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International Journal of Pharmaceutics 250 (2003) 45–50
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

An alternative paclitaxel microemulsion formulation: hypersensitivity evaluation and pharmacokinetic profile

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Received 18 April 2002; received in revised form 22 July 2002; accepted 22 August 2002

Abstract

Based on the clinical fact that paclitaxel injection (Taxol®) frequently causes hypersensitivity reactions, we prepared an alternative paclitaxel microemulsion with small particle size (17.2 nm). The hypersensitivity evaluation and pharmacokinetic behavior in rats were conducted to assess the new microemulsion. The results showed that the new microemulsion was negative and the placebo Taxol® solution was positive with regard to allergic reactions. In the pharmacokinetic study, five rats were administrated Taxol® or paclitaxel microemulsion. Blood samples were collected at 0, 0.25, 0.5, 1, 2, 4, 6, 8, 10, 12, 14 h and paclitaxel determined by HPLC. The area under the curve (AUC) was significantly higher in the microemulsion group (34.98 μg ml^{-1} h) than that in the Taxol® group (21.98 μg ml^{-1} h). Also, the K_{10} was much smaller in the microemulsion group (0.57 h^{-1}) compared with the Taxol® group (1.29 h^{-1}), showing the elimination rate was much slower in the former than in the latter. Compared with Taxol®, the paclitaxel microemulsion caused less toxicity and had a longer circulation time in rats.

Keywords: Paclitaxel; Microemulsion; Taxol; Cremophor EL; Hypersensitivity; Pharmacokinetics

1. Introduction

Paclitaxel, a white or white-like powder extracted from the bark of the Pacific yew tree *Taxus brevifolia*, is one of the most effective anticancer drugs against ovarian cancer, breast cancer, head and neck cancer, non-small lung cancer and prostatic cancer. Different from other anticancer drugs, paclitaxel prevents the growth of tumor cells by promoting polymerization and assembly of tubulin proteins and stabilizing the microtubules (Rowinsky et al., 1990; Wei et al., 2001). Paclitaxel injection (Taxol®) and compound yew capsules are the main dosage forms available for clinical application. Because of the lower bioavailability from the latter preparation, in most cases intravenous infusion with various administration protocols are preferred. However, the clinical application of paclitaxel injection was initially hampered by hypersensitivity reactions. It is generally considered that Cremophor EL, a solubilizer used in paclitaxel injection, can induce histamine release and thereby lead to hypersensi-

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0378-5173/02/$ - see front matter
PII: S0378-5173(02)00478-7

tivity reactions. In addition, Cremophor EL can leach diethylhexylphtalate from polyvinylchloride (PVC) infusion sets, necessitating the use of plasticizer-free containers or bags (e.g. glass, polypropylene, polyolefin or low-density polyethylene) and causing inconvenience to medical staff and pain to patients (Pahala and Dannenfelser, 1998; Panchagnula, 1998).

In our study, we prepared a paclitaxel microemulsion with much less solubilizer than used in Taxol® and compared it with Taxol® with regard to hypersensitivity and to its pharmacokinetic profile in rats.

2. Material and method

2.1. Material and instruments

Taxol® (Batch No. 0107192) and paclitaxel powder were purchased from Sihuan Pharmaceutical Factory of Beijing and Diazepam was obtained from Beijing Pharmaceutical Factory. The paclitaxel microemulsion used in the experiment was prepared in our own laboratory. In the formulation, we used egg phosphatidylcholine, pluronic F68 and Cremophor EL as the surfactants, and alcohol as the co-surfactant. But compared with Taxol®, Cremophor EL used in our formulation was much less. Methanol, acetonitrile and tertiary butyl methyl ether were all of HPLC grade from Fisher Co., Inc (USA). All other reagents were of analytical grade. The HP 1100 Series HPLC System (Agilent Co. Inc., USA) was composed of a solvent delivery pump, a variable UV detector, a manual injector and a HP 3395 integrator.

2.2. Appearance and morphorlogy

The paclitaxel microemulsion is a translucent solution with visible sky-blue opalescence. Spherical particles could be detected under electronic microscope.

2.3. Particle size and distribution

The particle size and its distribution profile were determined by quasielastic light-scattering.

2.4. Determination of paclitaxel in microemulsion

A fixed amount of paclitaxel microemulsion was diluted into suitable concentration with methanol. Then 50 μl was injected into HPLC system. For the quantitative determination of paclitaxel, a reverse-phase HPLC method was used (HP 1100 Binary LC pump liquid chromatograph; C-18 column, 250×4.6 mm, 5 μm, Dalian Elite Tech. Co., Inc). The mobile phase was acetonitrile–water (50:50, v/v). The analysis was performed at the flow rate of 1 ml min^{-1} with the UV detector at 227 nm and the sensitivity was 0.02 AUFS.

2.5. Hypersensitivity reaction

As described in Section 1, Cremophor EL, a surfactant employed in Taxol®, is the main ingredient causing hypersensitivity reaction. To evaluate the microemulsion's hypersensitivity, we prepared formulation A (Batch No 01111501), the blank paclitaxel injection in accordance with the formula of Taxol®, and formulation B (Batch No 01111502), the blank paclitaxel microemulsion.

Twelve guinea pigs (weight 250–350 g, provided by the Department of Animal, Peking University Health Science Center) were divided into two groups (Group A and Group B). Each group consists of six Guinea pigs, three of which are male and the others are female. Group A was administrated formulation A and Group B was administrated formulation B. At first, every other day 0.2–0.5 ml of the formulations were intramascularly injected. After three injections, those guinea pigs in Group A and B were then divided into Subgroup A1, A2 and Subgroup B1, B2 individually. As for subgroup A1 and B1, on the 14th day following the first injection, 2–3 ml dose was injected intraperitonealy to observe whether the guinea pigs would suffer from nosescratch, sneeze, erect hair, twitch, dyspnea, gatism, shock or death. As for subgroup A2 and B2, on the 21st day following the first injection, the formulation was

injected in the same way as above and observations were taken.

2.6. Determination of paclitaxel in plasma

2.6.1. Determination of blood samples

An aliquot of 150 μl blood sample was placed into a centrifuge tube and then 50 μl diazepam (Internal Standard) and 2 ml tertiary butyl methyl ether were added. After vortexed for 2 min, the mixture was centrifuged at 3000 rpm for 10 min. The supernatant was placed into water bath at 40 °C and dried under gentle nitrogen gas. The residue was reconstituted by 150 μl mobile phase. Fifty microliter was injected into HPLC system. The peak area of paclitaxel (Ap) and diazepam (Ad) were recorded and the content of paclitaxel was determined as the ratio of Ap/Ad. The HPLC condition is listed below: the mobile phase was composed of methanol: acetonitrile: water (25:40:39, v/v), flow rate of 1.0 ml min^{-1}, detecting wavelength of 227 nm and sensitivity of 0.02 AUFS.

2.6.2. Preparation of the standard curve

One hundred and fifty microliter blank plasma each was placed into centrifuge tubes. Then paclitaxel was added such of that the final paclitaxel concentrations were 0.06, 0.12, 1.2, 6.0 and 12 mg ml^{-1}. These concentrations were then treated as in Section 2.6.1 and the peak areas of paclitaxel and diazepam were recorded. The linear regression of paclitaxel concentration (C) versus peak area ratio served as the standard curve of paclitaxel concentration in plasma.

2.6.3. The determination of relative recovery and precision

One hundred and fifty microliter blank plasma each was placed into a centrifuge tube. Then add the paclitaxel of different concentration and make the final paclitaxel concentration 0.12 ± 1.2 and 12 mg ml^{-1}. Then procedures were taken according to the method described in Section 2.6.1. Also, with measured concentration as index of precision, the intra-day and between-day (consecutive 5 days) precision was calculated.

2.7. The pharmacokinetic study of Taxol® and microemulsion in rats

2.7.1. Design of the experiments

Ten SD male rats (Provided by the Department of Animals, Peking University Health Science Center), weighing 200 ± 10 g, were randomly assigned into two groups of five rats. All the animals were fasted for 12 h prior to the experiments. Taxol® and the paclitaxel microemulsion were injected via tail vein at a single dose of 8 mg kg^{-1}, respectively. Blood (0.5 ml) was collected via orbit at 0, 0.25, 0.5, 1, 2, 4, 6, 8, 10, 12, 14 h after injection. Blood samples were placed into heparinized tubes. After centrifugation, the obtained plasma was stored at -4 °C until determination.

2.7.2. Data analysis

The 3P87 computing program (produced by the Committee of Mathematic Pharmacology of the Chinese Society of Pharmacology) was employed to analyze those obtained data.

3. Results

3.1. The particle size, distribution and the content of paclitaxel in microemulsion

The mean particle size measured by quasielastic light-scattering is 17.2 nm. Most particles were in the range of 10–50 nm.

The content of paclitaxel in microemulsion was 98.9% of added paclitaxel.

3.2. Hypersensitivity reaction

As for blank paclitaxel injection (Control) group, the guinea pigs showed frequent nose-scratch, tremble, sneeze, erect hair, twitch, or dyspnea. Some even showed gatism. Compared with the control group, the guinea pigs in the blank paclitaxel microemulsion group (Test) only showed hair erect and several guinea pigs

trembled. According to the guideline of hypersensitivity reaction grades in guinea pigs, we evaluated the hypersensitivity grade of the two groups. When the grade >2, it can be considered that the agent used in hypersensitivity tests displays a positive reaction. Based on the results of Group A (grade = 3) and Group B (grade = 1), Group A (the blank commercial paclitaxel injection) caused a severe hypersensitivity reaction, while the hypersensitivity reaction of Group B (the blank paclitaxel microemulsion) was negative.

3.3. The qualification of HPLC method

The impurities in plasma did not interfere with the determination of the drug in the samples (Fig. 1). The t_R of palictaxel was about 9.8 min and the t_R of diazepam (Internal standard) was about 8.2 min. The detection limit was 0.02 μg ml^{-1}. There was a good linearity between C and Ap/Ad (Ap/Ad = 0.0957C+0.0166, $r = 0.9997$) ranging from 0.06 to 12 mg ml^{-1}. The recovery ranged from 90 to 110%. The R.S.D. of inter-day and between-day was 2.0–4.3 and 3.7–5.0%, respectively.

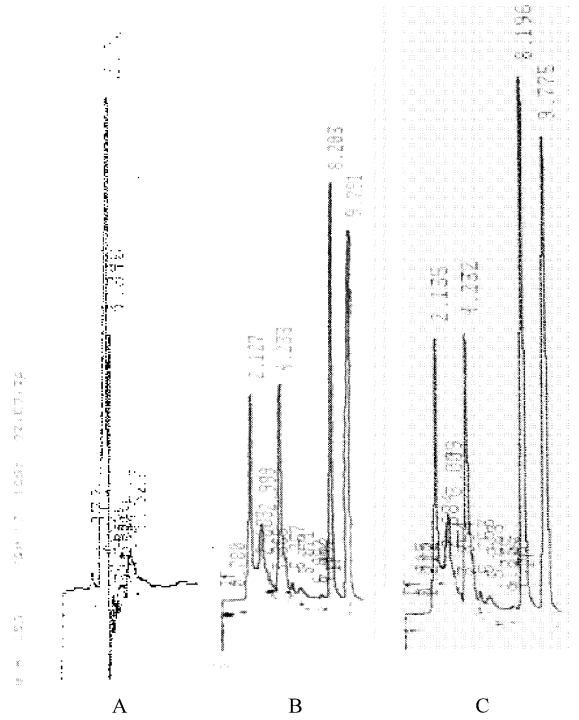

Fig. 1. Chromatograms of paclitaexl in plasma determined by HPLC method ((A) rat plasma blank; (B) after iv paclitaxel injection (C) after iv paclitaexl microemulsion).

3.4. The pharmacokinetic profile

The concentration–time curve after a single dose of paclitaxel microemulsion and injection in rats is shown in Fig. 2. Data fitting was conducted and the result showed that the pharmacokinetic behavior of paclitaxel microemulsion and injection fits a two-compartment model. The main pharmacokinetic parameters are listed in Table 1.

4. Discussion

Microemulsions belong to colloidal dispersion systems formed by 10–100 nm emulsion droplets dispersed in a dispersion medium. Microemulsion droplets are spherical, sometimes cylindrical with relatively uniform particle size. The microemulsion solution, always of homogeneous with uniform and transparent appearance, did not change, even when heated or centrifugated. It can be considered thermodynamically stable. So far, there is no consistent idea with regard to the nature and formation mechanism of microemulsions. Schulman and Prince proposed the hypothesis of instant negative interfacial tension formation, considering

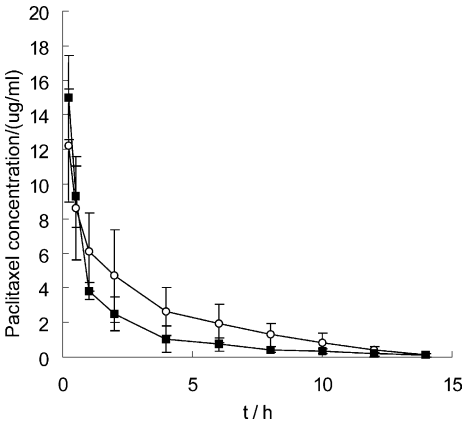

Fig. 2. Mean plasma concentration of paclitaxel after iv administration of paclitaxel injection (■) and paclitaxel microemulsion (○) in rats ($n = 5$).

Table 1
Comparison of pharmacokinetic parameters between paclitaxel injection and paclitaxel microemulsion

Parameter	Unit	Injection	Microemulsion
A	μg ml^{-1}	25.78±9.98	10.06±9.69*
α	h^{-1}	2.85±1.15	4.84±3.58**
B	μg ml^{-1}	2.99±1.97	8.23±6.07**
β	h^{-1}	0.22±0.07	0.24±0.11**
Vc	l	0.30±0.08	0.49±0.16*
$T_{1/2\alpha}$	h	0.27±0.09	0.21±0.13**
$T_{1/2\beta}$	h	3.46±1.50	3.30±1.13**
K_{21}	h^{-1}	0.51±0.26	3.08±4.04**
K_{12}	h^{-1}	1.27±0.87	1.44±1.75**
K_{10}	h^{-1}	1.29±0.16	0.60±0.25*
AUC	μg ml^{-1} h	21.85±6.14	34.98±12.60*
CL	l h^{-1}	0.38±0.09	0.25±0.09*

*, $P \leq 0.05$; **, $P > 0.05$. The data were mean±S.D. ($n = 5$). A and B, the hybrid parameter; α, distribution constant; β, elimination constant; Vc, apparent volume of distribution of the center compartment; $T_{1/2\alpha}$, half-time of phase I; $T_{1/2\beta}$, half-time of phase II; K_{21}, rate constant for drug leaving from compartment 2 to 1; K_{12}, rate constant for drug leaving from compartment 1 to 2; K_{10}, elimination constant of the center compartment; AUC, area under curve; CL, clearance from the two-compartment model.

interfacial tension plays an important role. Influenced by emulsifier and cosurfactant, the interfacial tension on the surface of microemulsion droplets is very low or even negative to ensure the stability of microemulsion system. However, some researchers do not agree with the above explanation (Lu, 1998).

Cremophor EL, a polyoxyethylene castor oil derivatives, is a formulation vehicle used for various lipophilic drugs, including the anticancer drug paclitaxel. However, Cremophor EL is not an inert vehicle, but exerts a series of biological and physiological effects involving clinical implication, such as severe hypersensitivity, hyperlipidaemia, abnormal lipoprotein patterns and peripheral neuropathy. The large amount of Cremophor EL in Taxol®, up to 0.5 ml Cremophor EL against 6 mg paclitaxel, has been reported to cause frequent and severe hypersensitivity during clinical application. The clinical adverse effects included vasodilation, dyspnea, hypotension, titillation and hives. It was reported that one volunteer without protection measures died in Phase I clinical trials. Furthermore, Cremophor EL can leach noxious substance from PVC infusion sets and necessitate the use of plasticizer-free containers or bags.

We successfully prepared a paclitaxel microemulsion with much less Cremophor EL, thereby decreasing the toxicity significantly, which was confirmed by the hypersensitivity test in guinea pigs.

From the Table 1, compared with Taxol®, the larger value of K_{10} of paclitaxel microemulsion indicates that, in rats, paclitaxel in microemulsion was eliminated slower from the central compartment than Taxol®. In addition, the area under curve (AUC) for Taxol® is smaller than that of microemulsion, and the CL is larger. These data suggest that with the same dosage, paclitaxel microemulsion can maintain a higher concentration during a longer period and thereby prolongs the circulation time of paclitaxel in rats. The pharmacokinetic profile of paclitaxel in microemulsion is influenced by the formulation characteristics. The drug molecules entrapped into O/W microemulsion droplets have to diffuse across the interfacial structure before they are released into system circulation. Furthermore, the small particle size of 10–50 nm enables the microemulsion droplets to escape from uptake and phagocytosis of RES. It has been reported that suitable modification of the surface of microemulsion droplets, such as a link to PEG–DSPE or PEG–PG, can improve the circulation time significantly (Zhang and Lu, 2001; Feng and Dexi, 1995). In order to achieve much longer circulation time as observed in these studies, further research will be conducted.

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