

Novel Paclitaxel Formulation (Genexol[®]-PM) : Preclinical Studies

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Abstract

Paclitaxel has demonstrated significant activity in clinical trials against a wide variety of tumors, including refractory ovarian cancer, metastatic breast cancer, non-small cell lung cancer, and AIDS-related Kaposi's sarcoma. Paclitaxel is a highly hydrophobic drug that is poorly soluble in conventional aqueous vehicles. Consequently, it is currently formulated as Taxol[®], a concentrated solution containing 6mg paclitaxel per milliliter of Cremophor[®] EL and dehydrated alcohol (1:1, v/v). Since paclitaxel is not absorbed from the GI tract due to multi-drug-resistant transporters, it is administered by intravenous administration. Taxol[®] is generally given at a dose of 135 to 175 mg/m² as a 3 or 24 hours infusion, every 3 weeks. The dose of paclitaxel is limited by toxic-side effects and Cremophor[®] EL itself is associated with patient toxicity. The Cremophor[®] EL has been considered as a cause of hypersensitivity reactions in some patients. To overcome these side effects, clinicians prolong infusion schedules or pretreat with corticosteroids, diphenhydramine and H₂-receptor antagonist.

Since one of the major obstacles for successful chemotherapy with paclitaxel is the toxic side effects due to the use of Cremophor[®] EL, effective chemotherapy using paclitaxel will rely on the development of new delivery systems.

In the present study, we developed a paclitaxel-containing biodegradable polymeric micellar system (Genexol[®]-PM) using a low molecular weight, non-toxic and biodegradable amphiphilic diblock copolymer, monomethoxy poly(ethylene glycol)-*block*-poly(D,L-lactide) [mPEG-PDLLA], and compared the anti-tumor efficacy, pharmacokinetics, tissue distributions, single dose toxicities, and 5-cycle toxicities in animal models with current clinical formulation of paclitaxel (Taxol[®]).

The *in vivo* anti-tumor efficacy of Genexol[®]-PM as measured by reduction in tumor volume of SKOV3 human ovarian cancer

implanted in CD-1 nude (nu/nu) athymic mice was greater than that of Taxol[®]. The maximum tolerated dose (MTD) of Genexol[®]-PM and Taxol[®] in CD-1 mice was determined to be 420 and 10.9-13.1mg/kg, respectively. The maximum tolerated dose of Genexol[®]-PM and Taxol[®] in beagle dogs over 3-hr period was 4.5 and <0.5mg/kg, respectively. After 5-cycle intravenous administration of Genexol[®]-PM in mice, 120mg/kg showed no important neurotoxic changes, while 280mg/kg produced neurotoxic seen around the end of 3rd cycle.

In 5-cycle toxicity studies in dogs, Genexol[®]-PM groups showed less and minor clinical signs without hypersensitivity reaction.

After intravenous administration of ¹⁴C-Genexol[®]-PM (120mg/kg) and ¹⁴C-Taxol[®] (9mg/kg) for distribution, metabolism and elimination studies in CD-1 mice, the ratio of the AUCs for plasma radioactivity were about 45:1 (Genexol[®]-PM:Taxol[®]).

When ¹⁴C-Genexol[®]-PM (120mg/kg) was administered, a much smaller proportion of the dose was present in the liver at 25 minutes than observed with ¹⁴C-Taxol[®] (9mg/kg). Comparison of the tissue radioactivity concentrations at this time for the two formulations shows that, in many tissues, the ratio of the concentrations corresponds well with the dose difference. Tissue distribution and pharmacokinetics of Genexol[®]-PM were investigated in xenografted C57BL/6 mice in comparison with Taxol[®]. Interestingly, Genexol[®]-PM (50mg/kg) reached the tumor sites slightly faster than Taxol[®] (20mg/kg) and had a higher tumor/plasma concentration ratio than Taxol[®].

Methods

Paclitaxel (Genexol[®])-containing biodegradable polymeric micellar system (Genexol[®]-PM) was prepared by a solid dispersion technique, conveniently modified to increase micelle stability. Briefly, paclitaxel and mPEG-PDLLA were dissolved in dehydrated

ethanol. After dissolving, the organic solvent was evaporated on a rotary evaporator under reduced pressure at 60°C to obtain a clear gel matrix. The resulting clear gel matrix was dissolved by the addition of water at 60°C to obtain a transparent paclitaxel incorporated micellar solution. After addition of lactose solution, the mixture was filtered through a 0.22 μ m filter and lyophilized by a freeze dryer.

The *in vivo* anti-tumor efficacy studies were conducted against human ovarian cancer cell line (SKOV3) in female CD-1 nude (nu/nu) athymic mice. The single dose toxicity study and 5-cycle toxicity study was conducted in CD-1 mice and beagle dogs. The pharmacokinetic and tissue distribution was investigated in CD-1 mice and female SPF C57BL/6 mice bearing murine B16 melanoma using ¹⁴C-Genexol[®]-PM and ¹⁴C-Taxol[®].

Results and Discussion

Treatment of nude mice bearing subcutaneous human SKOV3 ovarian tumors using i.v. treatment regime on days 0, 4 and 8 with Genexol[®]-PM and Taxol[®], produced marked, dose-dependent anti-tumor effects. Genexol[®]-PM at 20mg/kg and Taxol[®] at 20mg/kg were equally effective in slowing tumor growth, producing specific growth delays of 0.91 and 0.93, respectively, compared to their relevant control group. Genexol[®]-PM at the 60mg/kg dose level resulted in a statistically significant reduction in tumor size and a mean relative tumor volume of only 1.5 \pm 0.6 on day 70, the final day of the study (Figure 1).

A number of acute and chronic toxicology studies were carried out on Genexol[®]-PM compared to Taxol[®]. These studies are summarized in Table 2. A single dose toxicity study which followed a 21-day recovery period was performed in CD-1 mice. Genexol[®]-PM vehicle, Genexol[®]-PM at 35, 280 and 420mg/kg, Taxol[®] vehicle and Taxol[®] were tested. There were no deaths in Genexol[®]-PM treated groups. Deaths occurred in Taxol[®]-treated groups in a dose range of 13.1-35mg/kg. Since no deaths occurred even at the highest dose, the MTD of Genexol[®]-PM appears to be >420mg/kg. This finding reaffirms that Genexol[®]-PM is superior in safety in comparison with Taxol[®]. The single dose toxicity study was repeated in the second species, Beagle Dogs, and the animals were observed for mortality over 21-day period. The objective of this study was to determine and compare the systemic exposure and toxic

potential of Genexol[®]-PM infused over 3-hr period at 1.5, 3.0 and 4.5mg/kg. Taxol[®] was infused over a 30 minute period at 1.5 and 2.5 mg/kg. This study was performed in two separate phases due to adverse reactions found with Taxol[®] and/or its formulation. The 3-hr infusion of the first phase was stopped due to recurrent adverse reactions of the Taxol[®] group. Following a 19-day recovery period, the dogs were first given antihistamine and then given Taxol[®] at 1.5 and 2.5 mg/kg over a 30-minute infusion. Clinical signs were monitored. In general, clinical signs were comparable to Taxol[®].

In a pivotal 5-cycle study, Genexol[®]-PM and Taxol[®] were tested for tolerance and toxicokinetics along with appropriate vehicle controls in CD-1 mice. Genexol[®]-PM was tested at 120 and 280mg/kg, and Taxol[®] was tested at 9 mg/kg. The drug was injected in one bolus injection every 21 days for a total of five cycles. C_{max} for males was 395 and that for females was 459 μ g/ml at a dose of 120mg/kg. At a higher dose of 280mg/kg the C_{max} values were 4 times higher and appeared to be nonlinear, compared to the one low dose tested, as a function of doses. The MTD for Genexol[®]-PM was found to be <120mg/kg and that of Taxol[®] was founded to be approximately 9mg/kg. No toxicologically important changes were noted in groups treated with Genexol[®]-PM at 120mg/kg. However at a higher dose of 280mg/kg a few animals either died or were sacrificed due to symptoms (limited use of hinds limbs, unsteady gait, under activity and pallor) attributable to neurotoxicity.

Another 5-cycle pivotal study was performed in a non-rodent, second species of test animals. Thus, Genexol[®]-PM and Taxol[®] were tested for tolerance and toxicokinetics along with appropriate vehicle controls in Beagle Dogs. Genexol[®]-PM was administered at 2.5 and 4.5 mg/kg (3-h infusion, no premedication) and Taxol[®] was administered 2.5mg/kg (30-minute infusion, premedication) in five separate single intravenous infusions each apart by 21 days. It may be noted that dogs are, in general, highly sensitive and far less tolerant for this class of anti-tumor drugs. Vomiting, inappetence and loose faeces, generally consistent with anticancer drugs, were seen following the administration of each dose. Systemic exposure of male and female dogs to paclitaxel was about 10 times lower (C_{max} 331ng/ml of test drug vs 2,980ng/ml of Taxol[®] in male dogs) with Genexol[®]-PM than with Taxol[®] at identical infusion of 2.5mg/kg. In terms of acute effects, however, Genexol[®]-PM was better tolerated compared to Taxol[®].

Furthermore, none of the reactions of histamine release attributable to Taxol[®] vehicle (Cremophor[®] EL and Dehydrated alcohol) were found in the treatment with Genexol[®]-PM.

A comparative distribution, metabolism and excretion of ¹⁴C-Genexol[®]-PM and ¹⁴C-Taxol[®] were investigated in mice following a single intravenous dose. ¹⁴C-Genexol[®]-PM was prepared from ¹⁴C-paclitaxel and Genexol[®] using a method analogous to that used in the formulation intended for clinical use. A preparation representative of ¹⁴C-Taxol[®] was prepared from ¹⁴C-paclitaxel, Cremophor[®] EL and dehydrated alcohol. Each formulation was administered to male and female mice (CD-1 strain) intravenously at dose levels of 120mg/kg (¹⁴C-Genexol[®]-PM) and 9mg/kg (¹⁴C-Taxol[®]), these doses being expressed as the equivalent of paclitaxel. The doses are those in use, or to be used clinically. In the tissue distribution study, three male and three female mice dosed with either formulation were sacrificed at 25 minutes, 3, 8, 24, 48 and 96 hours after dosing. In the excretion study, the mice maintained during 96 hours were kept in metabolism cages for excreta collection. After evaluation of the distribution of total radioactivity in the tissues, plasma, kidney, liver, lungs, pancreas, spleen and testes (male) or ovaries (female) were selected for ¹⁴C-paclitaxel concentration measurement using a High Performance Liquid Radiochromatograph method.

When administered as ¹⁴C-Taxol[®], ¹⁴C-paclitaxel was rapidly extracted into the liver (in which almost half the dose was present at 25 minutes). This was followed by partial metabolism and almost total biliary-fecal excretion. Within 96 hours, excretion of radioactivity was almost entirely complete, with the radioactivity in the organs at this time mostly being present in liver. Persistence of radioactivity in the liver could be a consequence of enterohepatic circulation. The pattern of disposition of ¹⁴C-Taxol[®] was similar in both sexes, with some persistence of radioactivity in the testes in male animals. It was notable that almost all the systemic exposure resulting from ¹⁴C-Taxol[®] administrations would be from the unmetabolised ¹⁴C-paclitaxel itself.

When ¹⁴C-Genexol[®]-PM was administered, a much smaller proportion of the dose was present in the liver at 25 minutes than observed with ¹⁴C-Taxol[®]. Comparison of the tissue radioactivity concentrations at this time for the two formulations shows that, in many tissues, the ratio of the concentrations

(¹⁴C-Genexol[®]-PM:¹⁴C-Taxol[®]) corresponds well with the difference (13:1) in the dose levels. The ratios in liver (5:1) and plasma (31:1) were, however, much smaller and greater than the dose level ratio (13:1), respectively. The ratio in the pancreas was 13:1 in male as well as female mice. Although this ratio tends to represent the difference of the dose levels of Genexol[®]-PM/Taxol[®], it is noteworthy that Genexol[®]-PM is found distributed also in the pancreas. The ratio of AUCs for plasma radioactivity were, for both sexes, about 45:1 (Genexol[®]-PM:Taxol[®]). The proportion of radioactivity in the gastrointestinal tract is similar for the two formulations, leading to the view that the liver extraction for ¹⁴C-Genexol[®]-PM is less rapid than for ¹⁴C-Taxol[®]. This would result in the observed increase in the AUC and may also lead to a short-term 'depot' effect in fat (in which the radioactivity concentrations following ¹⁴C-Genexol[®]-PM administration are proportionately much greater than seen after ¹⁴C-Taxol[®] administration). By 96 hours after dosing, the concentration ratios for the two formulations reflect dose proportionality, with the notable exceptions of the testes in male mice (in which the concentration ratio is about 50:1) and ovaries in females (28:1). The overall excretion of ¹⁴C-paclitaxel when administered as ¹⁴C-Genexol[®]-PM was, as seen for ¹⁴C-Taxol[®], relatively rapid and almost entirely in the feces.

When considered as to the difference in dose level, systemic exposure to ¹⁴C-paclitaxel (and total radioactivity) was about three times greater when ¹⁴C-paclitaxel was administered as ¹⁴C-Genexol[®]-PM than when given as ¹⁴C-Taxol[®].

Tissue distribution and pharmacokinetics of Genexol[®]-PM were investigated in xenografted C57BL/6 mice in comparison with Taxol[®]. SPF female C57BL/6 mice were subcutaneously injected with murine B16F10 melanoma cells. When the tumors of appropriate size were formed, the animals were administered Genexol[®]-PM at 20 and 50mg/kg intravenously in a single dose. A separate group of animals was treated with i.v. injection of Taxol[®], 20mg/kg. Distribution of the test drug was monitored at timely intervals for up to 24 hours. Lowest amount of Genexol[®]-PM was found in plasma, followed by the heart. The highest concentration of Genexol[®]-PM, which was found in the tumor, was approximately 80 times higher than that found in the plasma (Figure 2). The second higher concentration was found in the liver. In

the previous study, the highest amount of radioactivity was found in the liver followed by pancreas, kidney, and small intestine. In the xenografted mice, the distribution was highest in the tumor followed by liver, kidney, spleen and lungs. Concentration in the pancreas was not measured in the xenografted study. Genexol[®]-PM and Taxol[®] were widely distributed immediately following intravenous administration. Interestingly, Genexol[®]-PM reached the tumor sites slightly faster than Taxol[®] and had a higher tumor/plasma concentration ratio than Taxol[®].

Overall, these DME (Distribution, Metabolism & Elimination) data indicated that the Genexol[®]-PM was successful in optimal delivery of the active entity at the tumor-bearing site.

The results of this study strengthen the validity of novel paclitaxel formulation (Genexol[®]-PM) using biodegradable amphiphilic diblock copolymers as solubilizer forming a polymeric micelle based drug delivery system for cancer therapy, combining reduction of acute and chronic toxicity and improvement of anti-tumor efficacy. Furthermore, for clinical purposes, the use of Genexol[®]-PM has advantageous over commercially available paclitaxel formulation (Taxol[®]) in terms of low toxicity levels and increased dose without premedication. The results of anti-tumor efficacy, acute toxicity, chronic toxicity, and DME studies suggest that Genexol[®]-PM may have better tolerability and efficacy over current chemotherapy with Taxol[®] and further clinical investigations of Genexol[®]-PM for the treatment of solid tumors are warranted.

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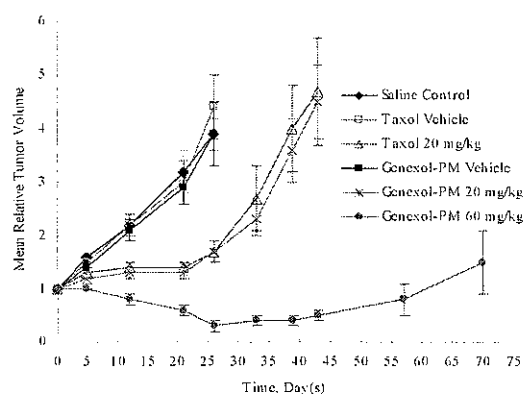


Figure 1. Anti-tumor efficacy of Genexol[®]-PM and Taxol[®] on CD-1 nude (nu/nu) athymic mice bearing SKOV3 human ovarian tumor xenograft. Tumors were allowed to establish and mice were treated on days 0, 4 and 8 with saline, Taxol[®] vehicle, Genexol[®]-PM vehicle, Taxol[®] and Genexol[®]-PM. Each point represents a mean \pm S.D.

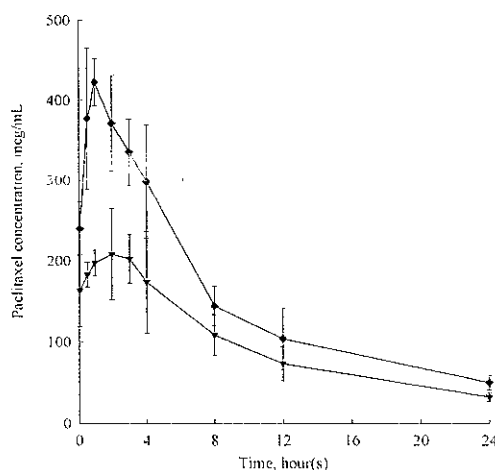


Figure 2. Time courses of paclitaxel levels in tumor of murine B16 melanoma induced mice after i.v. administration of 50 mg/kg dose of Genexol[®]-PM (◆) and 20 mg/kg dose of Taxol[®](▼). Each point represents the mean \pm S.D. of four mice per time point.