Cancer Chemother Pharmacol (2005) 56: 370–378
DOI 10.1007/s00280-005-1005-4

ORIGINAL ARTICLE

**Milly E. de Jonge · Alwin D. R. Huitema
Marjo J. Holtkamp · Selma M. van Dam
Jos H. Beijnen · Sjoerd Rodenhuis**

Aprepitant inhibits cyclophosphamide bioactivation and thiotepa metabolism

Received: 22 September 2004 / Accepted: 8 February 2005 / Published online: 19 April 2005

Abstract *Background*: Patients receiving the highly emetogenic high-dose chemotherapy regimen with cyclophosphamide, thiotepa and carboplatin (CTC) may benefit from the neurokin-1 receptor antagonist aprepitant in addition to standard anti-emetic therapy. As aprepitant has been shown to be a moderate inhibitor of the cytochrome P450 (CYP) 3A4 isoenzyme, its effect on the pharmacokinetics and metabolism of cyclophosphamide and thiotepa was evaluated. Moreover, preliminary results on the clinical efficacy of aprepitant in the CTC regimen are reported. *Patients and methods*: Six patients were enrolled in a protocol that employed a 4-day course of CTC high-dose chemotherapy with cyclophosphamide (1,500 mg/m^2/day), thiotepa (120 mg/m^2/day) and carboplatin (AUC 5 mg min/ml/day). Two patients received the tCTC protocol, which comprises two-third of the dose of CTC. In addition to standard anti-emetic therapy, the patients received aprepitant from one day before the start of their course until 3 days after chemotherapy. Blood samples were collected on days one and three of the course and analyzed for cyclophosphamide and its activated metabolite 4-hydroxycyclophosphamide, thiotepa and its main active metabolite tepa. The influence of aprepitant on the pharmacokinetics of cyclophosphamide and thiotepa was analyzed using a population pharmacokinetic analysis including a reference population of 49 patients receiving the same chemotherapy regimen without aprepitant and sampled under the same conditions. The frequency of nausea and vomiting in the six patients receiving CTC was compared with those of the last 22 consecutive patients receiving CTC chemotherapy without aprepitant. Inhibitory activity of aprepitant on cyclophosphamide and thiotepa metabolism was also tested in human liver microsomes. *Results*: In our patient population, the rate of autoinduction of cyclophosphamide ($P = 0.040$) and the formation clearance of tepa ($P < 0.001$) were reduced with 23% and 33% when aprepitant was co-administered, respectively. Exposures to the active metabolite 4-hydroxycyclophosphamide and tepa were therefore reduced (5% and 20%, respectively) in the presence of aprepitant. In human liver microsomes, the 50% inhibitory concentrations (IC_{50}) of aprepitant for inhibition of cyclophosphamide ($IC_{50} = 1.3$ μg/ml) and thiotepa ($IC_{50} = 0.27$ μg/ml) metabolism were within the therapeutic range. Patients receiving aprepitant experienced less frequently CINV both during and after the CTC course compared with the reference population (nausea 3.7 days vs. 5.8 days, $P = 0.052$; vomiting 0.5 days vs. 4.8 days, $P < 0.001$). *Conclusion*: Aprepitant inhibited both cyclophosphamide and thiotepa metabolism, most probably due to inhibition of the CYP 3A4 and/or 2B6 isoenzymes. The effects of this interaction are, however, small compared to the total variability. Addition of aprepitant may provide superior protection against vomiting in patients receiving the highly emetogenic high-dose CTC chemotherapy.

Keywords Aprepitant · Cyclophosphamide · Thiotepa · Pharmacokinetic drug–drug interaction · Nausea · Vomiting

M. E. de Jonge (✉) · A. D. R. Huitema
S. M. van Dam · J. H. Beijnen
Department of Pharmacy & Pharmacology,
The Netherlands Cancer Institute/Slotervaart Hospital,
Louwesweg 6, 1066 EC, Amsterdam, The Netherlands
E-mail: apmil@slz.nl
Tel.: +31-20-5124657
Fax: +31-20-5124753

M. J. Holtkamp · J. H. Beijnen · S. Rodenhuis
Department of Medical Oncology,
The Netherlands Cancer Institute, Plesmanlaan 121,
1066 CX, Amsterdam, The Netherlands

Introduction

High-dose chemotherapy regimens with cyclophosphamide, thiotepa and carboplatin (CTC), followed by peripheral blood progenitor cell transplantation, are

used in the treatment of breast and germ cell cancer [23–25]. Despite extensive anti-emetic therapy with a corticosteroid (dexamethasone) and a $5HT_3$-receptor antagonist (granisetron or ondansetron), this chemotherapy regimen is often associated with severe nausea and vomiting [26]. To increase the tolerance of the high-dose CTC regimen, anti-emetic therapy needs improvement.

Aprepitant (Emend, Merck & Co., Inc.) is a recently introduced neurokin-1 (NK_1) receptor antagonist developed for use in combination with a $5HT_3$-receptor antagonist and a corticosteroid to prevent both acute and delayed nausea and vomiting induced by highly emetogenic chemotherapy [4, 7, 8, 11, 19]. The recommended dosing regimen of aprepitant for the prevention of chemotherapy-induced nausea and vomiting (CINV) is 125 mg p.o. on the day of chemotherapy, followed by daily 80 mg p.o. on the 2 days following chemotherapy. Aprepitant may be useful in preventing CINV in the CTC regimen.

It has been shown that aprepitant not only has a direct inhibitory effect on cytochrome P450 (CYP) 3A4 [16, 27], but also an inducing effect on CYP 3A4 and 2C9 in the 2 weeks following administration [27]. When aprepitant was administered daily for 5 days together with the CYP 3A4 probe midazolam, the midazolam exposure increased 2.3-fold on day onefold and 3.3-fold on day 5 as compared with midazolam alone [16]. Using the same aprepitant regimen, a significant increase in the exposure to oral dexamethasone (2.2-fold) and oral methylprednisolon (2.5-fold), both CYP 3A4 substrates, was reported at days 1 and 5 upon co-administration with aprepitant compared with no aprepitant [18].

Cyclophosphamide and thiotepa are alkylating agents metabolized by CYP enzymes in to both active and inactive metabolites. Aprepitant might therefore influence cyclophosphamide and thiotepa disposition. Cyclophosphamide itself is an inactive prodrug, requiring activation, primarily by CYP2B6, to form its active metabolite 4-hydroxycyclophosphamide [2, 21, 22, 32]. The drug shows autoinduction after repeated administrations, which is caused by induction of CYP2B6 and 3A4 [9, 15, 17]. Thiotepa is metabolized by CYP2B6 and 3A4 iso-enzymes resulting in the formation of tepa [14]. Tepa has a longer elimination half-life than thiotepa, but similar pharmacologic properties [10, 28]. Individual differences in exposure to cyclophosphamide, thiotepa and their active metabolites might influence the outcome of treatment in the CTC regimen. It is generally assumed that exposure to the activated metabolites 4-hydroxycyclophosphamide and tepa contributes significantly to therapeutic outcome. These metabolites have also been shown to contribute to regimen-related toxicities in the CTC regimen [13]. For example, the occurrence of veno-occlusive disease of the liver may be related to exposure to 4-hydroxycyclophosphamide, while mucositis may be related to tepa exposure. We therefore considered it important to investigate whether co-administration of aprepitant influences cyclophosphamide bioactivation and thiotepa metabolism.

Patients and methods

Patients and treatment

Eight patients (M/F:1/7) were enrolled in protocols that employed the CTC high-dose chemotherapy regimen with peripheral blood progenitor cell transplantation. Five females were diagnosed with high-risk breast cancer [23], two females with metastatic breast cancer [25], and the male patient had advanced germ-cell cancer [24]. The full-dose CTC regimen [23, 24] consisted of 4 days of chemotherapy with cyclophosphamide (1,500 mg/m^2/day) as a 1 h infusion, immediately followed by carboplatin (dose calculated based on a modified Calvert formula, using a target area under the plasma concentration-time curve (AUC) value of 5 mg min/ml/day) as a daily 1 h infusion, and thiotepa (120 mg/m^2/day) divided over two 30-min infusions (the second daily dose of thiotepa was administered 12 h after the first dose). The male patient received two full-dose CTC courses while the five females received only a single course. The two other females received two courses of the tCTC regimen, which is the same as a full dose CTC regimen, except that the doses of cyclophosphamide, thiotepa and carboplatin during a course were two-third of those used in the full dose regimen [25]. Mesna (500 mg) was administered six times daily for a total of 36 doses, beginning 1 h prior to the first CP infusion. Starting 4 days before chemotherapy, the patients prophylactically received antibiotics (ciprofloxacin and fluconazole orally). Furthermore, the patients received ranitidine, fytomenadion and folic acid.

Standard anti-emetic treatment consisted of twice daily 1 mg granisetron i.v. and twice daily 10 mg dexamethasone i.v. on the 4 days of chemotherapy, followed by twice daily 1 mg granisetron i.v. and once daily 10 mg dexamethasone i.v. on the 2 days after chemotherapy. For this study, aprepitant was added to this regimen as a 125 mg loading dose at the day prior to start of chemotherapy. Every day of the CTC course, approximately 1 h before chemotherapy, until 3 days after the last chemotherapy dose, daily 80 mg aprepitant was administered. Two patients received 80 mg instead of 125 mg on the day prior to start of chemotherapy. Although it is known that aprepitant may increase dexamethasone plasma levels [18], dexamethasone doses in patients receiving aprepitant were not decreased.

The Committee on Medical Ethics of the Netherlands Cancer Institute had approved the high-dose chemotherapy protocols including pharmacokinetic sampling, and written informed consent was obtained from all patients. Patients were informed of the potential detrimental effects of inhibition by aprepitant.

Pharmacokinetic study

For pharmacokinetic analyses during the 4-day CTC course, blood samples were collected from a double

lumen intravenous catheter inserted in a subclavian vein. Collection took place during the course every day prior to the start of the infusions. On days 1 and 3, samples were withdrawn at 30 min after the start of cyclophosphamide infusion and at 60 (end of cyclophosphamide infusion and start of carboplatin infusion), 90, 120 (end of carboplatin infusion and start of thiotepa infusion), 150 (end of thiotepa infusion), 180, 210, 280, 390 and 660 min. Analytical methods for the determination of plasma concentrations of cyclophosphamide, 4-hydroxycyclophosphamide, thiotepa and tepa and carboplatin were used as reported previously [6, 30]. Pharmacokinetic analysis was performed in all courses for all six patients receiving the full-dose CTC with aprepitant. In the two patients receiving the tCTC courses, pharmacokinetics was monitored in one course with, and one course without, aprepitant for cross-over purposes.

Pharmacokinetic calculations were based on a previously published integrated population pharmacokinetic model of thiotepa (and its metabolite tepa) and cyclophosphamide (and its metabolite 4-hydroxycyclophosphamide) [5]. This model has been developed in our institute using the program NONMEM (double precision, version V 1.1) [1], and was based on data of 49 patients who received 86 courses of both the full-dose CTC chemotherapy regimen and the tCTC regimen with co-medication as described above. The first-order method was used with log-transformed data. The model was used exactly as published. Important aspects of the developed model were that it included the autoinduction process of cyclophosphamide, inhibition of the bioactivation of cyclophosphamide by thiotepa, and the inductive effect of cyclophosphamide on the metabolism of thiotepa [5].

In Fig. 1 the population model is schematically depicted. In brief, cyclophosphamide was eliminated by a non-inducible route ($CL_{CPnonind}$) and an inducible route (CL_{CPind}), the latter leading to formation of 4-hydroxycyclophosphamide. The apparent clearance of the inducible route leading to 4-hydroxycyclophosphamide was directly proportional to a hypothetical amount of enzyme (ENZ_{CPact}). Autoinduction led to a zero-order increase (k_{enzCP}) in the amount of this enzyme during treatment in the presence of cyclophosphamide. The inhibition of the conversion of cyclophosphamide to 4-hydroxycyclophosphamide in the presence of thiotepa was modeled by a thiotepa-concentration-dependent reversible deactivation of the enzyme resulting in the formation of inactive enzyme ($ENZ_{CPinact}$). Thiotepa itself was also eliminated by a non-inducible route ($CL_{TTnonind}$) and an inducible route (CL_{TTind}), the latter leading to formation of tepa. Metabolism of thiotepa in to its metabolite tepa was induced in the presence of cyclophosphamide. This process was modeled using a second hypothetical enzyme compartment (ENZ_{TT}) in which the amount increased with a zero-order rate constant (k_{enzTT}) in the presence of cyclophosphamide. The apparent thiotepa clearance of the inducible route was directly proportional to the amount in this second hypothetical enzyme compartment. A detailed description of the model has been published [5]. The final parameter estimates of the population pharmacokinetic model are summarized in Table 1.

This model was used for evaluating the influence of aprepitant on the pharmacokinetics of cyclophosphamide and thiotepa. Data of the 8 patients receiving aprepitant in our study were added to the data of the 49 previous patients that were used for model development. Use of aprepitant was included as a covariate in the

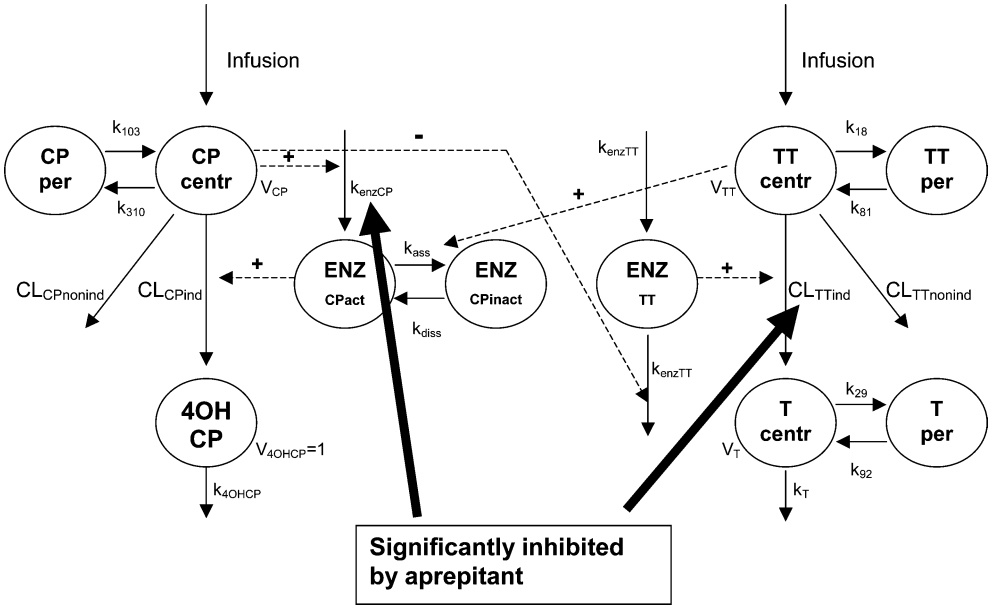

Fig. 1 Population pharmacokinetic model developed for cyclophosphamide (*CP*) and thiotepa (*TT*) and their metabolites 4-hydroxycyclophosphamide (*4OHCP*) and tepa (*T*), respectively, including three hypothetical enzyme compartments (ENZ_{CPact} = active enzyme pool involved in cyclophosphamide metabolism; $ENZ_{CPinact}$ = inactive enzyme pool involved in cyclophosphamide metabolism; ENZ_{TT} = enzyme pool involved in thiotepa metabolism) [5]

Table 1 Parameter estimates of the population pharmacokinetic model developed for cyclophosphamide and thiotepa

Parameter	Notation	Estimate (RSE %)	% IIV (RSE %)	% IOV (RSE %)
Non-inducible clearance of TT	$CL_{TTnonind}(l*h^{-1})$	17.0 (12.5)[a]	45.8 (47.2)	23.5 (31.4)
Initial inducible clearance of TT	$CL_{TTind}(l*h^{-1})$	12.4 (14.3)[a]	30.5 (35.3)	18.6 (31.4)
Volume of distribution of TT	$V_{TT}(l)$	44.5 (5.7)	24.6 (52.7)	15.4 (36.4)
Rate constant distribution TT from central to peripheral compartment	$k_{18}(h^{-1})$	0.314 (12.7)	45.1 (40.0)	
Rate constant distribution TT from peripheral to central compartment	$k_{81}(h^{-1})$	0.493 (11.6)		
First-order elimination rate constant of T	$k_T(h^{-1})$	0.555 (8.5)	22.1 (29.8)	
Rate constant distribution T from central to peripheral compartment	$k_{29}(h^{-1})$	3.49 (12.2)	35.4 (55.1)	
Rate constant distribution T from peripheral to central compartment	$k_{92}(h^{-1})$	1.01 (6.5)		
First-order formation and zero order elimination rate constant of the enzyme involved in TT metabolism	$k_{enzTT}(h^{-1})$	0.0343 (11.9)	200 (57.0)	
Maximal value of enzyme induction	E_{max}	0.361 (10.4)		
Volume of distribution of T	$V_T(l)$	14.2 (11.6)		
Non-inducible clearance of CP	$CL_{CPnonind}(l*h^{-1})$	1.76 (16.2)	54.0 (58.6)	32.4 (43.8)
Initial inducible clearance of CP	$CL_{CPind}(l*h^{-1})$	2.91 (8.5)		23.0 (30.8)
Volume of distribution of CP	$V_{CP}(l)$	31.9 (6.6)	15.9 (78.7)	16.5 (32.4)
Zero-order formation rate constant of the enzyme involved in CP metabolism	$k_{enzCP}(h^{-1})$	0.0220 (7.6)	35.5 (48.5)	
First-order elimination rate constant of 4OHCP	k_{4OHCP} (h^{-1})	169 (8.1)	24.0 (27.4)	
Rate constant of reversible enzyme inactivation	k_{ass} $(h^{-1}*\mu M^{-1})$	0.169 (9.4)	35.1 (46.3)	
Rate constant of reversible enzyme activation	k_{diss} (h^{-1})	0.405 (6.8)		
Rate constant distribution CP from central to peripheral compartment	$k_{310}(h^{-1})$	0.105 (27.5)	37.3 (44.2)	29.8 (49.4)
Rate constant distribution CP from peripheral to central compartment	$k_{103}(h^{-1})$	0.280 (24.0)		
Proportional error of TT (%)		21.7 (7.6)		
Additive error of TT (μM)		0.0645 (16.3)		
Proportional error of T (%)		16.7 (10.2)		
Additive error of CP (μM)		0.242 (13.9)		
Additive error of 4OHCP (μM)		0.270 (10.1)		

TT thiotepa, *T* tepa, *CP* cyclophosphamide, *4OHCP* 4-hydroxycyclophosphamide, *IIV* interindividual variability, *IOV* interoccasion (course-to-course) variability

[a]Estimated correlation (ρ) of $CL_{TTnonind}$– CL_{TTind} (RSE %) = −0.66 (45.0)

model on relevant pharmacokinetic parameters of cyclophosphamide and thiotepa, using:

$$V_{pop} = \theta_1 \times \theta_2^{APREP}$$

in which V_{pop} is the value of the parameter of interest, θ_1 is the population value in the absence of aprepitant (APREP = 0) and θ_2 is the fractional change in V_{pop} due to the presence of aprepitant (APREP = 1). The minimal value of objective function (OFV), which is equal to minus twice the log likelihood, was used for evaluating the goodness of fit after introduction of the covariate. The influence of aprepitant on a parameter was considered significant when the decrease in OFV of the model including this covariate was >3.8 ($P < 0.05$, log-likelihood ratio test) with regard to the model without the covariate.

Microsome study

The inhibitory capacity of aprepitant on the bioactivation of cyclophosphamide and the metabolism of thiotepa in to tepa was studied in pooled human liver microsomes. The experiments were performed for cyclophosphamide and thiotepa according to previous publications [12, 29]. Aprepitant originated from MSD (Haarlem, The Netherlands). Cyclophosphamide and 4-hydroperoxycyclophosphamide were a generous gift of Dr. Niemeyer, Baxter Oncology, Frankfurt, Germany (purity >95%). Thiotepa originated from the Department of Pharmacy (Slotervaart Hospital, Amsterdam) and tepa (purity >98%) was synthesized at the Faculty of Chemistry, Utrecht University, according to the method of Craig and Jackson [3]. All other chemicals used were of analytical grade. A 50 mM phosphate buffer pH 7.4 was prepared as well as a 2 M solution of semicarbazide hydrochloride and a 20 mg/ml magnesium chloride solution in 50 mM potassium phosphate buffer. Stock solutions of 2,000 μM cyclophosphamide or thiotepa were also prepared in 50 mM potassium phosphate buffer. Solutions of aprepitant (0, 5, 12.5, 25, 50, 125, 250 and 500 μM) were prepared in DMSO. Glucose-6-phosphate, glucose-6-phosphate dehydrogenase and β-NADP were obtained from Sigma (Zwijndrecht, The Netherlands). A NADPH regenerating solution (NRS)

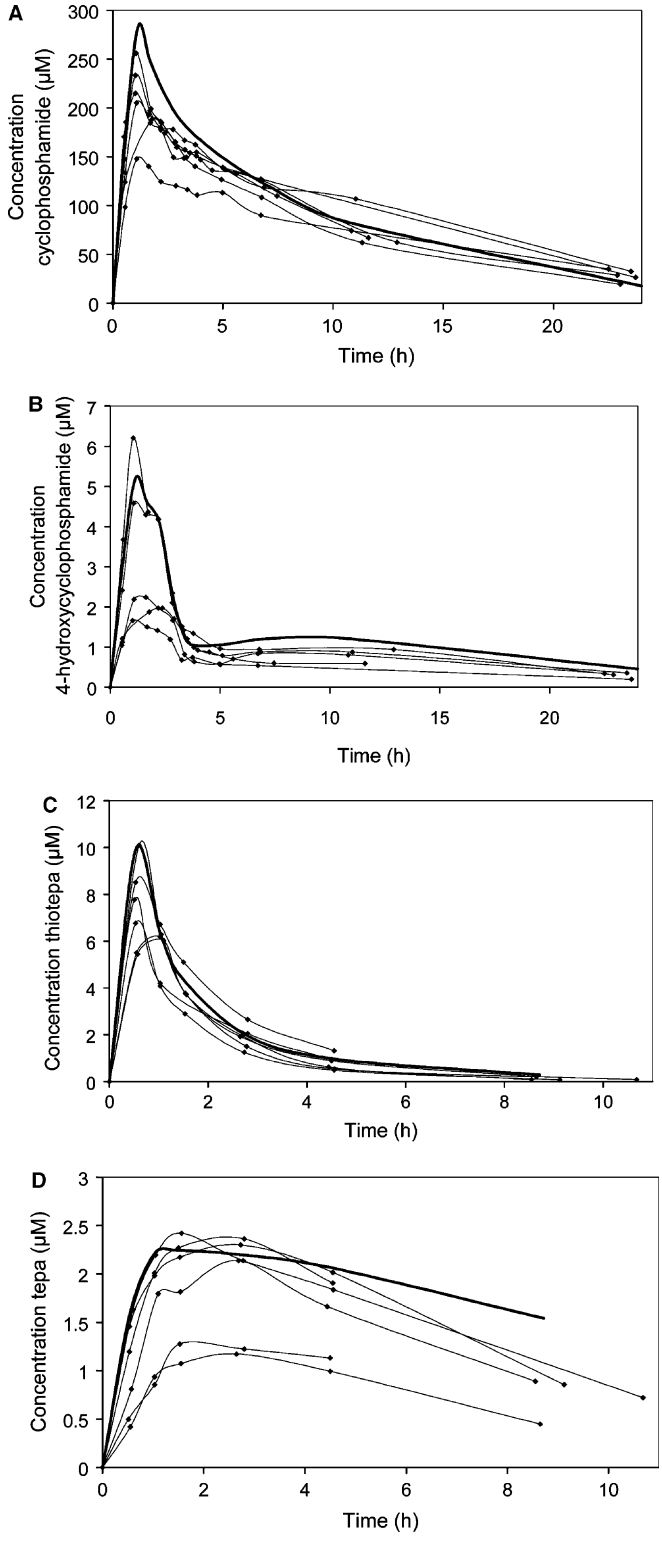

Fig. 2 Observed concentration-time curves of **a** cyclophosphamide, **b** 4-hydroxycyclophosphamide, **c** thiotepa and **d** tepa on the first day of a CTC course in the six patients receiving aprepitant compared to the population mean (without aprepitant; *thick line*)

was prepared in 50 mM phosphate buffer consisting of 500 μg/ml β-NADP, 2 mg/ml glucose-6-phosphate and 1.5 U/ml glucose-6-phosphate dehydrogenase. Pooled human liver microsomes (20 mg/ml in 250 mM sucrose) were obtained from Gentest (Woburn, MA, USA). Immediately before use, the pooled human liver microsomes were diluted with potassium phosphate buffer at 4°C, to reach a final protein concentration of 4 mg/ml solution.

A mixture of 250 μl NRS, 50 μl cyclophosphamide or thiotepa stock solution, 25 μl magnesium chloride solution, 75 μl potassium phosphate buffer and 50 μl aprepitant solution was pre-incubated at 37°C for 5 min. Reactions were initiated by addition of 50 μl of the 4 mg/ml microsome solution (stored at 4°C). Samples were incubated at 37°C for 1 h (cyclophosphamide) or 2 h (thiotepa). The reaction was terminated by the addition of 400 μl (cyclophosphamide) or 600 μl (thiotepa) of cold methanol. To the microsome incubates containing cyclophosphamide, 100 μl of the semicarbazide solution was added to stabilize the formed 4-hydroxycyclophosphamide. To initiate the reaction of semicarbazide with 4-hydroxycyclophosphamide, 50 μl of 4 M HCl was added, and after 10 min the solution was neutralized with 50 μl of 4 M NaOH solution, as validated previously [12]. A 100 μl volume of the final microsome incubates were analyzed for 4-hydroxycyclophosphamide and tepa [6]. All experiments were performed in duplicate.

The formation rate of 4-hydroxycyclophosphamide and tepa were calculated as amount (nmol) per unit time (h) per amount microsome protein (mg). Using the graph with the formation rate plotted versus inhibitor concentration, the 50% inhibitory concentration (IC_{50}) was the concentration causing half-maximal inhibition. Although 4-hydroxycyclophosphamide is unstable at 37°C, the absolute amount of 4-hydroxycyclophosphamide measured after 60 min incubation reflects the formation rate of this metabolite. Therefore, an apparent formation rate (V_{4OHCP}) was calculated for this conversion and used throughout. Tepa is a stable metabolite during the short incubation and therefore the formation rate of tepa (V_{tepa}) reflects the true rate of the enzyme reaction.

Efficacy study

The occurrence of nausea and vomiting was recorded by healthcare professionals on case record forms, which is a long-standing standard practice with CTC high-dose chemotherapy. Every day from day 6 (start of the 4-day chemotherapy course) to day 1 (the day after reinfusion of peripheral blood progenitor cells) it was recorded whether or not the patients experienced nausea and/or vomiting.

The nausea and vomiting scores in the six patients receiving CTC with aprepitant were compared with those in a reference group not receiving aprepitant. Nausea and vomiting were not monitored in the two patients receiving the tCTC regimen because lower chemotherapy doses were administered. As a reference

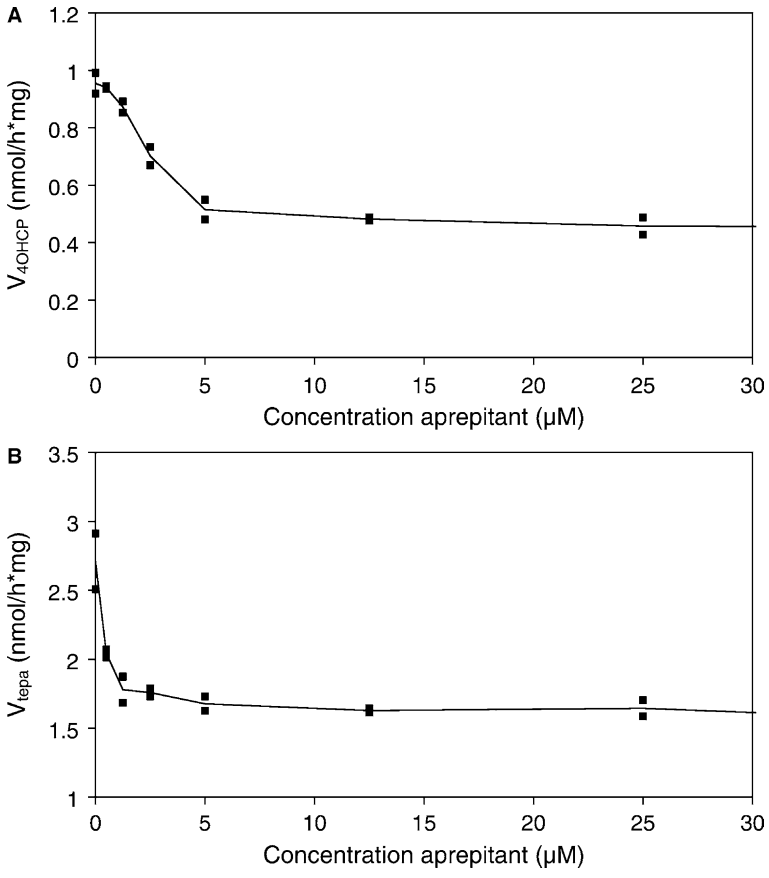

Fig. 3 Inhibition of the conversion of **a** cyclophosphamide to 4-hydroxycyclophosphamide ($IC_{50} = 2.5$ μM) and **b** thiotepa to tepa ($IC_{50} = 0.5$ μM) by aprepitant. The *solid line* represents the mean value for each data point

group, we selected the 22 consecutive patients (M/F:10/12) who had received exactly the same CTC chemotherapy regimen immediately before the first patient in our study [23, 24]. For all these 22 patients data on nausea and vomiting were available.

For comparisons of the nausea and vomiting scores between the two groups, the Mann–Whitney test was used. Only first courses were included in the analysis. The Statistical Service Solution for Windows version 11 (SPSS Inc., Chicago, IL, USA) was used with a two-sided significance level of 0.05.

Results

Pharmacokinetic study

In Fig. 2a–d, plasma-concentration time curves are shown of cyclophosphamide, 4-hydroxycyclophosphamide, thiotepa and tepa in the six patients receiving full-dose CTC with aprepitant. The population mean, without aprepitant, is also depicted. In the presence of aprepitant the plasma concentrations of 4-hydroxycyclophosphamide and tepa are clearly decreased.

Significant improvement of the cyclophosphamide pharmacokinetic model was seen when aprepitant was added as a covariate on the formation rate of the enzyme involved in cyclophosphamide bioactivation ($k_{enzCP\ \Delta}$ OFV = −4.2, $P = 0.040$). The formation rate of the enzyme was 23% lower in the presence of aprepitant (0.0170 h with and 0.0220 h^{-1} without aprepitant). This means that aprepitant significantly inhibits the autoinduction process of cyclophosphamide by inhibiting CYP enzyme induction. No improvement of the model was seen when the covariate was included on the formation clearance of 4-hydroxycyclophosphamide (CL_{CPind}). In spite of the significant interaction, inhibition of cyclophosphamide autoinduction resulted in only a 7% higher total cyclophosphamide exposure and a 5% lower exposure to 4-hydroxycyclophosphamide.

Inclusion of aprepitant as a covariate on the clearance of thiotepa to tepa (CL_{TTind}) in the population pharmacokinetic model, resulted in a significant improvement of the model (ΔOFV = −36, $P < 0.001$). Clearance of thiotepa to tepa was 33% lower in the presence of aprepitant (7.49 L/h with and 11.2 L/h without aprepitant). No model improvement was found when aprepitant was added as a covariate on the formation rate of the enzyme involved in thiotepa metabolism in (k_{enzTT}). Inhibition of thiotepa metabolism to tepa resulted in a 15% higher total thiotepa exposure and a 20% lower tepa exposure.

These results show that both the bioactivation of cyclophosphamide and the formation of tepa from thiotepa are significantly inhibited in the presence of aprepitant.

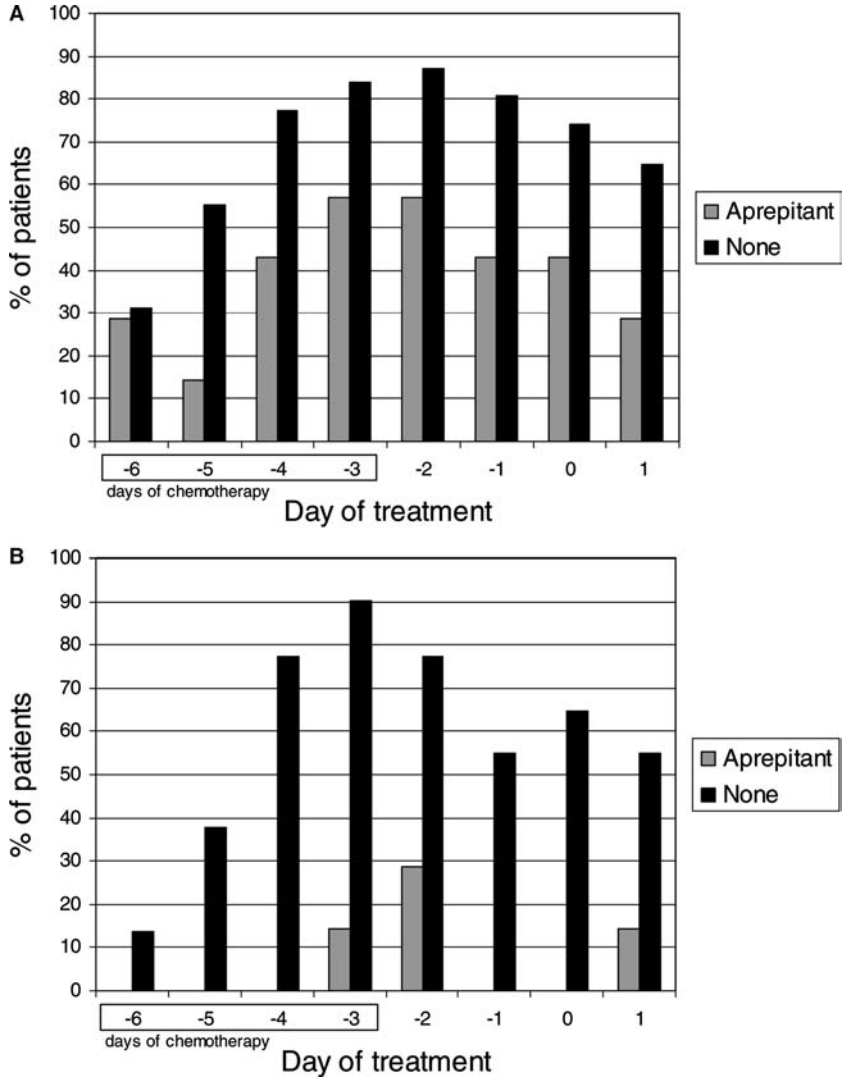

Fig. 4 Percentages of patients experiencing **a** nausea (any grade) and **b** vomiting (any grade) during and after CTC high-dose chemotherapy when receiving standard anti-emetic therapy with ($n = 6$) and without ($n = 22$) aprepitant (day 6 is the first day of the 4-day chemotherapy course)

Microsome study

In Figs. 3 a, b the inhibition of the bioactivation of cyclophosphamide and thiotepa by aprepitant is shown, respectively. The IC_{50} of aprepitant for inhibition of cyclophosphamide ($IC_{50} = 2.5$ μM = 1.3 μg/ml) and thiotepa ($IC_{50} = 0.5$ μM = 0.27 μg/ml) metabolism were within the therapeutic range (reported C_{max} values for aprepitant were 1.4–1.6 ± 0.22–0.36 μg/ml [20]) and thus considered of clinical relevance. Maximal inhibition of cyclophosphamide and thiotepa metabolism by aprepitant was 50%.

Efficacy study

In Figs. 4 a, b daily nausea and vomiting scores in both the six patients receiving aprepitant during their CTC chemotherapy regimen and the 22 reference patients are shown, respectively, stratified for each treatment day. It is evident that especially the frequency of vomiting was dramatically lower during and after chemotherapy in the six patients receiving aprepitant. This effect was clear on all days of the observation period. Also the frequency of nausea was lower in the aprepitant group. The total number of days the patients experienced vomiting during and after chemotherapy was significantly lower in the aprepitant group compared to the patients not receiving aprepitant (0.5 days vs. 4.8 days, $P < 0.001$). The data on nausea were only slightly better for the aprepitant group (3.7 days vs. 5.8 days, $P = 0.052$).

Discussion

In the majority of the patients receiving high-dose chemotherapy with cyclophosphamide, thiotepa and carboplatin, the therapy is complicated with severe nausea and vomiting despite anti-emetic therapy with dexamethasone and granisetron. In this small pilot study we observed impressive results of aprepitant added to standard anti-emetic therapy on prevention of CINV in high-dose CTC. Patients were significantly protected from vomiting both during the days of chemotherapy

and the days after the chemotherapy course compared to a historical reference population not receiving aprepitant. However, we also demonstrated significant inhibition of cyclophosphamide and thiotepa metabolism due to co-administration of aprepitant.

The observed interaction of aprepitant with cyclophosphamide and thiotepa may be explained by inhibition of CYP enzymes by aprepitant. Regarding the enzymes involved in cyclophosphamide autoinduction and thiotepa metabolism, as outlined in the introduction, both CYP3A4 and 2B6 are the most likely isoenzymes involved in this interaction. However, no studies have been performed to date showing inhibitory effects of aprepitant on CYP2B6. The drug–drug interaction between aprepitant and thiotepa not only resulted in delayed thiotepa metabolism (the formation clearance of the active tepa was decreased with 33%), but also in decreased exposures to tepa (−20%). The interaction with cyclophosphamide, however, resulted in a significant decreased rate of autoinduction (−23%), while the total reduction in exposure to the activated metabolite 4-hydroxycyclophosphamide was only 5%. This latter observation may be explained by the fact that 4-hydroxylation is the primary metabolic pathway of cyclophosphamide (70–80% of administered cyclophosphamide is bioactivated). Inhibiting this pathway will only result in delayed 4-hydroxylation since no abundant alternative escape routes for cyclophosphamide elimination are available. As a result, lower but more sustained plasma concentrations of 4-hydroxycyclophosphamide are obtained (Fig. 2b) resulting in only slightly decreased total exposures to this metabolite. Because of the large interindividual and intraindividual variability in clearance of both cyclophosphamide and thiotepa (Table 1), the relatively small effect of aprepitant on the pharmacokinetics of these compounds may be of minor clinical importance. Modified cyclophosphamide and thiotepa metabolism may potentially have contributed to improved outcomes of CINV in this study.

Regarding the efficacy part of the study, it should be noted that our study was small, used historical controls, and the techniques to score and quantify nausea and vomiting were relatively crude. To be able to use a historical group of patients as a control, we had to limit our observations to a set of data that had been collected previously, before aprepitant became available. Thus, although the effect of aprepitant appears to be impressive, more studies will be necessary to confirm these findings.

In addition to its potential role in the setting of high-dose therapy, aprepitant has recently been reported to be effective with conventional-dose adjuvant therapy for breast cancer as well [31]. In this study, a standard combination of cyclophosphamide and doxorubicin was used, but no pharmacokinetic determinations were performed. Based on our findings, aprepitant may reduce the activation-kinetics of cyclophosphamide in this setting, potentially influencing the efficacy of the treatment.

In conclusion, when added to standard anti-emetic therapy, aprepitant may significantly prevent both acute and delayed nausea and vomiting in the high-dose CTC chemotherapy regimen, and is therefore potentially useful in this regimen. However, since aprepitant significantly inhibits both cyclophosphamide and thiotepa metabolism resulting in decreased formation of their active metabolites 4-hydroxycyclophosphamide and tepa, respectively, more research is necessary to evaluate the impact of aprepitant on the efficacy of the CTC regimen.

Acknowledgements This research was supported with a grant from the Dutch Cancer Society (project NKI 2001–2420).

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