Preparation and Evaluation of Docetaxel-Nanoparticles for Intravenous Administration

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Introductory

- Docetaxel (DTX) is a semi-synthetic analogue of paclitaxel with significant antitumor activity against various human malignancies, including breast cancer, ovarian cancer, non-small cell lung cancer, and prostate cancer.
- Formulation development of DTX for intravenous delivery is challenging because the drug is practically insoluble in water.
- Administration of the currently marketed product, Taxotere®, in which DTX is stabilized with Tween 80, occasionally results in severe hypersensitivity reactions which are primarily attributed to the presence of Tween 80.
- In addition, the non-specific distribution of free DTX throughout the body contributes to drug-related side-effects, such as neurotoxicity, musculoskeletal toxicity, and nephrotoxicity.
- Nanoparticulate DTX formulations present less toxic and more targeted specific formulation vehicle for DTX.

Objective

- The objective of this study was to develop nanoparticles of DTX appropriate for intravenous administration using a template versus solvent evaporation process (Advanced EPAS) and to evaluate their suitability as a delivery system in cancer therapy.

Methods

Schematic of Advanced EPAS Process

Analitical Methods

Transmission Electron Microscopy
- DTX colloidal dispersions were deposited onto a carbon-coated copper specimen grid and stained using uranyl acetate.
- Size and morphology of particles were examined using a FEI Tecnai™ transmission electron microscope.

Particle Size
- Particle sizes of DTX colloidal dispersions were measured by photon correlation spectroscopy using a Malvern Zetasizer Nano ZS and are presented as the mean, hydrodynamic diameter (zeta averaged) and polydispersity index of three measurements.

Surface Charge
- Surface charge of DTX nanoparticles (DTX-NPs) was measured by determination of the zeta potential using a Malvern Zetasizer Nano ZS.
- Values of zeta potential were averaged over three repeated measurements.

In vitro Uptake
- T-47D cells were incubated with DTX-NPs or DTX Solution (Tween 80) (1:1) at 37°C and 5% CO2 for 0.5 and 4 hours.
- DTX was extracted with acetone and analyzed by HPLC.

In vitro Cytotoxicity
- In vitro cytotoxicity of NPs (without DTX), DTX-NPs, Solution (Tween 80) (1:1) and DTX Solution was evaluated in IL-1α stimulated murine mastocytoma cells (IC50) and human breast cancer (MDA-MB-468) cell lines over varying time periods.

In vivo Uptake Into Tumor Tissue
- Accumulation of DTX in tumors was evaluated in vivo by injecting either DTX-NPs or DTX Solution (Tween 80) (1:1) intravenously into C57BL/6 mice with pre-established mammary tumor-resistant tumor xenografts.

Pharmacokinetic Study
- The pharmacokinetic profile of DTX-NPs and DTX Solution (Tween 80) (1:1) was evaluated in mice by determining the concentration of DTX in plasma over time after intravenous bolus administration at a dose of 10 mg/kg, using typical drug metabolism and pharmacokinetics software.

Efficacy Study
- Mice randomly assigned to three groups (PBS, DTX Solution or DTX-NPs) were administered 20 mg/kg DTX/kg body weight by intravenous injections through the tail vein on day 5 and 10 after tumor implantation and tumor size was monitored.

HPLC-Assay
- Zorbax Eclipse Plus C18 (150 mm × 4.5 mm i.d., pore size 5 μm, Agilent).
- Mobile phase: acetonitrile/water (50:50, v/v) for standards, acetonitrile/water (5:95, v/v) for plasma and tissue samples.
- Flow rate: 1.0 mL/min, UV at 220 nm.

Conclusion

- In summary, DTX-NPs of appropriate size and stability were successfully prepared employing a template versus solvent evaporation process. Results from in vitro and in vivo studies indicate that DTX-NPs may act as an efficacious delivery system for DTX in cancer therapy.

Results

TEM Image

Short Term Stability of DTX Colloidal Dispersion

TEM image shows discrete sub-200 nm particles

DTX-NPs were stable for at least 7 days

In vitro Cytotoxicity

In vivo Uptake

Pharmacokinetic Study in Mice

DTX-NPs are effective in killing tumor cells

Accumulation of DTX in tumors was measured by in vivo imaging using IVIS Lumina II Imaging System.

NPs increase the accumulation of DTX in tumors compared to a solution

The pharmacokinetics of DTX-NPs in vivo after intravenous injection of DTX-NPs in C57BL/6 mice demonstrated a dose of 10 mg/kg.

DTX-NPs resulted in higher AUC and lower volume of distribution (Vd) than DTX-Solution.

Efficacy Study

Antitumor effect of DTX was measured using IVIS Lumina II Imaging System on tumor-bearing mice daily for 10 days.

DTX-NPs demonstrated significant antitumor activity.