Biology Contribution

Preclinical Evaluation of Genexol-PM, a Nanoparticle Formulation of Paclitaxel, as a Novel Radiosensitizer for the Treatment of Non-Small Cell Lung Cancer

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Summary

Genexol-PM was evaluated preclinically as a radio-sensitizer for chemoradiation therapy of non-small cell lung cancer (NSCLC). Using NSCLC cell lines and mouse xenograft models of NSCLC, we demonstrated that Genexol-PM is an effective radiosensitizer and is more effective than Taxol. We also found that Genexol-PM leads to lower paclitaxel dose in normal lung when compared with Taxol. Our findings support the clinical evaluation

Purpose: A key research objective in radiation oncology is to identify agents that can improve chemoradiation therapy. Nanoparticle (NP) chemotherapeutics possess several properties, such as preferential accumulation in tumors, that are uniquely suited for chemoradiation therapy. To facilitate the clinical translation of NP chemotherapeutics in chemoradiation therapy, we conducted preclinical evaluation of Genexol-PM, the only clinically approved NP chemotherapeutic with a controlled drug release profile, as a radiosensitizer using non-small cell lung cancer (NSCLC) as a model disease.

Methods and Materials: The physical characteristics and drug release profile of Genexol-PM were characterized. Genexol-PM's efficacy as a radiosensitizer was evaluated in vitro using NSCLC cell lines and in vivo using mouse xenograft models of NSCLC. Paclitaxel dose to normal lung and liver after Genexol-PM administration were quantified and compared with that after Taxol administration.

Results: Genexol-PM has a size of 23.91 ± 0.41 nm and surface charge of -8.1 ± 3.1 mV. It releases paclitaxel in a controlled release profile. In vitro evaluation of Genexol-PM as a radiosensitizer showed it is an effective radiosensitizer and is more effective than Taxol, its small molecule counterpart, at the half maximal inhibitory concentration. In vivo study of Genexol-PM as a radiosensitizer demonstrated that it is more effective as a radiosensitizer than Taxol. We also found that Genexol-PM leads to lower paclitaxel exposure to normal lung tissue than Taxol at 6 hours postadministration.

of Genexol-PM in chemo-
radiation therapy for NSCLC.

Conclusions: We have demonstrated that Genexol-PM is more effective than Taxol as a radiosensi-
tizer in the preclinical setting and holds high potential for clinical translation. Our data support the
clinical evaluation of Genexol-PM in chemoradiation therapy for NSCLC. © 2013 Elsevier Inc.

Introduction

The concurrent administration of chemotherapy and radiation
therapy, also called chemoradiation therapy, is an important treat-
ment paradigm in the curative management of many cancers (1).
Chemoradiation therapy has not only consistently shown improved
local tumor control but also improves survival when compared with
either sequential treatment or sole administration of chemotherapy
or radiation therapy in some malignancies (1, 2). However, che-
moradiation therapy is not without limitations. The concurrent use
of both chemotherapy and radiation therapy has significantly higher
toxicities compared with either treatment alone or sequential use
(3). Thus, the development of agents and approaches to further
improve the therapeutic index of chemoradiation therapy has been
a major research objective. A key challenge in this effort has been to
selectively deliver chemotherapeutics to tumors while minimizing
drug dose to normal tissue. Although traditional drug delivery
techniques have failed to address this challenge (4), the develop-
ment of nanoparticle (NP) formulations of chemotherapeutics
offers an unprecedented opportunity. NP therapeutics possess
important characteristics that are uniquely suited for chemo-
radiotherapy. NPs, as macromolecules, preferentially accumulate
in tumors through the enhanced permeability and retention (EPR)
effect, leading to high intratumoral drug concentrations (5). NPs are
also unable to penetrate normal vasculatures and capillaries, thus
leading to lower drug doses to normal tissues compared with their
small molecule counterparts. Moreover, NP therapeutics have been
found to have lower systemic toxicity than small molecules (6).
Lastly, NP formulations of chemotherapeutics can release their
cargo in a controlled fashion (5). Such prolonged release can
increase the synergistic effects of chemotherapy and radiation
therapy. Indeed, several preclinical studies have demonstrated that
NP chemotherapeutics can improve the therapeutic efficacy of
chemoradiotherapy (7, 8). However, there have been no reports
evaluating NP therapeutics that are clinically approved or under-
going clinical investigation for use in chemoradiation therapy. Such
studies are necessary for clinical translation of NP therapeutics in
chemoradiation therapy. To fill this knowledge gap, we conducted
a preclinical evaluation of Genexol-PM, the only clinically
approved second-generation NP chemotherapeutic with
a controlled drug release profile, in chemoradiation therapy using
non-small cell lung cancer (NSCLC) as a model disease.

Genexol-PM is a polymeric NP micelle formulation of pacli-
taxel that has been approved in South Korea for the treatment of
breast cancer and NSCLC (9, 10). It is composed of low-
molecular-weight amphiphilic diblock copolymer, monomethoxy
poly (ethylene glycol)-block-poly(D,L-lactide) (mPEG-PDLLA)
and paclitaxel (11). Genexol-PM has shown lower toxicity than
Taxol with its maximum tolerated dose identified as 2 to 3 times
that of Taxol (9-11). Given that chemoradiation therapy is often
used for locally advanced and unresectable NSCLC and paclitaxel
has been shown be an excellent radiosensitizer in NSCLC, we
chose to study Genexol-PM in chemoradiation therapy using
NSCLC as a model disease. Moreover, chemoradiation therapy for

of patients dying from treatment-related pulmonary toxicities (3,
12). Thus, there is a strong clinical need to improve chemo-
radiation therapy for NSCLC. In this study, Genexol-PM was
compared with Taxol in chemoradiation therapy in vitro using
NSCLC cell lines and in vivo using mouse flank xenograft models
of NSCLC. We also quantified the paclitaxel dose delivered to
normal lung and liver tissues after Genexol-PM and Taxol
administration.

Methods and Materials

Materials

Genexol-PM was provided by Samyang Biopharmaceuticals
Corporation (Seoul, Korea) as a gift. Each gram of Genexol-PM
contains 115 mg of paclitaxel. Genexol-PM was resuspended in
phosphate buffer saline based on clinical administration protocol
before use. Taxol 6 mg/mL injectable solution (Teva, Sellersville,
PA) was purchased from the University of North Carolina Hospital
pharmacy.

Characterization of Genexol-PM

Genexol-PM size (diameter, nm) and surface charge (ζ-potential,
mV) were characterized using a ZetaPALS dynamic light
scattering detector (Brookhaven Instruments, Holtsville, NY).
Transmission electron microscopy (TEM) images of Genexol-PM
were obtained at the University of North Carolina (UNC)
Microscopy Services Laboratory Core Facility. NPs were stained
using a phosphotungstate stain before TEM imaging.

Paclitaxel release characterization

To measure the release profile of paclitaxel from Genexol-PM, 0.1
mL of Genexol-PM solution at a concentration of 1 mg/mL was
aliquot equally into Slide-A-Lyzer MINI dialysis microtubes with
a molecular weight cut-off of 10 kDa (Pierce, Rockford, IL) and
subjected to dialysis as described previously (7). Paclitaxel
content was quantitatively analyzed using an Agilent 1100 HPLC
(Paolo Alto, CA) equipped with a C18 chromolith flash column
(Merck, Darmstadt, Germany). Paclitaxel absorbance was
measured by a UV-VIS detector at 227 nm and a retention time of
8.5 min in 0.25 mL/min gradient (from 0:100 to 100:0) of
acetonitrile/water.

Cell culture

NSCLC cell lines were acquired from the Lineberger Compre-
hensive Cancer Center Tissue Culture Facility. A549 cells were
cultured in Dulbecco's Modified Eagle Medium (DMEM) (Gibco,
Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine
serum (Mediatech, Manassas, VA) and penicillin/streptomycin

fetal bovine serum (FBS), 2 mM L-glutamine, 1.5 g/L Na bicarbonate, 10 mM HEPES (4-(2-hydroxyethyl)-1-piper-azineethanesulfonic acid) buffer, 1 mM Na pyruvate, penicillin/streptomycin (Mediatech), 4.5 g/L glucose.

In vitro x-ray irradiation

Radiation therapy was given using a Precision X-RAD 320 (Precision X-Ray, North Branford, CT) operating at 320 kvp and 12.5 mA as described previously (7).

Clonogenic survival assay

Cells were seeded at densities ranging from 100 to 200,000 cells in 4 mL of culture medium in 25-mL flasks 1 day before treatment. Cells were treated with IC_{50} doses of Taxol (1.77 ug/mL for A549 and 17.7 ug/mL for H460) or Genexol-PM (15 ug/mL for A549 and 150 ug/mL for H460) for 1 hour and washed 3 times with fresh media after incubation. Cells were irradiated with 2 fractions of 0, 1, 2, or 3 Gy separated by 12 hours. Cells were incubated and counted as described previously (7). Data were analyzed using Origin Pro 8.6 software. Polynomial curve fitting was performed using second polynomial order.

In vivo tumor assay

Tumors were established in the upper dorsal region of Nu/Nu mice by injecting 1×10^6 of H460 or A549 cells in a 1:1 RPMI:Matrigel solution. Tumors were incubated for 10 days (H460) or 14 days (A549) to reach approximate tumor volume of 100 mm³ before treatment. Mice (7 per experimental group) were treated intravenously with Taxol (2.5 mg/kg) or Genexol-PM (21.5 mg/kg) and subsequently irradiated 6 hours postinjection. The mice were randomized into experimental groups before treatment. The dose rate at a source-subject distance of 70 cm was 50 cGy/min. Mice were irradiated with 5 daily fractions of 3 Gy. The head and abdomen regions of mice were shielded using 0.5 cm of lead. Only the truncal region containing tumor xenograft was irradiated. Tumor volumes were calculated by measuring 2 perpendicular diameters with a caliper and using the formula of $V = 0.5 \times a \times b^2$ where, a and b are the larger and smaller diameters, respectively. The tumors were measured every 2 days, and the relative percent change in tumor volume was calculated using the relation $100 * (V_i - V_o)/V_o$, where V_i is the volume calculated and V_o is the initial volume on day 1.

Tumor volume was measured every other day until the tumor reached 3 times the initial volume or 2 cm in the maximum dimension, at which point the animal was euthanized. All animal work was approved and monitored by the UNC Animal Care and Use Committee.

Statistical methods

We used response features analysis to analyze our serial measures of tumor growth (13). This method allows for the comparison of individual growth delay profiles by using summary measures. The particular summary measure used was the AUC (or area under the curve), which was particularly appropriate for the growth-delay

a significant difference in AUCs between chemoradiation therapy with Genexol-PM compared with chemoradiation therapy with Taxol. Statistical analyses were performed using SAS statistical software, version 9.2, from the SAS Institute (Cary, NC).

Quantification of paclitaxel dose in tissue

Mice bearing H460 tumors (5 animals per group) were treated intravenously with equivalent paclitaxel dose of Taxol (2.5 mg/kg) or Genexol-PM (21.5 mg/kg). Mice were euthanized after 6 h or 24 h, and organs were collected and weighed. Organs were homogenized 4:1 (v/w) in chloroform to extract paclitaxel. Homogenate was centrifuged, and the organic layer was collected, evaporated, and reconstituted in 0.1% acetic acid in methanol for analysis via liquid chromatography-mass spectrometry using an Applied Biosystems API 4000 triple quadrupole mass spectrometer with an atmospheric pressure chemical ionization interface.

Results

Characterization of Genexol-PM

Genexol-PM's physical properties were characterized as these properties have not been described in previous publications. Genexol-PM has a size of 23.91 ± 0.41 nm by dynamic light scattering. The NPs are also monodisperse with a polydispersity index of 0.08 ± 0.02. The NP size and dispersity were confirmed by TEM (Fig. 1A). We found that Genexol-PM has a surface charge of -8.1
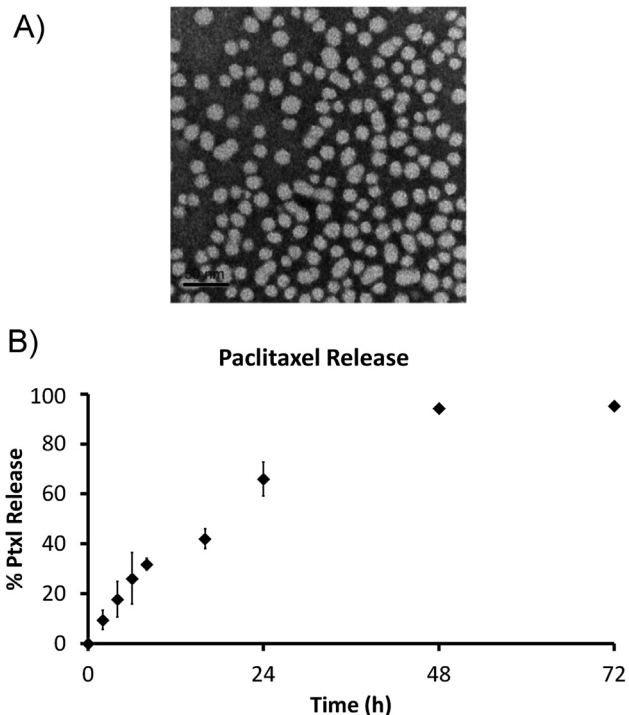

Fig. 1. Characterization of Genexol-PM. (A) Transmission electron microscopy image of Genexol-PM depicting a monodisperse population of particles with a narrow size distribution of 23.0 ± 4.5. (B) Drug-release curve of Genexol-PM. Genexol-PM

± 3.1 mV. We also studied the drug release profile of Genexol-PM. Paclitaxel release from the Genexol-PM demonstrated first-order controlled release kinetics with ~65% drug released at 24 hours and 95% drug release at 48 hours (Fig. 1B).

In vitro radiosensitization with Genexol-PM

The therapeutic efficacy of Genexol-PM as a radiosensitizer in vitro was evaluated in 2 commonly studied NSCLC cell lines H460 (large cell) and A549 (adenocarcinoma). To determine the sensitivity of each cell line to Genexol-PM, a dose-response curve was performed with no radiation therapy for each cell line (Fig. S1). Radiosensitization experiments were conducted using IC_{50} concentrations as well as equivalent paclitaxel concentrations of Genexol-PM and Taxol. Clonogenic survival curves of cells after combined Genexol-PM and fractionated radiation therapy were generated for each cell line. They were compared to that of fractionated radiation therapy alone. We demonstrated that despite the lack of EPR effect in vitro, Genexol-PM is an effective radiosensitizer and is more effective than Taxol in both cell lines at IC_{50} doses (Fig. 2). The sensitizer enhancement ratio (SER) of Taxol at 10% survival in H460 cells is 1.03, whereas the SER of Genexol-PM is 1.12. The SER is greater in A549 cells at 10%; with Taxol at 1.12 and Genexol-PM at 1.23. Also, Genexol-PM was as effective a radiosensitizer as Taxol at equivalent paclitaxel doses (Fig. S2). These results confirmed that Genexol-PM is an effective radiosensitizer.

In vivo radiosensitization with Genexol-PM

To validate the in vitro result and to evaluate the efficacy of Genexol-PM as a radiosensitizer in vivo, we used a murine flank xenograft model of NSCLC. Mice bearing either H460 or A549 cell xenograft tumors were treated with Genexol-PM, Taxol, or saline followed by radiation therapy. After treatment with 5 daily fractions of 3 Gy, the tumor growth delay curves demonstrated that chemoradiation therapy with Genexol-PM leads to significantly longer tumor growth delay compared with chemoradiotherapy with Taxol in H460 cells ($P=.008$; Fig. 3A). Although the difference between the 2 treatments does not appear as significant in A549 cells ($P=.18$; Fig. 3B), the impression remains that chemoradiation therapy with Genexol-PM is more effective. Since tumors that were treated with chemotherapy only (Genexol-PM or Taxol) did not show significant difference in growth delay, the difference between Genexol-PM+XRT and Taxol+XRT is likely due to more effective radiosensitization by Genexol-PM (Fig. S3). Our results showed the high potential of Genexol-PM in improving chemoradiation therapy.

Evaluation of normal tissue drug exposure

Pulmonary toxicity is the main cause of mortality and morbidity from chemoradiation therapy for NSCLC. It is caused by normal lung tissue receiving both chemotherapy and radiation therapy. To determine whether Genexol-PM can lead to lower normal lung paclitaxel exposure, we compared the paclitaxel concentrations in mouse lungs after Genexol-PM and Taxol administration. We found that Genexol-PM leads to significantly lower lung paclitaxel concentration ($P=.01$) acutely after injection (6 hours). This
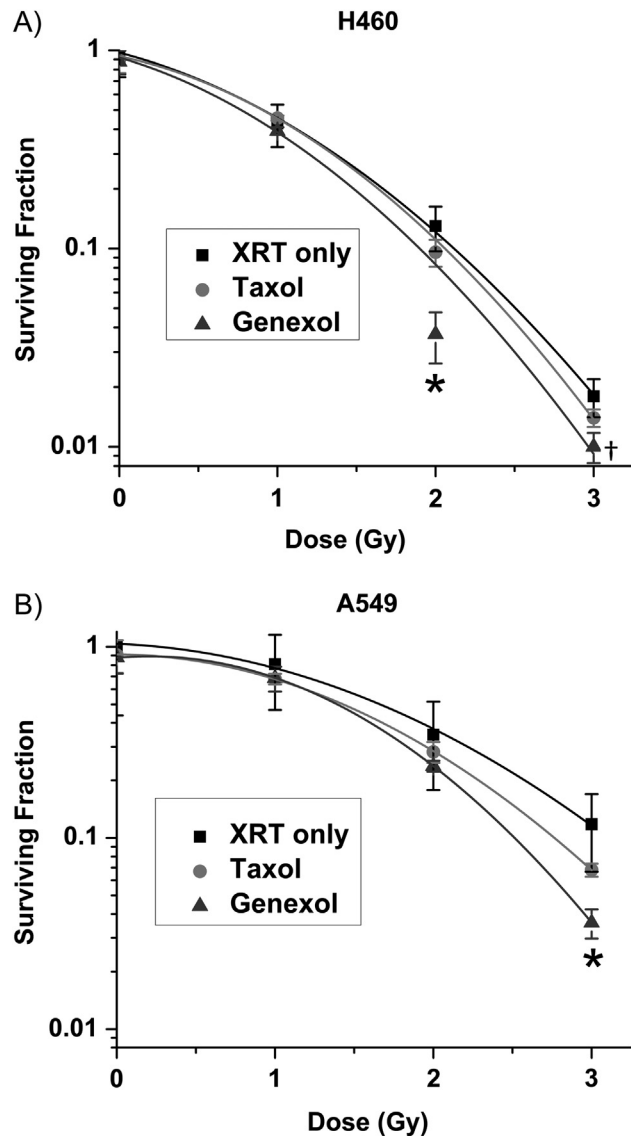

Fig. 2. Efficacy of Genexol-PM as a radiosensitizer in non-small cell lung cancer cell lines in vitro. Radiation survival curves for (A) H460 and (B) A549 non-small cell lung cancer cells treated with saline, Taxol, or Genexol-PM. Cells were irradiated with 2 fractions of indicated doses 12 hours apart. Significant differences between Taxol and Genexol-PM at a given dose are indicated (*$P<.01$, †$P<.05$). Error bars correspond to standard error of the mean (3 samples per time point). XRT = radiation therapy.

and the concern over hepatotoxicity, we also compared the paclitaxel concentration in mouse liver after Genexol-PM and Taxol administration. Although not statistically significant, Genexol-PM lead to lower paclitaxel exposure to mouse liver than paclitaxel at 6 hours ($P=.30$). Similar to the drug exposure in the lung, there was no difference in liver paclitaxel concentration between Genexol-PM and Taxol at 24 hours.
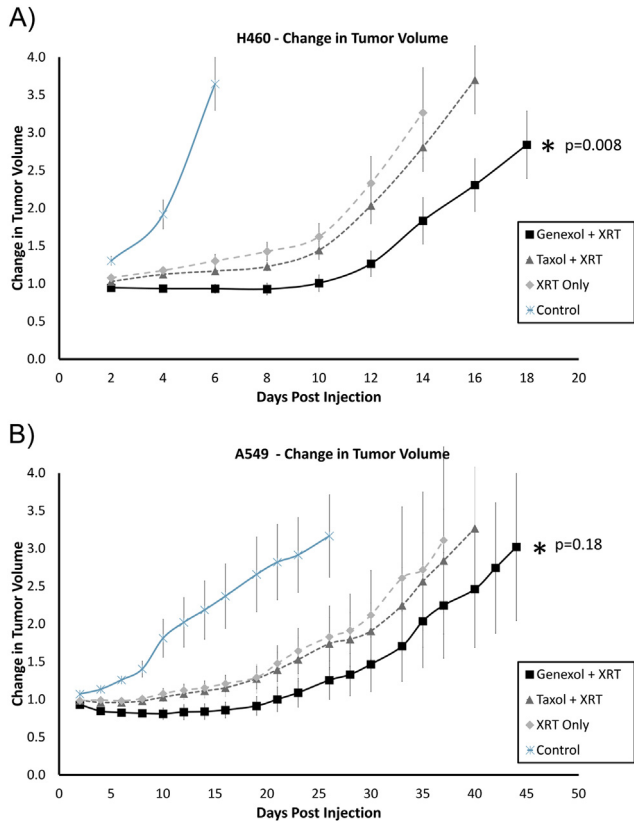
Discussion

A)

H460 - Change in Tumor Volume

* p=0.008

■ Genexol + XRT
▲ Taxol + XRT
◆ XRT Only
✕ Control

B)

A549 - Change in Tumor Volume

* p=0.18

■ Genexol + XRT
▲ Taxol + XRT
◆ XRT Only
✕ Control

Fig. 3.   Efficacy of Genexol-PM as a radiosensitizer in chemo-radiation therapy for non-small cell lung cancer cell cell lines in vivo. Mice bearing flank tumor xenografts were administered (via tail-vein intravenous injection) saline, Taxol, or Genexol-PM followed by radiation therapy (XRT). Tumors were irradiated in 5 daily fractions of 3 Gy beginning at 6 hours after chemotherapy injection. Changes in tumor volume were measured and the tumor growth delay curves for mice bearing (A) H460 and (B) A549 tumors were generated.

widely used as chemotherapeutics, there has been limited clinical evaluation of these drugs in chemoradiation therapy because of toxicity concerns (15). Recent nanomedicine clinical translation efforts have focused on the development of NP formulation of taxanes, a class of chemotherapeutics that is also used in chemoradiation. Today, there are 2 clinically approved NP taxane formulations, nab-paclitaxel (Abraxane) and Genexol-PM, with several additional therapeutics under clinical investigation (paclitaxel poliglumex [Opaxio], BIND-014 [biologically targeted polymeric NP docetaxel]) (5). Among these, Genexol-PM is the only NP therapeutic that possesses controlled drug release and is approved for clinical use. Furthermore, the only other approved NP taxane, nab-paclitaxel (nanoparticle albumin-bound pacli-taxel), dissociates upon administration and cannot take full advantage of the EPR effect (16). Therefore, Genexol-PM is the best NP therapeutic candidate for clinical translation in chemo-radiation therapy.

Preclinical studies have shown that the optimal NP physical characteristics for tumor targeting are sizes <100 nm with negative surface charges (17). Genexol-PM's properties (23.0 ± 4.5 nm and -8.1 ± 3.1 mV) suggest that it is excellent for tumor targeting. Another advantage of using Genexol-PM in chemo-radiotherapy is its controlled drug release. Although previous clinical investigations have demonstrated that prolonged drug exposure can increase the therapeutic efficacy of chemoradiation therapy (18), most small molecule chemotherapeutics, including taxanes, are too toxic to be administered continuously. NP therapeutics, conversely, can increase drug exposure time in tumors without increasing toxicity, both to local healthy tissue and systemically. In this study, we found Genexol-PM releases pacli-taxel in a controlled and slow fashion, with only 40% drug release after 16 hours and >90% of drug release after 48 hours. Such slow drug release should lead to increased synergistic effects between paclitaxel and radiation therapy, which in turn should result in improved therapeutic efficacy.

The efficacy of Taxol as a radiosensitizer has been evaluated previously (19). Our study compared the efficacy of Genexol-PM as a radiosensitizer with that of Taxol. At the IC_{50} dose,
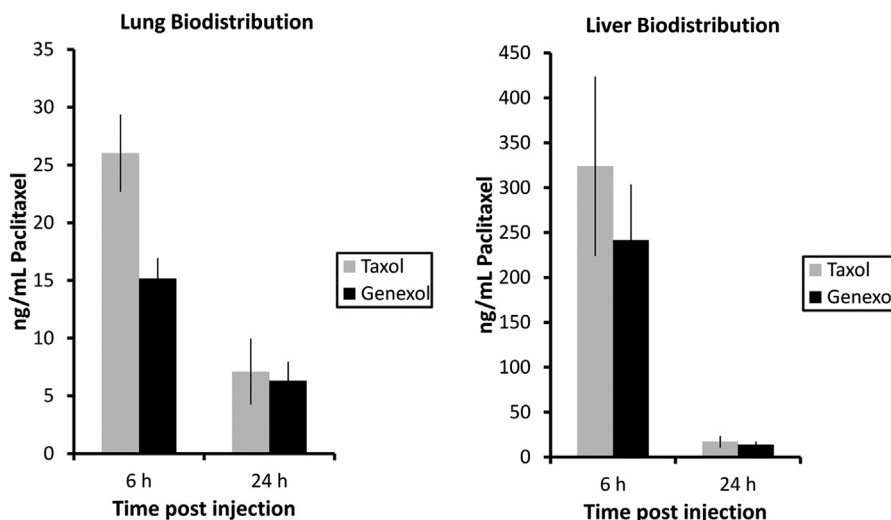

Lung Biodistribution

□ Taxol
■ Genexol

Liver Biodistribution

□ Taxol
■ Genexol

Fig. 4.   Quantification of paclitaxel dose in mouse lungs after Genexol-PM administration. Mice were administered equivalent paclitaxel

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