Toxicity Studies of Cremophor-free Paclitaxel Solid Dispersion Formulated by a Supercritical Antisolvent Process

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To evaluate the acute toxicity of a paclitaxel solid dispersion formulation, single dose studies in ICR mice were carried out for injectable excipients, paclitaxel solid dispersion powder, and Taxol®. In the dose range of excipients used for preparing paclitaxel solid dispersion, each excipient was clinically safe, and the LD₅₀ for excipients was higher than 2,000 mg/kg for both males and females. In this study, there were no remarkable clinical signs or deaths related to paclitaxel solid dispersion even at doses up to 160 mg/kg of paclitaxel. But Taxol® resulted in clinical signs when it contained more than 30 mg/mL paclitaxel. The LD₅₀ for paclitaxel solid dispersion was above 160 mg/kg and the LD₅₀ for Taxol® was 31.3 mg/kg, more than 5 times lower than that of paclitaxel solid dispersion. However, paclitaxel solid dispersion could not be administered i.v. at a dose exceeding 160 mg/kg, because of high viscosity. To evaluate the nephrotoxicity of paclitaxel solid dispersion, plasma level of creatinine and kidney weight were measured and compared to Taxol®. At the doses administered, paclitaxel solid dispersion did not change creatinine clearance, while Taxol® killed all animals at doses >15 mg/kg. To investigate membrane damage when paclitaxel formulations were injected, hemolytic activity was determined for different concentrations. Paclitaxel solid dispersion showed about 10% hemolytic activity, whereas Taxol® showed about 40% hemolytic activity when it contained 2 mg of paclitaxel. Comparisons with the LD₅₀ value, nephrotoxicity, and hemolytic activity of Taxol® suggested that Cremophor-free paclitaxel solid dispersion as an injectable formulation is a promising approach to increasing the safety and clinical efficacy of paclitaxel for treatment of cancer.

Key words: Paclitaxel, Solid dispersion, Toxicity, LD₅₀, Taxol, Nephrotoxicity

INTRODUCTION

Paclitaxel was identified as the active ingredient in the anti-tumor activity of crude ethanolic extracts of the bark of the Western Yew tree, Taxus brevifolia (Wani et al., 1971). Subsequently, development of the drug was suspended for more than a decade due to problems associated with the solubilization. Paclitaxel is insoluble in water (it has a solubility < 0.7 μg/mL), slightly soluble in octanol, propylene glycol and butanol, soluble in Cremophor EL, ethanol, methanol, chloroform, acetone and ether, and freely soluble in dimethyl acetamide. Therefore, an approach using 50% Cremophor EL and 50% dehydrated ethanol was chosen for further development (Adams et al., 1993). The pharmaceutical formulation of paclitaxel (Taxol®; Bristol-Myers Squibb) contains 30 mg paclitaxel dissolved in 5 mL of this (1:1, v/v) mixture. However the ethanol/Cremophor EL vehicle required to solubilize the paclitaxel in Taxol® is toxic. Although it has been used to administer other drugs, such as cyclosporine (Howrie et al., 1985) and teniposide (O’Dwyer et al., 1986), the amount of Cremophor EL necessary to deliver the required doses of Taxol® is significantly higher than that administered with any other marketed drug (Rowinsky et al., 1992). Thus the vehicle has been shown to cause serious, even fatal, hypersensitivity episodes (Dye and Watkins, 1980) at all steps in both preclinical and clinical testing (Weiss et al., 1990).

Paclitaxel is a drug with a low therapeutic index and...
therapy has always been associated with toxic side effects (Nightingale, 1992). However, the potential benefits of paclitaxel therapy, in general, outweigh the possible risks (for an excellent review describing different clinical aspects of paclitaxel therapy, see Rowinsky and Donehower, 1995). Paclitaxel is practically insoluble in water. Hence, the commercially available injection is a sterile solution of the drug in Cremophor EL and dehydrated alcohol. Five to six doses of paclitaxel are generally given at a dose of 135 or 175 mg/m² as a 3 or 24 h infusion, every 3 weeks (Kramer and Heuser, 1995).

Paclitaxel concentrate is a clear, colorless to slightly yellow viscous liquid. Paclitaxel is administered by i.v. infusion at a concentration of 0.3-1.2 mg/mL, after diluting the paclitaxel concentrate for injection with 0.9% sodium chloride injection solution or 5% dextrose injection solution or 5% dextrose and 0.9% sodium chloride injection solution, or 5% dextrose in Ringer’s injection solution (Kramer and Heuser, 1995). After dilution in an infusion solution, the drug may appear hazy due to the raw materials of the formulation vehicle rather than the precipitation of paclitaxel. Paclitaxel in aqueous solutions is chemically stable for 1-2 days. Inclusion of a hydrophilic, microporous in-line filter of a pore size not more than 0.22 µm is necessary during paclitaxel infusion. Contact of undiluted paclitaxel concentrate for injection with plasticized polyvinyl chloride (PVC) equipment or devices used to prepare solutions for infusion is not recommended because Cremophor EL causes leaching of diethylhexylphthalate (DEHP) from PVC containers. This leaching of DEHP is substantial and occurs in a concentration-dependent manner, and is also dependent on the type of administration set used (Allwood and Martin, 1996). In addition to plastic surfaces, rapid and nonspecific adsorption of paclitaxel also occurs on glass surfaces (Song et al., 1996). This problem can be overcome, to some extent, by increasing the organic component of the solvent system or using organic solvents. In order to minimize the exposure of patients to leached DEHP, diluted paclitaxel solutions should be stored carefully, preferably in glass or polypropylene bottles or in plastic (polypropylene or polyolefin) bags and administered through polyethylene lined administration sets (Allwood and Martin, 1996).

Paclitaxel therapy is associated with hypersensitivity reactions, so premedication is mandatory before paclitaxel administration. These hypersensitivity reactions may be due to Cremophor EL, rather than to the drug itself (Gregory and DeLisa, 1993). The premedication schedule includes; corticosteroids (e.g. dexamethasone), diphenhydramine or chlorpheniramine, H₂-receptor antagonists (e.g. ranitidine), and anti-emetics. Paclitaxel administration must be discontinued immediately in case of severe hypersensitivity reactions (Kohler and Goldspiel, 1994) and the patient must be treated with epinephrine and i.v. fluids. There is no known antidote for paclitaxel overdose (Panchagnula, 1998).

Based on experience with Cremophor EL in other experimental and marketed drugs, it was expected that the concentrations present in the Taxol® formulation would cause problems in animal toxicology studies (Adams et al., 1993; Rowinsky et al., 1990; Lassus et al., 1985). Dogs are known to be particularly sensitive to agents that cause histamine release, and the Cremophor-ethanol vehicle controls showed considerable toxic manifestations, even without Taxol®. The vehicle was administered to dogs in both large volume single doses and in repeated smaller dose schedules. Large volume single doses in beagles produced vasodilation, hypotension, difficulty in breathing, lethargy and ultimately death. The vehicle was much better tolerated in repeated smaller doses and showed no cumulative effects. It appeared from this data that the vehicle effects were due to peak blood levels of Cremophor and that attempts should be made in the clinical setting to control peak levels by either repeated smaller doses of Taxol® or by relatively slow infusion. With regard to the toxicity of Taxol® itself, the drug was studied in CD2F1 mice, Sprague-Dawley rats, and beagle dogs. Studies in mice and rats were done using the i.p. route since it was not possible to administer the large volumes of vehicle in the clinical formulation by vein. The major nonvehicle-related toxicity in both rats and dogs was myelo-suppression; lymphoid depletion was also noted. Gastrointestinal toxicity was moderately severe in dogs and included emesis, diarrhea, and mucosal ulcerations. There was also some toxicity to male reproductive organs seen in all species. Toxicities were cumulative in a 5-day schedule. These data indicated that a single injection schedule might be desirable to try in the clinic to minimize cumulative toxicity, but this would require fairly large volumes of vehicle which could cause problems; the alternative of daily dosing would largely avoid vehicle toxicity, but would create the potential for cumulative Taxol® toxicity. Consequently, the clinical plan was to try a variety of administration schedules in Phase I trials.

We developed a Cremophor-free paclitaxel solid dispersion powder with hydrophilic polymers and surfactant using a supercritical fluid process (Park et al., 2008) and expected that the prepared solid dispersion would be less toxic than Taxol®. Therefore, in this study, we report in detail the acute toxicity of paclitaxel formulations and its excipients by a single dose in mice in comparison to Taxol®. In addition, nephrotoxicity was measured and compared with the nephrotoxicity of Taxol®, and erythrocytic hemolysis was measured to evaluate the toxicity of paclitaxel formulations for injection.