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Anti-tumor efficacy and biodistribution of intravenous polymeric micellar paclitaxel

Xichen Zhang,[1] Helen M Burt,[1,2] Gina Mangold,[3] Daniel Dexter,[3] Daniel Von Hoff,[3] Lawrence Mayer[2,4] and William L Hunter[1]

[1]Angiotech Pharmaceuticals, Inc., 6660 NW Marine Drive, Vancouver, BC, Canada V6T 1Z4. Tel: (+1) 604 221-7676; Fax: (+1) 604 221-2330. [2]Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, BC, Canada, V5Z 4E6. [3]Cancer Therapy and Research Centre, Institute for Drug Development, San Antonio, TX 78245, USA. [4]BC Cancer Agency, Vancouver, BC, Canada V5Z 4E6.

The purpose of this study was to evaluate the diblock copolymer poly(DL-lactide)-block-methoxy polyethylene glycol as an i.v. delivery vehicle for paclitaxel. Nude mice were implanted s.c. with fragments of MV-522 lung carcinomas and treated with paclitaxel on a daily×5 schedule when tumors were approximately 5×5 mm in size. Cremophor paclitaxel or polymeric micellar paclitaxel were given i.p. or i.v. at the maximum tolerated dose (Cremophor paclitaxel MTD: 20 mg/kg/day i.v. or i.p.; micellar paclitaxel MTD: 25 mg/kg/day i.v. or 100 mg/kg/day i.p.). The tumors were measured using callipers during the experiment and accurately weighted at the end. Two biodistribution studies were carried out. In one study, the nude mice were given micellar paclitaxel at a dose of 25 mg/kg i.v. or 100 mg/kg i.p. In another study, BDF-1 mice were given either micellar paclitaxel or Cremophor paclitaxel at a dose of 20 mg/kg i.v. The mice were sacrificed after a given time and the organs were harvested. Paclitaxel in the organs was extracted with acetonitrile and analyzed using HPLC. Tumor growth inhibitions of 98.5 and 98.7% were obtained from i.v. administered micellar paclitaxel and Cremophor paclitaxel at their MTDs, respectively. Micellar paclitaxel was more efficacious i.p. (98.7% tumor growth inhibition) than Cremophor paclitaxel i.p. (83.0% tumor growth inhibition) at their MTDs. The highest concentrations of paclitaxel were found in the liver after administration of paclitaxel formulations. Paclitaxel was also found in spleen, kidney, lung and blood, in order of decreasing concentration. The preliminary results indicate that polymeric micellar paclitaxel could be a clinically useful chemotherapeutic formulation.

Key words: Biodistribution, *in vivo* efficacy, micellar paclitaxel.

Introduction

Paclitaxel, a naturally occurring taxane which is extracted from *Taxus brevifolia*, has shown high activity against a wide range of tumors, and has been used clinically in the treatment of metastatic breast cancer, refractory ovarian cancer and several other malignancies.[1,2] Paclitaxel is a highly hydrophobic drug with very low water solubility. In order to enhance paclitaxel solubility, a mixture of 50:50 Cremophor EL® (a polyoxyethylated caster oil) and ethanol is used in the current cliical formulation. The formulation is diluted 5- to 20-fold with saline or other aqueous i.v. solutions before i.v. infusion. This results in the administration of significant amounts of Cremophor (about 80 mg Cremophor for 1 mg paclitaxel). Serious side effects (such as hypersensitivity and extraction of plasticizer from the i.v. infusion line) attributable to this agent have been observed.[3,4]

To circumvent these problems, a great deal of effort has been given to develop new systemic formulations for paclitaxel which are Cremophor-free and are based on an aqueous vehicle. Cosolvents (ethanol/polysorbate-80, polyethylene glycol or polyvinylpyrrolidone), oil-in-water emulsion, liposomes, cyclodextrins and surfactants (pluronic L64) have all been employed to enhance paclitaxel solubility.[5–8] Unfortunately, the problems of low paclitaxel solubility and drug precipitation upon dilution have not been completely resolved. We have developed polymeric micellar paclitaxel formulations using amphiphilic diblock copolymers of poly(DL-lactide)-block-methoxy polyethylene glycol (PDLLA–MePEG).[9] These copolymers form micelles of hydrophobic PDLLA cores and hydrophilic MePEG shells in water. A solid paclitaxel/polymer matrix is prepared containing up to 25% paclitaxel (250 mg paclitaxel/750 mg polymer) and solubilized to form a polymeric micellar paclitaxel system (maximum paclitaxel concentration 50 mg/ml) without precipitation upon dilution. In this paper, the *in vivo* anti-tumor efficacy and the biodistribution of polymeric micellar paclitaxel following i.v. and i.p. administration are reported.

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Correspondence to WL Hunter

Materials and methods

Materials

PDLLA–MePEG was synthesized from DL-lactide (PURAC, Lincolnshire, IL) and MePEG (Sigma, St Louis, MO) through ring opening bulk polymerization.[9] Paclitaxel was obtained from Hauser Chemicals (Boulder, CO). Paclitaxel formulation in Cremophor/alcohol (referred to as 'Cremophor paclitaxel') was purchased from Bristol-Myers Squibb (Princeton, NJ). Acetonitrile was obtained from Fisher Scientific (Nepean, Ontario, Canada). PDLLA-MePEG 2000–40/60 (i.e. molecular weight of MePEG: 2000, weight ratio of PDLLA to MePEG: 40/60) was used to formulate paclitaxel. Paclitaxel and the copolymer were dissolved in acetonitrile followed by evaporation of the solvent under a stream of nitrogen at 60°C for about 2 h. The resulting paclitaxel/copolymer matrix was solubilized by adding hot (60°C) water to the preheated matrix followed by vortexing. The paclitaxel loading was 10% by weight of the paclitaxel/copolymer matrix.

In vivo efficacy

Nude mice (female, type *nu/nu*, 17–20 g) were implanted s.c. by trocar with fragments (1 mm^3) of MV-522 lung carcinomas harvested from s.c. growing MV-522 tumors in nude mice hosts. When tumors were approximately 5×5 mm in size, the animals were pair-matched into treatment and control groups with 10 mice in each group. At the same day of grouping (day 1), the mice were treated with paclitaxel formulations or vehicle on a daily $\times 5$ schedule. Cremophor paclitaxel was given i.p. or i.v. at its maximum tolerated dose (MTD, 20 mg/kg/day). Polymeric micellar paclitaxel was also given i.p. (MTD 100 mg/kg/day) or i.v. (MTD 25 mg/kg/day). The MTDs were found in initial dosing experiments using tumor-free mice, where a criteria of 10–20% body weight loss without death was used to determine MTD. The tumor sizes were measured using callipers during the experiment and converted to tumor weight by equation of tumor weight = (length^2 × width)/2. The experiment was terminated when control tumors reached 1 g (day 25). The tumors were then excised and accurately weighed.

Biodistribution

In the first set of experiments, the nude mice were given micellar paclitaxel at a dose of 25 mg/kg i.v. or 100 mg/kg i.p. The mice were sacrificed at 5 and 30 min and 1.5, 3, 6, 12 and 24 h after the injection. Samples of plasma, liver, kidney, lung and spleen were harvested and stored at −20°C before analysis. In the second set of experiments, BDF-1 mice (female, 9 weeks, 20 g) were given either micellar paclitaxel or Cremophor paclitaxel at a dose of 20 mg/kg i.v. Samples of blood, liver, kidney, spleen, lung and muscle were collected at 15 and 30 min and 1, 4, 8 and 24 h after the injection.

Paclitaxel was extracted from the tissue sample using acetonitrile.[10,11] Typically, acetonitrile with a volume (ml) of no less than twice the tissue sample weight (g) was added to the sample. This was followed by homogenization using a Polytron homogenizer. The supernatant was separated by centrifugation. The paclitaxel concentration in the supernatant was analyzed directly using reverse-phase HPLC.[10,11] HPLC analysis was performed using a 110A pump and C-18 ultrasphere column (Beckman), and a SPD-6A UV detector set at 232 nm, a SIL-9A autoinjector and a C-R3A integrator (Shimadzu). The injection volume was 40 μl and the flow rate was 1.5 ml/min. The mobile phase was 50:50 acetonitrile and water. The peak area was used to calculate paclitaxel concentration in the supernatant, according to a standard calibration line obtained from pure paclitaxel 60:40 acetonitrile:water solutions. New calibration was done for every set of sample measurements. The interday variability of the calibration coefficient was less than 15%. The detection limit was about 1 μg/ml. The assay was linear in the range of 1–100 μg/ml.

The area under the curve (AUC) was calculated by the trapezoidal rule. The maximum paclitaxel concentration (C_{max}) in the tissue and the time for reaching the C_{max} (t_{max}) are taken directly from the biodistribution data.

Results

In vivo efficacy

The body weight changes of the nude mice are shown in Figure 1. The weight increased in the control groups to about the same extent (saline control +3.5% day 8, polymer vehicle control +5.5% day 8). In the paclitaxel-treated groups, the body weight decreased until day 8 and then increased. Micellar paclitaxel 100 mg/kg i.p. had greater weight loss (−24.8% day 8) than Cremophor paclitaxel 20 mg/kg i.p. (−4.2% day 8). After i.v. administration, the weight loss of micellar paclitaxel 25 mg/kg (−9.9% day 8) and Cremophor paclitaxel 20 mg/kg i.v. (−11.1% day 8) were

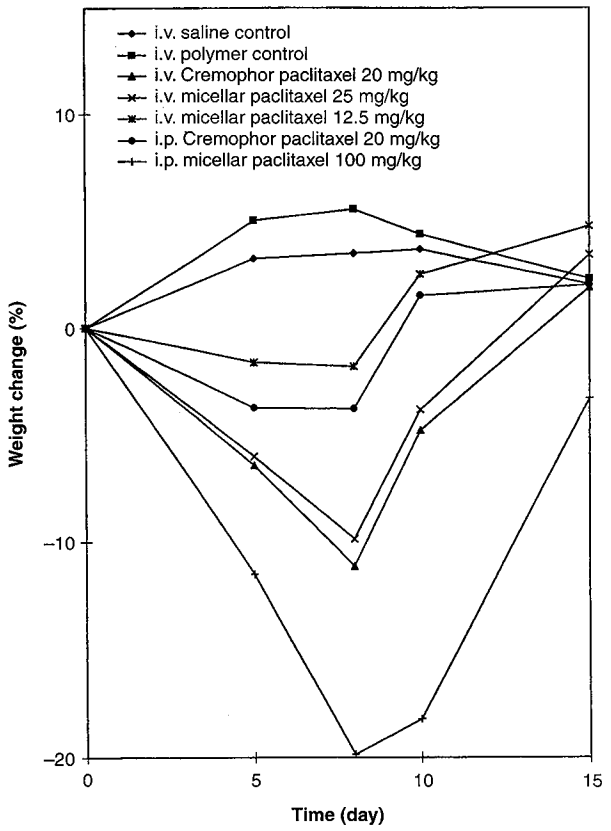

Figure 1. The average weight change of nude mice after administration of vehicle, Cremophor paclitaxel or micellar paclitaxel.

comparable, the weight loss of micellar paclitaxel 12.5 mg/kg i.v. was much lower (−1.8% day 8). One toxic death occurred in the i.p. 100 mg/kg group during the study (25 days). There was no death in the other groups. No sign of stress related to toxicity was observed in all the groups.

The anti-tumor efficacy results are given in Table 1 and Figure 2. Both micellar paclitaxel and Cremophor paclitaxel inhibited tumor growth, while the polymer vehicle alone did not inhibit tumor growth significantly ($p = 0.39$). Tumor growth inhibition was calculated as (1 − mean treated tumor weight/mean control tumor weight) × 100%. Some mice experienced tumor regression. With these mice, a mean percent tumor regression was calculated by (1 − final mean tumor weight/initial mean tumor weight) × 100%.

At the MTDs for i.v. administration, the observed tumor growth inhibition and tumor regression were the same ($p = 0.18$) for both Cremophor (98.7% inhibition, 71.2% regression) and micellar paclitaxel (98.5%, 73.2%). However, at the MTDs for i.p. Cremophor and micellar paclitaxel, both tumor growth inhibition and regression were significantly greater ($p = 0.020$) for the micellar paclitaxel (98.7%, 85.5%) compared to Cremophor paclitaxel (83.0%, 51.1%). The i.v. micellar paclitaxel at 1/2MTD (92.5%, 39.9%) was less efficacious than at MTD ($p = 0.024$).

Biodistribution

The results of paclitaxel biodistributions after i.v. 25 mg/kg or i.p. 100 mg/kg administration of micellar paclitaxel to nude mice are given in Figure 3 and Table 2. Paclitaxel was detected in tissue samples of plasma, liver, kidney, spleen and lung. After i.v. administration, paclitaxel concentrations decreased exponentially, while after i.p. administration, paclitaxel concentrations reached their peak in about 0.5 h. In both i.v. and i.p. routes, the highest paclitaxel concentration

Table 1. Efficacy of Cremophor paclitaxel and micellar paclitaxel against MV-522 human lung tumor xenograft (n=10)

Group	Dose/route	Final tumor weight (mg)	Tumor growth inhibition (%)	Mice with partial tumor regression	Mice with complete tumor regression	Tumor regression (%)
Control	saline/i.v.	702.6±127.9	0	0	0	—
Control	polymer/i.v.	548.2±117.9	24.9	0	0	—
Cremophor paclitaxel	20 mg/kg/i.p. (MTD)	119.5±42.3	83.0	6	0	51.1
Cremophor paclitaxel	20 mg/kg/i.v. (MTD)	9.5±3.5	98.7	5	5	71.2
Micellar paclitaxel	100 mg/kg/i.p. (MTD)	9.5±2.3	98.7	3	6	85.5
Micellar paclitaxel	25 mg/kg/i.v. (MTD)	10.9±3.4	98.5	8	2	73.2
Micellar paclitaxel	12.5 mg/kg/i.v.	52.4±13.9	92.5	6	2	39.9

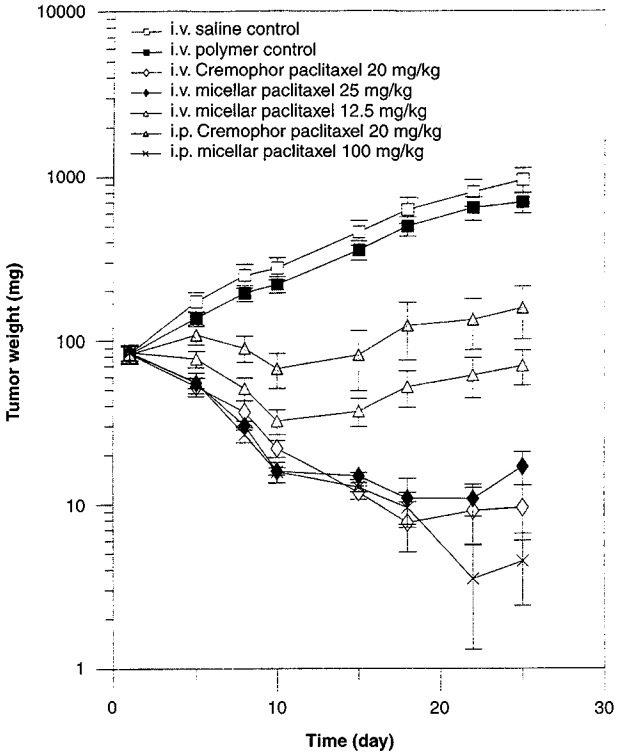

Figure 2. The weight change of MV-522 human lung tumor in nude mice after administration of vehicle, Cremophor paclitaxel or micellar paclitaxel.

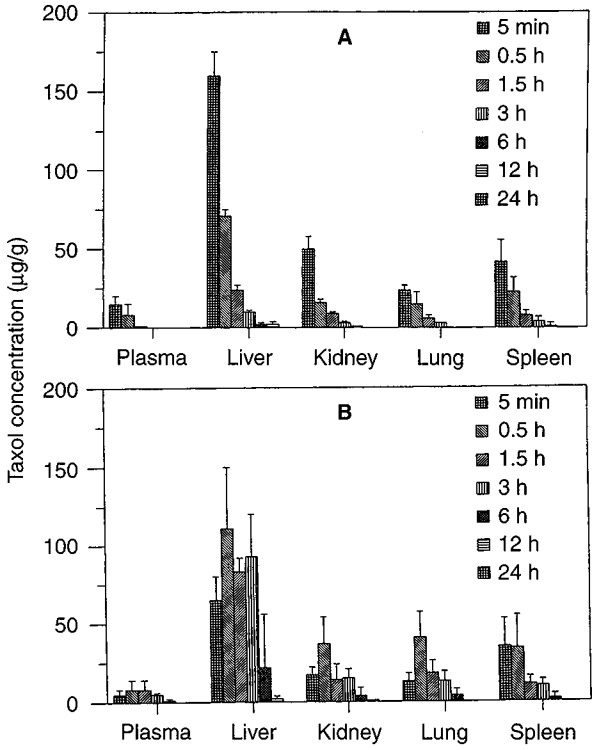

Figure 3. Tissue distribution of paclitaxel in plasma, liver, kidney, lung and spleen after i.v. 25 mg/kg injection (A) or i.p. 100 mg/kg injection (B) of micellar paclitaxel to nude mice ($n=3$).

and AUC values were found in the liver. The lowest paclitaxel concentrations and AUC were found in the plasma. For examples, after i.v. administration, the AUCs in plasma, liver, kidney, spleen and lung were 11.1, 159.3, 44.9, 49.8 and 29.5 μg·h/g, respectively. In the case of i.p. injection, the AUCs in plasma, liver, kidney, spleen and lung were 30.0, 522.3, 106.9, 78.9 and 103.7 μg·h/g, respectively. After dose adjustment, the $AUC_{i.p.}/AUC_{i.v.}$ were calculated as 67.6, 82.0, 59.5, 39.6 and 87.9% in the plasma, liver, kidney, spleen and lung, respectively.

The biodistribution of micellar paclitaxel and Cremophor paclitaxel after i.v. injection (20 mg/kg) in BDF-1 mice is shown in Figure 4 and Table 3. In both cases, paclitaxel concentrations in each tissue decreased exponentially. The half-lifes of micellar paclitaxel and Cremophor paclitaxel were about 1.2 and 0.8 h (calculated by semi-log regression), respectively. The highest concentration and AUC were found in the liver, followed by (in decreasing order) kidney, spleen, lung, blood and muscle. With the exception of the spleen, Cremophor paclitaxel resulted in higher AUC values than micellar paclitaxel in the corresponding tissue. For example, in blood, Cremophor paclitaxel gave 5.5 times higher AUC than micellar paclitaxel.

Table 2. The t_{max}, C_{max} and AUC of paclitaxel in nude mice after injection of micellar paclitaxel (i.v. 25 mg/kg or i.p. 100 mg/kg, $n=3$)

	Plasma	Liver	Kidney	Spleen	Lung
$AUC_{0.08-24}$, i.v., μg·h/g	11.1	159.3	44.9	49.8	29.5
$AUC_{0.08-24}$, i.p., μg·h/g	30.0	522.3	106.9	78.9	103.7
t_{max}, i.p., h	0.5	0.5	0.5	0.08–0.5	0.5
C_{max}, i.p., μg/g	8.5	110.6	36.8	34.0	41.4

Table 3. The $ACU_{0.25-24}$ (μg·hr/g) of paclitaxel in BDF-1 mice after i.v. injection of micellar paclitaxel or Cremophor paclitaxel (20 mg/kg, $n=3$)

	Plasma	Liver	Kidney	Spleen	Lung	Muscle
Micellar paclitaxel	7.4	274.4	72.3	103.7	55.1	11.9
Cremophor paclitaxel	40.6	361.9	138.7	85.4	128.5	30.6

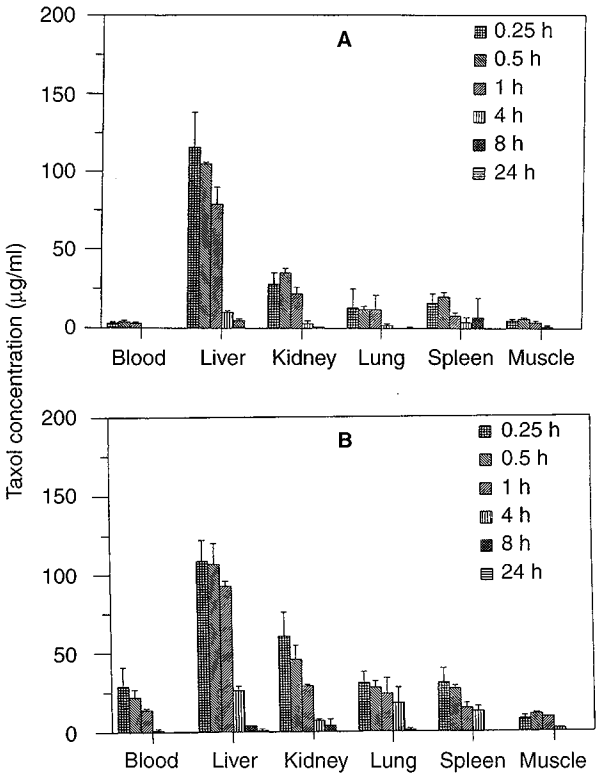

Figure 4. Tissue distribution of paclitaxel in blood, liver, kidney, lung, spleen and muscle after i.v. 20 mg/kg injection of micellar paclitaxel (A) or Cremophor paclitaxel (B) to BDF-1 female mice ($n=3$).

Discussion

Amphiphilic diblock copolymers can form micelles which are effective carriers for hydrophobic drugs.[9,12–21] Polyaspartic acid-block-polyethylene glycol has been studied extensively as a micellar carrier for adriamycin.[12–17] High drug carrying capacity, prolonged circulation time in blood and good anti-tumor efficacy have been shown with this drug delivery system.[12–18] Poly(DL-lactide)-block-methoxy polyethylene glycol has recently been evaluated as a micellar carrier for drugs.[9,18–21]

In this study, the potency of therapeutic efficacy between i.v. Cremophor paclitaxel and i.v. micellar paclitaxel were comparable. The MV-522 human lung carcinoma xenograft responded very well to i.v. administered micellar paclitaxel or Cremophor paclitaxel on a daily × 5 dosing schedule. All mice either experienced complete tumor growth inhibition or significant tumor regression at the MTD. Micellar paclitaxel was more efficacious at MTD than at 1/2MTD. Similar paclitaxel biodistributions were also observed from the formulations except the drug level in blood of micellar paclitaxel was lower (Figure 4). Cremophor and micellar paclitaxel formulations were comparably tolerated after i.v. injection, as indicated by the similar MTD values and body weight loss (Figure 1).

Intraperitoneal micellar paclitaxel at its MTD 100 mg/kg had better anti-tumor efficacy than i.p. Cremophor paclitaxel at its MTD 20 mg/kg. Although the weight loss was larger (Figure 1), the micellar formulation could be injected i.p. at a dose 5-fold higher than the Cremophor paclitaxel. The higher MTD of micellar paclitaxel may reflect less vehicle toxicity of the micellar paclitaxel or be due to slower adsorption of paclitaxel to the blood system.

Conclusion

In summary, comparable therapeutic efficiacy, potency and biodistribution behaviors between i.v. micellar paclitaxel and i.v. Cremophor paclitaxel were observed. Micellar paclitaxel was more effacious than Cremophor paclitaxel after i.p. injection at their MTDs. Currently, little is known about the toxic effects of the diblock copolymers and the correlation between toxicity and copolymer composition. Therefore, further studies are ongoing to identify copolymer compositions with low toxicity, long blood circulation time, high paclitaxel loading capacity and good stability of micellar paclitaxel.

References

1. Spencer CM, Faulds D. Paclitaxel: a review of its pharmacodynamic and pharmacokinetic properties and therapeutic potential in the treatment of cancer. *Drugs* 1994; **48:** 794–847.

2. Onetto N, Canett R, Winograd B, *et al.* Overview of paclitaxel safety. *J Natl Cancer Inst Monogr* 1993; **15:** 131–9.
3. Dorr RT. Pharmacology and toxicology of Cremophor EL diluent. *Ann Pharmacother* 1994; **28:** S11–4.
4. Goldspiel BR. Pharmaceutical issues: preparation, administration, stability and compatibility with other medications. *Ann Pharmacother* 1994; **28:** S23–6.
5. Strubinger RM. Biopharmaceutics of paclitaxel (paclitaxel): formulation, activity, and pharmacokinetics. In: Suffness M, ed. *Paclitaxel—science and applications.* Boca Raton, FL: CRC Press, 1995: 237–58.
6. Sharma A, Straubinger RM. Novel paclitaxel formulations: preparation and characterisation of paclitaxel-containing liposomes. *Pharmacol Res* 1994; **11:** 889–96.
7. Alkan-Onyuksel H, Ramakrishnan S, Chai HB, Pezzuto IM. A mixed micellar formulation suitable for the parenteral administration of paclitaxel. *Pharmacol Res* 1994; **11:** 206–12.
8. Dordunoo SK, Burt HM. Solubility and stability of paclitaxel: effects of buffers and cyclodextrins. *Int J Pharmac* 1996; **133:** 191–201.
9. Zhang X, Jackson JK, Burt HM. Development of amphiphilic diblock copolymers as micellar carriers of paclitaxel. *Int J Pharmac* 1996; **132:** 195–206.
10. Eiseman JL, Eddington ND, Leslie J, *et al.* Plasma pharmacokinetics and tissue distribution of paclitaxel in CD_2F_1 mice. *Cancer Chemother Pharmacol* 1994; **34:** 465–71.
11. Innocenti F, Danesi R, Paolo AD, *et al.* Plasma and tissue disposition of paclitaxel (paclitaxel) after intraperitoneal administration in mice. *Drug Metab Disp* 1995; **23:** 713–7.
12. Kataoka K, Kwon GS, Yokoyama M, Okano T, Sakurai Y. Block copolymer micells as vehicles for drug delivery. *J Controlled Rel* 1993; **24:** 119–32.
13. Kwon GS, Kataoka K. Block polymer micelles as long-circulating drug vehicles. *Adv Drug Del Rev* 1995; **16:** 295–309.
14. Kwon GS, Naito M, Kataoka K, Yokoyama M, Sakurai Y, Okano T. Block copolymer micelles as vehicles for hydrophobic drugs. *Colloids Surfaces B: Biointerfaces* 1994; **2:** 429–34.
15. Kwon G, Suwa S, Yokoyama M, Okano T, Sakurai Y, Kataoka K. Enhanced tumor accumulation and prolonged circulation times of micelle-forming poly(ethylene oxide-aspartate) block copolymer–adriamycin conjugates. *J Controlled Rel* 1994; **29:** 17–23.
16. Kwon GS, Naito M, Yokoyama M, Okano T, Sakurai Y, Kataoka K. Physical entrapment of adriamycin in AB block copolymer micelles. *Pharmacol Res* 1995; **12:** 192–5.
17. Yokoyama M, Okano T, Sakurai Y, Kataoka K. Improved synthesis of adriamycin-conjugated poly(ethylene oxide)–poly(aspartic acid) block copolymer and formation of unimodal micellar structure with controlled amount of physically entrapped adriamycin. *J Controlled Rel* 1994; **32:** 269–77.
18. Churchill JR, Hutchinson FG. Biodegradable amphiphilic copolymers. European Patent Application, 1986; no. 85304489.9.
19. Piskin E, Kaitian X, Denkbas EB, Kucukyavuz Z. Novel PDLLA/PEG copolymer micelles as drug carriers. *J Biomater Sci Polym Ed* 1995; **7:** 359–73.
20. Hagan SA, Dunn SE, Garnett MC, Davies MC, Illum L, Davis SS. PLA-PEG micelles—a novel drug delivery system. *Proc Int Symp Control Rel Bioact Mater* 1995; **22:** 194–5.
21. Hagen SA, Davis SS, Illum L, *et al.* Estimation of the poly(ethylene glycol) chain length of L-polyactide–polyethylene glycol in aqueous dispersions using viscoelastic measurements. *Langmuir* 1995; **11:** 1482–5.

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