

***In Vivo* Pharmacokinetic and Tissue Distribution Studies in Mice of Alternative Formulations for Local and Systemic Delivery of Paclitaxel: Gel, Film, Prodrug, Liposomes and Micelles**

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Abstract: The aim of this study was to increase the understanding on the pharmacokinetic and tissue distribution of paclitaxel as influenced by formulation approach. For this purpose, various formulations investigated in Swiss mice included liposomes, poloxamer 407 gel and chitosan film for subcutaneous route; and water-soluble methacrylate prodrug, liposomes and poloxamer micelles for systemic administration. During this study, the currently marketed formulation of Cremophor EL of paclitaxel was used as the reference. A highest plasma concentration following intravenous administration of paclitaxel was observed for rigid and 'Stealth' liposomes containing the prodrug while, least was for covalently incorporated paclitaxel micelles. Further, poloxamer micelles demonstrated both the highest mean residence time of 7.34 h and volume of distribution ($V_{ss}=4.82$ and $V_z=5.87$ L/kg) for paclitaxel. This was followed by prodrug loaded 'Stealth' liposomes, which showed a mean residence time of 4.96 h but were least distributed into apparent physiological volume ($V_{ss}=2.12$ and $V_z=3.16$ L/kg). These results clearly signify the role of formulation/excipient in drug disposition and possible interactions. Importantly, due to decrease in the clearance rate of drug, the area under curve values of paclitaxel increased by 1.64- and 2.5-fold for micellar and prodrug loaded 'Stealth' liposomal formulations, respectively over reference formulation. While thermoreversible gels served to decrease plasma concentration of paclitaxel (8-fold) after subcutaneous administration, systemic levels were totally absent after implantation of films. In tissue distribution studies, maximum percent of paclitaxel was observed in liver for reference formulation, conventional liposomes and micelles whereas highest levels of prodrug and 'Stealth' liposomes were in kidney and spleen, respectively. The novel formulations significantly altered tissue accumulation profiles of paclitaxel relative to the reference formulation, for example, reduction in uptake by heart from liposomes and micelles, as well as the major recognition mechanism for elimination. It is proposed that a combination therapy with liposomes and micelles of paclitaxel for systemic delivery along with implantation of chitosan film for local delivery, may serve not only to improve patient compliance by obliterating the need to administer Cremophor EL, but also increase patient survival.

Keywords: Paclitaxel delivery, systemic, local, pharmacokinetics, tissue distribution.

INTRODUCTION

The understanding of behavior of drugs in biological system has always been the subject of primary importance in treatment of diseases. This comprehension has immensely contributed to optimizing dosage regimens, potentiation of therapeutic efficacy and tailoring of drug delivery systems to meet specific needs, and to reduce side effects. In this regard, development of animal models, interspecies scaling and physiologic based pharmacokinetic modeling concepts have brought about pragmatic changes in establishing pharmacokinetic/pharmacodynamic relationships [1]. Presently, pharmaceutical industry is faced with the challenge of introducing drug delivery systems with reliable performance but also with a greater emphasis on patient compliance [2, 3]. Following discovery of potent molecules, these challenges of drug development go through successive stages, such as, design of suitable delivery systems and *in*

vivo evaluation. In this context, preclinical pharmacokinetic, tissue distribution and metabolism studies provide valuable information on safety profile since, usually good correlation exists between pharmacokinetic and toxicological profile [4-6].

Therapeutic concepts in the treatment of diseases have undergone thorough refinement with emerging novel drug delivery systems, which are marked with an ability to alter pharmacokinetics of drugs [7,8]. The utility of these novel systems has always demanded establishment of proof of concepts on performance since, technology is also associated with inherent complexities [9]. For example, the efficacy of presently available promising anticancer agents like doxorubicin, paclitaxel and camptothecin is limited by toxic side effects due to non-specific distribution, especially to the rapidly proliferating cells in body. Also, limitations result from the fact that, these agents *per se* fail to selectively localize in tumors, which emphasizes the need for novel drug delivery systems. To suppress toxic effects and to improve efficacy (drug burden on malignant cells), numerous delivery systems based on specific carrier properties are being extensively studied and evaluated in the field of cancer

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chemotherapeutics [10-13]. It is the alteration in pharmacokinetics brought about by these novel strategies, which reduces access to normal cells but at the same time offer opportunities for tumor targeting (passive or active).

Paclitaxel is one of the most extensively investigated anticancer drug in the last 3 decades as evident from the quantum of literature available both in terms of delivery and in the clinical scenario. Though the novel formulations developed to overcome the well established limitations (life-threatening hypersensitivity reactions of surfactant (Cremophor EL; CrEL) in formulation) of present day prescription encompass almost all possible avenues to deliver the promising molecule, indeed, only a few of them have reached clinical trials [14-31]. The lack of much needed and awaited success with hydrophobic anticancer drugs like paclitaxel still stands as impetus for instigating research in this area, since most of the attempts merely served as alternative means of paclitaxel delivery without significant improvement in clinical efficacy of the molecule. Hence, the task of delivering this antineoplastic agent by alternative and safer means is impending with a huge scope, as clinicians are extensively evaluating its efficacy, alone or in combination, in treatment of a variety of cancers [17, 32-34]. In addition, a combination therapy of local and systemic delivery of antineoplastic agents is emerging as an effective treatment modality to minimize spread of cancer and prevent development of multidrug resistance [35-37]. The present investigation involved a preclinical pharmacokinetic evaluation of novel formulations in mice along with tissue distribution studies for local and systemic delivery of paclitaxel, with a view to overcome some of the limitations associated with current paclitaxel based chemotherapy, and provide insight into the possibilities for better treatment modalities.

MATERIALS AND METHODS

Paclitaxel was kindly provided by Dabur India Ltd. Radioactive paclitaxel (^{14}C) of specific activity 42.5 mCi/mmol was obtained from Sigma (USA). Paclitaxel concentrate in CrEL-alcohol solvent system (Intaxel, non-aqueous injection concentrate; 6 mg/ml) supplied by Dabur India Ltd. was reconstituted in saline solution and administered within 15 min. Tetrabutyl ammonium hydroxide (40% solution in methanol) and *tert*-butyl methyl ether were procured from Hi-Media Laboratories (India). Acetic acid, hydrochloric acid, toluene and Triton X were purchased from LOBA Chemie (India). Diethyl ether was obtained from Central Drug House Ltd. (India). Thiopentone sodium and saline were of parenteral grade.

The formulations evaluated for systemic delivery of paclitaxel were prodrug, liposomes and micelles. The prodrug of paclitaxel (a methacrylic acid based ester conjugate), with 1657 Da molecular weight was synthesized and confirmed by Fourier-transform infrared spectroscopy, nuclear magnetic resonance spectroscopy and MALDI techniques. The liposomes of paclitaxel, as well as its prodrug were prepared by film hydration method, and the obtained multilamellar liposomes were size reduced by extrusion through 0.2 μm membrane filters. Paclitaxel incorporated liposomes contained 3 mol% of drug with

respect to lipid, and a lipid composition of soya phosphatidylcholine: phosphatidylglycerol mole ratio at 9:1. Prodrug incorporated liposomes contained 3 mol% of drug (expressed as equivalents of paclitaxel) with respect to lipid, and a lipid composition of fully hydrogenated phosphatidylcholine:distearoyl phosphatidylglycerol:cholesterol at 9:1:4.5 and 5 mol% polyethylene glycol (2000 Da) conjugated distearoyl phosphatidylethanolamine. Micelles containing covalently entrapped paclitaxel were prepared (based on the same esterification reaction via prodrug approach using methacrylic acid linker) with poloxamer 407 as micelle former and paclitaxel:poloxamer at 1.9:1 on molar basis (separate communication).

The formulations evaluated for localized delivery of paclitaxel were liposomes, poloxamer 407 gel and chitosan films. Poloxamer 407 thermoreversible gels (30% w/w) were prepared by incorporating liposomes containing paclitaxel (of the composition mentioned above) by cold method at drug concentration of 0.27% (w/v) [38]. Films were cast with >85% deacetylated chitosan, which contained chitosan: glycerol:poloxamer 407:paclitaxel with weight ratio of 2.5:1:1:2 [39].

Animal Treatment and Biodistribution Studies

In bred, adult male Swiss mice (26-31 g) were obtained from central animal facility of institution and were allowed water and rat chow *ad libitum*. The general procedures of animal care and housing met the standard Institution's rules. All experiments were carried out in accordance with the protocols approved by the Institutional animal ethical committee. All formulations were administered parenterally either by intravenous (i.v.) or subcutaneous (s.c.) route and destructive sampling method was adopted for acquisition of biological samples (blood and tissues) for analysis. Mice were given a single bolus injection of formulation by tail i.v. route, which contained a dose of paclitaxel (196 μL containing 0.3-0.5 μCi of radiolabelled compound) or by s.c. route in the neck or upper back just below the neck (200 μL containing 0.4-0.6 μCi of radiolabeled compound). In addition to injectable formulations, films were also evaluated for local delivery of paclitaxel by s.c. implantation under thiopentone anesthesia (40 mg/kg) in the neck region. The following formulations were tested by i.v. route (dose of paclitaxel administered is shown in parentheses) and time points of sampling were 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6 and 9 h:

- (i) CrEL-paclitaxel (10 mg/kg)
- (ii) paclitaxel-liposome (10 mg/kg)
- (iii) prodrug (10 mg/kg)
- (iv) prodrug-liposome (5 mg/kg)
- (v) micelles (10 mg/kg)

The following formulations were tested by s.c. route:

- (i) paclitaxel-liposome formulation (10 mg/kg; at 0.5, 2, 4, 6, 8, 10, 12, 18, 24 and 48 h)
- (ii) liposome-gel (20 mg/kg; at 1, 2, 4, 6, 10, 15, 20, 25 and 50 h)
- (iii) paclitaxel-film (666 mg/kg; at 2, 15, 37, 49, 53, 62, 100 days)

Always animals were anesthetized with ether inhalation and blood was drawn from retro-orbital plexus (terminal bleeding). Subsequently, blood samples were centrifuged at 5000 rpm for 10 min, plasma was separated and stored at -20°C . Immediately after obtaining blood sample, mice were sacrificed by cervical dislocation and dissected to obtain various tissues (brain, heart, kidney, liver, lung and spleen). Each tissue was weighed and stored in vial at -20°C until analysis.

Bioanalysis

The estimation of paclitaxel concentrations in plasma and tissues is important in order to understand the altered disposition caused due to variability in formulation and route of administration. The plasma and tissue concentrations of paclitaxel were determined by radiochemical method of analysis as described below. From the known ratio of paclitaxel to radiolabeled paclitaxel in each formulation, plasma and tissue levels of radioactivity were then converted into equivalents of paclitaxel. In all cases, concentration of paclitaxel was expressed as ng/ml for plasma and ng/mg for tissue. The blood and tissue levels represent the total sum of free, carrier- and/or tissue-associated paclitaxel, which include parent drug, metabolites and/or other structurally modified forms. Preliminary studies with tissue homogenates and high performance liquid chromatography studies proved that paclitaxel is generated from prodrug (chromatograms not shown).

Plasma Concentration Determination

At the above mentioned time intervals, blood samples were obtained from mice after administration of various formulations as mentioned above. The plasma was separated from blood sample by centrifugation at 5000 rpm for 10 min. To a measured quantity of plasma sample in plastic scintillation vial, 3 ml of scintillation cocktail was added and kept overnight. Subsequently, vials were centrifuged at 6000 rpm for 30 min to settle any interfering substances before liquid scintillation counting.

Tissue Distribution Studies

Prior to the inception of *in vivo* studies, ascertaining complete recoveries of paclitaxel from all tissues after administration of paclitaxel validated the extraction method involved in biodistribution studies. Frozen tissue samples were thawed in acetonitrile-water solvent system (1:1 v/v) for 45 min and homogenized with the help of Teflon pestle fixed to an overhead stirrer. To the obtained homogenate (of total volume 6-8 ml), 5 ml of *tert*-butyl methyl ether was added and shaken for 1 h on an oscillating shaker. For complete recovery of paclitaxel, the ether extract was transferred into plastic scintillation vial and the extraction was repeated from homogenate with an additional 5 ml of ether. Subsequently, the combined extracts were dried and 3 ml of scintillation cocktail was added for counting. To confirm complete recoveries of paclitaxel, tissue residue of each organ (the remnant tissue residue obtained as a result of homogenization) after ether extraction was separately solubilized with tissue solubilizer and the amount of

radioactivity remaining was also estimated by radiochemical method of analysis. Since drug recoveries were not complete from liver, the tissue residue after ether extraction was solubilized using 5-8 ml of tissue solubilizer (24 ml tetrabutyl ammonium hydroxide, 12 ml toluene, 18 ml Triton X 100, 6 ml water) at 50°C for 48 h followed by the addition of 300 μl of acetic acid and 8 ml of cocktail. Following addition of tissue solubilizer, samples were allowed to stand for 12 h, and the supernatant obtained by centrifugation at 5000 rpm for 30 min was taken for liquid scintillation counting. The combined counts of both ether extract and tissue solubilize were taken for calculation purposes. However, the above extraction method was slightly modified in the case of chemically altered paclitaxel containing formulations, namely, prodrug, liposomes containing prodrug and micelles. The differences were, ether extraction was performed thrice instead of twice and 500 μl of 1 N hydrochloric acid was added to the homogenate (in 2nd and 3rd extractions). This procedure was suitable for obtaining 95% recoveries in all tissues studied with the exception of liver. Hence, extraction of paclitaxel from liver always involved ether extraction followed by tissue solubilization step to maximize recoveries.

Pharmacokinetic Analysis

In order to have a comparative evaluation of *in vivo* performance, pharmacokinetic analysis was performed on all plasma concentration time profiles of formulations (after i.v. and s.c. routes of administration). The area under concentration time profile was calculated using linear trapezoidal rule with extrapolation to infinity and individual profiles were analyzed by compartment model independent method using PCNONLIN software (version 4.2) to obtain pharmacokinetic parameters such as maximum plasma concentration (C_{max}), apparent plasma terminal half-life ($t_{1/2}$), area under curve extrapolated to infinity ($\text{AUC}_{0-\text{inf}}$) and area under first moment curve extrapolated to infinity ($\text{AUMC}_{0-\text{inf}}$), mean residence time (MRT), apparent volume of distribution at steady state (V_{SS}), total body clearance (CL_{T}) and apparent volume of distribution in terminal phase (V_{Z}).

Statistical Methods

The pharmacokinetic data of formulations was analyzed using Sigma Stat version 4 by *Student-Newman-Keuls* procedure for multiple comparisons. A statistically significant difference was considered at $p < 0.05$.

RESULTS

Plasma Profiles

The plasma concentration time profiles following i.v. (Fig. 1) and s.c. administration were described by noncompartment model and pertinent pharmacokinetic parameters along with administered dose in each case are shown in Table 1. The maximum plasma concentration was observed after i.v. administration of liposomes followed by CrEL-paclitaxel, prodrug and micelles. At the administered dose, C_{max} values attained after i.v. administration of CrEL-paclitaxel, paclitaxel-liposome, prodrug, prodrug-liposome and micelles were 9292, 7366, 9954, 6623 and 5930 ng/ml,

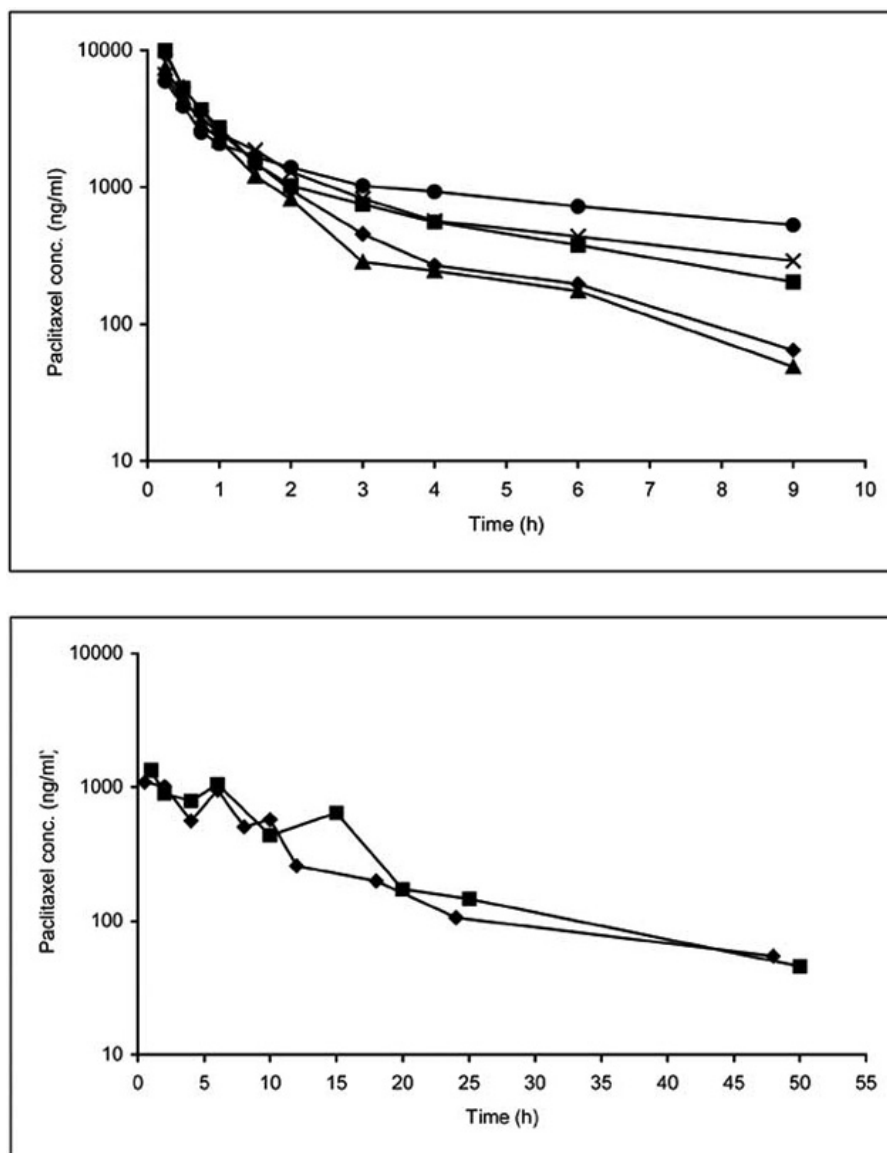


Fig. (1). Comparative plasma concentration time profiles of paclitaxel after i.v. administration (top panel; Key: ◆ CrEL-paclitaxel; ■ Paclitaxel-liposome; ▲ Prodrug; × Prodrug-liposome; ● Micelle) and s.c. administration (bottom panel; Key: ◆ Paclitaxel-liposome; ■ Liposome-gel) of different formulations in mice. Radiochemical method of analysis was used for determination of radioactivity of plasma samples which was subsequently converted into equivalents of paclitaxel whose concentration is expressed as ng/ml (data is mean, n=3; error bars are not shown for clarity).

respectively. The $AUC_{0-\infty}$ values when normalized for dose (assuming dose-proportionality) were 23340, 15225, 11138, 9286 and 6568 ng.h/ml for prodrug-liposome, micelles, paclitaxel-liposome, CrEL-paclitaxel and prodrug, respectively.

When apparent $t_{1/2}$ of formulations was compared, the parameter was of the following order: 6.2, 5.1, 3.4, 2.4 and 2.1 h for micelles, prodrug-liposome, paclitaxel-liposome, CrEL-paclitaxel and prodrug, respectively, suggesting that in spite of low initial plasma concentrations micelles were cleared slowly from plasma. Further, prodrug-liposome exhibited a lowest clearance rate of 0.43 L/h/kg, while micelles were cleared at a rate of 0.65 L/h/kg, followed by the same sequence as observed for $t_{1/2}$. In addition, the most

extensively distributed formulation was micelles with V_{SS} and V_Z of 4.82 and 5.87 L/kg, respectively, whereas the least was observed to be prodrug-liposome (2.12 and 3.16 L/kg, respectively) (see Table 2 for a statistical comparison of various pharmacokinetic parameters).

Following s.c. administration of liposomes and gel-liposome, initial plasma concentrations of paclitaxel were 8-14 fold lower than compared with i.v. route (Table 1). However, when i.v. route of administration of paclitaxel-liposome was compared with s.c. administration of liposome-gel, $AUC_{0-\infty}$ for paclitaxel was decreased from 11138 to 9187 ng.h/ml (after normalization for dose, assuming dose-proportionality). In contrast to liposome-gel and liposomes, films have not shown any detectable levels in

Table 1. Pharmacokinetic Parameters of Paclitaxel in Different Formulations Obtained from *In Vivo* Studies in Mice*

Route	i.v. ^a					s.c. ^b	
	Formulation ^c	CrEL-paclitaxel	Prodrug	Paclitaxel-liposome	Prodrug-liposome ^d	Micelle	Paclitaxel-liposome
Dose (mg/kg)	10	10	10	5	10	10	20
<i>Pharmacokinetic parameter^e</i>							
C _{max} (ng/ml)	9292	7366	9954	6623	5930	1095	1334
t _{1/2} (h)	2.4	2.1	3.4	5.1	6.2	18.0	15.0
AUC _{0-inf} (ng.h)/ml	9286	6568	11138	11670	15225	11454	18373
AUMC _{0-inf} (ng.h ²)/ml	25012	18322	34787	57858	111794	110380	356862
MRT (h)	2.69	2.79	3.12	4.96	7.34	9.64	19.42
V _{ss} (L/kg)	2.90	4.25	2.80	2.12	4.82	8.41	21.14
CL _T (L/h/kg)	1.07	1.52	0.89	0.43	0.65	0.87	0.54
V _Z (L/kg)	3.71	4.61	4.40	3.16	5.87	22.67	11.78

*Pharmacokinetic parameters calculated by compartment model independent method using PCNONLIN. All data is mean n=3. Standard deviation was, in general, less than 8%

^a196 µl was administered by tail i.v. route

^b200 µl was administered in the neck region

^cAll formulations were tested within 48 h of preparation

^dDue to viscosity of formulation, only 5 mg/kg dose of paclitaxel was administered

Note: A dose of 666 mg/kg was administered s.c. by films. Pharmacokinetic parameters for film formulation are not shown here since blood levels were not detected.

plasma in spite of delivering 66-fold higher dose of paclitaxel. At the same time, both s.c.-administered formulations demonstrated an increased volume of distribution for paclitaxel.

Tissue Distribution Profiles

The mean paclitaxel tissue concentration time profiles after i.v. administration of CrEL-paclitaxel, paclitaxel-liposome, prodrug, prodrug-liposome and micelles are shown in Fig. 2. For all formulations administered, highest and lowest levels of drug were found in liver and brain, respectively with the exception of prodrug and prodrug-liposome where highest concentrations were in kidney and spleen, respectively. The maximum concentrations attained in liver for CrEL-paclitaxel, paclitaxel-liposome, micelles, prodrug and prodrug-liposome were 74.5, 91.5, 58.6, 6.9 and 15.6 ng/mg, respectively. In spleen tissue, maximum concentrations of paclitaxel were 44.2, 15.7, 18.1, 10.6 and 1.6 ng/mg for prodrug-liposome, CrEL-paclitaxel, paclitaxel-liposome, micelles and prodrug, respectively. At the same time, highest levels of paclitaxel observed in heart for various formulations were as follows: CrEL-paclitaxel 11.8, paclitaxel-liposome 8.7, micelles 9.4, prodrug 2.76 and prodrug-liposome 4.4 ng/mg. The highest paclitaxel concentration in kidney relative to other organs corresponds to prodrug as evident from Fig. 2C. Accumulation of paclitaxel in lung demonstrated a similar trend to that of liver in the sense maximum concentrations were detected for CrEL-paclitaxel followed by prodrug-liposome. Further, maximum paclitaxel concentrations were achieved in brain after administration of CrEL-paclitaxel.

Apart from bio-distribution studies of formulations administered by i.v. route, tissue accumulation of paclitaxel was also studied after s.c. administration of liposomes and liposome-gel (Fig. 3). Though a similar trend in tissue distribution of paclitaxel was observed for both formulations (highest in liver, lowest in brain), gel-liposome also resulted in considerably higher concentrations of paclitaxel in lung and brain. For example, after normalization of dose (assuming dose-proportionality), a highest paclitaxel concentration of 17.5 ng/mg was observed in lung (almost equivalent to that of i.v.-administered formulations) with gel and that of liposomes was 6.21 ng/mg. In addition, paclitaxel levels in brain were greatest after s.c. administration of gel with a maximum of 1.6 ng/mg when compared with any formulation irrespective of the route of administration. Further, after s.c. implantation of films no tissue concentrations of paclitaxel were detected in agreement with the earlier observation of lack of plasma levels.

DISCUSSION

The objective of the present investigation was to evaluate different formulations loaded with native as well as chemically modified form of paclitaxel *via* intravenous and subcutaneous routes for parenteral delivery. These formulations include prodrug, liposomes, micelles, poloxamer gel and chitosan films while the commercial CrEL formulation served as control. After i.v. administration, apparent terminal elimination half-life in increasing order showed the following sequence for formulations: prodrug < CrEL-paclitaxel < paclitaxel-liposome < prodrug-liposome < micelle which depends on solubility, hydro-

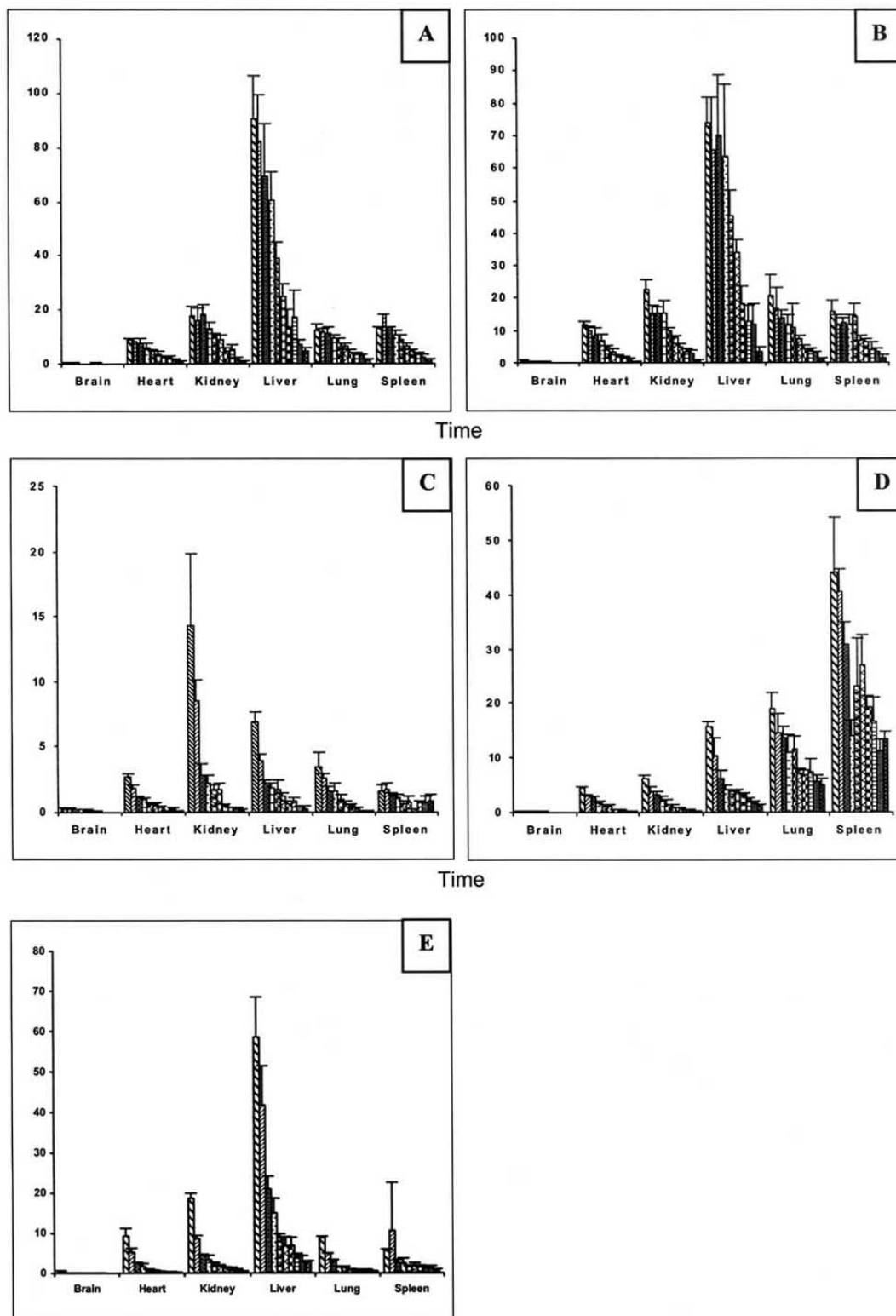


Fig. (2). Tissue distribution profiles of paclitaxel (ng/mg) after i.v. administration of (A) Commercial formulation, (B) paclitaxel-liposome, (C) prodrug, (D) prodrug-liposome, and (E) micelles in mice (data is mean \pm SD; n=3). For clarity reasons, error bars are unidirectional. Tissues were homogenized using acetonitrile-water (1:1 v/v) solvent mixture and extracted with *tert*-butyl methyl ether; ether phase was evaporated and radioactivity was determined by liquid scintillation counting. Only in the case of liver, the remanant tissue after ether extraction was separately solubilized with tissue solubilizer for determination of radioactivity.

15min 30min 45min 1h 1.5h 2h 3h 4h 6h 9h

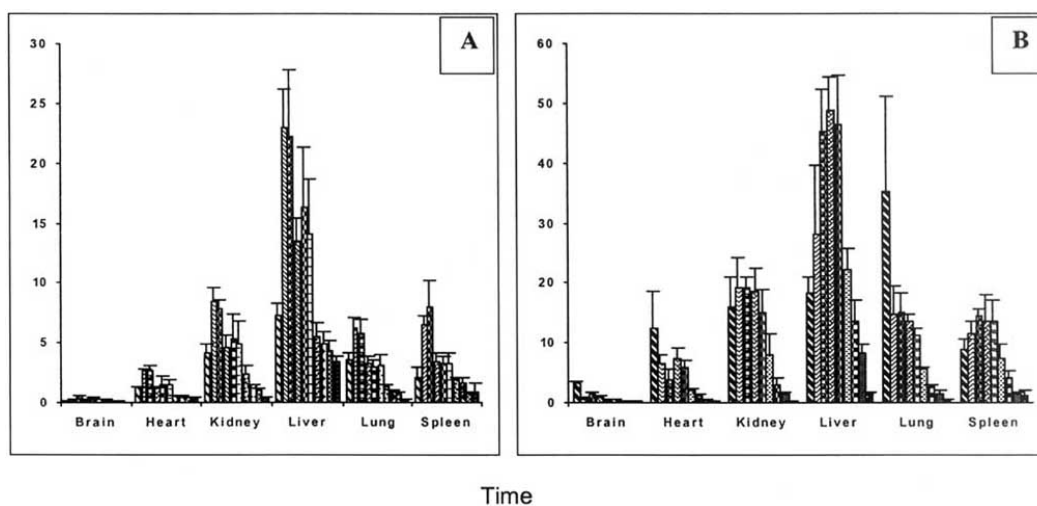


Fig. (3). Tissue distribution profiles of paclitaxel after s.c. administration of (A) paclitaxel-liposome and (B) liposome-gel formulation in mice (data is mean ± SD; n=3). For clarity reasons, error bars are unidirectional. Tissues were homogenized using acetonitrile-water (1:1 v/v) solvent mixture and extracted with *tert*-butyl methyl ether; ether phase was evaporated and radioactivity was determined by liquid scintillation counting. Only in the case of liver, the remanant tissue after ether extraction was separately solubilized with tissue solubilizer for determination of radioactivity.

Key: (paclitaxel-liposome)
 (liposome-gel).

Table 2. Statistical Comparison of Various Pharmacokinetic Parameters After i.v. Administration of Different Paclitaxel Formulations in Mice*

Comparison ^a	C _{max}	t _{1/2}	AUC _{0-inf}	CL _T
CrEL-paclitaxel vs. Prodrug	Yes	No	No	Yes
CrEL-paclitaxel vs. Paclitaxel-liposome	No	Yes	No	No
CrEL-paclitaxel vs. Prodrug-liposome	Yes	Yes	Yes	Yes
CrEL-paclitaxel vs. Micelle	Yes	Yes	Yes	Yes
Prodrug vs. Paclitaxel-liposome	Yes	Yes	Yes	Yes
Prodrug vs. Prodrug- liposome	Yes	Yes	Yes	Yes
Prodrug vs. Micelle	No	Yes	Yes	Yes
Paclitaxel-liposome vs. Prodrug-liposome	Yes	Yes	Yes	Yes
Paclitaxel-liposome vs. Micelle	Yes	Yes	Yes	No
Prodrug-liposome vs. Micelle	Yes	Yes	Yes	No

*Multiple comparisons were done by *Student-Newman-Keuls* method

^aYes = statistically significant difference exists (p<0.05), No = statistically significant difference does not exist (p>0.05)

phobicity, molecular weight, size, charge, surface characteristics; and the ability of body to recognize either as soft or hard drug. In contrast to paclitaxel which is primarily eliminated by hepatic biotransformation (Fig. 2A) [40], kidney followed by liver were involved in elimination of its water soluble prodrug (Fig. 2C). However, prodrug-liposome formulation upon i.v. administration has apparently served to increase t_{1/2} of paclitaxel by 3 h and the increase is

attributed to saturated lipids and polyethylene glycol (PEG) coating which rendered the formulation more ‘biostable’ compared to conventional paclitaxel liposomes. Further, micellar formulation of paclitaxel demonstrated highest apparent t_{1/2} of 6.2 h probably because of its size and ability to impart better ‘Stealth[®]’ features as compared to prodrug-liposome.

The AUC is an important pharmacokinetic parameter to correlate exposure time of drug with pharmacodynamic activity and those formulations, which are long circulating are expected to show higher AUC values. This holds an important consequence in the field of cancer chemotherapy where novel approaches are directed towards developing long circulating delivery systems that facilitate accumulation of drug in tumors by enhanced permeation and retention mechanism. The water soluble prodrug of paclitaxel exhibited not only diminished area under plasma profile but also tissue levels, which is attributed to relatively rapid elimination/distribution from central compartment. In the present study, micelles (coated with poloxamer) and prodrug-liposomes (coated with PEG) categorized as 'Stealth[®]' colloidal carrier systems, demonstrated highest AUC_{0-inf} values after i.v. administration and hence these hold a promising delivery strategy for further evaluation in tumor models, and as a feasible alternative to the currently available commercial CrEL formulation. In contrast to liposomes and micelles, AUC values were either considerably reduced after s.c. administration of liposome-gel or were completely absent as in the case of films (Table 1). Hence, these formulations clearly demonstrate their ability to reduce systemic burden of paclitaxel but at the same time provide local release and maximize tumor concentrations.

Plasma profile analysis is an important aspect in understanding biological handling of a given drug. All profiles after i.v. administration, of paclitaxel show at least one distribution phase suggesting that more than one compartment exists, which signify the pharmacokinetic parameter of volume of distribution (Fig. 1). After i.v. administration of formulations, a rapid decline in plasma concentration is indicative of rapid distribution of drug into highly perfused organs (as evident from tissue distribution studies), such as, liver, lung, spleen and kidney. For carrier-based colloidal delivery systems, clearly, the quick distribution is either due to rapid release of entrapped drug followed by partitioning into tissues, or due to tissue interstitial spaces accumulation/macrophage uptake of carrier along with entrapped drug. An approximate 10-fold reduction in plasma concentrations of paclitaxel in a 4 h-post i.v. administration period of albumin conjugate [41], liposomes [42] and micelles [43] in mice was previously reported. A similar profile characterized with 4 h distribution phase was observed with liposomal and micellar formulations in the present investigation. Interestingly, in spite of the 10-fold fall in blood levels during distribution phase, the formulations were reported to be more efficacious than CrEL-paclitaxel. Hence, it is anticipated on similar grounds, and by virtue of improved pharmacokinetic profile that the presently described formulations would at least match, or be more efficacious in performance than CrEL formulation in tumor models.

For a given drug and formulation, elevated plasma profile is usually obtained by increasing the administered dose. However, keeping the dose constant, an elevated terminal part of curve (at the end of 9 h; see Fig. 1) is observed after administration of liposomes and micelles resulting in increased AUC_{0-inf} values. Similarly, a gradual decline of paclitaxel levels due to increase in apparent $t_{1/2}$ was

demonstrated by liposomes and gel after s.c. administration indicating a sustained release pattern. Amongst the formulations evaluated, the apparent volume of distribution increased further after entrapment in polymeric micelles suggesting that paclitaxel is most extensively distributed when conjugated chemically to poloxamer, whereas encapsulation of paclitaxel in the form of prodrug in PEG coated liposomes reduced its distribution. These results clearly signify the role of carrier system in influencing distribution of drugs *in vivo*, and the differences are a result of the manner in which body handles these colloidal systems based on their physicochemical properties. Similar increases in volumes of distribution at steady state were previously reported for carrier-entrapped paclitaxel [42-43].

Tissue distribution studies were performed, which may be helpful in providing comprehensive picture of possible relationships between plasma levels, drug levels in tissues and toxicity. Tissue distribution studies of i.v.-administered, ³H labeled CrEL-paclitaxel (20mg/kg) in female C3Hf/Kam mice indicated that maximum concentrations were in liver followed by kidney, lung, heart, spleen and brain in the decreasing order [44]. In another investigation, the AUCs of CrEL-paclitaxel in liver, kidney, spleen and lung after i.v. route of administration were 11.1, 159.3, 44.9 and 29.5 µg.h/g, respectively, whereas for intraperitoneal route they were found to be 522.3, 106.9, 78.9 and 103.7 µg.h/g, respectively [45]. The trend was exactly same for biodistribution of CrEL-paclitaxel in the present study. In comparison to CrEL-paclitaxel, paclitaxel-liposomes demonstrate increased accumulation in liver, lung and spleen due to uptake by monomolecular phagocyte system. A striking difference in tissue accumulation of paclitaxel between conventional and modified liposomes is that, the former are taken up predominantly by liver followed by spleen and lung, while highest percentage of 'Stealth[®]' liposomes was in spleen, followed by lung and liver. It is also possible that prodrug was prematurely liberated from liposomes due to destabilization by biological components during transit in the blood stream. But, tissue distribution studies have revealed that maximum concentrations of paclitaxel were attained in spleen, lung and liver. Hence, it could be argued that, if prodrug were prematurely liberated from these carrier systems, substantial levels of radioactivity would be detected in kidney, which strongly demonstrates the circulation stability of PEG-liposomes and polymeric micelles. However, blood components seems to have significantly destabilized conventional liposomes since, the distribution profile of paclitaxel is similar to that of CrEL formulation. Importantly, reduced accumulation of paclitaxel in heart was observed with liposomes and micelles, which has a clinical significance since paclitaxel therapy is associated with cardiotoxicity [46,47].

Polymer drug conjugates and micellar formulations have been investigated as carriers for paclitaxel in an attempt to direct the molecule to tumors and reduce side effects [45]. Earlier, it was reported that paclitaxel physically entrapped with poly(D,L-lactide)-block-methoxy polyethylene glycol micelles was more efficacious than CrEL-paclitaxel and highest concentration of paclitaxel was found in lung after administration of the formulation followed by distribution in kidney, liver, heart and spleen [43]. A comparative account

Table 3. Comparison of Maximum Paclitaxel Levels Localized in Different Tissues After i.v. Administration of Various Formulations*

Tissue ^a (ng/mg)	Kim <i>et al.</i> 2001 (micelles) ^b	Li <i>et al.</i> 2000 (conjugate) ^c	Present study
Heart	14 (2)	8 (1)	9 (2)
Kidney	47 (14)	30 (4)	18 (2)
Liver	42 (6)	45 (17)	58 (10)
Lung	54 (9)	16 (3)	9 (1)
Spleen	13 (3)	42 (7)	10 (11)

*Comparison of polymeric conjugate of paclitaxel and physically entrapped micellar paclitaxel formulation with current study micellar formulation. Standard deviation is shown in parentheses.

^aKim *et al.* used HPLC method of analysis while Li *et al.* and present study was based on radiochemical method

of analysis. The tissue concentrations are expressed as equivalents of paclitaxel when radiochemical method of analysis was used.

^bKim *et al.* (2001) physically entrapped paclitaxel in polymeric micellar system. Tissue distribution studies were conducted at 50mg/kg dose of paclitaxel in female SPF C57BL/6 tumour bearing mice. Hence, for comparison purpose, reported drug levels in tissues were normalized for dose (reported value/5) assuming dose proportionality.

^cLi *et al.* (2000) conjugated paclitaxel to poly(L-glutamic acid) carrier. Tissue distribution studies were conducted at 20mg/kg dose of paclitaxel in female C3H/Kam tumour bearing mice. Hence, for comparison purpose, reported drug levels in tissues were normalized for dose (reported value/2) assuming dose proportionality.

(after normalization for dose) of maximum tissue levels of physically entrapped micellar formulation with the present covalently entrapped formulation is shown in Table 3. The figures clearly suggest similar accumulation in heart, liver and spleen but substantially lower values in kidney and lung. In another report, tissue distribution studies of a poly(glutamic acid) paclitaxel conjugate indicated a maximum affinity to liver followed by spleen and kidney [44]. However, in comparison to the polymer conjugate, the micellar formulation of paclitaxel has displayed significantly lower values in kidney, lung and spleen in the present investigation (Table 3).

Apart from liposomes and gels that have been evaluated for local and sustained parenteral delivery of paclitaxel *via* s.c. administration, the present study focused on evaluation of chitosan films. These studies proved very impressive, since neither detectable blood nor tissue levels of paclitaxel were seen after s.c. implantation of films at dose of 666 mg/kg in mice. The lack of systemic levels clearly indicates sustained and local release of drug that was further confirmed by a time dependent study involving morphological observation of films obtained after implantation in mice. These studies revealed that films soften with loss of structure and integrity with time suggesting that they undergo biodegradation at the site of implantation to release drug locally without any systemic side effects (separate communication).

In addition to antiproliferative activity, paclitaxel has demonstrated dose dependent, antimetastatic, antiangiogenic, apoptotic effects, as well as, synergism with radiation therapy in tumor models [48]. The radiosensitizing effect of paclitaxel has been ascribed to the ability of the molecule to make tumor cells susceptible to ionizing radiation at G₂/M phase of cell cycle, which has been demonstrated in a variety of human cell lines [49]. Hence, it is proposed that intratumoral injection of gel based formulation, and placement of paclitaxel films in tumor resection cavity following surgery would be effective in augmentation of combination therapy in dissemination of cancer, for

example, interstitial drug delivery in the treatment of brain tumors along with radiation therapy [50].

CONCLUSIONS

The results of this study signify the ability of CrEL-free (patient compliance) novel formulations to alter pharmacokinetics paclitaxel. The novel formulations have demonstrated atleast similar, or superior pharmacokinetic and biodistribution profiles in mice in comparison to CrEL formulation. The entrapment of paclitaxel in the form of its prodrug in long circulating liposomes and micelles using biocompatible materials has resulted in increased apparent half-life of paclitaxel. In particular, the covalently entrapped micellar formulation needs special attention since covalent bonds were stable enough to impart circulation stability in blood when tested in mice. On the other hand, chitosan films of paclitaxel seem to be very promising in treatment of localized tumors since they release drug locally with lack of systemic side effects. However, in order to translate the useful pharmacokinetic parameters observed in the present investigation for clinical purposes, additional studies in tumor models are required to confirm the above findings with a pharmacodynamic basis, and with an emphasis on the role of drug carrier systems *per se* in augmenting therapeutic efficacy of the promising anticancer drug.

ABBREVIATIONS

AUC	=	Area under curve
AUMC	=	Area under first moment curve
CL _T	=	Clearance total
C _{max}	=	Concentration maximum
CrEL	=	Cremophor EL
i.v.	=	Intravenous
s.c.	=	Subcutaneous
PEG	=	Polyethylene glycol

$t_{1/2}$	=	Half-life
MRT	=	Mean residence time
V_{ss}	=	Volume of distribution at steady state
V_z	=	Volume distribution in terminal phase

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