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Report

A New Parenteral Emulsion for the Administration of Taxol

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Taxol (NSC-125973) is a poorly soluble plant product that exhibits excellent antimitotic properties. This study involves the development of a new formulation for taxol. The stability of taxol in a 50% triacetin emulsion as well as possible methods of intravenous administration of this dosage form was examined. A stable emulsion was found at taxol concentrations of 10 and 15 mg/ml of emulsion. The 50% triacetin emulsion showed an intravenous LD50 of 1.2 ml/kg in Swiss-Webster mice. The 10 mg/ml taxol formulation was demonstrated to be stable upon addition to 5% dextrose iv fluids provided that small packing systems were used. The taxol–triacetin emulsion can also be intravenously injected at various rates, and it may prove to be a useful formulation for taxol.

KEY WORDS: taxol; formulation; water-soluble emulsion; toxicity of triacetin.

INTRODUCTION

Taxol is a poorly water soluble plant product isolated from several species of Western yew. It exhibits excellent antimitotic properties and is presently undergoing phase I clinical trial in cancer treatment. Taxol shows confirmed activity against several tumor systems including leukemia, colon, melanoma, sarcomas, and Lewis lung tumor systems (1–3). In addition to its antitumor activity, taxol may also be helpful as a tumor cell synchronizing agent. The mechanism of its antimitotic activity appears to be due to its ability to alter normal microtubule assembly and thus cause mitotic arrest (4).

At present intravenous dosage formulations of taxol consist of ethanol:cremophor EL:isotonic saline (5:5:90). The drug's solubility in this vehicle does not exceed 0.6 mg/ml, and it remains physically stable only for a short time (3 hr) (5). Therefore, large volumes of these formulations, with limited solubility, would have to be infused to obtain a desired dose of 30 mg. Another undesirable effect of a vehicle containing cremophor EL is that it can produce an anaphylactoid reaction in certain individuals (6–8).

Commonly, water-miscible cosolvents are used as a method of formulating intravenous non-water-soluble drugs. However, often the drugs precipitate upon the addition of the cosolvent mixture to iv fluids or blood, and extremely slow infusions are needed to prevent this precipitation (9). Another, perhaps more convenient method of drug administration involves the use of O/W emulsions.

Intravenous emulsions have been produced containing fats, carbohydrates, and vitamins to provide nutrition for patients unable to assimilate the nutritional needs by the normal oral route. Contrast media for X-ray examinations have also been administered in the form of an intravenous emulsion (10). However, relatively few drug dosage formulations are available as intravenous emulsions.

This study involves the formulation of an emulsion as a possible alternative to the use of cosolvents in taxol administration.

MATERIALS AND METHODS

Emulsion Formulation

Emulsions were formulated with triacetin (Sigma Chemical Co., St. Louis, Mo.) levels ranging from 10 to 70% using a hand homogenizer. Different combinations of L-α-lecithin (Sigma Chemical Co., St. Louis, Mo.), pluronic F-68 (BASF Wyandotte Corp., Parsippany, N.J.), polysorbate 80 (Sigma Chemical Co., St. Louis, Mo.), and ethyl oleate (Eastman Kodak Co., Rochester, N.Y.) were used in amounts ranging from 0 to 2%. Glycerol (Fischer Scientific Co., Fair Lawn, N.J.) was used to prevent creaming at concentrations between 5 and 30%. The best formulation was determined by the most stable mean 1-μm droplet size and the least amount of surfactants needed to obtain this product.

Hemolysis Testing of the Emulsion

Intralipid (Miles Laboratories Inc., Berkeley, Calif.) and the prototype triacetin emulsion containing 10 mg taxol/ml were tested for hemolysis by the method of Reed and Yalkowsky (11) utilizing an emulsion-to-blood ratio of 1:2.

Acute Toxicity of the Triacetin Emulsion

The LD50 for the 50% triacetin emulsion was determined in Swiss-Webster mice (25 g). Range finding was accomplished by injecting 10 mice with doses ranging from

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0.02 to 0.1 ml of the 50% triacetin emulsion. For the final LD50 doses of 0.02, 0.03, 0.04, and 0.05 ml of the emulsion were injected intravenously into the tail vein using a 1-ml tuberculin syringe equipped with a 27-gauge, 0.5-in. needle. Eight mice were tested at each dose and toxic signs and symptoms were noted. Five mice were also given doses of surfactants two or three times greater than those used for the LD50 determination of triacetin emulsion.

Emulsion Administration Through an iv Piggyback

Stability of the taxol emulsion (10 and 15 mg/ml) was tested in D5W. Taxol–triacetin emulsion (1, 2, 3, or 4 ml) was added with shaking to beakers containing 25 ml of D5W. Samples were examined every 15 min through a polarizing microscope for precipitation or crystallization of taxol. Time to precipitation was recorded.

Emulsion Administration as an iv Bolus

The plausibility of an intravenous bolus injection of taxol–triacetin emulsion was tested. A flow-through system similar to that used by Yalkowsky *et al.* (12) was used to measure precipitation of taxol except for the following alteration: the flow cell was exchanged for a beaker with stir-bar. Isotonic saline was allowed to flow at a rate of 10 ml/min for 1 min prior to the injection of 0.5 ml taxol emulsion at rates varying between 0.05 and 4.0 ml/min. The length of tubing between injection and beaker was 30 cm. The beaker reservoir was examined microscopically at 1, 3, 5, 10, 15, and 30 min after injection for precipitation. To test further the possibility of an intravenous injection, five Swiss-Webster mice (25 g) were injected with 0.02 ml of the 20 mg/ml taxol emulsion, intravenously, and toxic effects were noted.

Physical Stability

The 50% triacetin emulsions were tested for particle size distribution, and mean particle sizes were determined. The emulsions were diluted 20-fold with water in order to separate the particles further. One hundred particles were then measured using a micrometer and a light microscope. Particle size measurements were taken at room temperature at 1, 2, 3, and 7 days, then weekly for the next 6 months.

Chemical Stability of Taxol in the Triacetin Emulsion

The disappearance of taxol in the 50% triacetin, 1.5% soy lecithin, 1.5% pluronic F68, and 2.0% ethyl oleate emulsion was monitored by high-performance liquid chromatography (HPLC). A 0.10-ml volume of taxol–triacetin emulsion was diluted 100-fold with acetonitrile. This mixture was injected (20 μl) into the HPLC using a mobile phase of ACN:water (65:35) and a flow rate of 1.0 ml/min. The column was 4-mm i.d. by 250 mm with 5-μm C18 reverse-phase packing material (Econosphere, Alltech Assoc., Los Altos, Calif.). Detection was accomplished at 254 nm with a variable-wavelength ultraviolet spectrophotometer. The emulsions were analyzed in duplicate at three different temperatures (4, 22, 40°C) for 0, 30, 60, 90, 120, 150, and 180 days. Peak areas were determined through the use of a Hewlett Packard 3390A Integrator.

Analysis of pH was conducted on the emulsion by centrifugation (3000*g*) to separate the water phase. The analysis was then accomplished with a Corning pH meter 140 (Medfield, Mass.) and pH paper.

Table I. Hemolysis of Solutions in Comparison to Normal Saline

Solution	% hemolysis
Normal saline	0
Intralipid emulsion (20%)	78.9
Taxol–triacetin emulsion[a]	12.5

[a] This formulation consisted of 1.5% soy lecithin, 1.5% pluronic F68, 2.0% ethyl oleate, 50% triacetin, and 10 mg taxol/ml.

RESULTS AND DISCUSSION

Intralipid, a commonly used parenteral emulsion, was found to be inadequate as a vehicle for taxol. The reason for this inadequacy is the poor solubility of taxol in soybean oil (0.3 mg/ml). Triacetin proved to be a more appropriate vehicle because of its greater solubility of taxol (75 mg/ml).

Development of the Triacetin Emulsion

After testing a variety of different excipients and triacetin concentrations for physical stability it was determined that the best emulsion consisted of 1.5% purified soybean lecithin, 1.5% pluronic F68, 2.0% ethyl oleate. Glycerol can be added to the emulsion at a concentration of 10% to prevent creaming, however, physical stability is only minimally improved. Glycerol had little effect on physical stability and, therefore, was not in the formulation testing.

Evaluation of the Emulsion

Hemolysis Testing

Hemolysis testing of the taxol–triacetin emulsion (10 mg/ml) proved it to be less hemolytic than 20% Intralipid (Table I).

Acute Toxicity

The acute toxicity of the 50% triacetin emulsion in mice showed an intravenous LD50 of 1.2 (95% confidence limits of 0.7 to 1.5) ml/kg body weight (Table II). Toxic signs and symptoms included lethargy, ataxia, and respiratory depression. The excipients alone showed no toxicity when administered at a dose five times greater than that of the concentration in the triacetin emulsion. The injection of 0.02 ml of the 20 mg/ml taxol–triacetin emulsion showed no visible toxic side effects. Preliminary data indicate an intraperitoneal LD50 of about 2 ml/kg with similar signs and symptoms. This suggests that the toxicity of the emulsion is

Table II. The Dose–Lethality Data of the 50% Triacetin Emulsion in Mice

Dose (ml/kg)	% dead
0.8	25
1.2	50
1.6	75
2.0	87

Table III. Intravenous Piggyback Administration of Emulsion

% emulsion in D5W	Emulsion (ml) in 50 ml of D5W	Time to crystallization (min)	
		Taxol, 10 mg/ml	Taxol, 15 mg/ml
3.85	2.0	15	15
4.76	2.5	15	15
7.41	4.0	30	15
9.09	5.0	30	30
10.7	6.0	150	60
11.5	6.5	240	—
12.3	7.0	420	—
13.8	8.0	>1200	120

not due to some larger emulsion droplets trapping in the capillaries of the lungs but, rather, is caused by the triacetin alone.

Injection into iv Bag

The administration of the taxol triacetin emulsion to an intravenous piggyback system was tested. The emulsion containing 10 mg taxol/ml was found to be suitable for addition to an iv piggyback system (Table III). The emulsion concentration must be greater than 15% of the volume in order to prevent precipitation of taxol within 4 hr. For example, the addition of 3 ml of taxol–triacetin emulsion (10 mg/ml) to 25 ml of D5W would show initial signs of precipitation in 150 min. Thus, the infusion of this solution should be completed in 2 hr or less.

Bolus Injection

The iv bolus injection of the taxol–triacetin emulsion (10 and 15 mg/ml) was examined. No precipitation was evident until 5 min after the bolus injection. At 5 min a slight precipitate developed. The amount of precipitate increased greatly at 10 min. The injection rate made little difference on the time to precipitation and no difference in the amount of precipitation when taking into account dilutional differences. This gradual precipitation probably has no clinical significance since a great deal of dilution would occur in the body within 5 min.

Injection rates of less than 0.1 ml/min caused emulsion breakage and formation of large oil droplets. However, emulsion breakage was no worse than that produced by Intralipid under similar conditions. Since Intralipid is given by slow infusion, it appears likely that the taxol–triacetin emulsion can also be infused slowly.

Physical Stability

Initially, the emulsion gave a 1-μm-average diameter droplet, with the droplet size ranging from 0.5 to 5 μm. The average droplet size grew to 2 μm within 1 week. In a 2-month period the mean particle size increased to 4 μm. The droplet sizes continued to increase with time until at 6 months two separate phases existed. However, vigorous shaking of this two-phased emulsion for 30 sec reduced the droplet size to 2 μm. The size range of these droplets varied from 0.8 to 5 μm, with a few droplets found to be 10 μm. The addition of this emulsion to a large volume of normal saline or D5W resulted in a decrease in droplet size. This decrease in droplet size is probably due to the water solubility of triacetin.

Triacetin, being a water-soluble oil (1 part triacetin in 14 part water), has emulsification stability properties that are largely dependent on the dispersion of droplet sizes. Ostwald ripening of water-soluble oils is characteristic and is dependent on emulsifying equipment. By producing a monodispersed emulsion system it would be impossible to have any Ostwald ripening (13). Thus, the emulsion instability is probably the result of inadequate emulsification equipment.

The rapid dissolution of water-soluble oil in the body is expected to prevent phagocytosis of the oil droplets even though they may be initially 3 μm in diameter. Phagocytosis of oil droplets is a major problem in the use of emulsions as drug delivery vehicles, since it leads to accumulation of drug by the reticuloendothelial system and may prevent the drug from reaching the targeted tissues or organs. Further, macrophage consumption of large particulates reduces their response to infectious agents and, thus, compromises the immune system (14,15).

Chemical Stability

Taxol eluted from the HPLC column at a retention time of 6.5 min. Triacetin and other constituents eluted within 5 min. Taxol remained chemically stable in the emulsion for 6 months with no significant loss (Table IV). The initial pH of

Table IV. Stability of Taxol in a 50% Triacetin Emulsion as Measured by Percentage Taxol Remaining

Temperature (°C)	Months						
	0	1	2	3	4	5	6
4	100	—	103	107	102	102	107
4	100	—[a]	94.4	97.9	96.4	97.9	101
22	100	—	89.4	91.4	93.1	87.9	98.3
22	100	106	105	97.1	100	103	104
22	100	101	101	102	97.2	101	96.2
22	100	100[a]	96.4	92.8	96.4	97.9	96.9
40	100	—[a]	59.9	40.7	—	—	—

[a] These formulations crystallized at this time and remained crystallized throughout the study.

4.64 of the emulsion at room temperature slowly decreased over the 6-month period, ending with a pH of 3.10. When stored at 4°C the emulsion's pH remained unchanged over the 6-month study. At 40°C the pH dropped in 1 month to 2.9, and the emulsion formed a single phase. An acetic acid smell was also evident at the higher temperature.

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