Paclitaxel Solubility in Aqueous Dispersions and Mixed Micellar Solutions of Lecithin

Malgorzata Sznitowska,* Malgorzata Klunder, and Marcin Placzek

Department of Pharmaceutical Technology, Medical University of Gdansk; 80–416 Gdansk, Hallera 107, Poland.
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The aim of this study was to find a biocompatible, lecithin-based carrier for paclitaxel (PTX) suitable for intravenous infusion and ensuring a soluble PTX concentration of 100 mg/100 ml or higher for at least 24 h. Aqueous dispersions of egg or soya lecithin (water–lecithin dispersions, WLD), mixed micellar (MM) solutions of egg lecithin and sodium deoxycholate, and formulations containing lecithin plus the co-surfactants and co-solvents poloxamer, polysorbate, Span, benzalkonium chloride, and macrogol were investigated. Amorphous PTX was prepared by lyophilization. PTX co-lyophilized with surfactants was also studied. Unlike crystalline PTX, the drug in an amorphous form is easily soluble in 1—5% (w/w) WLD or in MM. The highest solubility (up to 570 mg/100 ml) was achieved in 5% WLD. Dissolved PTX precipitated from all tested formulations over 24 h. Despite this, concentrations of dissolved PTX of 100 mg/100 ml or higher were observed after 24 h in 5% egg WLD, 1—5% soya WLD, and in 5% MM (lecithin : deoxycholate ratio 1 : 1 w/w). When four different batches of 5% egg WLD were prepared, containing PTX in clinically relevant concentration of 100 mg/100 ml, no precipitation of PTX was observed within 24 h and this formulation is the most promising candidate for further *in vivo* studies. Neither additional surfactants nor co-lyophilization increased PTX solubility in the lecithin-based carriers. The use of parenteral emulsions as solvents for the co-lyophilized PTX also failed to increase the solubility of the drug up to the target concentration.

Key words paclitaxel; lecithin; mixed micelle; lyophilization

Paclitaxel (PTX) is a very effective antineoplastic agent used to treat a variety of tumors. It was discovered in the 1960s in a screening study by the National Cancer Institute designed to develop new natural substances with anticancer activity. PTX brought much hope to people with cancer, but its poor solubility in water and insufficient supply have slowed clinical studies of the drug.[1)]

After much investigation, an appropriate PTX formulation containing Cremophor EL (ethoxylated castor oil) and dehydrated ethanol (1 : 1 v/v) and suitable for intravenous infusion was found. The Bristol-Myers Squibb Company owned the first patent on a PTX formulation that was introduced to the market in 1993 under the name Taxol. At present, as the patent has expired, many generic products are on the market, all containing Cremophor EL. This solubilizing agent ensures sufficient drug solubility and stability but evokes many severe side effects.[2,3)] Cremophor EL is responsible for acute hypersensitivity reactions that affect about 30% of patients. It also contributes to peripheral neuropathy and leaches the plasticizers from polyvinyl chloride (PVC) bags and infusion sets. As a result, PTX solutions must be prepared and administered in non-PVC infusion systems with in-line filtration, and pre-medication must be given to patients. These issues have provoked considerable interest in the development of taxanes with improved aqueous solubility and in formulations devoid of Cremophor EL.

Increased solubility of the drug, which is necessary for intravenous delivery, can be achieved by, for example, using co-solvents or preparing an emulsion,[1,4)] by modification of the PTX molecule into prodrugs[5,6)] or analogs,[7,8)] and by preparing liposomes[4)] or micelles.[9)] However, none of these formulations has been introduced to clinical practice yet, due to the lack of sufficient biocompatibility to meet the requirements of intravenous preparations. However, in 2005 the FDA (Food and Drug Administration) approved Abraxane for injection (Abraxis BioScience), the first alternative to the Cremophor-containing preparation. It is a nanosuspension of PTX conjugated with human albumin.[10)] Despite its better clinical profile, Abraxane is generally not replacing Taxol in oncological practice, mostly due to its high cost. Hence, further research into alternative formulations suitable for parenteral infusion is justified.

The aim of our study was to develop a new PTX formulation using excipients that are already in use for parenteral administration. Since PTX must be given to the patients as an infusion, the choice of suitable carriers is very limited. Unfortunately, neither submicron, lecithin-based emulsions nor liposomes ensure appropriate PTX solubility and stability. For example, the solubility of PTX in soybean oil, which is used in parenteral emulsions, is about 180 mg/100 g, and this allows preparation of an emulsion with PTX at a concentration of only 18 mg/100 ml, if the oil content in the emulsion is 10%. PTX is soluble in some types of emulsions, but they generally either contain toxic ingredients or are insufficiently stable. Vitamin E emulsions were recently proposed as suitable and possibly non-toxic carriers for PTX that offer high drug solubility (1000 mg/100 ml).[11,12)]

PTX is administered in doses of 135 mg/m^2 (24-h infusion) or 175 mg/m^2 (3-h infusion). The latter dose is more often recommended, and equals about 300 mg of PTX for an adult patient. Taxol and its generics are sold as concentrates containing 6 mg of PTX in 1 ml. The concentrate is diluted ex tempore with 0.9% sodium chloride or 5% glucose for infusion, to concentrations of 30—120 mg/100 ml. Thus, depending on concentration, volumes of 250—1000 ml are required for an individual infusion. Another serious drawback of the PTX formulation is that the diluted concentrate is stable for a maximum of 27 h, due to recrystallization of the drug.

Our goal was to find a suitable carrier for PTX that would

* To whom correspondence should be addressed. e-mail: msznito@amg.gda.pl

allow its intravenous administration as an infusion, with a concentration of dissolved drug of at least 100 mg/100 ml maintained for at least 24 h. Lecithin, a non-toxic and biocompatible substance, was chosen as the main solubilizing agent. Lecithin is very well tolerated when given intravenously in the form of parenteral emulsions, in which its concentration is usually 1.2% (w/v), or sometimes 2.4% (w/v). Patients receive up to 6 g of lecithin per day, even in long-term parenteral nutrition. Unfortunately, as mentioned above, PTX is insufficiently soluble in parenteral soybean emulsion, so we investigated other systems containing lecithin, namely, aqueous lecithin dispersions (WLD) and mixed micellar solutions (MM), as potential carriers for PTX. MM systems composed of egg lecithin and cholic acids are already in clinical use as carriers for fat-soluble vitamins (Cernevit) and vitamin K (Konakion MM). Cholic acids are combined with lecithin in order to solubilize the latter. Lecithin does not dissolve completely in water, but in our previous study we found that lecithin alone, as an aqueous dispersion, is a solubilizing carrier for some substances.[13] WLD can be prepared in a simple process and its physicochemical characteristics indicate that it may be suitable for parenteral delivery.

PTX is a crystalline substance, but for the purpose of our study we used amorphous PTX, which we prepared according to a patented method that includes freeze-drying.[14] We also attempted to increase the solubility of PTX by lyophilizing it with the solubilizing agents.

Experimental

Materials and Reagents PTX was donated by Warsaw Pharmaceutical Works "Polfa" (Poland). Its identity and purity were certified. The main excipients were egg lecithin (Lipoid E80, Lipoid, Ludwigshafen, Germany) and soya lecithin (Lipoid S100, Lipoid, Ludwigshafen, Germany). Additional excipients used to increase the solubility of the drug were sodium deoxycholate, macrogol 6000 (PEG 6000), polysorbate 80 (Tween 80), Span 80 (all from Sigma-Aldrich, Steinheim, Germany), poloxamer (Synperonic F68) (Serva Electrophoresis, Heidelberg, Germany), and benzalkonium chloride (FeF Chemicals, Køge, Denmark). 1,4-dioxan was obtained from Merck (Darmstadt, Germany). Lyophilizates were dissolved in 10% and 20% Ivelip parenteral emulsion (Baxter Poland, Warsaw, Poland). Highly purified water was produced in an Elix 3 apparatus (Millipore, Bedford, U.S.A.). All organic solvents used for the chromatographic analysis were HPLC grade, and purchased from POCh (Gliwice, Poland) or Merck (Darmstadt, Germany). Amorphous PTX was prepared according to the patented method.[14] Shortly, 30 mg of PTX was dissolved in 8.0 ml of dioxan, then 2.0 ml of water was added and the solution was frozen and lyophilized.

Preparation of Non-lyophilized Carriers Containing Lecithin or Lecithin and Sodium Deoxycholate Dispersions of egg or soya lecithin (10% and 5% w/w WLD) in an isotonic solution of glycerol (2.76%) were prepared. The lecithin was first dispersed in water at 60 °C by use of a thermostated magnetic stirrer (IKA Werke, Staufen, Germany). Next, the dispersion was stirred for 2 min at the same temperature with a high-shear mixer (Ultra-Turrax, Janke & Kunkel, Staufen, Germany) and sonicated at 20 kHz for 10 min (Ultrasonic disintegrator UD-20, Techpan, Pulawy, Poland). The dispersion was filtered through a 0.8 mm cellulose acetate filter (Sartorius AG, Goettingen, Germany), dispensed into vials in a nitrogen atmosphere, and sterilized by autoclaving (121 °C, 15 min). Typically the batch size of WLD was 200 ml.

WLD containing 1% or 3% lecithin were prepared by diluting the 5% (w/w) WLD with isotonic glycerol. In addition, a 3% (w/w) WLD containing 0.75% (w/w) Span 80 was prepared by dissolving the surfactant in WLD.

Formulations containing egg lecithin and sodium deoxycholate (MM) were prepared by mixing suitable amounts of 10% (w/w) sodium deoxycholate solution and 10% (w/w) egg WLD. The vials were shaken gently and water was added as necessary. The ratios of the mixed excipients in the final preparations are given in Tables 1 and 2.

Preparation of Lyophilized Formulations Surfactants or PEG were dissolved in water to obtain the following solutions (% w/w): 10% and 30% sodium deoxycholate, 10% PEG 6000, 6.6% poloxamer, 0.5% benzalkonium chloride, and 10% Span 80.

Suitable amounts of the solutions were added to a vial containing an aqueous dispersion of egg or soya lecithin (10% or diluted WLD) and shaken gently. The ratios of mixed excipients are given in Tables 1—3. If necessary, water was added to obtain 5 ml of the mixture. A solution of PTX (2.5 ml) in dioxan was slowly added. The mixed solutions were shaken carefully, and freeze-dried.

Formulations without PTX were also prepared. They contained egg lecithin, egg lecithin and sodium deoxycholate, and egg lecithin and Span 80. They were prepared as described above, without adding the organic solution of the drug in the final step.

Solubility Studies Amorphous PTX was dissolved in an isotonic egg (3%, 5%) or soya (1%, 5%) WLD. Crystalline PTX was dissolved in an isotonic dispersion of egg lecithin (3%, 5%, 10%) or in a mixed micellar solution (MM) composed of egg lecithin and sodium deoxycholate. Typically the amount of PTX was 60 mg or 100 mg and 15 ml of the liquid was added. When the solvent was added, the suspension was mixed by shaking and rotating gently a vial for 5 min. The amount of solubilized drug was determined by sampling the dispersion after this time ($t=0$) and after 6 and 24 h. During this period, the vials were shaken gently in a water bath shaker at room temperature (20±2 °C). To separate undissolved PTX, the dispersions were centrifuged at 930×$\boldsymbol{g}$ for 5 min (WF6, Zaklady Mechaniki Precyzyjnej, Warsaw, Poland), and the supernatant was sampled.

If the excipients without PTX were lyophilized, the lyophilized powders were dissolved first in an appropriate solvent and then amorphous PTX was added. Water (or water with glycerol), a 1% solution of poloxamer, and a 1.7% solution of Tween 80 were used to reconstitute the lyophilizate (Table 2).

The solvents for PTX co-lyophilized with excipients were water, an isotonic solution of glycerol (2.76%), a 1% solution of poloxamer, and 10% and 20% Ivelip parenteral emulsion. After reconstitution, the samples were shaken and treated as described above.

The solubility of PTX in each investigated system was measured in at least two separate experiments.

Assay of the Solubilized Paclitaxel The amount of dissolved PTX was determined by using HPLC (Merck Hitachi apparatus, Darmstadt, Germany). The supernatant was diluted with a mobile phase consisting of acetonitrile, methanol, and water (58 : 5 : 37 w/w). Each sample was analyzed on a column at least twice. A Vydac (Hespera, U.S.A.) C18 column, 4.6 mm×250 mm with a 5 mm pore size, was used. The flow rate of the mobile phase was 1 ml/min. Detection was by UV absorption measurement (UV–Vis detector: type L-4250, Merck Hitachi, Darmstadt, Germany) at 228 nm. The peak areas were measured by interfacing the detector to an integrator (D-2500A, Merck Hitachi, Darmstadt, Germany). The concentration of PTX (retention time 3.5 min) was calculated from standard curves. The method was validated, and its linearity ($r^2=0.9999$) and reproducibility ($s=2.63\%$) were satisfactory.

Microscopic Observation All formulations were observed using a microscope (Motic B1-220A, Centrum Mikroskopii, Warsaw, Poland) to determine the changes in shape and diameter of the dispersed particles. The microscope was interfaced with a camera (Panasonic, GP-KR222E, Matsushita Communication Industrial, Japan) and a computer running MultiScanBase v. 12.07 software (Computer Scanning System, Warsaw, Poland).

Results and Discussion

Solubility of PTX in WLD The solubility of crystalline PTX in WLD is very low, and the substance dissolves slowly (Table 4). After 24 h, depending on the lecithin concentration, only 29—45 mg/100 ml concentrations of dissolved PTX were achieved. Even lower solubility of crystalline PTX than that attained in egg WLD was observed in an MM system composed of egg lecithin and sodium deoxycholate (Table 1).

In contrast to the crystalline form, amorphous PTX dissolved very quickly in both systems and the solubility determined after short shaking (5 min) was 90—583 mg/100 ml (Tables 1, 4). The amorphous form of PTX can be obtained

(according to the patented method) by freeze-drying a solution of PTX in a mixture of dioxan and water.[14] As observed under the microscope, the particles in the lyophilized powder are amorphous, irregular, and 3—5 μm in size. Microcalorimetric studies and electron microscopy observations have also demonstrated the amorphous nature of the substance.[14]

The initially high concentration of the dissolved amorphous drug decreased when the observation was continued for up to 24 h (at ambient temperature, with shaking), what demonstrates the instability of the saturated systems. For example, the initial concentration of PTX dissolved in 5% WLD prepared with soya lecithin was about 570 mg/100 ml for at least 6 h but decreased to 103.5 mg/100 ml after 24 h, and precipitation of PTX in the form of long needles was observed (microscopic observation). However, after 24 h, in all investigated WLD systems, the solubility of the amorphous PTX was maintained at a concentration of approximately 100 mg/100 ml or higher (Table 4).

Table 1. The Effect of the Mixed Micellar System Composed of Egg Lecithin (eL) and Sodium Deoxycholate (DCh) on Aqueous Solubility [mg/100 ml] of Amorphous, Crystalline or Co-lyophilized Paclitaxel

Paclitaxel	eL : Dch ratio (final concentration)	Time		
		$t=0$	6 h	24 h
Crystalline	1%–1%	—	14.5	9.8
	2%–0.5%	—	10.7	8.0
Amorphous	2%–2%	128.8±13.5	114.4±13.0	17.2±9.3
	3%–0.75%	17.3	18.2	10.4
Co-lyophilized[a)]	0.5%–0.5%	74.9	69.6	31.3
	1%–0.2%	101.8	8.4	7.1
	1%–0.5%	109.4	87.5	44.9
	1%–0.8%	126.4±10.2	54.7±15.1	61.5±17.3
	1%–1.5%	115.5	89.3	25.5
	1.6% : 1.6%	160.5	139.8	28.5
	2% : 2%	174.8±20.2	151.5±19.2	24.1±13.3
	2.5%–2.5%	173.4	151.0	109.8

Average±standard deviation ($n=3$—5) or the average of 2 experiments is given (the individual values from two experiments were within the range not exceeding 30% of the mean value). *a*) Total concentration of the co-lyophilized paclitaxel in the reconstituted system was 0.2%.

The 5% WLD formulations prepared with either egg lecithin or soya lecithin exhibited similar solubilizing potential towards amorphous PTX, when the initial solubility was compared (Table 4). However, after 24 h the PTX solubility in 5% egg WLD was still high (475 mg/100 ml), while re-crystallization of PTX in 5% soya WLD resulted in a large decrease in concentration of the dissolved drug (103 mg/100 ml). On the other hand, amorphous PTX was more soluble in 1% soya WLD than in 3% egg WLD. The chemical composition of soya and egg lecithins is different, with higher amount of phosphatidylcholine and smaller concentration of total unsaturated fatty acids in the latter,[15,16] and this chemical diversity is the most probable reason that, depending on concentration, different structures may be present in the investigated egg and soya WLD, what may influence the solubilization potential. However, the nature of these variations is unknown.

Solubility of PTX in MM As with other anticancer drugs, slow infusion of a diluted PTX solution is required to avoid severe toxic effects. This is why the drug is administered in volumes larger than are required for injections. Consequently, the infusion fluid should be biocompatible, and high concentrations of synthetic surfactants or novel excipients are not acceptable. Lecithin-based preparations, even with small amounts of added co-surfactants or co-solvents, are carriers that can be regarded as safe and non-toxic, especially when the drug is intended only for incidental administration (a maximum of six infusions with 21-d intervals). Sodium deoxycholate, polysorbate, benzalkonium chloride, poloxamer as well as PEG were combined with lecithin to improve solubility of PTX.

The solubility of amorphous PTX in MM depended on the ratio of lecithin to deoxycholate. In MM composed of egg lecithin and deoxycholate in equal concentrations 2% (w/w) the initial solubility of PTX was about 128 mg/100 ml, while only 17.8 mg/100 ml of PTX dissolved in MM prepared with 3% egg lecithin and 0.75% deoxycholate (Table 1). The solu-

Table 2. The Effect of Solvent Used for Reconstitution of Lyophilized Excipients on Solubility [mg/100 ml] of Paclitaxel When Used as an Amorphous (A) or Co-lyophilized (L) Substance (for the Comment Regarding Reproducibility See Table 1)

Excipients (final concentration)	A/L	Time	Water	2.25% Glycerol	1% Poloxamer	1.7% Polisorbat	10%[c)] Emulsion
Egg lecithin	A	$t=0$	—	n.s.[d)]	—	—	n.s.
(3%)		6 h	13.5	n.s.	27.6	38.9	n.s.
		24 h	12.4	n.s.	32.3	36.0	n.s.
Egg lecithin and deoxycholate	A	$t=0$	—	n.s.	—	—	n.s.
(3%–3%)		6 h	56.6	n.s.	61.7	57.6	n.s.
		24 h	55.1	n.s.	53.2	29.5	n.s.
Soya lecithin and deoxycholate	L[a)]	$t=0$	164.6	163.1	n.s.	n.s.	170.5
(2.5%–1.25%)		6 h	163.3	161.5	n.s.	n.s.	154.9
		24 h	17.9	11.6	n.s.	n.s.	0.19
Egg lecithin and Span	A	$t=0$	—	n.s.	—	—	n.s.
(3%–0.75%)		6 h	17.1	n.s.	25.6	37.9	n.s.
		24 h	14.0	n.s.	27.9	33.8	n.s.
Soya lecithin and poloxamer	L[b)]	$t=0$	n.s.	1.6	1.3	n.s.	29.2 (34.6)
(3.3%–0.66%)		6 h	n.s.	1.3	1.1	n.s.	30.4 (36.4)
		24 h	n.s.	1.2	1.2	n.s.	36.3 (38.0)
Egg lecithin and poloxamer	L[b)]	$t=0$	49.6±20.5	36.5	28.1	n.s.	42.5±5.3
(3.3%–0.66%)		6 h	54.3±10.5	47.3	40.7	n.s.	45.1±10.8
		24 h	51.0±9.5	49.4	33.1	n.s.	48.3±12.3

a) Total concentration of the co-lyophilized paclitaxel was 0.2%. *b*) Total concentration of the co-lyophilized paclitaxel was 0.1%. *c*) In parentheses solubility in 20% emulsion is given. *d*) n.s., not studied.

tions of amorphous PTX in MM were thermodynamically unstable over 24 h and decrease of the concentration of the dissolved drug, with precipitation even faster than in WLD, was observed. For example the initial solubility of PTX 128 mg/100 ml decreased to 17 mg/100 ml within 24 h.

In order to achieve better solubility, PTX was co-lyophilized with various ratios of egg lecithin and sodium deoxycholate and the dry substance was dissolved in water, with the final concentrations of lecithin and deoxycholate within the range 0.5—2.5% and 0.2—2.5%, respectively (Table 1). When the concentration of lecithin in the reconstituted MM was 1%, the initial solubility of PTX was in a narrow range between 100—125 mg/100 ml, irrespective of the amount of deoxycholate added (0.2—1.5%). Increasing the concentration of lecithin in the MM from 1.0% up to 1.6—2.5% led to a 1.5-fold increase in PTX solubility (from 101—126 to 160—175 mg/100 ml). The most promising MM system was composed of 2.5% lecithin and 2.5% deoxycholate: it maintained a soluble concentration of PTX of at least 110 mg/100 ml for 24 h. Comparison of the results obtained for the formulations containing 2% egg lecithin and 2% deoxycholate indicate that co-lyophilization improved the solubility of PTX in MM only slightly (Table 1).

The Effect of Other Co-surfactants and Solvents Egg lecithin was not only combined with sodium deoxycholate in an MM system, but was mixed with other co-surfactants and co-solvents and lyophilized with PTX. The results for systems containing poloxamer, PEG, or benzalkonium chloride are presented in Table 3. Table 3 also presents the solubility of PTX co-lyophilized with lecithin alone. The solubility of co-lyophilized PTX was low, not exceeding 54 mg/100 ml, with the lowest solubility in the presence of a small amount of the cationic surfactant benzalkonium chloride. It is concluded that co-lyophilization of PTX with lecithin alone or together with sodium deoxycholate, poloxamer, or Span did not improve the solubility of PTX or the stability of the solution (Tables 1, 3).

Neither the solubility of PTX nor the stability of the solution was satisfactorily improved by dissolving the lyophilizates and co-lyophilizates in solvents other than water, accepted for administration as intravenous infusion, namely: water with glycerol, diluted poloxamer or polysorbate solutions, or parenteral fat emulsions (Table 2). Similar results were obtained when the solvent was water or water with glycerol (isotonic solution). The use of 1% poloxamer or 1.7% polysorbate as the solvent for reconstitution of the lyophilizate increased the solubility of PTX in the reconstituted solutions of egg lecithin and egg lecithin with Span. The final solubility was still low, however, not exceeding 39 mg/100 ml. The very low solubility of amorphous PTX in 3% egg WLD resulted from the fact that, in this case, the WLD was prepared *ex tempore* from lyophilized egg lecithin.

When compared with water, neither the poloxamer nor the polysorbate solutions used for reconstitution increased the solubility of PTX in the MM system or in co-lyophilizates with lecithin and poloxamer. Precipitation of the dissolved PTX was delayed, however, in the presence of poloxamer (Tables 2, 3), confirming the fact that this polymer effectively stabilizes nanodispersed systems.[17] On the other hand, the type of lecithin influenced the solubility of co-lyophilized PTX in the presence of poloxamer. When the results obtained in solutions prepared with isotonic glycerol as a solvent are considered, the solubility of PTX in the egg lecithin–poloxamer and soya lecithin–poloxamer systems was 36 mg/100 ml and 1.6 mg/100 ml, respectively (Table 2).

Interestingly, parenteral emulsion used as a solvent did not allow for increased solubility of PTX co-lyophilized with the MM system or the egg lecithin and poloxamer. A significant difference was observed, however, when the emulsion was used for dissolving co-lyophilizates prepared with soya lecithin and poloxamer. However, the solubility was still low, not exceeding 38 mg/100 ml, with only slight further improvement when 20% parenteral emulsion was added. In a separate experiment, the solubility of amorphous PTX

Table 3. Solubility [mg/100 ml] of Paclitaxel (PTX) Co-lyophilized with Egg Lecithin (eL) and Other Surfactants or PEG (X) Determined after Reconstitution in Water (for the Comment Regarding Reproducibility See Table 1)

Co-surfactant	PTX : eL : X (%)[a)]	Time		
		$t=0$	6 h	24 h
None	0.1 : 3.3 : 0	51.7	51.1	54.6
Poloxamer	0.1 : 3.3 : 0.66	49.6±20.5	54.3±10.5	51.9±9.5
	0.1 : 3.3 : 0.16	42.0	43.9	50.7
PEG	0.1 : 3.3 : 1.0	22.3	23.5	17.3
Benzalkonium chloride	0.1 : 3.3 : 0.01	12.4	8.4	5.4

a) Concentration in reconstituted formulation.

Table 4. Solubility [mg/100 ml] of Amorphous and Crystalline Paclitaxel in Aqueous Dispersions (WLD) of Egg and Soya Lecithin (for the Comment Regarding Reproducibility See Table 1)

Lecithin concentration	Amorphous paclitaxel			Crystalline paclitaxel		
	Time			Time		
	$t=0$	6 h	24 h	$t=0$	6 h	24 h
Egg lecithin WLD						
3%	90.1±13.3	92.2±19.9	90.2±19.0	6.2	20.2	28.9
5%	511.5±31.9	583.6±39.3	475.4±69.8	n.s.	19.3	38.1
10%	n.s.[a)]	n.s.	n.s.	n.s.	19.4±2.2	45.5±8.1
Soya lecithin WLD						
1%	211.0±30.2	182.3±15.5	155.5±22.5	n.s.	n.s.	n.s.
5%	579.8±25.1	565.7±40.1	103.5±8.0	n.s.	n.s.	n.s.

a) n.s., not studied.

in a 20% submicron emulsion was determined to be 155 mg/100 ml, decreasing to 40 mg/100 ml after 24 h.

The target solubility, *ca.* 100 mg/100 ml, was achieved initially in a few preparations, but it was maintained for at least 24 h only in 3—5% egg WLD, 1—5% soya WLD, and a co-lyophilized MM formulation (2.5% egg lecithin and 2.5% deoxycholate). Among these, the WLD formulations are the most promising, as the presence of sodium deoxycholate does not further improve PTX solubility and the results indicate that, depending on the lecithin–deoxycholate ratio, the solubility of PTX may even be reduced in MM in comparison to WLD formulations (Tables 1, 4). Since the intravenous dose of deoxycholate should be limited (LD_{50} in rats 150 mg/kg), the use of lecithin alone as a solubilizing excipient is advantageous.

The precipitation of dissolved PTX observed in WLD formulations occurs when the solution is supersaturated. Such formulations were prepared in the presence of an access of PTX. However, if the initial concentration of PTX in WLD is only 100 mg/100 ml, the system is not saturated and no change in the concentration occurs within at least 24 h, what was confirmed in separate experiments, when PTX was dissolved at this concentration in four different batches of 5% egg WLD. Thus, using WLD the PTX solution at concentration required for clinical practice can be prepared and no danger of crystallization during infusion is expected.

WLD prepared by the proposed method is a yellowish, non-transparent homogenous liquid, free of undissolved particles, when visually inspected. Using a laser diffractometer, we detected particles in the range of 0.05—2 μm. Generally, particles larger than 5 μm are believed to cause adverse reactions in particular emboli in lungs, however even larger lipid particles present in parenteral emulsions are not considered as dangerous.[18] Thus the range of the particle size observed in WLD is not a limitation for the future use *in vivo*. In the literature no data is found neither on application of WLD as a drug carrier nor on the type of the particles present in the dispersion prepared with the method proposed for WLD. Our preliminary electron microscopic observation revealed the presence of structured, aggregated particles (data not presented). Neither the more detail structure of the particles, nor the mechanism of solubilization of PTX in WLD has not been revealed so far. The system requires further structural studies, but the results presented in this article as well as good tolerance of the infusion in rabbits in a pilot study (unpublished data) indicate that WLD is a promising formulation for intravenous administration of PTX.

Conclusions

The study achieved the aim of maintaining the solubility of PTX at least 100 mg/100 ml, when egg or soya WLD was used as a carrier for the amorphous drug. The immediate solubility of PTX in 5% egg WLD is almost five times higher than concentration actually required in clinical practice (namely 100 mg/100 ml) and in several batches of the preparation no precipitation of PTX was observed during 24 h, when solutions containing PTX at this concentration were prepared. The resulting formulation is suitable for intravenous infusion because (1) it uses a low and clinically accepted concentration of lecithin as the biocompatible solubilizing agent, (2) it lacks particles larger than 5 μm, and (3) it is physically stable for at least 24 h. Broader physicochemical characterization of the WLD is necessary, however, to confirm its suitability for clinical use.

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