both drugs had superior control of symptoms compared with patients who received one or the other. The difference in the time course of emesis blockade observed with two different classes of receptor antagonists provides substantial evidence for involvement of separate pathophysiological mechanisms in chemotherapy-induced vomiting. Serotonin mediates the early vomiting process that occurs within 8–12 h following cisplatin-based chemotherapy, after which time substance P acting at $NK_1$ receptors becomes the dominant mediator of vomiting


*Keywords:* Antiemetic; Substance P; Cancer; Neurokinin

## 1. Introduction

Vomiting is a fundamental protective reflex mediated by the central nervous system to prevent the harmful consequences of ingested, potentially toxic substances. The vomiting reflex is mediated by several distinct brainstem nuclei which integrate afferent inputs from diverse sources, including: the area postrema (a medullary site which contains the chemoreceptor trigger zone); the vestibular system; the pharynx and gastrointestinal and cardiovascular systems; and higher brainstem or cortical sites [1]. The peripheral afferent input from the gastrointestinal tract is mediated predominantly by the vagus nerve. Afferent inputs to the vomiting centre are coordinated by the brainstem neuronal network of the dorsal vagal complex which includes the nucleus tractus solitarius (NTS). The NTS is a site for convergence of afferent input into the final common efferent pathway, via the dorsal motor nucleus of the vagus, that produces the various visceral and

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skeletal muscular contractions necessary to produce oral expulsion of gastrointestinal contents, as well as changes in gut motility [2].

Many neurotransmitters have been implicated in the pathogenesis of vomiting, including dopamine, acetylcholine, histamine, opiates, serotonin and substance P [1,3,4]. A more refined understanding of the relative importance of these neurotransmitters and their interrelationships in the regulation of vomiting is necessary for the development of more effective approaches for the treatment or prevention of vomiting.

### 1.1. Chemotherapy-induced vomiting (CIV)

In addition to its continued importance as a clinical problem, vomiting induced by cancer chemotherapy may serve as an important model for understanding the physiology of vomiting in general. Cisplatin is the single most emetogenic chemotherapeutic agent currently in use and may be considered the benchmark for evaluation of preventive strategies for CIV. At doses $>50$ mg/m$^2$ and in the absence of prophylactic therapy, cisplatin causes vomiting in virtually all patients [5]. This vomiting typically follows a biphasic time course. Following initiation of the cisplatin infusion there is a latency period of 1–3 h before the onset of vomiting. The peak frequency of vomiting tends to occur at 6–8 h post-initiation of the cisplatin infusion, and this first phase of vomiting diminishes at approximately 12 h. There follows a tendency for less emesis over approximately 4 h, after which the second phase of vomiting begins (approximately 16 h post-initiation of cisplatin). This second phase peaks between 24 and 72 h, although vomiting frequently occurs for several more days. In the setting of clinical studies of cisplatin-induced emesis, CIV has historically been described as occurring in two arbitrarily defined phases: the acute phase (from 0 to 24 h following the initiation of chemotherapy) and the delayed phase (from 24 h onwards); although the definitions are arbitrary, we present our findings in the context of this convention.

### 1.2. Mechanisms of CIV: serotonin

The neurotransmitter serotonin (5-hydroxytryptamine or 5-HT) has been shown both clinically and also in relevant animal assays to be an important mediator of the early ("acute") phase of CIV. Preclinical studies have shown that cisplatin causes a calcium-dependent exocytic release of serotonin from enterochromaffin cells in the gastrointestinal tract, possibly as a result of free radical generation [8–10]. The released serotonin then activates receptors on vagal afferent fibres, which stimulates the CNS centres that mediate the emetic response [8,9]. These receptors are known to be of the 5-HT$_3$ subtype, as 5-HT$_3$ receptor antagonists (RAs) inhibit the acute emetic response in a ferret CIV model [6,7], an observation which generated further interest in the role of serotonin.

Cisplatin administration in humans has also shown clear evidence for the involvement of serotonin. After cisplatin administration, there ensues a large increase in the urinary output of the serotonin metabolite 5-hydroxyindoleacetic acid (5-HIAA) within 24 h [11], indicating the release of intracellular serotonin. The time course of this response in the acute CIV period appears to correlate well with the clinical efficacy profile of 5-HT$_3$ RAs, which are most active against the acute-phase CIV associated with cisplatin-based chemotherapy [11].

Although the predominant therapeutic effect of 5-HT$_3$ RAs is believed to be antagonism of peripherally released serotonin, a central effect cannot be completely excluded. 5-HT$_3$ receptors have been shown to exist in the area postrema, NTS, subnucleus gelatinosus and in lower densities in the dorsal motor nucleus of the vagus and the spinal trigeminal tract of a number of species including man [12–19]. It has been shown in dogs that antagonism at 5-HT$_3$ receptors located within the blood–brain barrier can block cisplatin-induced emesis [20], but the relevance of this finding to the pathophysiology of CIV in humans is unclear, as plasma levels of 5-HIAA are not increased by cisplatin in dogs [21]. Higgins and colleagues reported that in the ferret, emesis induced by cisplatin was attenuated, but not blocked, by infusions of 5-HT$_3$ receptor antagonists into the area postrema [22], although the 5-HT$_3$ selective agonist 2-methyl 5-HT did not reproducibly induce emesis when infused via the same route. It is therefore unclear whether 5-HT$_3$ RAs have any central activity in humans. It is widely assumed that the major antiemetic activity of 5-HT$_3$ RAs occurs through inhibition of afferent vagal stimulation in the periphery. Underscoring the fact that interpretation of the effects of 5-HT$_3$ RAs is not a simple matter, Andrews and colleagues have reported that although ferrets are initially refractory to radiation-induced emesis following vagotomy, the emetic reflex returns in some animals [23]. This finding suggests that emetic reflex pathways may have some plasticity, and could help to explain why the antiemetic effects of 5-HT$_3$ RAs are more pronounced in some patients than in others.

Although important in the acute phase, serotonin is not believed to be a significant mediator of emesis occurring more than 24 h after chemotherapy (historically known as delayed vomiting). Delayed CIV responds poorly to 5-HT$_3$ antagonists in both humans and animal models [11], and it is therefore highly likely that other neurotransmitters are involved in the pathogenesis of delayed-phase symptoms.

### 1.3. Mechanisms of CIV: substance P

Substance P, a member of the tachykinin family of neuropeptides, was first implicated as a potential mediator

of vomiting when Amin and colleagues described high levels of this peptide in the area postrema of dogs [24]. Subsequently, studies in ferrets showed that the potent capsaicin analogue resinferatoxin blocked the emetic response to both centrally and peripherally acting emetic agents [23]. It was suggested that this antiemetic effect was mediated by resinferatoxin-induced depletion of sensory neurotransmitters such as substance P in the NTS in the brainstem. In support of this concept, animal studies using both centrally and peripherally active emetogenic stimuli demonstrated that vomiting was prevented by non-peptide antagonists of the neurokinin-1 ($NK_1$) receptor, a site at which substance P is thought to act [4,25,26]. Substance P is co-localised with serotonin in enterochromaffin cells in the gastrointestinal tract, and substance P levels in the peripheral circulation have been reported to be elevated following cisplatin administration in patients [27]. Substance P has been shown in animals to cross the blood-brain barrier, which raises the possibility that substance P of peripheral origin may act centrally to induce emesis [28]. CNS penetration by the $NK_1$ RAs has been shown to be essential for the prevention of vomiting in the first 4 hours following cisplatin-based chemotherapy, which suggests that the antiemetic effect of $NK_1$ RAs is mediated centrally, probably in region of the NTS [29].

The spectrum of antiemetic activity observed with $NK_1$ RAs in preclinical studies was broader than with other antiemetics such as 5-$HT_3$ RAs. Specifically, $NK_1$ RAs prevented both acute and delayed vomiting induced by cisplatin in the ferret [4,30], whereas 5-$HT_3$ RAs prevented only acute vomiting in this model. These preclinical data, especially those derived from the ferret model of CIV, were compelling enough to justify clinical evaluation of $NK_1$ RAs.

## 2. Patients and methods

### 2.1. Clinical data with aprepitant (MK-869) and L-758, 298

Aprepitant (MK-869) is a powerful and selective brain-penetrant $NK_1$ RA that can be administered orally, and L-758,298 is an intravenous pro-drug for aprepitant. Four studies have been published demonstrating the efficacy of these $NK_1$ RAs in the prevention of CIV associated with high-dose cisplatin [31–34]. Their particular efficacy in delayed CIV represents a potentially important medical advance in the treatment of a condition for which current therapy is sub-optimal. Using the conventional definitions of the acute and delayed phases, the published studies showed that the efficacy of the $NK_1$ RAs was not significantly different from that of the 5-$HT_3$ RAs during the acute phase, but the $NK_1$ RAs were clearly superior during the delayed phase. The existing evidence, driven by the effectiveness of the 5-$HT_3$ RAs and the $NK_1$ RAs, suggests that serotonin therefore may be more influential in the acute phase and substance P more significant in the delayed phase.

To establish a framework for clarifying in detail the roles of serotonin and substance P in the pathogenesis of CIV, the time course of acute-phase vomiting in two of the previously published studies was analysed.

## 3. Results

### 3.1. Single-agent comparison of L-758,298 and ondansetron

The time course of emesis was initially analysed in a study comparing a single dose of L-758,298 with a single dose of ondansetron in patients receiving their first course of high-dose cisplatin ($\geqslant 50$ mg/m$^2$) (efficacy and tolerability results previously published) [31].

A *post hoc* analysis of data collected up to 7 days post-cisplatin in this study showed that a differential pattern of emesis occurred in the two treatment groups. Over the entire observation period of 168 h, 31.0% of patients had no emetic episodes in the L-758,298 treatment group and 21.7% of patients had no emetic episodes in the ondansetron group as shown in Table 1. The Kaplan–Meier curve in Fig. 1 depicts the cumulative percentage of patients who remained emesis-free since the initiation of cisplatin-based chemotherapy. Historically, the frequency of acute emesis following

Table 1
Percentages of patients with no emesis during intervals post-cisplatin in aprepitant study protocol #004 [31]

| Treatment group | 0–8 h | 0–16 h | 0–24 h | 0–168 h |
|---|---|---|---|---|
| Ondansetron ($n=23$) | 82.6 | 69.6 | 52.2 | 21.7 |
| L-758,298 ($n=30$) | 36.7 | 36.7 | 36.7 | 31.0 |

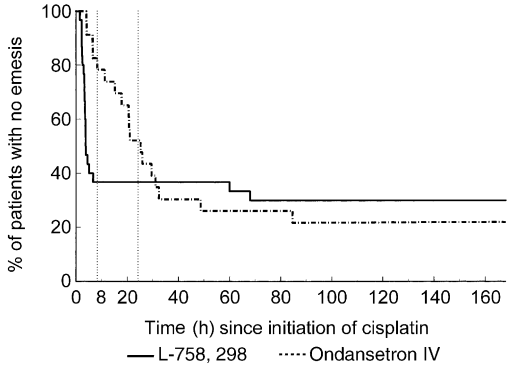


Fig. 1. Percentage of patients with no emesis in the overall study period (0–168 h = 7 days) in aprepitant study protocol #004 [31]. Group 1 = L-758,298 (intravenous prodrug for aprepitant); Group 2 = ondansetron.

this dose of cisplatin without antiemetic prophylaxis is virtually 100%, with a median time to first emesis of less than 2 h. Therefore, the $NK_1$ RA and the 5-$HT_3$ RA demonstrated equivalent antiemetic efficacy against cisplatin-associated CIV in this trial.

Closer examination of the time course of responses showed that within the first 24 h after cisplatin, more patients in the L-758,298 group experienced their first episode of emesis compared with patients who received ondansetron. The time-course of initial emetic episodes in the two treatment groups in the first 24 h was quite distinctive, as shown in Table 1 and Fig. 2. Notably, all treatment failures in the L-758,298 group occurred in the first 8 h of the acute phase; the no-emesis rate for the 0–8 h interval was 36.7%, and no patients in this treatment group had their first episode of vomiting between 8 and 24 h post cisplatin. By contrast, few patients in the ondansetron group experienced emesis in the first 8 h after cisplatin, as demonstrated by the no-emesis rate of 82.6% for this interval. After the first 8 h, however, and unlike patients in the L-758, 298 group, additional patients in the ondansetron group experienced their first episode of emesis between 8 and 24 h post-cisplatin. No-emesis rates in the ondansetron group declined to 69.6% in the 0–16 h period and 52.2% in the 0–24 h period, whereas the no-emesis rate for the L-758,298 group remained unchanged between 8 and 24 h post-cisplatin. Thus, the relative benefit of treatment in the first 8 hours appeared to be much greater with ondansetron, whereas L-758,298 was more effective during the latter part of the acute phase.

### 3.2. Time course of emesis with aprepitant and granisetron used in combination

The time course of emesis was also examined in a subsequent trial evaluating the oral $NK_1$ RA aprepitant and the 5-$HT_3$ RA granisetron used in combination for the prevention of cisplatin-induced emesis [33]. All patients were cisplatin-naïve and scheduled to receive a cisplatin dose $\geqslant 70$ mg/m$^2$. Patients were randomised in a blinded fashion to four parallel groups (Table 2). The proportions of patients with no emesis during the entire 120-h study period are shown in Table 3. In the groups receiving aprepitant without granisetron (Groups III and IV), 36% of patients (31 of 89 patients in Group III and 31 of 84 patients in Group IV) had no emesis, in contrast to 23% (21/90) of the patients in Group I who had no emesis after having received granisetron without aprepitant.

Of the 111 patients who had emesis after taking aprepitant without granisetron, 82% (91/111) had their initial emetic episode during the first 8 hours. In marked contrast, of the 69 patients treated with granisetron but not aprepitant and who had emesis, only 10% (7/69) had their first emetic episode during the first 8 h; 90% of the initial emetic episodes in these patients occurred between 8 and 120 h. These time courses are illustrated by a Kaplan–Meier curve in Fig. 3.

As in the previously described analysis, a closer examination of the acute phase was also performed for this study (Table 3). Consistent with the results of the

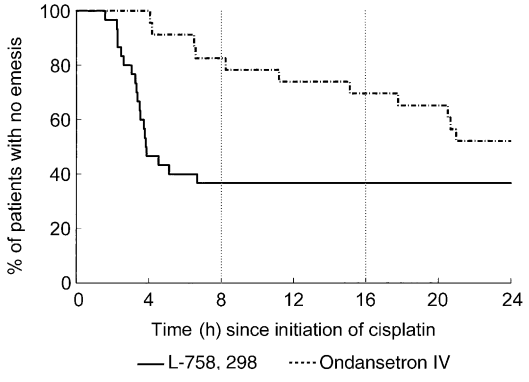


Fig. 2. Percentage of patients with no emesis in the acute phase (0–24 h) in aprepitant study protocol #004 [31]. Group 1 = L-758,298 (intravenous prodrug for aprepitant); Group 2 = ondansetron.

Table 2
Aprepitant protocol #012 study design [33]

| Treatment group | Day −1 | Day 1 | Days 2–5 |
|---|---|---|---|
| I<br>Granisetron + Dexamethasone | Placebo | Granisetron (i.v., 10 μg/kg)<br>Dexamethasone (20 mg p.o.) | Placebo |
| II<br>5 days Aprepitant + Granisetron + Dexamethasone | Placebo | Granisetron (i.v., 10 μg/kg)<br>Dexamethasone (20 mg p.o.)<br>Aprepitant (400 mg p.o.) | Aprepitant (300 mg p.o.) |
| III<br>6 days Aprepitant + Dexamethasone | Aprepitant (400 mg p.o.) | Dexamethasone (20 mg p.o.)<br>Aprepitant (400 mg p.o.) | Aprepitant (300 mg p.o.) |
| IV<br>5 days Aprepitant + Dexamethasone | Placebo | Dexamethasone (20 mg p.o.)<br>Aprepitant (400 mg p.o.) | Aprepitant (300 mg p.o.) |

i.v., intravenous; p.o., orally.

Table 3
Percentages of patients with no emesis during intervals post-cisplatin in aprepitant study protocol #012 [33]

| Treatment group[a] | 0–8 h | 0–16 h | 0–24 h | 0–120 h |
|---|---|---|---|---|
| I ($n = 90$) | 92.2 | 81.1 | 56.7 | 23.3 |
| II ($n = 84$) | 88.1 | 85.7 | 79.8[b] | 57.1 |
| III ($n = 89$) | 49.4 | 47.2 | 46.1 | 35.2 |
| IV ($n = 84$) | 45.2 | 44 | 42.9 | 36.9 |

[a] Dose regimens are specified in Table 2.
[b] Significantly different from Group I (95% confidence interval (CI) on the between-treatment difference does not include 0).

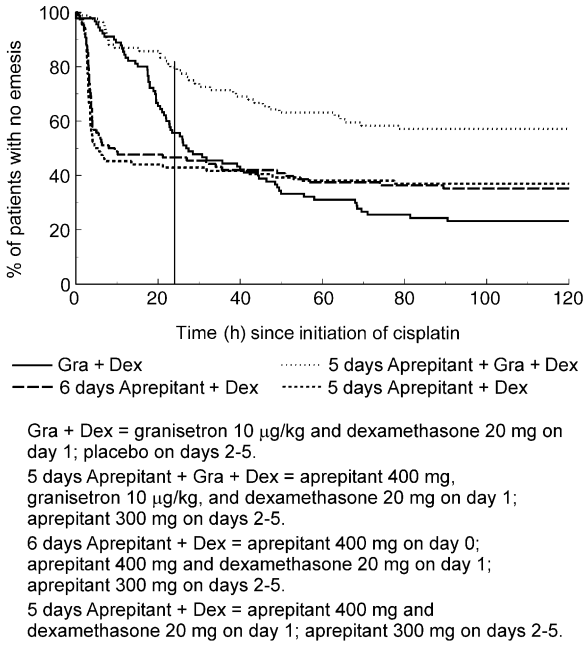


Fig. 3. Percentage of patients with no emesis in the overall study period (0–120 h) in aprepitant study protocol #012 [33]. Gra, Granisetron; Dex, Dexamethasone.

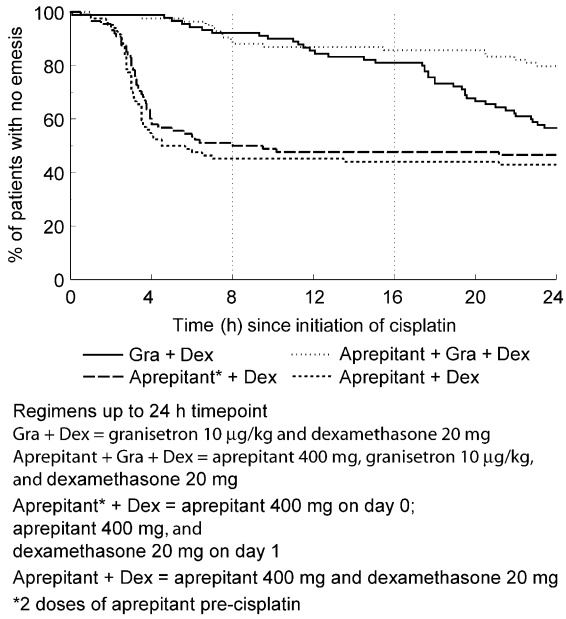


Fig. 4. Percentage of patients with no emesis in the acute phase (0–24 h) in aprepitant study protocol #012 [33].

single-agent comparison, acute emesis in the 173 patients who received aprepitant but not granisetron (Groups III and IV) occurred most often in the first 8 h in this study. Administration of an additional dose of aprepitant on the evening prior to cisplatin (Group III) did not prevent these early acute failures. Only 5 of these 173 patients in Groups III and IV (3%) experienced their first episode of emesis 8–24 h after cisplatin treatment. Conversely, patients receiving granisetron (Groups I and II) did not generally experience emesis in the first 8 h. Among patients in the granisetron groups, the no-emesis rate during the first 8 h was 90%. In Group I, in which aprepitant was not coadministered with granisetron, 32 of the 90 patients (36%) experienced their first episode of emesis in the period of 8–24 h. By contrast, granisetron combined with aprepitant (Group II) provided the best control of acute emesis; only 20% of patients (17/84) in this group had emesis during the first 24 h. These findings are illustrated graphically in Fig. 4, which depicts the Kaplan-Meier curve for time to first emesis in the acute phase. The treatment failures in the groups receiving aprepitant but not granisetron were concentrated in the first 8 h post-cisplatin, whereas the slope indicating treatment failures in the granisetron groups occurred in a notably more gradual fashion during the first 8 h. Furthermore, the survival curve for the granisetron groups revealed a similarity in slope between the curves for Groups I and II to approximately 16 h, after which time the slope of the curve for Group I, in which aprepitant was not coadministered, becomes much steeper and diverges substantially from the curve for Group II, in which patients received both granisetron and aprepitant.

## 4. Discussion

The role of serotonin-related mechanisms in cisplatin-induced emesis is now well established. 5-$HT_3$ RAs are the cornerstone of current preventive regimens for CIV, given their efficacy and excellent tolerability. When used with cisplatin, the 5-$HT_3$ RAs are most effective in the prevention of emesis occurring in the first 24 h (acute emesis) although they appear to be distinctly less effective for emesis developing after 24 h (delayed emesis). Only recently have the mechanisms underlying delayed emesis become amenable to detailed mechanistic study with the description of relevant animal models [35,36]. In the ferret model proposed by Rudd and colleagues [35], 5-$HT_3$ RAs had limited efficacy in preventing cisplatin-induced delayed emesis. In contrast, the $NK_1$ receptor antagonist CP-99,994 was effective in minimising both early and late cisplatin-induced emesis. In addition, it has been shown that aprepitant prevents emesis in this ferret model [30]. These observations suggested a potential clinical role for this class of agents in CIV.

Subsequent clinical data convincingly points to the efficacy of the $NK_1$ RAs in the control of cisplatin-induced emesis. In the two studies in which $NK_1$ RAs were compared directly with 5-$HT_3$ RAs, a strikingly consistent pattern of efficacy was observed. Patients treated with the $NK_1$ RA had less emesis over the entire period of observation than did patients treated with a

5-$HT_3$ RA. Because 100% of patients would have been anticipated to have emesis in the absence of antiemetic therapy, these data provide compelling evidence that $NK_1$ RAs have antiemetic efficacy. In the acute phase of both studies, the relative responses in the $NK_1$ RA and 5-$HT_3$ RA treatment groups were similar: fewer patients treated with the 5-$HT_3$ RA had emesis compared with those treated with the $NK_1$ RA. The superior prevention of emesis over the entire observation period following $NK_1$ RA therapy, despite less control of acute symptoms, is due to the distinctive efficacy of the $NK_1$ RA in preventing emesis in the delayed phase.

Beyond the contrasting therapeutic profiles of the $NK_1$ RA and the 5-$HT_3$ RA over the acute and delayed phases in both these studies, these agents also appear to have distinctive patterns of efficacy within the acute phase itself, especially in the first 8 h. In both studies, the preponderance of initial acute emetic events following $NK_1$ RA therapy in the absence of concomitant 5-$HT_3$ RA therapy occurred within 8 h of cisplatin (100% in the first study [19 of 19 patients with acute emesis in Group I]; and 95% in the second study [45 of 48 patients with acute emesis in Group III and 46 of 48 patients with acute emesis in Group IV]). By contrast, the initial episodes of acute emesis following 5-$HT_3$ RA therapy in the absence of concomitant $NK_1$ RA therapy were not concentrated in the first 8 h (36% in the first study [4 of 11 patients with acute emesis in Group I] and 18% in the second study [7 of 39 patients with acute emesis in Group I]). This differential antiemetic effect early in the acute phase was nearly identical in both studies, as illustrated by the corresponding Kaplan–Meier curves (Figs. 2 and 4). It is notable that the pattern of early emesis was also identical in the 2 $NK_1$ RA treatment groups in the second study, in which 1 of these 2 groups received an additional dose of the $NK_1$ RA on the evening prior to cisplatin. This finding demonstrates that the relatively inferior effect of the $NK_1$ RA in the first 8 h probably reflects the intrinsic biology of emesis rather than low tissue levels of the $NK_1$ RA. The differential time curves of emesis in the two trials strongly suggest that the greatest benefit of the $NK_1$ RAs is observable more than 8 h after cisplatin administration.

When the two 5-$HT_3$ RA-containing groups in the second trial were examined, emetic control was better in the group which also received aprepitant. The improved control became apparent only after approximately 12 h as illustrated in Fig. 3. Likewise, in a third study of aprepitant versus a standard therapy regimen conducted recently, the pattern of first emetic events was similar across treatment groups until approximately 16 h post-cisplatin, after which those treatment regimens which included aprepitant emerged as clearly superior [37].

Our clinical data suggest that serotonin-dependent mechanisms predominate in the first 8–12 h post-cisplatin, but thereafter, neurokinin-1 dependent mechanisms appear to have relatively greater importance. Specifically, early acute events responsive to 5-$HT_3$ RAs are likely to be mediated by peripheral serotonin release, whereas later acute and delayed events responsive to $NK_1$ RAs are more likely to be mediated by substance P acting centrally at $NK_1$ receptors. Further research is required to clarify why an initial delay tends to precede those emetic events particularly amenable to prevention by $NK_1$ RAs. These observations, by providing insight into the pathophysiology of emesis during the various time intervals after cisplatin administration, support the rationale for combination therapy in the clinical setting to enhance control of CIV. This mechanistic analysis of the differential effects of highly selective receptor antagonists on a complex CNS process may serve as a model for the future study of such processes.

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