Pharmaceutical Research, Vol. 23, No. 6, June 2006 (© 2006)
DOI: 10.1007/s11095-006-0022-2

Research Paper

Enhanced Oral Paclitaxel Bioavailability After Administration of Paclitaxel-Loaded Lipid Nanocapsules

Sandra Peltier,[1] Jean-Michel Oger,[1] Frédéric Lagarce,[1] William Couet,[2] and Jean-Pierre Benoît[1,3]

Received November 24, 2005; accepted January 18, 2006

Purpose. The aim of this study was to evaluate the pharmacokinetics of paclitaxel-loaded lipid nanocapsules (LNC) in rats to assess the intrinsic effect of the dosage form on the improvement of paclitaxel oral exposure.
Methods. Paclitaxel-loaded LNC were prepared and characterized in terms of size distribution, drug payload, and the kinetics of paclitaxel crystallization. Taxol®, Taxol® with verapamil, or paclitaxel-loaded LNC were administered orally to rats. The plasma concentration of paclitaxel was determined using liquid chromatography mass spectrometry.
Results. The average size of LNC was 60.9 ± 1.5 nm. The drug payload of paclitaxel was 1.91 ± 0.01 mg/g of aqueous dispersion. The encapsulation efficiency was 99.9 ± 1.0%, and 1.7 ± 0.1% of paclitaxel was crystallized after 24 h. The oral bioavailability of Taxol® alone was 6.5%. After oral administration of paclitaxel-loaded LNC or paclitaxel associated with verapamil, the area under the plasma concentration–time curve was significantly increased (about 3-fold) in comparison to the control group ($p < 0.05$).
Conclusions. The results indicated that LNC provided a promising new formulation to enhance the oral bioavailability of paclitaxel while avoiding the use of pharmacologically active P-gp inhibitors, such as verapamil.

KEY WORDS: nanocapsule; oral bioavailability; paclitaxel; pharmacokinetics; P-glycoprotein inhibitor.

INTRODUCTION

Paclitaxel is an antineoplastic agent that was originally derived from the bark of the Pacific yew tree (*Taxus brevifolia*). It inhibits cellular growth by promoting and stabilizing microtubule assembly by noncovalent interaction with tubulin, thereby blocking cell replication in the late G2 mitotic phase of the cell cycle (1). It is a potent anticancer drug with proven activity against a number of human, solid tumors and has become standard treatment as the single agent or in combination chemotherapy for the management of advanced breast, ovarian, and non-small-cell lung cancer (2). Paclitaxel possesses a high molecular weight (MW; 854 Da) and a very low aqueous solubility (<0.03 mg/mL) (3). The compound does not contain any functional groups that can be ionized by altering pH or that allow salt formation to increase its solubility. The development of a paclitaxel formulation has therefore been a challenge, and many approaches have been tested or are under investigation (4).

The currently marketed forms (Taxol®, Paxene®) of paclitaxel (6 mg/mL) for intravenous (IV) infusion are formulated in a 1:1 v/v mixture of Cremophor® EL (polyoxyethylated castor oil derivatives) and dehydrated alcohol. However, IV administration is associated with a number of major drawbacks including morbidity related to IV access, risk of catheter-related infection, potential thrombosis and extravasation, and the presence of particulate matter in the infusional preparations. Moreover, severe hypersensitivity reactions, with vasodilatation, labored breathing, lethargy, and hypotension, have been observed after IV infusion of paclitaxel, and it is now well established that the pharmaceutical vehicle Cremophor® EL contributes largely to this effect (5). Thus, much research is being carried out to identify alternative intravenous formulations without Cremophor® EL (4,6–9).

Oral administration of paclitaxel is thus very attractive because it improves patients' quality of life. Indeed, oral drug treatment is practical and convenient to patients, and it facilitates the development of chronic treatment schedules. Finally, with the aim of decreasing costs of anticancer therapy, oral treatment of cytotoxic agents is interesting because oral administration eliminates the need for hospitalization, medical and nursing assistance, and infusion equipment. Unfortunately, paclitaxel has a very low level of oral bioavailability at less than 10% (10–14). This poor bioavailability level results from limited aqueous solubility and

[1] U646, Angers, F-49100 France, Université d'Angers, Angers F-49035 Cedex1 France.
[2] EA3809, Pharmacologie des Agents Anti-Infectieux, Faculté de Médecine & Pharmacie 86005 Poitiers Cedex, France.
[3] To whom correspondence should be addressed. (e-mail: jean-pierre.benoit@univ-angers.fr)

ABBREVIATIONS: LNC, lipid nanocapsules; P-gp, P-glycoprotein.

0724-8741/06/0600-1243/0 © 2006 Springer Science + Business Media, Inc.

dissolution, affinity for the intestinal and liver cytochrome P450 (like CYP3A4) metabolic enzymes, and the multidrug efflux pump P-glycoprotein (P-gp), which is present abundantly in the gastrointestinal tract (1,11,15–17). In a proof-of-concept study, the oral bioavailability of paclitaxel increased from only 11% in wild-type mice to 35% in mice that were homozygous for a disruption of the *mdr1a* gene, demonstrating that P-gp plays a major role in reducing the bioavailability of this drug (16).

Numerous studies have clearly shown that in both animals and patients, the oral bioavailability of paclitaxel was greatly improved when the drug was administered with P-gp inhibitors, such as cyclosporin A (CsA) or its analogs, or verapamil or its analog KR30031 (12,14,18). CsA, which inhibits the functions of both P-gp and CYP3A4, has been shown to improve paclitaxel oral bioavailability *in vivo* by enhancing oral absorption and decreasing elimination (12). If calculated relative to the area under the plasma concentration–time curve (AUC) of intravenously administered paclitaxel (Taxol®) in mice treated without CsA, the oral bioavailability of paclitaxel increased from 9 to 67% with the coadministration of CsA (12). The combined treatment of oral paclitaxel with SDZPSC833, a non-immuno-suppressive cyclosporine analog, resulted in an approximately 10-fold increase in the AUC of paclitaxel (18). The calcium channel blocker verapamil was the first agent found to inhibit the P-gp efflux pump *in vitro* (17). In an *in vivo* study in rats, the AUC of paclitaxel was multiplied by 5 and 7.5 with the coadministration of verapamil or KR30031, respectively (14). However, the usefulness of pharmacologically active compounds, such as verapamil or cyclosporin A, is limited in humans, especially for repeated administration, because of the risk of cardiac and immunosuppressive adverse effects (13,14).

Another strategy for the improvement of the oral bioavailability of paclitaxel investigated the development of lipidic formulations, such as supersaturable, self-emulsifying drug delivery systems (S-SEDDS) or self-microemulsifying drug delivery systems (SMEDDS), to increase paclitaxel solubilization and lymphatic absorption that help avoid liver degradation (10,19). The paclitaxel S-SEDDS and SMEDDS formulations have shown 5-fold and 2-fold increases in AUC, respectively, compared with that of an orally dosed Taxol® formulation (10,19).

New lipid nanocapsules (LNC) were developed in our laboratory (20,21). These LNC, interestingly obtained as a suspension in saline water, were constituted by medium-chain triglycerides and hydrophilic/lipophilic surfactants. These lipid particles have the advantage of being formulated without the need for heavy equipment and can be formed in the absence of organic solvent. Their oily, liquid, triglyceride core allows the solubilization and encapsulation of paclitaxel and could increase lymphatic absorption. Moreover, Solutol® HS15, which is a key excipient in the composition of LNC, has been shown to inhibit P-gp (22). A recent study has shown that LNC are able to reverse multidrug resistance (MDR) mechanisms (23).

The objective of the current study was therefore to evaluate in rats the pharmacokinetics of paclitaxel-loaded LNC to assess the intrinsic effect of the dosage form on the improvement of paclitaxel oral exposure.

MATERIALS AND METHODS

Materials

Paclitaxel powder used for LNC formulation was from Bioxel Pharma (Quebec, Canada). Captex® 8000 (tricaprylin) was a gift from Abitec Corp. (Colombus, OH, USA) via Unipex (Rueil-Malmaison, France). Lipoid® S100-3 (soybean lecithin at 94% of phosphatidylcholine) and Solutol® HS15 (mixture of free polyethylene glycol 660 and polyethylene glycol 660 hydroxystearate) were gifts from Lipoid Gmbh (Ludwigshafen, Germany) and BASF (Ludwigshafen, Germany), respectively. NaCl was obtained from Prolabo VWR International (Fontenay-sous-Bois, France). An inject-able solution of paclitaxel at 6 mg/mL (Taxol®) was obtained from Bristol-Myers Squibb (Rueil-Malmaison, France). Verapamil powder was supplied by Sigma-Aldrich (Saint Quentin Fallavier, France). Docetaxel powder was obtained from ARC Inc. (Saint Louis, MO, USA) via Isobio (Fleurus, Belgium). Water was obtained from a Milli RO System (Millipore, Paris, France). Methanol, *tert*-butylmethylether, and tetrahydrofurane were of high-performance liquid chromatography (HPLC) grade and were from Fischer Scientific (Elancourt, France), Sigma-Aldrich, and Carlo Erba Reactifs (Val de Reuil, France), respectively.

Preparation of Paclitaxel-Loaded Lipid Nanocapsules

LNC were prepared by modifications of the original process described by Heurtault *et al.* (21). Briefly, 88 mg of paclitaxel was dissolved in 4.2 g of Captex® 8000 in the presence of ethanol. Ethanol was evaporated at 85°C. After cooling, 375 mg of Lipoid® S100-3 was then added to the mixture and heated to 85°C. A solution of Solutol® HS15, NaCl, and water (2.4 g, 220 mg, and 5.4 g, respectively) was added, and the mixture was homogenized under magnetic stirring. Three cycles of progressive heating and cooling in between 70 and 90°C were then carried out, followed by an irreversible shock induced by dilution with 33.6 mL of 2°C deionised water added to the mixture at 78°C. Afterward, slow, magnetic stirring was applied to the suspension of LNC for 5 min at room temperature.

Characterization of Paclitaxel-Loaded Lipid Nanocapsules

Size Distribution

LNC were analyzed for size distribution by photon correlation spectroscopy using a Beckman Coulter N4 Plus (Beckman Coulter S. A., Miami, FL, USA).

Drug Payload and Encapsulation Efficiency

Three samples of each batch of paclitaxel-loaded LNC were prepared by dissolving an exact quantity of LNC dispersion in a 96/4 (v/v) methanol/tetrahydrofurane solution. Filtration was performed using a Millex® HV 0.45-μm filter (Fischer Scientific). A 20-μL aliquot of each filtrate was injected in triplicate into the HPLC column. Chromatography was performed using a Waters Alliance® 2690 system (Waters SA, Saint Quentin en Yvelynes, France) with a

Uptisphere® C_{18}-ODB 150 × 2.0 mm, 5 μm column (Interchrom, Montluçon, France) and a UV detector at 227 nm. The column temperature was 35°C and the flow rate was 0.2 mL/min. The gradient profile of mobile phase of water/acetonitrile used was generated according to a gradient timetable. Quantification was achieved by comparing the observed peak area ratios of paclitaxel samples to a calibration curve made under the same conditions. The mean drug payload (milligrams of paclitaxel per gram of LNC dispersion) of each batch of LNC dispersion and the standard deviation were calculated from the three samples. The encapsulation efficiency (percent) was calculated as the ratio between the drug amount that has been encapsulated in the LNC after formulation and that added in the formulation process.

Paclitaxel Precipitation

Possible drug precipitation 24 h after preparation (storage at 7 ± 2°C) was followed up using the same analytical conditions as for drug payload determination.

A sample of paclitaxel-loaded LNC dispersion was filtered with a nylon Magna® 5.0-μm membrane filter (Fischer Scientific). Paclitaxel remaining on the filter was dissolved in 20 mL of a 96/4 (v/v) methanol/tetrahydrofurane solution. Filtration was performed using a Millex® HV 0.45-μm filter (Fischer Scientific). A 20-μL aliquot of filtrate was injected into the HPLC column.

Animal Study

Animals in this study were handled in accordance with the provisions of the *Principles of Laboratory Animal Care* (NIH publication #85-23, revised 1985). This experiment was also carried out in accordance with the "Good practice guide to the administration of substances and removal of blood, including routes and volumes" adopted by the European Federation of Pharmaceutical Industries Associations (EFPIA) and the European Centre for the Validation of Alternative Methods (ECVAM) in 2001 (24). Experiments were performed on male Sprague–Dawley (SD) rats weighing 321 ± 41 g (mean ± standard deviation) that were given no food overnight for 12–14 h, but with access to water. Experiments were conducted in a parallel study design with the following treatment groups: treatment A, oral dose of 10.0 mg/kg paclitaxel with Taxol® form diluted with water (1.7 mg paclitaxel/g); treatment B, oral dose of 10.0 mg/kg paclitaxel with Taxol® form + 20.0 mg/kg verapamil diluted with water (1.7 mg paclitaxel/g); treatment C, oral dose of 10.0 mg/kg paclitaxel with LNC dispersion form; treatment D, IV dose of 2.5 mg/kg paclitaxel with Taxol® diluted with normal saline solution (1 mg paclitaxel/g) (Table I).

Animals were surgically prepared the day before the study. While under general anesthesia, each rat was fitted with a mixed catheter PE10-PE50 (Phymep, Paris, France) implanted in the femoral artery, exteriorized at the back of the neck, and filled with heparin saline (100 U heparin/mL). This cannula was used to simplify blood sampling on conscious rats and to avoid the potential effects of anesthetic on intestinal processing and transit.

For oral administration (treatments A, B, C), preparations were administered by gastric intubation after weighing in a syringe to achieve a target dose of paclitaxel of 10 mg/kg, i.e., a dosing volume of ~1.5–2 mL. For intravenous bolus experiments, paclitaxel was administered into the dorsal penis vein with an approximate volume of 1 mL of preparation to achieve a target dose of paclitaxel of 2.5 mg/kg (treatment D).

Blood samples were collected over 12.5 h via the cannula implanted into heparinized tubes at predose, then after 20, 40, 60, 90, 120, 180, 270, 390, 540, and 750 min for oral experiments, and at predose, then after 2, 8, 15, 30, 60, 120, 180, 270, 390, 540, and 750 min for intravenous experiments. Each blood sample was replaced with an equal volume of normal saline solution. Blood samples were centrifuged for 15 min at 10,000 rpm. The supernatant plasma fraction was transferred to a clean vial and stored at −20°C for analysis.

Quantification of Paclitaxel in Plasma Samples

An aliquot (100 μL) of plasma sample was mixed with 50 μL of methanol and 50 μL of internal standard solution (docetaxel, 100 ng/mL in methanol). After vortex mixing, extraction was accomplished by adding 1.0 mL of *tert*-methylbuthylether following gentle agitation with a tube rotator SB1 Stuart Scientific at 33 rpm (VWR International Merck Eurolab S. A., Fontenay-sous-Bois, France). The mixture was then centrifuged for 3 min at 10,000 rpm, after

Table I. Composition of Paclitaxel Formulations Evaluated *in Vivo*

Component	Formulation group A (mg/g)	Formulation group B (mg/g)	Formulation group C (mg/g)	Formulation group D (mg/g)
Paclitaxel	1.7	1.7	1.9	1.0
Verapamil	–	3.4	–	–
Cremophor® EL	149.3	149.3	–	91.5
Solutol® HS15	–	–	51.9	–
Absolute ethanol	112.2	112.2	–	68.7
Water	736.8	733.4	842.6	–
NaCl 0.9% solution	–	–	–	838.8
Captex® 8000	–	–	90.7	–
Lipoid® S100-3	–	–	8.1	–
NaCl powder	–	–	4.8	–
Name of formulation	Diluted Taxol® for oral treatment	Taxol® + verapamil	Paclitaxel-loaded LNC	Diluted Taxol® for IV treatment

which 800 μL of the organic layer was transferred to a clean vial and evaporated until dry. The residue was then dissolved in 160 μL of reconstitution solution (70% methanol in water containing 0.4% formic acid) and transferred to 0.3-mL microinserts in autosampler vials. A 15-μL aliquot of each sample was injected onto the HPLC column.

Chromatography was performed using a Waters Alliance® 2695 system (Waters S. A.) with an Uptisphere® C_{18}-ODB 150 × 2.0 mm, 5 μm column (Interchrom, Montluçon, France). The gradient profile of mobile phase of 0.1% formic acid in water/0.1% formic acid in methanol used was generated according to a gradient timetable. The flow rate was 0.300 mL/min and the column temperature was set at 40.0°C. The total HPLC effluent was directed into a Quattro Micro® triple quadruple mass spectrometer (Waters S. A.). Ionization was achieved using a turbo ion spray in the positive ion mode. The mass spectrometer operated in the multiple reaction monitoring (MRM) mode. The $(M–H)^+$ m/z transitions for each compound were 854.60 → 286.10 for paclitaxel and 808.30 → 226.10 for docetaxel (internal standard). Typical retention times for paclitaxel and internal standard were 8.25 and 8.34 min, respectively.

Quantification was achieved by comparing the observed peak area ratios of paclitaxel and internal standard of the samples to a weight ($1/X$), best-fit quadratic regression curve determined from drug-fortified plasma standards. The range of linear response was 1–5000 ng/mL. The lower limit of detection was 0.3 ng/mL and the lower limit of quantification was 1 ng/mL for paclitaxel.

Pharmacokinetic Data Analysis

The concentration–time data were analyzed by Kinetica® (v4.2; Innaphase, Philadelphia, PA, USA) using noncompartmental analysis to obtain the pharmacokinetic parameters of paclitaxel.

The area under the plasma paclitaxel concentration–time curve (AUC) and the area under the first moment curve (AUMC) were calculated using the linear trapezoidal method. Then the AUC was calculated by dividing the concentration of last point (C_{750}) by the elimination rate constant (λ_z) as follows:

$$AUC_{0-\infty} = AUC_{0-750} + C_{750}/\lambda_z.$$

The percentage of extrapolated AUC was below 30%.

The AUMC was calculated as follows:

$$AUMC_{0-\infty} = AUMC_{0-750} + (T_{750} \cdot C_{750})/\lambda_z + C_{750}/\lambda_z^2.$$

The mean residence time (MRT), the plasmatic clearance (Cl), and the apparent volume of distribution at steady state (V_{ss}) were calculated using the IV data set:

$$MRT_{0-\infty} = AUMC_{0-\infty}/AUC_{0-\infty}$$
$$CI = Dose/AUC_{0-\infty}$$
$$V_{ss} = (Dose \cdot MRT_{0-\infty})/AUC_{0-\infty}$$

The terminal elimination half-life ($t_{1/2}$) observed for paclitaxel following IV administration was calculated using the slope from linear regression analysis of log-transformed concentration values from the last four time data points.

Statistical analysis of the data was performed using the nonparametric Mann–Whitney *U* test. The *a priori* level of significance was $p = 0.05$.

RESULTS

Characterization of Paclitaxel-Loaded Lipid Nanocapsules

Lipid nanocapsules loaded with paclitaxel had a monomodal particle size distribution of around 60.9 ± 1.5 nm with a narrow distribution (polydispersity index $P < 0.2$). The mean drug payload of all batches was 1.91 ± 0.01 mg of paclitaxel/g of LNC dispersion. The incorporation of paclitaxel in LNC was very efficient as demonstrated by the high encapsulation efficiency (99.9 ± 1.0%). After storage for 24 h at 7 ± 2°C, 1.7 ± 0.1% of paclitaxel was crystallized in the aqueous phase.

Pharmacokinetics of Paclitaxel-Loaded Lipid Nanocapsules

Paclitaxel plasma concentration data after intravenous administration were analyzed by noncompartmental analysis. Figure 1 shows the drug logarithmic concentration–time profiles corresponding to individual animals after intravenous administration of diluted Taxol® solution at a dose of 2.5 mg/kg. The mean AUC was 1478 ± 382 ng h/mL and the AUMC was 3349 ± 146 ng h^2/mL. The maximum concentration (C_{max}) was 3802 ± 1357 ng/mL. The MRT was 2.4 ± 0.8 h. The Cl and V_{ss} were 29.7 ± 8.7 mL min^{-1} kg^{-1} and 4552 ± 2894 mL/kg, respectively. The $t_{1/2}$ observed for paclitaxel was 4.5 ± 0.2 h.

Raw plasma concentration–time data obtained for each animal used for evaluating various oral paclitaxel treatments (formulation groups A, B, and C) were plotted in Figs. 2, 3, and 4. Mean pharmacokinetic parameters corresponding to these treatment groups are summarized in Table II. The values of C_{max} for paclitaxel coadministered with verapamil and for paclitaxel-loaded LNC were 237 ± 121 and 368 ± 326 ng/mL, respectively, which were higher than the C_{max} of Taxol® alone (103 ± 82 ng/mL). As shown in Table II, the administration of 10 mg/kg of paclitaxel formulated in LNC resulted in a significantly enhanced level of AUC compared with the control group ($p = 0.04$). This nearly 3-fold increase in AUC was of the same order as that obtained with the coadministration of verapamil.

The interindividual variability was higher for paclitaxel administered orally compared with the drug given intrave-

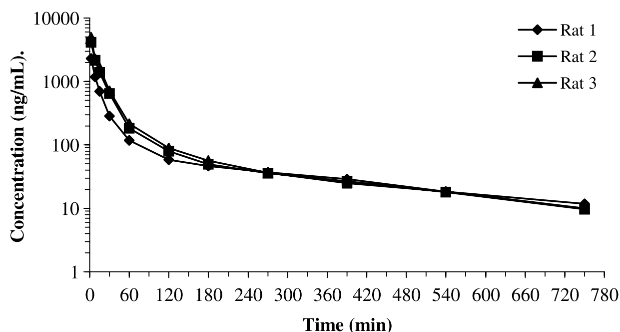

Fig. 1. Plasma paclitaxel logarithmic concentration–time profiles after the intravenous administration of a 2.5-mg/kg dose of Taxol® ($n = 3$).

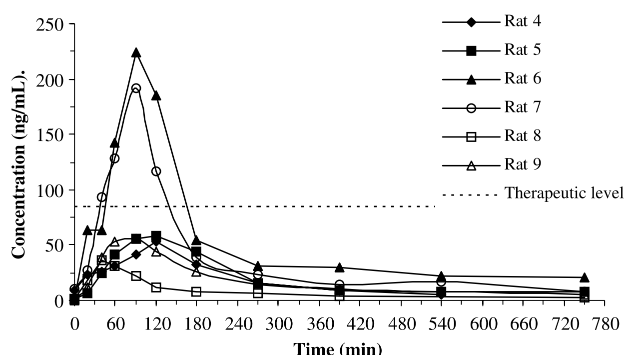

Fig. 2. Plasma paclitaxel concentration–time profiles after the oral administration of a 10-mg/kg dose of paclitaxel using diluted Taxol® solution (n = 6).

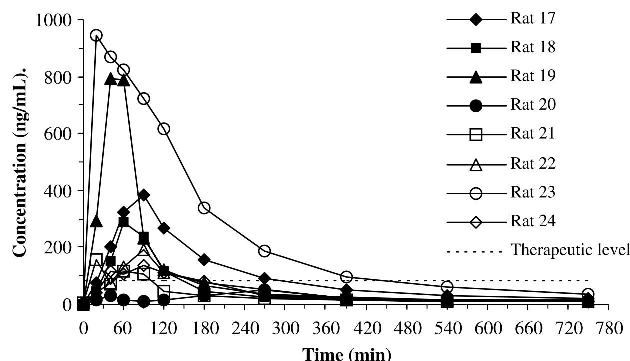

Fig. 4. Plasma paclitaxel concentration–time profiles after the oral administration of a 10-mg/kg dose of paclitaxel loaded in LNC (n = 8).

nously. This high oral variability was not much improved with LNC but was reduced when verapamil was coadministered.

DISCUSSION

Formulation

Paclitaxel was solubilized in Captex® 8000 in the presence of ethanol as a solubilization auxiliary. Indeed, some solubility assays had shown that paclitaxel was more soluble in Captex® 8000 than in Labrafac® (data not shown), an excipient originally used in the original process (21). Solutol® HS15 was a key excipient of the formulation because of its importance in the inversion phase process. The hydrophilic/lipophilic balance of Solutol® HS15 probably changed with temperature, as was the case for ethoxylated surfactants, becoming less hydrophilic on heating (21). This change took place in the phase inversion zone. A fast cooling and dilution in the state close to the beginning of the phase inversion zone led to the formation of nano-objects (21). Very low standard deviations of physicochemical parameters of paclitaxel-loaded LNC were observed, which showed a good reproducibility of the formulation method. The incorporation of paclitaxel in LNC was very efficient as demonstrated by the high encapsulation efficiency of 99.9%. This encapsulation yield was higher than the encapsulation yield

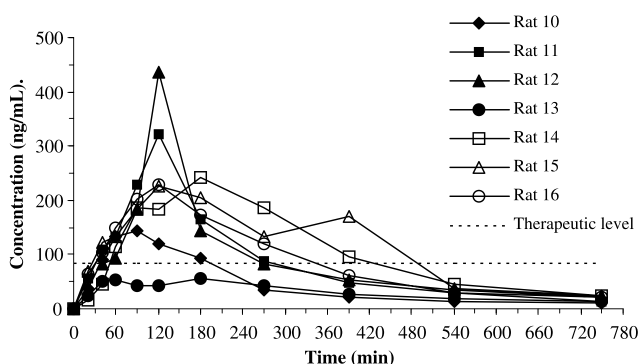

Fig. 3. Plasma paclitaxel concentration–time profiles after the oral administration of a 10-mg/kg dose of paclitaxel using diluted Taxol® solution associated with 20 mg/kg of verapamil (n = 7).

obtained with amiodarone, which was 92–94%, or with indinavir, which was 50% (25,26). This could be explained by the very high lipophilicity of paclitaxel. The stability study showed that only 1.7% of paclitaxel had crystallized after 24 h of storage at 7°C. This instability resulted from the release of paclitaxel from the lipid nanocapsules and the formation of crystals of paclitaxel in its external aqueous phase because of its very low aqueous solubility. To overcome this problem, lyophilization of paclitaxel-loaded LNC could be carried out in the presence of a cryoprotectant such as trehalose, which seems to be the best additive for the lyophilization of LNC (27).

Pharmacokinetics of Paclitaxel

The range of paclitaxel oral doses found in the literature is large. In most studies, doses ranging from 10 to 100 mg/kg were used (10–16,18).

The underlying assumption for bioavailability assessment from AUCs comparison is that clearance is the same during the various treatments. This may actually be a problem in the presence of nonlinear elimination when dose administered are the same and bioavailability varies importantly between formulations. This is the reason why it was decided that an oral dose four times higher (10 mg/kg) than the IV (of 2.5 mg/kg) be used. Yet this is, of course, an *a priori* choice that cannot be optimal until F is known. Nevertheless, it can be observed that AUCs after oral treatment, especially following the new formulation that we are mostly interested in, are in the same order of magnitude, demonstrating that this *a priori* dose adjustment was appropriate.

The apparent volume of distribution for paclitaxel administered intravenously was calculated to be 4552 mL/kg. This value was much higher than normal total body water in rats (670 mL/kg (10)), suggesting that the drug is extensively distributed into tissues outside the plasma compartment. The mean terminal half-life ($t_{1/2}$) of the drug was 4.5 h and the AUC was 1478 ng h/mL. These values were consistent with values reported in the literature of 3.85 h for $t_{1/2}$ and 1160 ng h/mL for AUC of paclitaxel administered at the same dose of 2.5 mg/kg (10).

The oral Taxol® formulation produced a low mean C_{max} (103 ± 82 ng/mL). When the mean AUC obtained with the

Table II. Pharmacokinetic Parameters (Mean Values ± Standard Deviation) of Paclitaxel in Rats Following Oral (10.0 mg/kg) Administration

Parameters	Treatment group		
	Diluted Taxol® for oral treatment (group A)	Taxol® + verapamil (group B)	Paclitaxel-loaded LNC (group C)
Dose (mg/kg)	10	10	10
$AUC_{0-\infty}$ (ngh/mL)	382 ± 267	1071 ± 386*	1051 ± 940*
$AUMC_{0-\infty}$ (ngh^2/mL)	2779 ± 2404	5692 ± 1836	4708 ± 3386
C_{max} (ng/mL)	103 ± 82	237 ± 121*	368 ± 326^{NS}
t_{max} (h)	1.5 ± 0.5	2.2 ± 0.6	1.4 ± 1.3

C_{max}, maximum plasma concentration; t_{max}, time to reach maximal plasma concentration; * $P < 0.05$ (*vs* group A); ^{NS}, not statistically significant (*vs* group A).

oral Taxol® formulation was compared with the mean AUC obtained after intravenous administration, the absolute oral bioavailability was found to be 6.5%, which was very low. This poor oral exposure of paclitaxel from the Taxol® formulation in rats was similar to values reported in the literature (10,14,19,28). Indeed, the oral bioavailability of paclitaxel in male Sprague–Dawley rats was reported to be 4.6% when a paclitaxel dose of 25 mg/kg was administered with the use of a formulation vehicle containing dimethylisosorbide, Tween® 80, and *dl*-α-tocopheryl acetate (concentration of 6 mg/mL paclitaxel) (14). Another study reported an oral bioavailability of paclitaxel of 2.0% when a paclitaxel dose of 10 mg/kg was administered with the use of a formulation containing Cremophor® EL (concentration of 6.8 mg/g paclitaxel) (10). This low oral bioavailability was caused by the poor aqueous solubility and by the high affinity of paclitaxel for P-gp, which is present in the gastrointestinal tract (11,17,29). P-gp can actively transport paclitaxel out of the enterocytes and toward the gut lumen. The action of P-gp could also explain a part of the high interindividual variability observed. Indeed, Lown *et al.* had shown that P-gp accounted for 30% of the interindividual variability of the peak blood concentration of oral cyclosporine (30). In our research, we observed that the oral variability of paclitaxel (variation of 70% in AUC) was probably caused principally by a variation in bioavailability (F) and not by a variation in clearance because this variability was reduced when paclitaxel was administered intravenously (variation of 26% in AUC). In addition to the action of P-gp on paclitaxel absorption, presystemic elimination in the intestinal wall and liver by the CYP isoenzymes 3A4 and 2C8 might also play a role in the low oral bioavailability of paclitaxel (17,31).

Coadministration of paclitaxel with verapamil produced an AUC of 1071 ± 386 ng h/mL, which was significantly higher than the AUC (382 ± 267 ng h/mL) obtained with paclitaxel alone ($p = 0.01$). This 3-fold improvement was in agreement with values reported by Woo *et al.* (14). They had shown that the oral AUC of paclitaxel (oral dose of 25 mg/kg) was increased from 448 to 2231 ng h/mL by the coadministration of verapamil. This increase in AUC was probably produced by the ability of verapamil to inhibit the P-glycoprotein, which was also shown in *in vitro* experiments (14). Moreover, when paclitaxel was associated with cyclosporin A (CsA), another P-gp inhibitor, Van Asperen *et al.* demonstrated that the increase in oral AUC was not only caused by enhanced absorption but also by decreased elimination (12). This reduced clearance of paclitaxel in mice treated with CsA may have several causes: (a) the direct secretion via the gut wall may be reduced due to an inhibition of P-gp; (b) this also may result from a diminished hepatic clearance as a result of metabolic competition because CsA and paclitaxel are both metabolized by cytochrome P450 3A4 enzymes (12). Therefore, inhibition of intestinal P-gp by verapamil could increase AUC via two mechanisms: absorption enhancement and a decrease in elimination by reducing direct secretion by the gut wall. Another effect of verapamil on paclitaxel pharmacokinetics was the reduction in interindividual variability (variation of 36% in AUC) in relation to the control group.

When paclitaxel was loaded in LNC and administered orally, the maximal plasmatic concentration was increased from 103 ± 82 (paclitaxel alone) to 368 ± 326 ng/mL and the AUC was significantly raised from 382 ± 267 to 1051 ± 940 ng h/mL ($p = 0.04$). After oral administration of paclitaxel alone, plasma concentrations were below the therapeutic range of 0.1 μmol/L (equivalent to 85 ng/mL), which was the threshold value of cytotoxic activity *in vitro*, for four animals ($n = 6$) (32). Therapeutic plasma concentrations of paclitaxel above 0.1 μmol/L were achieved for six and seven animals after association with verapamil ($n = 7$) and with paclitaxel-loaded LNC ($n = 8$), respectively. For a single oral dose of 10 mg/kg of paclitaxel, the administration of paclitaxel within LNC increased the number of animals for which therapeutic plasma concentrations have been reached. Unfortunately, LNC presented no beneficial effect on the reduction in interindividual variability (variation of 89% in AUC).

The improvement of oral paclitaxel exposure when it was loaded in LNC may be caused by the inhibition of the P-glycoprotein by LNC. Indeed, Sparreboom *et al.* have shown that P-gp in the epithelium of the gut limits the bioavailability of orally administered paclitaxel (16). After the administration of 10 mg paclitaxel/kg body weight, the plasma AUC was about 6-fold higher in mice, with disruption of the *mdr1a* gene (encoding for the major P-gp of the intestine), than in wild-type mice (16). We have further demonstrated, through *in vitro* and *in vivo* studies, the ability of LNC to inhibit the P-gp (23). Firstly, LNC inhibit *in vitro* ATPase activity from P-gp inside-out membrane vesicle preparation. Moreover, LNC enhance the uptake of $^{99}Tc^{m}$-methoxyisobutylisonitrile ($^{99}Tc^{m}$-MIBI), a surrogate P-gp substrate, by glioma cells, expressing multidrug efflux pumps *in vitro* and *in vivo*. This ability of LNC to inhibit P-gp activity could probably be

attributed to Solutol® HS15, a nonionic surfactant of the external layer of nanocapsules (21). Indeed, the effectiveness of Solutol® HS15 in reversing multidrug resistance has previously been demonstrated (22,33).

No inhibition effects of Solutol® HS15 have been described on cytochrome P450 enzymes, which are also involved in the limited oral bioavailability of paclitaxel. However, Woo *et al.* have shown that the low absorption of paclitaxel may be more dependent on the P-gp efflux pump in the intestinal mucosa than on the intestinal metabolism by CYP3A (14). Indeed, when ketoconazole, a potent inhibitor of CYP3A, was combined with paclitaxel, the AUC improvement was limited in comparison with AUC enhancement obtained with the coadministration of a P-gp inhibitor.

The lipidic nature of the nanocapsules could also explain the improvement of oral exposure of paclitaxel, which is a lipophilic compound. The administration of paclitaxel loaded in a lipidic formulation could increase its absorption by two mechanisms. Firstly, the presence of lipids and surfactants could facilitate paclitaxel dissolution (a "physicochemical" mechanism). Secondly, bioavailability could be increased by an improvement of intestinal lymphatic transport in the presence of a lipid formulation (34). The primary pharmaceutical interest of this absorption pathway is to avoid hepatic first-pass metabolisms. Nevertheless, this lymphatic transport is probably limited because triglycerides used in LNC are medium-chain triglycerides, and it has been shown that lymphatic transport is greater with long-chain triglycerides (35).

Heurtault *et al.* have demonstrated that the oily phase, in which paclitaxel is soluble, is surrounded by a tensioactive cohesive interface (21). Therefore, the molecular structure of paclitaxel, when encapsulated in LNC, is masked from P-gp and cytochrome P450 enzymes. This hypothesis, which could also explain the improvement of oral exposition to paclitaxel when it loaded in LNC, supposes that LNC were able to resist the mechanic and enzymatic action of the gastrointestinal tract. In this case, three possible uptake mechanisms have been suggested for oral absorption of nanoparticles (36): (a) uptake via a paracellular pathway (37), (b) intracellular uptake and transport via the intestinal mucosa, and (c) lymphatic uptake via M cells and Peyer's patches.

Development of an oral formulation of paclitaxel could permit the reduction of paclitaxel-related hypersensitivity reactions observed when paclitaxel is administered intravenously. These reactions are caused by the presence of Cremophor® EL and impose a premedication with corticosteroids and both H_1- and H_2-histamine antagonists. When Taxol® was administered orally (with cyclosporin A) to patients without premedication, the potential hypersensitivity reactions observed were very mild because the co-solvent Cremophor® EL was not very absorbed (38). Moreover, the absence of Cremophor® EL in our LNC could still decrease the risk of hypersensitivity reactions. Unfortunately, oral administration of an anticancer drug is usually exposed to a risk of local toxicity on the gastrointestinal tract. Nevertheless, after oral administration of paclitaxel in mice, Van Asperen *et al.* have made a histological examination and have not observed any symptoms of toxicity in the tissues examined (12). Some studies are still necessary to determine the local toxicity of paclitaxel when it is administered within LNC.

CONCLUSION

Paclitaxel loaded in lipid nanocapsules has been prepared and administered orally for the first time. Its encapsulation in LNC has led to an improvement of oral paclitaxel exposure compared to the control group. The surfactant Solutol® HS15 contained in LNC might inhibit the P-gp efflux system. Lymphatic transport of paclitaxel and the nanocapsule structure might also be beneficial to the oral absorption of paclitaxel. This particular pathway will be investigated in further studies.

It seems that lipid nanocapsules may be a promising delivery system for enhancement of oral paclitaxel exposure. Our next objective is to modify the formulation of these LNC to optimize the bioavailability of paclitaxel associated with these vehicles.

ACKNOWLEDGMENTS

We are very grateful to Pierre Legras and Jerome Roux from the animal house of the Medical College of Angers for skilful technical support. We would also like to thank Drs. Claire Dulieu and Didier Bazile from Mainelab/Ethypharm for supplying the batches of lipid nanocapsules. This work was supported by grants from the "Fondation pour la Recherche Médicale", the "Comité départemental du Maine-et-Loire de la Ligue contre le Cancer", and the "Association pour la Recherche sur le Cancer".

REFERENCES

1. E. K. Rowinsky. Clinical pharmacology of Taxol. *J. Natl. Cancer Inst. Monogr.* **15**:25–37 (1993).
2. E. K. Rowinsky and R. C. Donehower. Paclitaxel (taxol). *N. Engl. J. Med.* **332**:1004–1014 (1995).
3. K. C. Nicolaou, W. M. Dai, and R. K. Guy. Chemistry and biology of taxol. *Angew. Chem. Int. Ed. Engl.* **33**:15–44 (1994).
4. A. K. Singla, A. Garg, and D. Aggarwal. Paclitaxel and its formulations. *Int. J. Pharm.* **235**:179–192 (2002).
5. L. van Zuylen, J. Verweij, and A. Sparreboom. Role of formulation vehicles in taxane pharmacology. *Invest. New Drugs* **19**:125–141 (2001).
6. S. A. Wissing, O. Kayser, and R. H. Muller. Solid lipid nanoparticles for parenteral drug delivery. *Adv. Drug Deliv. Rev.* **56**:1257–1272 (2004).
7. D. Le Garrec, S. Gori, L. Luo, D. Lessard, D. C. Smith, M. A. Yessine, M. Ranger, and J. C. Leroux. Poly(N-vinylpyrrolidone)-block-poly(D,L-lactide) as a new polymeric solubilizer for hydrophobic anticancer drugs: *in vitro* and *in vivo* evaluation. *J. Control. Release* **99**:83–101 (2004).
8. T. K. Yeh, Z. Lu, M. G. Wientjes, and J. L. Au. Formulating paclitaxel in nanoparticles alters its disposition. *Pharm. Res.* **22**:867–874 (2005).
9. L. Mu and S. S. Feng. A novel controlled release formulation for the anticancer drug paclitaxel (Taxol): PLGA nanoparticles containing vitamin E TPGS. *J. Control. Release* **86**:33–48 (2003).
10. P. Gao, B. D. Rush, W. P. Pfund, T. Huang, J. M. Bauer, W. Morozowich, M. S. Kuo, and M. J. Hageman. Development of a supersaturable SEDDS (S-SEDDS) formulation of paclitaxel with improved oral bioavailability. *J. Pharm. Sci.* **92**:2386–2398 (2003).
11. M. M. Malingre, J. H. Beijnen, and J. H. Schellens. Oral delivery of taxanes. *Invest. New Drugs* **19**:155–162 (2001).
12. J. van Asperen, O. van Tellingen, M. A. van der Valk, M. Rozenhart, and J. H. Beijnen. Enhanced oral absorption and

decreased elimination of paclitaxel in mice cotreated with cyclosporin A. *Clin. Cancer Res.* **4**:2293–2297 (1998).
13. H. A. Bardelmeijer, J. H. Beijnen, K. R. Brouwer, H. Rosing, W. J. Nooijen, J. H. Schellens, and O. van Tellingen. Increased oral bioavailability of paclitaxel by GF120918 in mice through selective modulation of P-glycoprotein. *Clin. Cancer Res.* **6**:4416–4421 (2000).
14. J. S. Woo, C. H. Lee, C. K. Shim, and S. J. Hwang. Enhanced oral bioavailability of paclitaxel by coadministration of the P-glycoprotein inhibitor KR30031. *Pharm. Res.* **20**:24–30 (2003).
15. M. Ramaswamy, X. Zhang, H. M. Burt, and K. M. Wasan. Human plasma distribution of free paclitaxel and paclitaxel associated with diblock copolymers. *J. Pharm. Sci.* **86**:460–464 (1997).
16. A. Sparreboom, J. van Asperen, U. Mayer, A. H. Schinkel, J. W. Smit, D. K. Meijer, P. Borst, W. J. Nooijen, J. H. Beijnen, and O. van Tellingen. Limited oral bioavailability and active epithelial excretion of paclitaxel (Taxol) caused by P-glycoprotein in the intestine. *Proc. Natl. Acad. Sci. USA* **94**:2031–2035 (1997).
17. C. M. Kruijtzer, J. H. Beijnen, and J. H. Schellens. Improvement of oral drug treatment by temporary inhibition of drug transporters and/or cytochrome P450 in the gastrointestinal tract and liver: an overview. *Oncologist* **7**:516–530 (2002).
18. J. van Asperen, O. van Tellingen, A. Sparreboom, A. H. Schinkel, P. Borst, W. J. Nooijen, and J. H. Beijnen. Enhanced oral bioavailability of paclitaxel in mice treated with the P-glycoprotein blocker SDZ PSC 833. *Br. J. Cancer* **76**:1181–1183 (1997).
19. S. Yang, R. N. Gursoy, G. Lambert, and S. Benita. Enhanced oral absorption of paclitaxel in a novel self-microemulsifying drug delivery system with or without concomitant use of P-glycoprotein inhibitors. *Pharm. Res.* **21**:261–270 (2004).
20. B. Heurtault, P. Saulnier, J. P. Benoit, J. E. Proust, B. Pech, and J. Richard. Nanocapsules comprising (semi-) liquid lipid core and solid lipid shell, prepared by phase inversion methods, useful for administration of lipophilic or hydrophilic drugs by injection, orally or intranasally, 2000. Patent WO 01/64328.
21. B. Heurtault, P. Saulnier, B. Pech, J. E. Proust, and J. P. Benoit. A novel phase inversion-based process for the preparation of lipid nanocarriers. *Pharm. Res.* **19**:875–880 (2002).
22. J. S. Coon, W. Knudson, K. Clodfelter, B. Lu, and R. S. Weinstein. Solutol HS 15, nontoxic polyoxyethylene esters of 12-hydroxystearic acid, reverses multidrug resistance. *Cancer Res.* **51**:897–902 (1991).
23. J. P. Benoit and A. Lamprecht. Use of P-glycoprotein inhibitor surfactants at the surface of a colloidal carrier, 2004. Patent WO2004071498.
24. K. H. Diehl, R. Hull, D. Morton, R. Pfister, Y. Rabemampianina, D. Smith, J. M. Vidal, and C. van de Vorstenbosch. A good practice guide to the administration of substances and removal of blood, including routes and volumes. *J. Appl. Toxicol.* **21**:15–23 (2001).
25. A. Lamprecht, Y. Bouligand, and J. P. Benoit. New lipid nanocapsules exhibit sustained release properties for amiodarone. *J. Control. Release* **84**:59–68 (2002).
26. M. Pereira de Oliveira, E. Garcion, N. Venisse, J. P. Benoit, W. Couet, and J. C. Olivier. Tissue distribution of indinavir administered as solid lipid nanocapsule formulation in mdr1a (+/+) and mdr1a (−/−) CF-1 mice. *Pharm. Res.* **22**:1898–1905 (2005).
27. C. Dulieu and D. Bazile. Influence of lipid nanocapsules composition on their aptness to freeze-drying. *Pharm. Res.* **22**:285–292 (2005).
28. J. S. Choi, B. W. Jo, and Y. C. Kim. Enhanced paclitaxel bioavailability after oral administration of paclitaxel or prodrug to rats pretreated with quercetin. *Eur. J. Pharm. Biopharm.* **57**:313–318 (2004).
29. J. D. Adams, K. P. Flora, B. R. Goldspiel, J. W. Wilson, S. G. Arbuck, and R. Finley. Taxol: a history of pharmaceutical development and current pharmaceutical concerns. *J. Natl. Cancer Inst. Monogr.* **15**:141–147 (1993).
30. K. S. Lown, R. R. Mayo, A. B. Leichtman, H. L. Hsiao, D. K. Turgeon, P. Schmiedlin-Ren, M. B. Brown, W. Guo, S. J. Rossi, L. Z. Benet, and P. B. Watkins. Role of intestinal P-glycoprotein (mdr1) in interpatient variation in the oral bioavailability of cyclosporine. *Clin. Pharmacol. Ther.* **62**:248–260 (1997).
31. B. Monsarrat, P. Alvinerie, M. Wright, J. Dubois, F. Gueritte-Voegelein, D. Guenard, R. C. Donehower, and E. K. Rowinsky. Hepatic metabolism and biliary excretion of Taxol in rats and humans. *J. Natl. Cancer Inst. Monogr.* **15**:39–46 (1993).
32. J. M. Meerum Terwogt, M. M. Malingre, J. H. Beijnen, W. W. ten Bokkel Huinink, H. Rosing, F. J. Koopman, O. van Tellingen, M. Swart, and J. H. Schellens. Coadministration of oral cyclosporin A enables oral therapy with paclitaxel. *Clin. Cancer Res.* **5**:3379–3384 (1999).
33. L. E. Buckingham, M. Balasubramanian, R. M. Emanuele, K. E. Clodfelter, and J. S. Coon. Comparison of solutol HS 15, Cremophor EL and novel ethoxylated fatty acid surfactants as multidrug resistance modification agents. *Int. J. Cancer* **62**:436–442 (1995).
34. W. N. Charman. Lipids, lipophilic drugs, and oral drug delivery—some emerging concepts. *J. Pharm. Sci.* **89**:967–978 (2000).
35. S. M. Caliph, W. N. Charman, and C. J. Porter. Effect of short-, medium-, and long-chain fatty acid-based vehicles on the absolute oral bioavailability and intestinal lymphatic transport of halofantrine and assessment of mass balance in lymph-cannulated and non-cannulated rats. *J. Pharm. Sci.* **89**:1073–1084 (2000).
36. Y. Jiao, N. Ubrich, M. Marchand-Arvier, C. Vigneron, M. Hoffman, T. Lecompte, and P. Maincent. *In vitro* and *in vivo* evaluation of oral heparin-loaded polymeric nanoparticles in rabbits. *Circulation* **105**:230–235 (2002).
37. M. Aprahamian, C. Michel, W. Humbert, J. P. Devissaguet, and C. Damge. Transmucosal passage of polyalkylcyanoacrylate nanocapsules as a new drug carrier in the small intestine. *Biol. Cell* **61**:69–76 (1987).
38. M. M. Malingre, J. H. Beijnen, H. Rosing, F. J. Koopman, O. van Tellingen, K. Duchin, W. W. Ten Bokkel Huinink, M. Swart, J. Lieverst, and J. H. Schellens. A phase I and pharmacokinetic study of bi-daily dosing of oral paclitaxel in combination with cyclosporin A. *Cancer Chemother. Pharmacol.* **47**:347–354 (2001).