Cancer Chemother Pharmacol (2005) 55: 61–71
DOI 10.1007/s00280-004-0824-z

ORIGINAL ARTICLE

**Candice Jamois · Emmanuelle Comets · France Mentré
Sylvie Marion · Robert Farinotti
Laurence Bonhomme-Faivre**

Pharmacokinetics and neutrophil toxicity of paclitaxel orally administered in mice with recombinant interleukin-2

Received: 22 October 2003 / Accepted: 18 March 2004 / Published online: 16 September 2004

Abstract *Purpose*: Intrinsic P-glycoprotein (P-gp) expression in the gut limits paclitaxel uptake and, thus, its bioavailability when administered orally. Interleukin-2 has been reported to be a P-gp modulator in vitro and in vivo in mice. In the work described here, the effects of interleukin-2 pretreatment on pharmacokinetics and toxicity of paclitaxel orally administered were investigated. *Methods*: For the pharmacokinetic study, 96 mice were allocated to two groups receiving either 10 mg/kg of paclitaxel by the oral route alone or 16.5 μg of human recombinant interleukin-2 (rIL2) by the intraperitoneal route twice daily for 3 days and then paclitaxel. Pharmacokinetic profiles were analysed first by the Bailer method, and then using a compartmental approach. For the toxicity study, 90 *Swiss* mice were allocated to three groups receiving paclitaxel (10 mg/kg orally), rIL2 alone (16.5 μg i.p. twice daily for 3 days, control group), or both treatments. Haematological parameters were measured and the three groups were compared using the Bailer method. A Bonferroni correction was applied to the test. *Results*: A complex absorption of paclitaxel was revealed. The Bailer method showed that the mean area under the curve (AUC) values over 0–24 h were not significantly different in the two groups, despite a trend to reduced AUC in the pretreated group. The AUC over 0–0.5 h was significantly higher in the group pretreated with rIL2, but represented only a fraction of total exposure. These results were confirmed by the compartmental analysis. The elimination rate constant remained the same across both groups. rIL2 thus increased paclitaxel absorption for the 15 min following oral intake of the drug but did not enhance the overall exposure. *Conclusion*: We found that a 3-day pretreatment with rIL2 had some in vivo inhibitory effects on P-gp activity for a short period after oral dosing of paclitaxel. Those results encourage further investigation of the effect of rIL2 on the overall exposure of paclitaxel. On the other hand, it seems that the joint administration of the two drugs did not increase the risk of myelosuppression, which might be worth knowing to treat advanced cancers.

Keywords Paclitaxel · Oral administration · Recombinant interleukin-2 · Pharmacokinetic · Neutrophil toxicity

C. Jamois · L. Bonhomme-Faivre (✉)
Laboratoire de Pharmacologie, Service Pharmacie,
Assistance Publique des Hôpitaux de Paris–Hôpital
Paul Brousse, 14 avenue Paul Vaillant Couturier,
94800 Villejuif, France
E-mail: laurence.bonhomme-faivre@pbr.ap-hop-paris.fr
Fax: +33-1-45593728

C. Jamois · R. Farinotti · L. Bonhomme-Faivre
Unité Propre de Recherche de l'Enseignement Supérieur 2706,
Barrière et passage des médicaments,
Faculté de Pharmacie de Chatenay Malabry,
Université Paris XI, Paris, France

E. Comets · F. Mentré
INSERM E 0357, Département d'épidémiologie,
biostatistiques et recherche clinique,
Assistance Publique des Hôpitaux de Paris–Hôpital
Bichat-Claude-Bernard, 46 rue Henri Huchard,
75018 Paris, France

S. Marion
Service d'Hématologie,
Assistance Publique des Hôpitaux de Paris–Hôpital
Paul Brousse, 14 avenue Paul Vaillant Couturier,
94800 Villejuif, France

R. Farinotti
Service de Pharmacie Clinique et des Biomatériaux,
Assistance Publique des Hôpitaux de Paris–Hôpital Bichat,
46 rue Henri Huchard, 75018 Paris, France

Introduction

In the past years an increasing interest can be seen towards an oral administration of cytotoxic agents with several new oral analogues or oral formulations of commonly used cytotoxic drugs [10]. Examples are etoposide and analogues, topotecan and related compounds, cyclophosphamide and trophosphamide, idarubicin, vinorelbine, miltefosine and several prodrugs of 5′-fluorouracil (5-FU) [25]. Oral treatment with

cytotoxic agents is preferred since this route of administration is more convenient for patients, allowing a better quality of life [20]. Oral treatment can be taken at home eliminating the need for hospital admission. In addition, oral treatment avoids the discomfort of an injection and the risks of infection and extravasation that are associated with intravenous access lines. Furthermore, it reduces administration costs and facilitates the use of long-term treatment [23].

The taxanes paclitaxel and docetaxel are potent anticancer drugs with proven activity against a broad range of human malignancies, including ovarian and breast cancer and non-small-cell lung carcinoma [16, 28]. The oral bioavailability of taxanes is low and limits extensive use of oral administration [11]. P-glycoprotein (P-gp) is held to be responsible of this poor bioavailability in vivo. Preclinical studies with mdr1a P-gp knock-out mice, which lack functional P-gp activity in the gut, have shown significantly higher bioavailability of orally administered paclitaxel [33]. P-gp is an active drug transporter belonging to the ATP-binding cassette transporter family with a very wide substrate range. It is abundant in the apical membrane of many pharmacologically important epithelial barriers, such as the intestinal epithelium and the blood–brain barrier [22, 37]. In the intestine, P-gp transports its substrates in an extracellular direction, thus limiting their absorption from the intestinal lumen and contributing to the low bioavailability of such drugs [36, 44]. In humans, P-gp is encoded by *MDR1*, whereas in mice, *mdr1a* is the major RNA transcript expressed in the small intestine [30].

The bioavailability of paclitaxel is also decreased by metabolism via a phase I metabolism involving cytochrome P450. CYP3A (CYP3A4) and CYP2C8, extensively expressed in the liver and intestine of humans and rodents, are mainly responsible for phase I metabolism of such a drug [1, 9, 44]. A sticking overlap of substrate specificity for CYP3A and P-gp has been observed [31, 43]. Furthermore, P-gp and CYP3A share many inhibitors such as cyclosporin A, ketoconazole, and ritonavir. Hence, in most human and animal studies, simultaneous inhibition of P-gp and CYP3A by these compounds increases the bioavailability of P-gp/CYP3A cosubstrates [19, 24, 29].

It has been shown that recombinant interleukin-2 (rIL2) is able to decrease P-gp expression in cultured cells of human colon carcinoma [35]. In addition, studies in wild-type mice revealed good bioavailability of paclitaxel after oral administration when combined with P-gp blockers such as cyclosporin A or SDZ PSC 833 [32, 38, 39]. Furthermore, a previous study has also suggested that rIL2 pretreatment before intraperitoneal administration of paclitaxel induces a decrease in the protein expression of intestinal P-gp and leads to a decrease in intestinal P-gp activity in vivo in mice. This decreased P-gp activity could be associated with increased paclitaxel bioavailability in mice treated with rIL2 [3]. Interleukin-2 is a glycoprotein lymphokine used in humans, either alone or in combination with lymphokine-activated killer cells, to treat advanced cancer [21, 26, 27, 45]. Moreover, the suppression of hepatic drug metabolism induced by interleukin-2 in mice, is directly related to a decrease in the level of cytochrome P-450 [6].

To further study the feasibility of a clinically effective oral formulation of paclitaxel, we investigated in the present study whether a 3-day pretreatment with intraperitoneal rIL2 had a pharmacokinetic effect on the profile of paclitaxel given orally in *Swiss* mice. We also studied whether it affected the haematological toxicity in the mice.

Materials and methods

Chemicals

Paclitaxel was obtained from Bristol Myers Squibb (Puteaux, France), docetaxel (the internal standard) from Bellon Rhône Poulenc Rorer (Montrouge, France) and rIL2 from Chiron (Suresnes, France). Methanol and absolute ethanol were obtained from Merck (Nogent sur Marne, France) and acetonitrile, ammonium acetate, triethylamine, *n*-hexane and 1-ml Cyano Bond Elut Varian columns were obtained from Prolabo (Fontenay sous Bois, France).

Pharmacokinetic study

A total of 96 young *Swiss* mice (6 weeks of age) with a mean body weight of 30 g were purchased from Charles River (France). The animals were housed in cages with food and water ad libitum. Animals were acclimated for 1 week prior to the start of the experiments. The mice were allocated to two groups: the first group were treated with paclitaxel (10 mg/kg orally) alone, and the second group were treated with rIL2 (16.5 μg twice daily from day 1 to day 3 (at 9.00 a.m. and 4.30 p.m.) and paclitaxel (10 mg/kg orally) on day 4. Prior to the administration, Taxol (paclitaxel, vehicle Cremophor EL and ethanol 1:1 v/v) was diluted with isotonic sodium chloride to a final concentration of 2 mg/ml. Blood samples were collected from both groups on sodium heparin at 15, 30, 60, 90 min and 2, 3, 5, 7, 16 and 24 h after oral administration of paclitaxel. Four mice of each group were sacrificed at each time point, except day 24, when eight mice were sacrificed.

Analytical methods

Plasma was separated by centrifugation (20 min at 1500 *g*), delivered in aliquots and stored at −20°C until analysis. Plasma paclitaxel concentrations were determined by high-performance liquid chromatography (HPLC) after solid–liquid extraction using a modified

version of the method of Willey et al. [46]. Plasma (100 µl) was mixed with 100 µl ammonium acetate 0.2 *M* and 50 µl docetaxel used as the internal standard for 20 s. Paclitaxel was extracted from plasma by solid phase extraction onto 1-ml Cyano Bond Elut Varian columns. The columns were first conditioned with 1 ml methanol, then with 1 ml 0.01 *M* ammonium acetate. The samples were loaded onto the Cyano Bond Elut Varian columns and washed with 2 ml 0.01 *M* ammonium acetate, 2 ml 20% methanol in 0.01 *M* ammonium acetate and 1 ml *n*-hexane. The columns were dried under vacuum for 1 min. Paclitaxel and the internal standard were eluted using 2 ml 0.1% triethylamine in acetonitrile. The eluents were evaporated under nitrogen at 0°C. The residues were reconstituted in 100 µl of a water–acetonitrile mixture (55:45, v/v) and vortexed for 30 s. Finally, 50 µl of each sample was injected onto the HPLC column.

The chromatographic system consisted of a Shimadzu LC6A pump, a Shimadzu SPD6A detector and a Shimadzu CR5A recorder (Touzard et Matignon, Les Ulis, France). Reverse-phase HPLC was performed using a Nucleosil C18 column (250×4.6 mm; 5 µm). The mobile phase, a water–acetonitrile mixture (55:45, v/v), was delivered at a flow rate of 1.5 ml/min, and UV detection was set at 227 nm. The quantification limit was 0.05 µg/ml. Intraday and interday coefficients of variation calculated at two concentrations were equal to or less than 10%.

Noncompartmental pharmacokinetic analysis

We first estimated the global area under the curve (AUC) over 0–24 h in each group, using a noncompartmental approach. Because the absorption profile was complex, we separated the pharmacokinetic profile into three parts, according to the observed profile. We then estimated the AUC of each part separately.

As each animal provided only one blood sample for each time point, the data from animals belonging to the same group were pooled using a naive-averaging-data approach [5]. We decided to substitute the values below the limit of quantification (BLQ) with a value equal to the limit of quantification divided by 2 (LOQ/2) to best incorporate plasma samples which fell below the assay's lower limit of quantification [13]. Where r_i is the number of mice sampled at time t_i following drug administration, the mean concentration y_i at time t_i ($i=0,1,\cdots,n$) was computed as the experimental mean of the r_i observed concentrations. The mean AUC_{0-24} in each group was estimated using the linear trapezoidal rule without extrapolation to infinity from time t_0 to t_n as follows:

$$\overline{\mathrm{AUC}}_{t_0}^{t_n} = 0.5 \times (t_1 - t_n) \times y_0 + \sum_{i=2}^{n} [0.5 \times (t_i - t_{i-2}) \times y_{i-1}] + 0.5 \times (t_n - t_{n-1}) \times y_i$$

The method developed by Bailer for destructive sampling was used to compare the AUCs between the two treatment groups [2]. The standard error of the mean concentrations y_i at time t_i, denoted sem_i, was estimated as the empirical standard deviation divided by $\sqrt{r_i}$. Thus, the standard error of the mean AUC was estimated according to the equation:

$$\mathrm{se}^2\left[\overline{\mathrm{AUC}}_{t_0}^{t_n}\right] = [0.5 \times (t_1 - t_0) \times \mathrm{sem}_0]^2 + \sum_{i=2}^{n} [0.5 \times (t_i - t_{i-2})]^2 + [0.5 \times (t_n - t_{n-1}) \times \mathrm{sem}_n]^2$$

The test for equality of the mean AUCs between treatments A and B was performed using the standard Wald statistic:

$$z_{\mathrm{obs}} = \frac{\left[\overline{\mathrm{AUC}}_A - \overline{\mathrm{AUC}}_B\right]}{\left[\sqrt{\mathrm{se}^2\left(\overline{\mathrm{AUC}}_A\right) + \mathrm{se}^2\left(\overline{\mathrm{AUC}}_B\right)}\right]}$$

Under the null hypothesis that the mean AUCs are equal, this statistic follows a normal distribution. The null hypothesis was rejected if $|z_{\mathrm{obs}}|$ was greater than 1.96.

Modelling the pharmacokinetics of paclitaxel

We also modelled the paclitaxel concentration vs time profile in the two groups using a compartmental approach. The error model was assumed to be additive. We used all the observed concentrations at each time point and in each group. The variance at each time point t_i was modelled as the empirical variance sem_i, as defined above, and the estimation was performed using weighted least squares [15].

The model was fitted to the two groups simultaneously using nonlinear regression, and we then tested whether certain parameters could be taken as identical in the two groups. Nested models were compared using the log-likelihood ratio test, using the objective function value (which is −2 times the log-likelihood value) because differences in this value between hierarchical models are approximately χ^2 distributed. For a one-parameter difference between the models, a difference of 3.84 in the objective function values corresponded to the more complex model being significantly better at α level of 0.05. We used the software R [17], a freeware equivalent of the statistical language Splus, with the library nls2 [15], to perform parameter estimation and all subsequent analyses. Standard errors of the estimations were obtained for all the parameter estimates. The goodness of fit of the model was assessed by examining plots of the standardized residuals (predicted minus observed concentrations divided by the variance) vs time and vs predicted concentrations.

Toxicity study

A total of 90 *Swiss* mice were allocated to three groups treated with paclitaxel (10 mg/kg) alone (group 1), rIL2 (16.5 μg twice daily from day 1 to day 3 at 9:00 a.m. and 4:30 p.m.) then paclitaxel (10 mg/kg on day 4) (group 2), or rIL2 alone (16.5 μg twice daily from day 1 to day 3) (group 3). Blood samples were collected on days −3 (3 days before oral treatment with paclitaxel, first day of treatment with rIL2), 0 (oral treatment with paclitaxel), 1, 3, 4, 5, 6, 7, 8 and 10. Haematological parameters including neutrophils (g/l), white blood cell counts (g/l), red blood cell counts (T/l), haematocrit, haemoglobin (g/l), platelets (g/l), lymphocytes (g/l), monocytes (g/l), basophils (g/l) and eosinophils (g/l) were measured at the haematology laboratory of Paul Brousse Hospital (Villejuif, France) on a SYSMEX NE-1500 TOA (Medical Electronic Japan).

To allow quantitative assessment of neutropenia, we evaluated the difference in the evolution of neutrophil counts between the three groups. We used the mean of the neutrophil counts over the 3 days before the oral dose of paclitaxel as a reference level (N_0). We calculated the mean neutrophil count for each group and each time point (N_i), thus allowing the evolution with time of the neutrophil count from baseline (N_i–N_0) to be assessed. The mean AUC of the change in neutrophil count was then compared between the three groups using the Bailer method. A Bonferroni correction was applied to take into account the fact that three tests were performed. The significance level of the pairwise tests was therefore 0.017, and the null hypothesis of equality of treatments was rejected if $|z_{obs}|$ was greater than 2.394.

To assess the effect of administering rIL2 for 3 days on haematological parameters, we compared the baseline neutrophil counts, lymphocytes, red cells and haemoglobin on day 0 in the two groups receiving rIL2 vs the third group. A nonparametric Mann-Whitney *U*-test was performed (Stat View software) and a significance level of 5% was selected.

Results

Pharmacokinetic analysis

Noncompartmental analysis

The plasma concentration time profiles of paclitaxel in the two groups studied are displayed in Fig. 1. Two mice died before sampling at 16 h and two at 24 h, in group 2. The figure shows the complexity of the pharmacokinetic absorption profile with three successive phases. Because of this complexity of the absorption processes, the

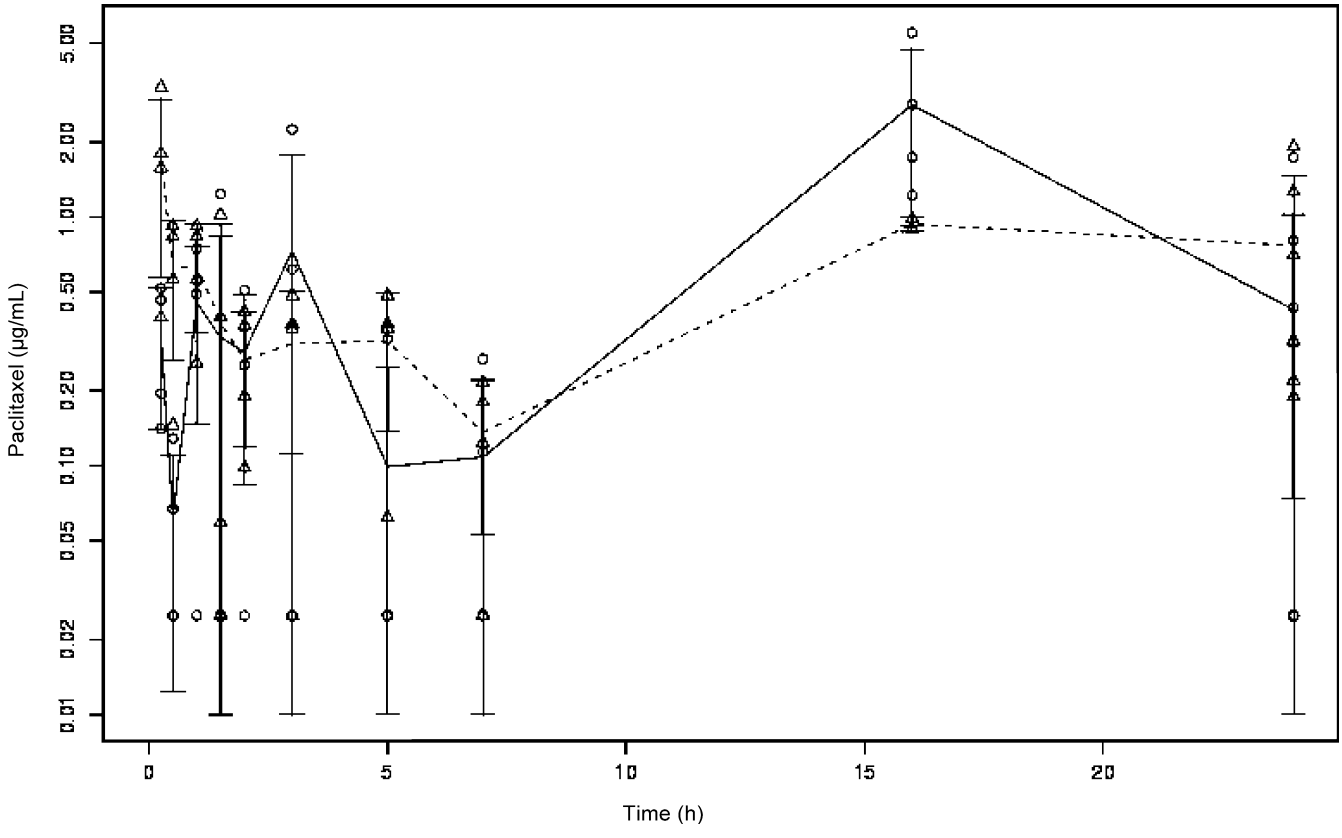


Fig. 1 Plasma concentration–time profiles of paclitaxel in *Swiss* mice treated with paclitaxel alone (10 mg/kg), (*full line*, *circles*) and treated with paclitaxel after a 3-day pretreatment by rIL2 (16.5 μg twice daily from day 1 to day 3) (*dotted line*, *triangles*).The symbols show the individual data, while the lines connect the mean paclitaxel concentrations at each time, and the segments indicate plus and minus the standard deviation. The *y*-axis is in logarithmic scale

Table 1 AUC of paclitaxel in the two groups estimated by non-compartmental analysis. Results are presented as mean values and SE. Also shown are the corresponding CV values expressed as percentages (CV = SE/mean)

Period (h)	AUC (μg/ml) h	
	Paclitaxel alone, mean ± SE (CV%)	rIL2 + paclitaxel, mean ± SE (CV%)
0–24	28.33 ± 8.22 (29)	14.17 ± 1.28 (9)
0–0.5	0.09 ± 0.02 (27)*	0.52 ± 0.15 (29)*
0.5–5	1.81 ± 0.81 (45)	1.87 ± 0.36 (19)
5–24	26.43 ± 8.18 (31)	11.86 ± 1.22 (10)

*Significant difference between groups.

pharmacokinetic profile was studied both globally, by estimating the AUC over 0–24 h, and part by part, by estimating the AUC over 0–0.5 h, over 0.5–5 h and over 5–24 h.

Table 1 shows the mean and standard error of the AUC in the two groups of mice, first over 0–24 h and then for each part of the pharmacokinetic profile. Although there was a trend to higher AUC over 0–24 h in the group without pretreatment, the z_{obs} value of the Wald statistic was 1.701, lower than 1.96, so that the difference in overall AUC between the two groups was not significant. However, for the first part 0–0.5 h, z_{obs} was equal to 3.983, indicating a significant difference between the AUC over 0–0.5 h between the two groups, while the z_{obs} values from 0.5 to 5 h, and from 5 to 24 h were 0.02921 and 1.762, respectively. From these results, it seems that rIL2 is able to increase early paclitaxel absorption in pretreated mice.

Modelling the pharmacokinetics of paclitaxel

A pharmacokinetic model combining three stages was built to reproduce the observed pharmacokinetic profile, modelling the underlying complex absorption processes [8]. Figure 2 depicts this model.

During the first stage, plasma paclitaxel concentrations reach their maximum values before the first sampling point 15 min after the oral administration of paclitaxel. The first phase is characterized by apparently immediate absorption and fast elimination, while the two later phases show a slower absorption. The first peak was therefore modelled as a fraction of the dose absorbed immediately and eliminated with a first-order process, while the two later peaks were modelled as first-order absorption and elimination. Figure 1 also suggests a delay before the onset of the two later peaks. To reflect this, the model included an absorption time lag $T_{lag,i}$ for process i = 2 and 3.

Where $C_p(t)$ is the concentration of paclitaxel in plasma at time t, and $Q_{dig,2}(t)$ and $Q_{dig,3}(t)$ the amounts of paclitaxel remaining in the digestive tract at time t for the second and third phase, respectively, the equations describing the system are as follows:

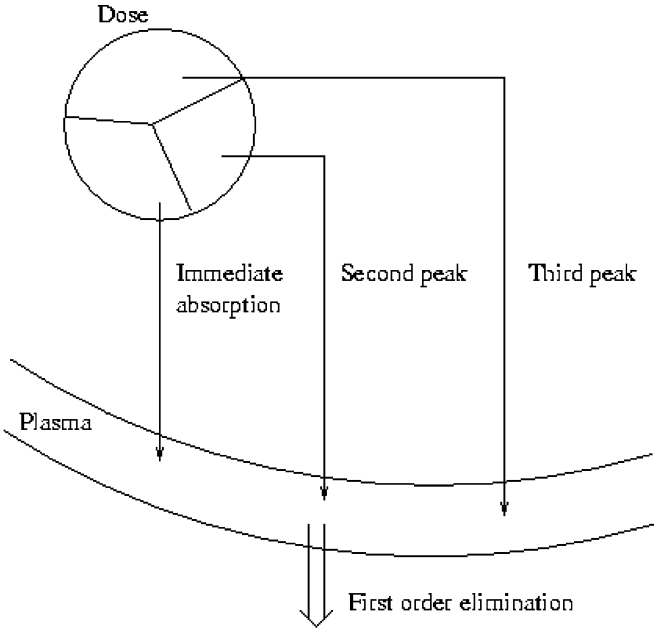


Fig. 2 Scheme of the pharmacokinetic model describing the complex absorption profile

$$\begin{aligned}
\frac{dQ_{dig,2}(t)}{dt} &= -k_{a,2} \times Q_{dig,2}(t) \quad \text{if } t > T_{lag,2} \\
&= 0 \quad \text{otherwise} \\
\frac{dQ_{dig,3}(t)}{dt} &= -k_{a,3} \times Q_{dig,3}(t) \quad \text{if } t > T_{lag,3} \\
&= 0 \quad \text{otherwise}
\end{aligned} \tag{1}$$

$$\frac{dC_p(t)}{dt} = \left[\frac{1}{(V/F)} \times \frac{dQ_{dig,2}(t)}{dt}\right] + \left[\frac{1}{(V/F)} \times \frac{dQ_{dig,3}(t)}{dt}\right] - \left(k_{el} \times C_p(t)\right)$$

where $k_{a,2}$ and $k_{a,3}$ are the absorption rate constants for the second and third phase, respectively, and k_{el} is the elimination rate constant from the plasma compartment. In this model, we estimated $C_{i,0}$, the total concentration of paclitaxel in plasma due to process i (i.e. the total amount absorbed in process i divided by the volume of distribution of the plasma compartment), for i = 1, 2 and 3, as well as the rate constants k_{el}, $k_{a,2}$ and $k_{a,3}$. The same apparent volume of distribution V/F was assumed for the three processes, and was derived from $C_{i,0}$ by:

$$\frac{V}{F} = \frac{D}{(C_{1,0} + C_{2,0} + C_{3,0})} \tag{2}$$

The time lags represent breakpoints for the model, and we therefore performed the minimization for the two time lags using a grid-search method. $T_{lag,2}$ was allowed to vary from 0.25 to 1 by increments of 0.05, and $T_{lag,3}$ to vary from 3 to 15 by increments of 0.25. For each point ($T_{lag,2}$, $T_{lag,3}$) on the grid thus defined, a minimization with respect to the other parameters was performed, and the couple ($T_{lag,2}$, $T_{lag,3}$) yielding the lowest log-likelihood was selected. This model provided

a good description of the observed concentration vs time profile. The time lag for the second process was estimated to be 0.5 regardless of the value taken for $T_{lag,3}$. However, we encountered a problem when fitting the third process. There were too few data points collected during the third process, and as a result $T_{lag,3}$ was not identifiable, with all values of $T_{lag,3}$ yielding the same log-likelihood for a given $T_{lag,2}$. We fixed $T_{lag,3}$ to 15 in the following, the value which for the fitted profiles appeared the most reasonable.

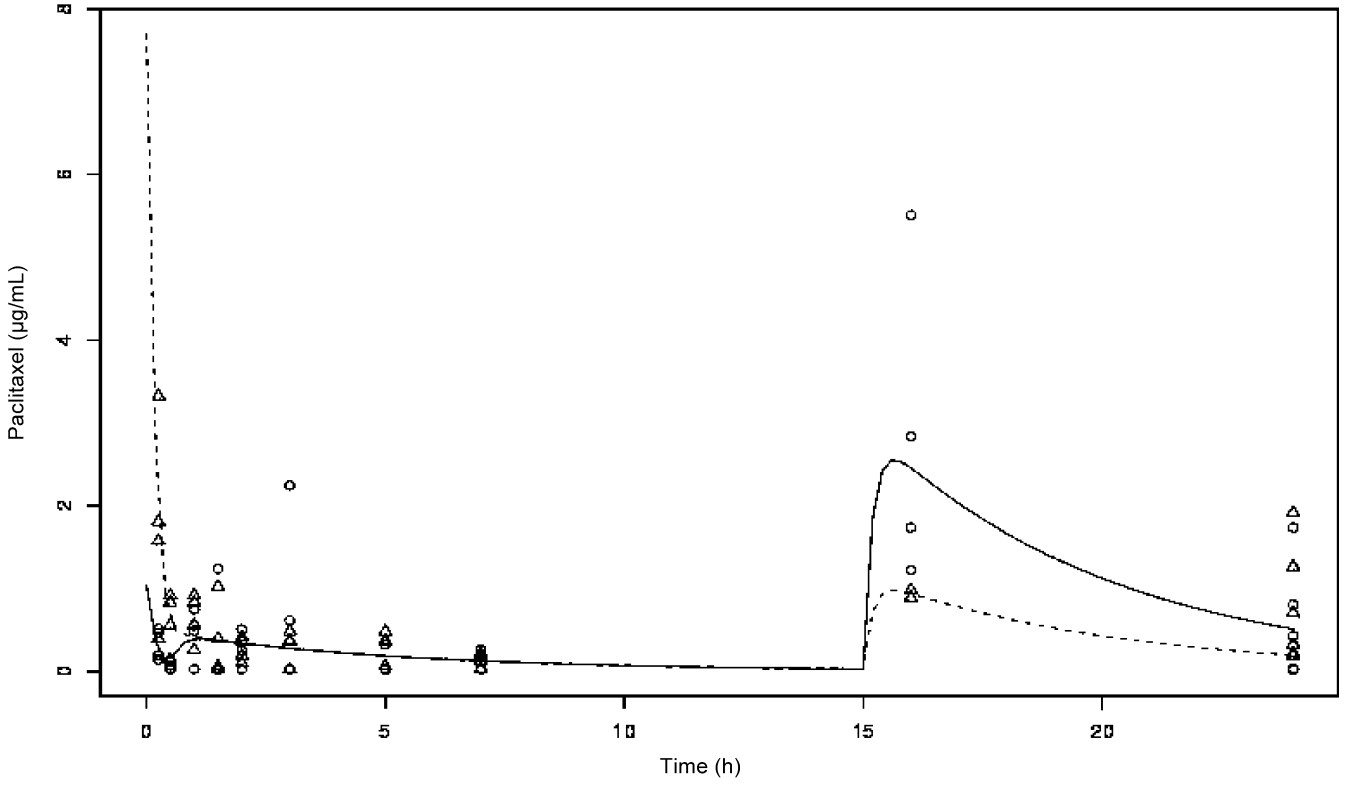
We first tested the assumption that the absorption rate constant was the same for the two later phases, that is, $k_{a,2} = k_{a,3} = k_a$. This assumption did not cause a significant drop in the log-likelihood, so it was kept in the following. We then compared the parameters of the model between the two groups. We found that the rate constant k_{el} characterizing the elimination of paclitaxel could be assumed to be the same, and there was also no difference between the two groups in the total amount of drug absorbed during the second peak, so that $C_{2,0}$ could be assumed identical. On the other hand, assuming the same $C_{1,0}$ or $C_{3,0}$ for the two groups yielded a significantly lower likelihood ($P < 0.0002$ for $C_{1,0}$ and $P < 0.05$ for $C_{3,0}$, respectively). The fit of the final model is depicted in Fig. 3 and the scatter plot of standardized residuals in Fig. 4. As seen in these figures, there was no obvious trend indicating model misfit; however, there was a large variability in the observed concentrations of paclitaxel, especially at late times in the group without rIL2.

Table 2 shows the parameters estimated in each group for the final model. In the second part of this table, some derived parameters have been calculated. $\sum_i C_{i,0}$ represents the sum of the total amounts of paclitaxel released during the three phases divided by the volume of distribution. The apparent volume of distribution V/F, given by Eq. 2, was larger in the group pretreated with rIL2. Because the drug was administered orally, this could in fact reflect a lower bioavailability in this group. The relative amount of drug absorbed during the first phase was 16% in the pretreated group vs 1% in the other group, in accordance with the hypothesis of enhanced early absorption after rIL2 administration. This confirms the result of the noncompartmental analysis. On the other hand, the relative amount of drug absorbed during the last phase was 60% in the pretreated group vs 86% in the other group, since a larger proportion of the drug was absorbed during the first phase in the pretreated group.

Toxicity analysis

Figure 5 displays the time-course of individual neutrophil counts observed in the three groups of mice. The interindividual variability was low, except on day 6 for the group that received only pretreatment with rIL2, where one outlier showed a very high value. The nine mice sampled 3 days before oral administration of paclitaxel and which did not receive any other treatment,

Fig. 3 Predicted concentration vs time according to the final model (parameters of Table 2), overlaid on the observations. The observations for mice from the two groups are shown (*circles* group 1, *triangles* group 2)



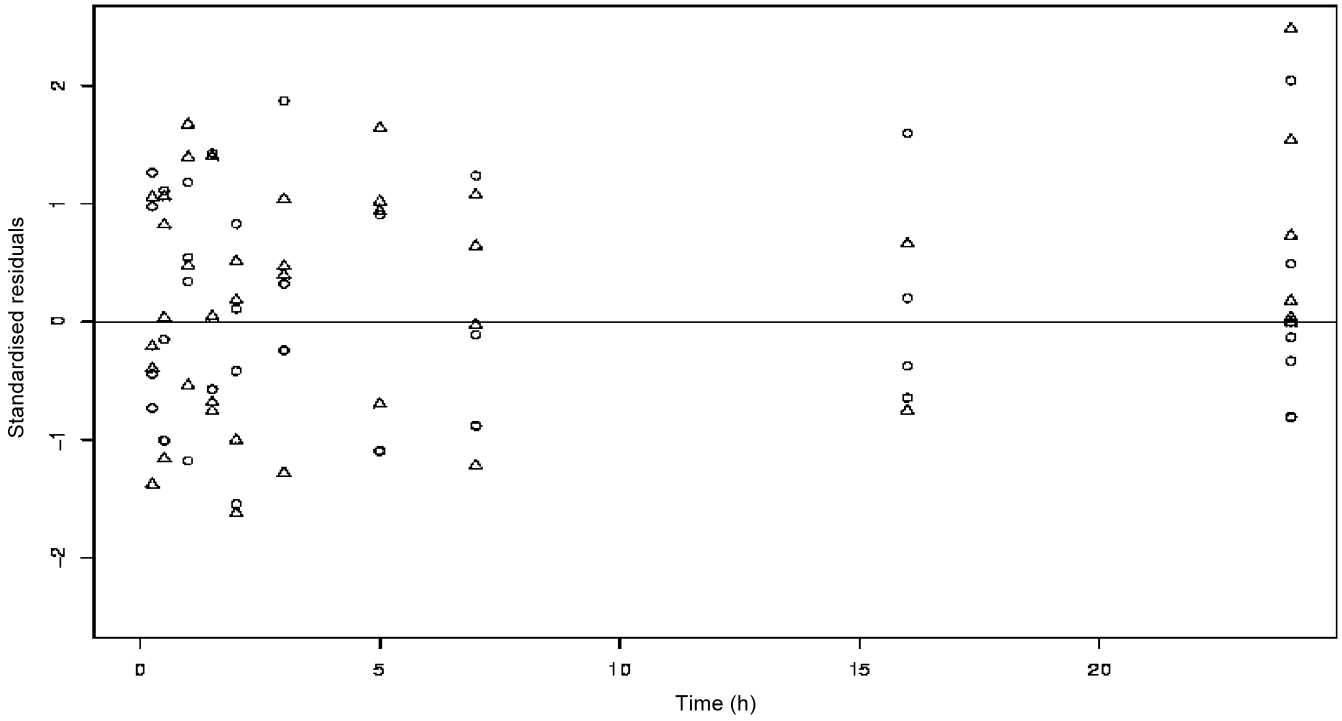


Fig. 4 Standardized residual scatter plot for the final model. The residuals for mice from the two groups are shown (*circles* group 1, *triangles* group 2)

Table 2 Estimated pharmacokinetic parameters and derived pharmacokinetic parameters for the complete pharmacokinetic profile in each group. Standard errors of the estimations are given in parentheses

Parameters	Estimated value (SE) paclitaxel alone	Estimated value (SE) rIL2 + paclitaxel
Estimated parameters		
$C_{1,0}$ (mg/l)	1.0 (0.6)	7.7 (3.9)
$C_{2,0}$ (mg/l)	11.8 (3.4)	11.8 (3.4)
$C_{3,0}$ (mg/l)	77.0 (29)	29.0 (8.7)
$k_{a,2} = k_{a,3}$ (h^{-1})	0.2 (0.1)	0.2 (0.1)
k_{el} (h^{-1})	5.3 (1.3)	5.3 (1.3)
$T_{lag,2}$ (h)	0.5[a]	0.5[a]
$T_{lag,3}$ (h)	15*	15*
Derived parameters		
$\sum C_{i,0}$ (mg/l)	89.8 (31.5)	48.5 (15.0)
V/F (l)	3.34 (1.2)	6.19 (1.9)

[a]Estimated from a grid search and then fixed.

were considered as a reference. The mean neutrophil level on day −3, N_0, plus or minus SEM, was 332 ± 184 g/l. The mean neutrophil value was then calculated for each group and each sampling point (N_i). The time-course of the change in neutrophils ($N_i - N_0$) is represented in Fig. 6 for each group. The times on the *x*-axis correspond to time from day −3.

The mean AUC values and their standard errors, as estimated by the Bailer method, are shown on Table 3. The AUC values estimated in the three groups of mice were then compared using pairwise tests, with a critical value of 2.394. None of the three tests was significant, so we did not find significant differences between the AUCs of the three groups in this study, despite the apparently large differences between group 1 and the two others shown in Table 3.

The administration of rIL2 increased the neutrophil counts on day 0, before administration of paclitaxel ($P = 0.04$ according to the nonparametric Mann-Whitney *U*-test) in these mice. There were no significant difference in the baseline mean values of the other haematological parameters between the groups with or without rIL2 (nonparametric Mann-Whitney *U*-test).

Discussion

In this study the effects of IL2 pretreatment on the pharmacokinetics and toxicity of paclitaxel orally administered to *Swiss* mice were investigated.

In both groups, with and without rIL2, there was a large interindividual variability in the observed paclitaxel concentrations, probably either related to the high variability of P-gp expression known to occur in both humans and mice [14, 42], or to the variability in the expression of CYP2C8, abundantly expressed in the small intestine [1, 9]. The concentration vs time profile of paclitaxel showed complex absorption in three phases in both groups of mice. The first stage was equivalent to an i.v. bolus with zero-order rate constant and first-order elimination, as if a small amount of the paclitaxel had been absorbed very quickly, similar to that following sublingual administration. The two later stages could be considered processes with first-order absorption and elimination.

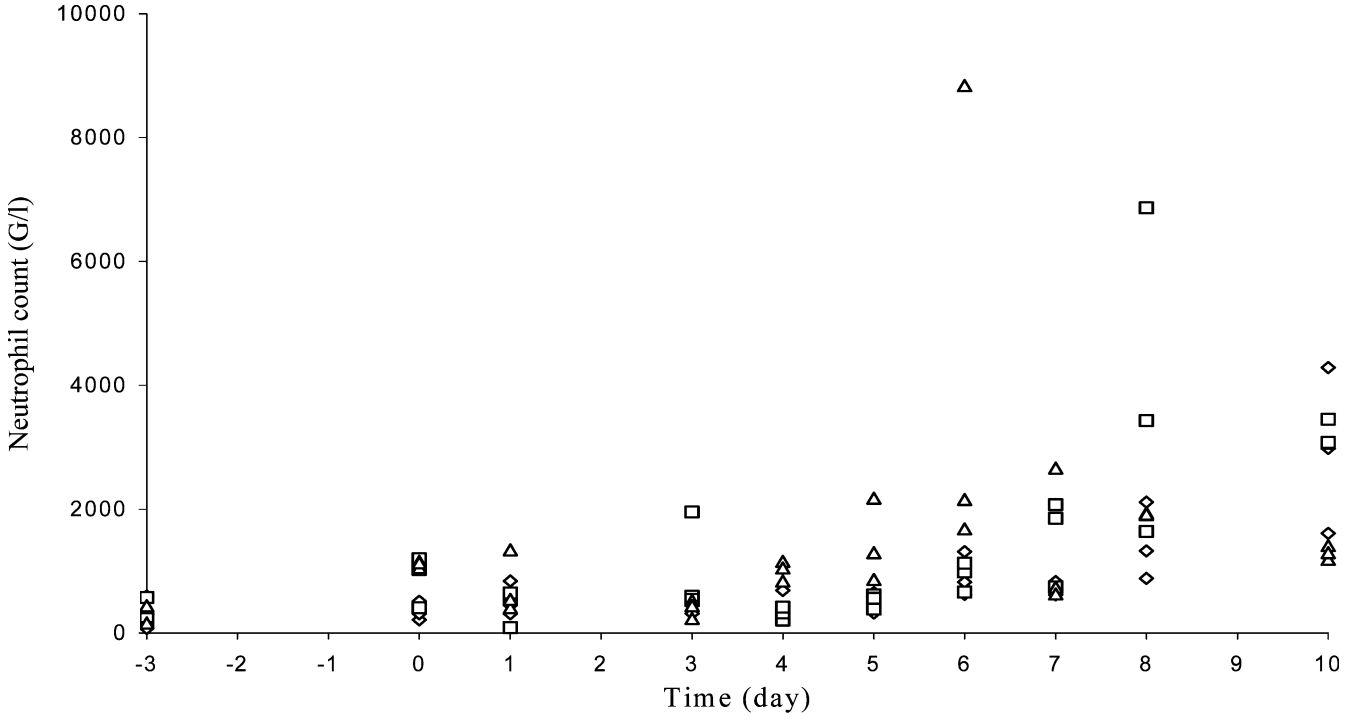


Fig. 5 Individual neutrophil counts vs time in *Swiss* mice treated with paclitaxel alone (10 mg/kg), (*diamonds*), with paclitaxel after a 3-day pretreatment with rIL2 (16.5 μg twice daily from day −3 to −1), (*squares*), and with only rIL2 (*triangles*)

This complex absorption has many explanations. First, Cremophor EL, the vehicle used in the clinical formulation of paclitaxel, has been shown to cause nonlinear pharmacokinetics after i.v. administration of paclitaxel in mice [32] and humans [34]. Although several studies have shown that Cremophor EL levels are undetectable at all oral paclitaxel dose levels, so that Cremophor EL is not absorbed after oral administration of the paclitaxel i.v. formulation [32, 41], it is possible that Cremophor EL interferes with the absorption of paclitaxel in the gut, but such an effect has not yet been demonstrated [47]. A second explanation of the complex absorption profile is the technique of oral force-feeding itself, as the same relationships between concentrations and time have not been observed with intraperitoneal administration of paclitaxel during previous work in our laboratory [3].

Fig. 6 Time-course of mean change in neutrophil counts in *Swiss* mice treated with paclitaxel alone (10 mg/kg), (*diamonds*, *dashed line*), with paclitaxel after a 3-day pretreatment with rIL2 (16.5 μg twice daily from day −3 to −1), (*squares*, *dotted line*), and with only rIL2 (*triangles*, *full line*)

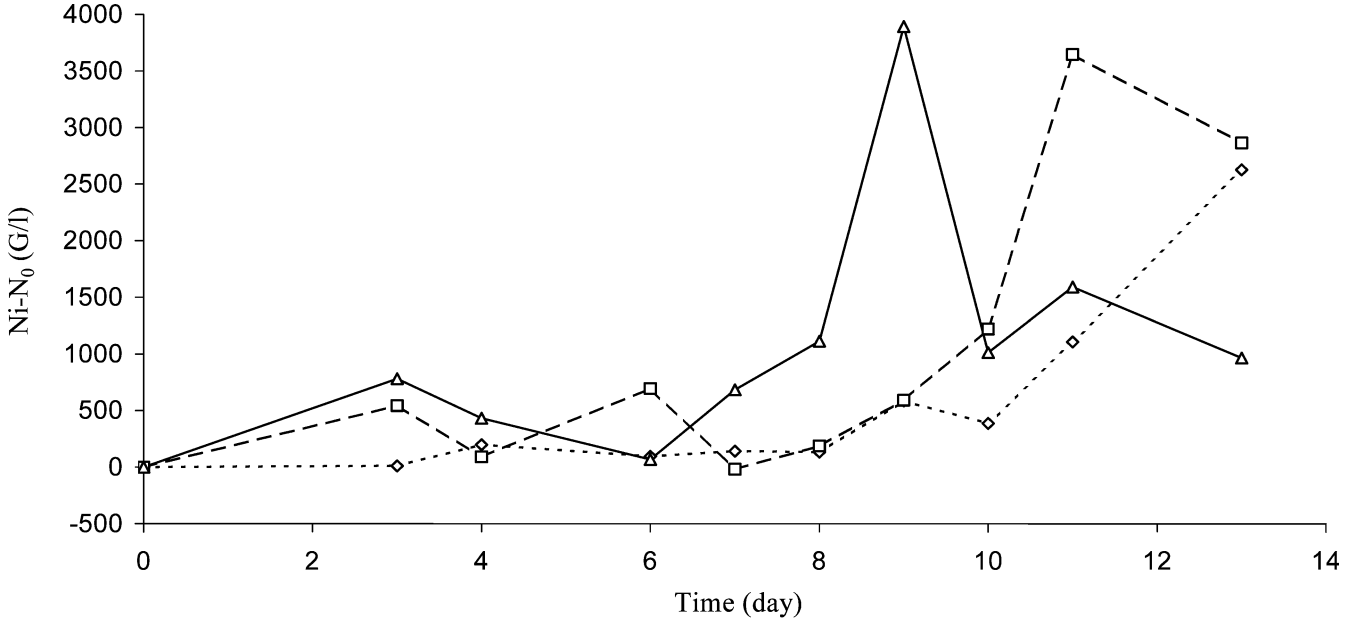


Table 3 AUC of change in neutrophil counts from 0 to 13 days in the three groups of mice. The values presented are means ± SE. Also shown in parentheses are the corresponding CVs expressed in percentages (CV = SE/mean)

AUC_{0-13} (g/l day)		
Paclitaxel alone	rIL2 + paclitaxel	rIL2 alone
5.987 ± 1.036 (17)	11.771 ± 3.775 (32)	11.196 ± 2.481 (22)

The focus of this study was to investigate the effect of pretreatment with rIL2 before paclitaxel administration. The noncompartmental approach showed that the mean AUC values of the two groups were not significantly different over 0–24 h, but that there was a significant difference between the two groups when comparing the $AUC_{0-0.5}$. rIL2 increased the early paclitaxel absorption for the first 15 min after oral intake of paclitaxel. Owing to the large interindividual variability at 16 h, the AUC_{0-24} is larger in group 1 than in group 2, but this difference was not statistically significant.

These results were confirmed by the compartmental analysis. The final model provided a good description of the observed concentrations for the two first processes of release. The AUC over the first peak was also found to be higher in the group pretreated with rIL2. In fact, the fraction of drug absorbed during the first peak, $C_{1,0}/\sum_i C_{i,0}$,was significantly increased in the group pretreated by rIL2. This might be explained by an inhibitory effect of rIL2 on intestinal P-gp activity enhancing early absorption of paclitaxel. The fraction of drug absorbed during the second peak, $C_{2,0}/\sum_i C_{i,0}$,was not significantly different between the two groups, but again the variability in the measurements was large. The elimination rate constant (k_{el}) was the same in the two groups, which means that rIL2 pretreatment did not modify paclitaxel elimination.

A study has recently demonstrated that human rIL2 decreases P-gp expression in vivo in intestine and brain of Swiss mice, thus increasing the oral digoxin bioavailability [7]. Therefore, it is possible that rIL2 enhances the early absorption of paclitaxel by a mechanistic action inhibiting P-gp without affecting its elimination, consistent with the increased AUC during the first period. Without i.v. data, we cannot ascertain whether the oral bioavailability was higher in group 2, or if the difference was due to a different absorption pattern over time. However, our results do seem to correlate with those of Veau et al., who observed that rIL2 pretreatment increases plasma AUC of orally administered saquinavir by 80% but does not modify plasma levels of saquinavir administered intravenously. The increased bioavailability of saquinavir following rIL2 pretreatment could be explained by an effect on the absorption process, and more specifically by a decrease of the intestinal P-gp-mediated efflux of saquinavir as a consequence of a lower P-gp protein expression, but not by a decrease in hepatic first-pass metabolism of orally administered saquinavir [41]. On the other hand, even if rIL2 indeed increases the early absorption of paclitaxel, the overall AUC was not significantly different in the group pretreated with rIL2, so that we would need to further investigate the effect of a pretreatment with rIL2 on the overall bioavailability of paclitaxel using a more appropriate study design to reduce the interindividual variability.

Modelling the third phase of the concentration vs time profile, we found that the fraction of drug absorbed during the third phase was higher in the group without rIL2. The same absorption rate constant $k_{a,3}$ could be assumed for both groups, and this parameter did not differ significantly from $k_{a,2}$, the absorption rate constant of the second phase. Identical $k_{a,2}$ and $k_{a,3}$ would suggest that paclitaxel is released in two steps in the digestive tract, each fraction being subsequently absorbed with the same absorption characteristics. The rebound effect observed after 16 h could in this case have been a result of enterohepatic recycling, and this phenomenon has been described previously [3]. However, these findings may be a result of the impossibility of estimating parameters rather than a feature of the drug. Indeed, there were a number of parameters that could not be estimated in this setting. First, we had to fix the value of the absorption time delay $T_{lag,3}$. Second, the apparent volume of distribution depends on the estimates of the amounts of drug released in the three phases, and $C_{3,0}$ was found to be highly dependent on the value of $T_{lag,3}$ chosen. As result, the parameter estimates for the third phase and for the volume of distribution are to be regarded with caution.

Figure 3 suggests that the difficulty in estimating the parameters related to the absorption during the third phase was probably due to the small number of data points at late times. With only one sampling time between the end of the second peak (at 7 h) and the end of the sampling (at 24 h), it was impossible to assess precisely the shape of the last absorption phase. In this study, we fixed $T_{lag,3}$ to a reasonable value, but the third phase observed here would need further investigation. However, because the administration of rIL2 only enhance the early absorption of paclitaxel, without modifying the overall AUC, the study could have been pursued at this point using a more adequate design to investigate this issue. Furthermore, as discussed above the Cremophor used in this study may be replaced in future studies by a different vehicle, so that there was no clinical incentive to pursue the modelling of the pharmacokinetics of this combination in this case. Also, because the parameters $C_{i,0}$ are defined up to a constant, the conclusions regarding the differences between the two groups depending only on the assumption that $T_{lag,3}$ is identical in the two groups remain true.

One component in optimizing cancer paclitaxel therapy is to establish relationships between drug concentrations and myelosuppression, which is dose limiting for this drug. The haematological toxicity of anticancer drugs is assessed by monitoring the decrease in haematological parameters. Typically, a summary variable of

drug exposure, e.g. area under the concentration vs time curve, is related to the grade of toxicity or to the observed nadir value. Therapeutically relevant information regarding the time-course of exposure and the duration of neutropenia, which is directly related to the risk of infection, are in such cases wasted. Models relating the concentration or the AUC of anticancer drugs to the decrease in neutrophils or leucocytes have been proposed [4, 12, 18]. Consequently, models that could explain and predict both the degree and duration of haematological toxicity after different schedules of administration of paclitaxel would be of a particular value. In this study, we measured the neutrophil counts over 10 days after administration of paclitaxel alone or in combination with rIL2, or after administration of rIL2 alone. Regarding this toxicity study, neutrophils were significantly increased on day 0 by rIL2 pretreatment whereas lymphocytes, red blood cells and haemoglobin were unaffected. No significant differences were observed in AUC between the three groups over the 10-day sampling period. This leads to the conclusion that the joint administration of the two drugs did not increase the risk of neutropenia and more generally, the risk of myelotoxicity, which might be worth knowing in the treatment of advanced cancers.

In this study, a compartmental approach was used and was found to be helpful in characterizing the underlying absorption profile of paclitaxel. While classical descriptive noncompartmental kinetics and statistical analyses are normally performed in preclinical studies, modelling of drug kinetics can greatly enhance the basic understanding of the behaviour of the drug and serve as a foundation for later analyses.

Our working hypothesis was that intrinsic P-gp expression in the gut could limit paclitaxel uptake and, thus, its bioavailability when administered orally. However, in this study the overall exposure to paclitaxel in *Swiss* mice after pretreatment with rIL2 seemed rather decreased compared to the group without pretreatment, despite a transient early increase. Although the design of the pharmacokinetic study was not optimal, and despite possible interference of Cremophor EL with the pharmacokinetics of paclitaxel, the results encourage further investigation of the effect of rIL2 on the bioavailability of oral paclitaxel using a more adequate study design.

In the future, to further study the effectiveness of rIL2 pretreatment before oral administration of paclitaxel, it would first be necessary to exclude the possibility that Cremophor EL interferes with paclitaxel pharmacokinetics. In fact, there may be a therapeutic advantage from using paclitaxel formulations in which Cremophor EL is absent. Such new formulations would be desirable to allow better control of the systemic Cremophor EL-mediated toxicity and pharmacokinetic interactions observed with numerous agents given in combination with the taxane. A large variety of new formulation vehicles for paclitaxel are now in preclinical development, including cosolvent systems (Tween 80/ethanol/Pluronic L64), water-soluble polymers (e.g. triacetin), liposomes, cyclodextrins, nanocapsules and microspheres [40].

In addition, it would be useful to perform the same study in tumour-bearing mice in order to illustrate the therapeutic response to paclitaxel after pretreatment with rIL2. The aim of a future study would then be to develop a model able to describe the common transient decrease and rebound in neutrophils usually observed after chemotherapy with paclitaxel. The P-gp genotype could also be studied and incorporated in a pharmacokinetic–pharmacodynamic model.

Acknowledgements We thank Jacques Ortega, and all the members of the Clinical Pharmacology Laboratory of Paul Brousse Hospital, for kindly helping with this project.

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