ENHANCED ORAL BIOAVAILABILITY OF PACLITAXEL BY RECOMBINANT INTERLEUKIN-2 IN MICE WITH MURINE LEWIS LUNG CARCINOMA

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SUMMARY

The effect of recombinant interleukin-2 (rIL-2) pretreatment on the pharmacokinetics of paclitaxel was investigated in the murine Lewis lung carcinoma model in C57Bl/6 mice. Paclitaxel 15 mg/kg was administrated orally to mice, either alone or after 3 days pretreatment with twice daily dose of 16.5 μg rIL-2. Plasma concentrations of paclitaxel were estimated by reversed phase HPLC. Pharmacokinetic parameters were determined using MicroPharm software. Using Bailer's method, a significant difference was observed in the AUCs of paclitaxel administrated alone and with rIL-2 pretreatment (928.2 ± 136.8 vs 2549.6 ± 131.3 $ng.h.ml^{-1}$, $p < 0.0001$). Pretreatment with rIL-2 resulted in a 3-fold increase in the oral bioavailability of paclitaxel without altering its elimination half-life (0.798 vs 0.747 h). This could be due to the inhibition of P-glycoprotein (P-gp) mediated transport, thus enhancing paclitaxel intestinal absorption. The combination of

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these two drugs could be of interest in clinical practice due to their activity in pulmonary cancer.

KEY WORDS

paclitaxel, recombinant interleukin-2, P-glycoprotein, pharmacokinetics, bioavailability

INTRODUCTION

Paclitaxel (Taxol®), one of the most important anticancer drugs developed in the past two decades, is active against multiple types of human solid tumors /1/. Oral administration of paclitaxel is convenient and practical for patients because cremophor EL, which is responsible for the non-linear pharmacokinetics of i.v. paclitaxel and for sever hypersensitivity reactions, is not absorbed following oral administration /2-5/. Moreover, oral administration may enable the development of chronic treatment schedules, resulting in sustained plasma concentrations of paclitaxel above the threshold level of pharmacological activity. Preclinical studies have shown the oral bioavailability of paclitaxel is low (<10%) due to its affinity for the membrane-bound drug efflux pump P-glycoprotein (P-gp) in the gastrointestinal tract. In addition, presystemic extraction in the liver by the cytochrome P450 system may also play an important role /6,7/.

It has been shown that paclitaxel is a substrate for the mdr1 P-gp /8,9/. Enhanced P-gp expression, leading to enhanced drug efflux and reduced drug accumulation, is considered a major mechanism of resistance to paclitaxel; several *in vitro* studies have shown that co-treatment with P-gp inhibitors, such as verapamil, cyclosporin A or zosuquidar (LY335979), results in reversal of paclitaxel resistance in P-gp expressing cells /10,11/.

Recombinant interleukin-2 (rIL-2) is instrumental in mounting an immune response: it stimulates NK cell and cytotoxic T lymphocyte activity. P-gp is also involved in the transport of IL-2 /12/. Furthermore, rIL-2 has been shown to decrease mdr1 mRNA as well as P-gp expression in cultured cells from human colon carcinoma /13/.

A previous study, already published, was carried out to investigate the impact of rIL-2 on the pharmacokinetics of paclitaxel in mice. This

experiment showed that rIL-2 increased the bioavailability of paclitaxel administrated by the intraperitoneal route. This augmentation was associated with decreased P-gp expression in brain and intestinal tissues /14/. However, no direct improvement of paclitaxel oral bioavailability was demonstrated when it was administrated after pretreatment with rIL-2, whereas such an improvement was shown when it was administrated with other P-gp inhibitors /15-18/.

The aim of this study was to investigate the effects of pretreatment with rIL-2 on pharmacokinetic parameters and oral bioavailability of paclitaxel in a mouse tumor model.

MATERIALS AND METHODS

Chemicals

Acetonitrile (HPLC grade) and methanol (HPLC grade) were purchased from VWR (Fontenay sous Bois, France). Paclitaxel stock solution (1 mg/ml) was obtained by diluting a Taxol® (30 mg) flacon (Bristol Mayers Squibb, Puteau, France) in methanol. Docetaxel (internal standard [I.S.]) stock solution (1 mg/ml) was obtained by diluting a Taxotere® (20 mg) flacon (Aventis Pharma, Montrouge, France) in methanol. Triethylamine (Prolabo, Fontenay sous Bois, France), ammonium acetate (Sigma-Aldrich, France) and n-hexane (VWR, Fontenay sous Bois, France) were analytical grade agents. Throughout the study, water for injections was used (C.D.M. Lavoisier, Paris, France).

Animals

Murine Lewis lung (3LL) tumors were maintained by serial passages in C57Bl/6 mice (Charles Rivers, France). Five days before beginning treatment, tumors were explanted, mechanically dissociated, and 10^6 3LL cells were injected into the left flanks of 6-8 week-old female C57Bl/6 mice. Each experimental group comprised 24 mice with tumors of 100 mm^3 on average.

Treatment, sampling time and collection

Taxol®, paclitaxel formulated in cremophor EL and ethanol (1:1 v/v), was diluted with isotonic sodium chloride solution to obtain a

final concentration of 2 mg/ml. Two groups of mice were compared. In the first group, from day 1 to 3, mice received 16.5 µg of Proleukin® (Chiron, Suresnes, France) by intraperitoneal injections twice daily then, at day 4, 15 mg/kg of Taxol® orally. In the second group, mice received 15 mg/kg of Taxol® orally alone (the mice of this group did not receive sham i.p. injections).

For paclitaxel assay, blood samples were collected in sodium heparin tubes before Taxol® administration and at 0.5, 1, 2, 3, 6, 16, 24 and 48 h after administration, using six mice per time point (three mice from each group). Blood samples were centrifuged for 10 min at 3,500 rpm and the plasma harvested into clean glass tubes, then stored at -20°C until analysis.

Drug analysis

Paclitaxel plasma concentrations were measured using a revised RP-HPLC/UV method /19/. This quantification followed solid-phase extraction. For analysis, 100 µl of plasma was mixed with 100 µl of ammonium acetate 0.2 M and 50 µl of a docetaxel (I.S.) solution at 5 µg/ml. The mixture was vortexed for 20 seconds. Sample extraction was accomplished using SPE cyano 1 ml Bond Elut cartridges (Prolabo, Fontenay sous Bois, France). Cartridges were conditioned using 1 ml of methanol and 1 ml of water. After sample loading, cartridges were washed using 1 ml of water, 1 ml of 20% methanol in 0.01 M ammonium acetate solution, followed by 1 ml of n-hexane. The cartridges were then dried under vacuum for 2 min. Paclitaxel and the I.S. were then eluted using 0.5·2 ml of 0.1% triethylamine in acetonitrile.

The eluent was then evaporated under a gentle stream of nitrogen at 30°C. The residue was reconstituted in 100 µl of a water/acteonitrile mixture (1:1, v/v) and vortexed for 20 seconds. An aliquot of 50 µl was then injected into the chromatographic system.

Chromatographic analysis was accomplished using a Nucleosil C_{18} column (4.6·250 mm, 5µ I.D.) (Interchim, Montlucon, France) with a mobile phase composed of acetonitrile and water (1:1, v/v), delivered at a flow rate of 1.2 ml/min using a Shimadzu LC6A pump (Touzard et Matignon, Les Ulis, France). The eluent was monitored using a Shimadzu SPD6A UV detector set at 227 nm. All chromatograms were processed using a Shimadzu CR5A integrator.

Data analysis

The pharmacokinetic analysis was achieved using MicroPharm software (MicroPharm Research) /20/. As each animal provided only one blood sample, data from animals belonging to the same group were pooled using a naïve averaging data approach /21/. Plasma concentration-time data were analyzed using a non-compartmental approach. The area under the plasma concentration versus time curve (AUC) was calculated using the linear-trapezoidal rule. The apparent plasma clearance (CL/F), apparent volume of distribution (V_d) and half-life of elimination ($t_{½}$) were then determined using the equations:

$$CL/F = Dose / AUC \quad \text{Eq. 1}$$

$$V_d = CL/K_e \quad \text{Eq. 2}$$

$$t_{½} = ln2/K_e \quad \text{Eq. 3}$$

where K_e is the slope of the log-linear terminal portion of the plasma concentration versus time curve, determined using weighted linear least-squares regression analysis.

Furthermore, the mean AUCs of the two groups were compared using Bailer's method /22/. The mean AUCs and their standard errors were calculated using the equations:

$$\overline{AUC}_{t_0}^{t_n} = 0.5(t_1-t_0)y_0 + \sum_{i=2}^{n} [0.5(t_i-t_{i-2})y_{i-1}] + 0.5(t_n-t_{n-1})y_n \quad \text{Eq. 4}$$

$$SE = \sqrt{[0.5(t_1-t_0)SE_0]_2 + \sum_{i=2}^{n} [0.5(t_i-t_{i-2})SE_{i-1}]^2 + [0.5(t_n-t_{n-1})SE_n]^2} \quad \text{Eq. 5}$$

where y_i, SE_i are the mean paclitaxel concentration and its standard error at time i (i=0,1,2, …, n). The test for the equality of the mean AUC between treatments A and B is performed using the standard Wald statistic:

$$Z_{obs} = [\overline{AUC}_A - \overline{AUC}_B] / [\sqrt{SE^2(\overline{AUC}_A) + SE^2(\overline{AUC}_B)}] \quad \text{Eq. 6}$$

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

The pharmacokinetic parameters were also determined from a non-linear curve fitting using a one-compartmental model with first order

absorption (absorption constant K_a), a lag-time (T_{lag}), and first order elimination (elimination constant K_e).

RESULTS

Drug analysis

The quantification method was validated according to FDA recommendations /23/.

The standard curve of paclitaxel was satisfactorily described by unweighted least-squares linear regression. Over paclitaxel plasma concentration range 50-5,000 ng/ml, the regression coefficient R^2 of the calibration curves remained ≥ 0.99. Based on the quality control samples, the overall relative standard deviation (an index of precision) was less than 12%. The overall relative error (an index of accuracy) was less than 10%. The lower limit of quantification (LLOQ) was 50 ng/ml. The stability of plasma samples at -20°C was confirmed by analyzing six quality control samples at the end of the study.

Non-compartmental analysis

As shown in Figure 1, plasma paclitaxel concentrations were higher in the rIL-2 pretreated group than in the other group (paclitaxel alone).

Pretreatment with rIL-2 caused an increase in the area under the curve, and a drop in the apparent clearance, accompanied by a decrease in the volume of distribution due to the augmentation of plasma concentrations in this group. Nevertheless, the elimination half-life did not change (Table 1).

Using Bailer's method, the AUCs in the two groups were shown to be significantly different (928.2 ± 136.8 vs 2549.6 ± 131.3 ng.h.ml^{-1}, $p < 0.0001$).

Compartmental analysis

A one-compartmental model with a lag time was chosen to describe the pharmacokinetic profile of paclitaxel in the two groups. Figure 2 shows paclitaxel mean plasma concentrations versus time profiles simulated according to the chosen model. A visual evaluation of the suitability of the model for the observed data, and examination

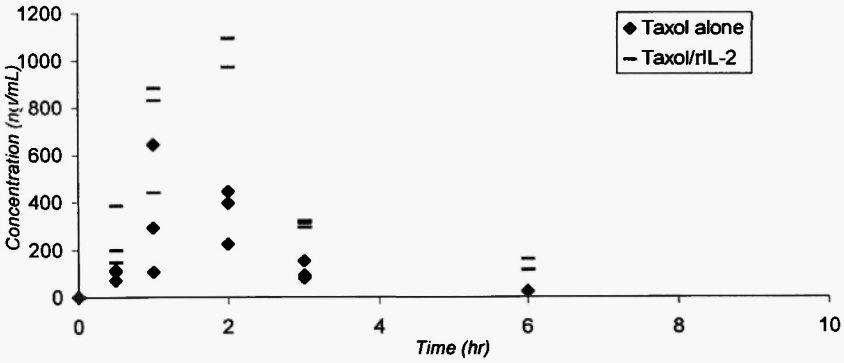


Fig. 1: Plasma concentration versus time profiles of paclitaxel in mice treated with paclitaxel alone (diamonds) and mice treated with paclitaxel after 3 days pretreatment with rIL-2 (squares).

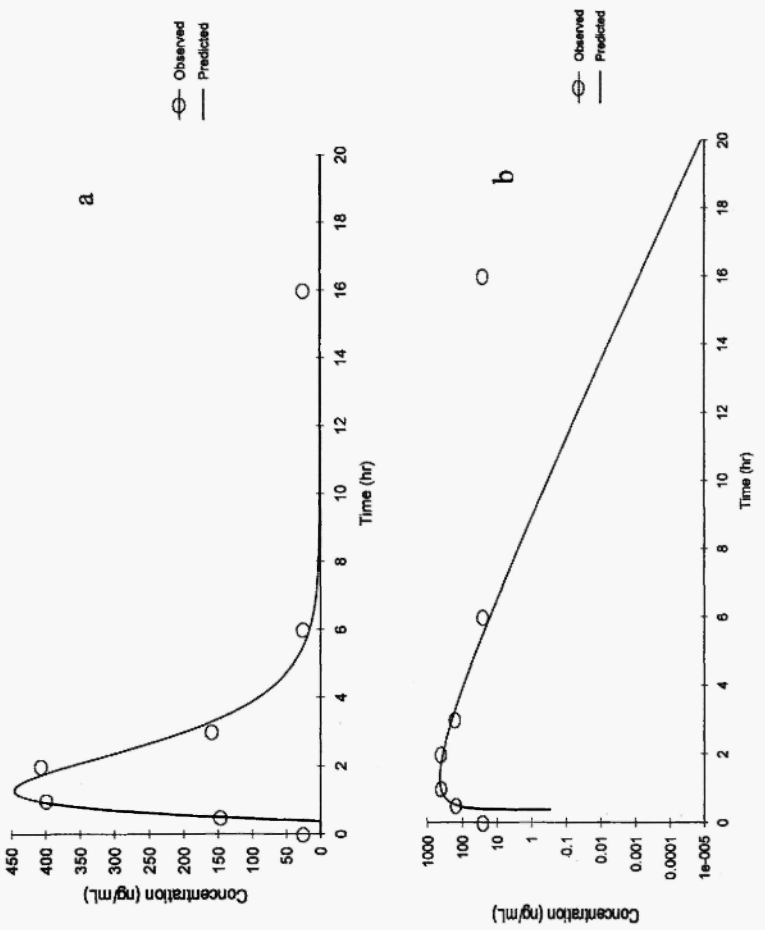
TABLE 1

Pharmacokinetic parameters obtained after non-compartmental analysis

	Taxol® alone	**Taxol®/rIL-2**
$AUC_{0\to t}$ ($ng.h.ml^{-1}$)	752.8	2424.5
$AUC_{0\to\infty}$ ($ng.h.ml^{-1}$)	781.5	2548.4
CL/F ($m/.h^{-1}.kg^{-1}$)	19926.9	6186.8
VD ($ml.kg^{-1}$)	19804.8	6650.8
$t_{½}$ (h)	0.798	0.747

of the goodness of fit plots (predicted values vs observed values, and weighted residuals vs predicted values) were the criteria of the choice of the model.

The pharmacokinetic parameters estimated using the model described above are detailed in Table 2. When the concentrations were simulated, the augmentation of paclitaxel AUC in the pretreated group was confirmed as already demonstrated using non-compartmental analysis.



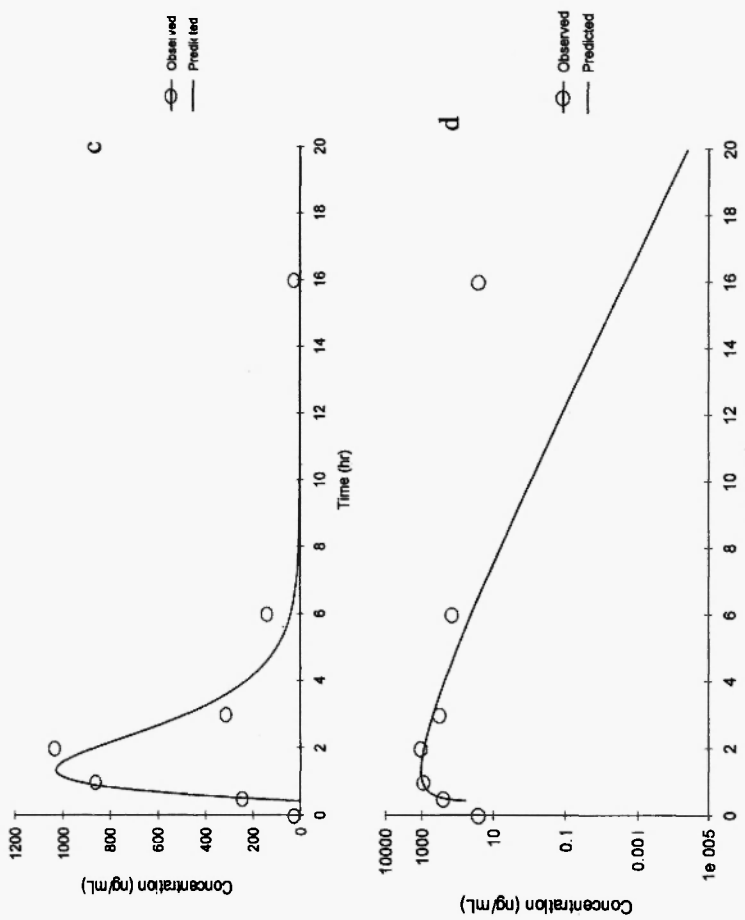


Fig. 2: **a, c:** Predicted paclitaxel mean concentration versus time according to the chosen model overlaid on the mean observations in mice treated with paclitaxel alone (a) and after 3 days pretreatment with rIL-2 (c). **b, d:** Semi-logarithmic presentation of mean plasma concentrations versus time profiles according to the chosen model in mice treated with paclitaxel alone (b) and after 3 days pretreatment with rIL-2 (d).

TABLE 2

Pharmacokinetic parameters obtained after a model dependant analysis

	Taxol® alone	**Taxol®/rIL-2**
$\mathbf{AUC_{Model}}$ (ng.h.ml^{-1})	825.2	2287.4
CL/F (m/.h^{-1}.kg^{-1})	18177.9	6557.8
$\mathbf{K_e}$ (h^{-1})	0.869	0.928
$\mathbf{K_a}$ (h^{-1})	1.048	1.089
$\mathbf{T_{lag}}$ (h)	0.385	0.387

DISCUSSION

In the experiment, mice of the control group (Taxol® alone) did not receive sham i.p. injections (vehicle alone). It was thought that these injections could not have a placebo effect on the mice, and thus would have no effect on the results obtained from the study.

This study has shown that pretreatment with rIL-2 resulted in a 3-fold increase in paclitaxel systemic exposure without altering its elimination half-life. The most likely mechanisms for this interaction are inhibition of P-gp mediated paclitaxel transport by rIL2, and/or inhibition of CYP isoenzymes 3A4 and 2C9 presystemic elimination in the intestinal wall and liver /24-26/.

Nevertheless, when inhibition of P-gp transport is the only mechanism implicated in the phenomena of increasing a drug's oral bioavailability (coadministration of ritonavir with digoxine) /27/, an augmentation of C_{max} is observed, while the elimination half-life seemed unchanged. Otherwise, the inhibition of CYP isoenzymes results in an augmentation of C_{max} accompanied by prolongation of the terminal elimination phase (coadministration of ritonavir with lopinavir) /28/.

The results obtained from this study showed that pretreatment with rIL-2 increased paclitaxel oral bioavailability without changing its elimination half-life, thus the most probable mechanism could be that rIL-2 inhibits P-gp mediated paclitaxel transport, without excluding the likely, but minor, cytochrome inhibition, otherwise not demon-

strated, probably due to the small number of points available for the construction of plasma concentration versus time curves. These results are in agreement with a previous study which suggested that pretreatment with rIL-2 had no effect on the first pass metabolism of orally administrated saquinavir /29/. Nevertheless, the quantification of paclitaxel metabolite 3′-*p*-hydroxypaclitxel, not accomplished during this study, would help to indicate the inhibition of CYP3A4 /16/.

Several preclinical studies have been initiated with P-gp inhibitors in combination with paclitaxel in order to enhance the oral bioavailability. Studies in mice revealed that coadministration of SDZPSC833, a cyclosporine D analogue and a potent P-gp inhibitor, with paclitaxel resulted in a 10-fold increase in systemic exposure /14/. A similar study was performed with cyclosporine A, and paclitaxel has shown comparable effects /15/. However, in this study, we have demonstrated that pretreatment with rIL2 resulted in a smaller increase in paclitaxel systemic exposure (3-fold vs 10-fold). Nevertheless, the combination of paclitaxel and rIL2 could be preferable, due to rIL-2's own antitumor activity.

In conclusion, we have found that a 3-day pretreatment with rIL-2 in mice results in an increase in paclitaxel oral bioavailability, by enhancing its absorption and probably decreasing its metabolism. This combination of two drugs that are active against pulmonary cancer could be of importance in clinical practice.

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