Preparation of nano-particulated docetaxel using NUFS (nanoparticulation using fat and supercritical fluid) technique and its preclinical evaluation

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ABSTRACT SUMMARY

The application of nanotechnology in human cancer treatment is offering many exciting possibilities including enhancement of anti-cancer efficacy and reduction of side effects. The taxanes have been widely used in cytotoxic treatment of many solid tumors as a family of very efficient anticancer drugs, but the current commercial formulation for the two main taxanes-Taxol for paclitaxel and Taxotere for docetaxel (DTX)-have shown dramatic side effects. DTX has been known to be superior to paclitaxel in clinical efficacy against many cancers, but it also exhibits severe side effects. Taxotere, the most famous commercial formulation of DTX contains the non-ionic surfactant Tween 80 (polysorbate 80) and 13% ethanol; the side effects caused by DTX and the solvent have significantly limited its clinical use. To improve the dissolution of DTX and expect enhanced permeability and retention (EPR) effect, a novel technique of nanoparticulation using fat and supercritical fluid (NUFS) was employed. The nanoparticulated DTX without any carrier, named Nufs-DTX, was prepared and reconstituted in 1-mlial status of water-soluble. The particle size and zeta potential were confirmed with dynamic light scattering (DLS). In vitro efficacy study of Nufs-DTX was compared to Taxotere by clonogenic assay. To evaluate in vivo efficacy, tumor growth delay was performed using A549 xenograft mouse model. The purpose of this study was to carry out a detailed evaluation of an intravenous lipid emulsion for docetaxel without Tween 80 before clinical administration. These results lead us to conclude that Nufs-DTX would have a great potential for the improvement of efficacy and toxicity in human lung cancer treatment compared to the crude Taxotere.

EXPERIMENTAL METHODS

Nano docetaxel (Nufs-DTX) was prepared NUFS technology from Bio-Synetics. The particle size and zeta potential were confirmed with Dynamic Light Scattering (DLS).

To evaluate in vitro efficacy, clonogenic assay was performed using A549 and MDA-MB231 cells. Colony formation was analyzed by plating 500 cells or 100 cells per well in six-well culture plates. Cells were treated with 0.05 or 0.1 ng/ml of Nufs-DTX or Taxotere and then incubated at 37°C for 10 days. Colonies were stained with 0.5% crystal violet in methanol. Stained colonies were washed with water, air-dried, and counted when they consisted of more than 50 cells. The survival fraction was calculated as (mean colonies counted)/(cells plated) x (plating efficiency), where plating efficiency was defined as (mean colonies counted)/(cells plated). All values were normalized to untreated cells.

To evaluate in vivo efficacy, tumor growth delay was performed using A549 xenograft mouse model. A suspension of 1x10⁶ cells in a 50ul volume was injected subcutaneously into the right hindlimb of mice. Tumors were grown for 2 weeks until average tumor volume reached 80~100mm³. Mice were treated with 10mg/kg of Nufs-DTX by intravenous injection one or three times. Body weights and tumor volumes were monitored for 4 weeks. Tumor volume was calculated using the formula

\[ V = \frac{(L \times W^2)}{2} \times 0.5, \]  
where \( V \) = volume, \( L \) = length,
and W = width. All experiments were performed following the protocol approved by the Institutional Animal Care and Use Committee of the Asan Institute for Life Science.

RESULTS AND DISCUSSION
Nufs-DTX was prepared the 1 vial status of water-soluble. The particle size and size distribution were determined using zetasizer (Fig.1). The particle size was 170±20nm. Nufs-DTX was well dispersed in water and stably maintained at room temperature for 4 weeks or more.

Figure1. Size distribution of Nufs-DTX

A clonogenic assay showed that Nufs-DTX was resulted in similar cell killing effect with Taxotere in A549 cells and equal or greater anticancer effect in MDA-MB231 cells (Fig.2).

Figure2. The survival fraction of Nufs-DTX in lung or breast cancer cells by clonogenic assay.

To evaluate anti-cancer effect of Nufs-DTX, tumor growth delay was performed using A549 xenograft mouse model by single injection compared to original drug (Taxotere). Nufs-DTX was confirmed to have same anticancer effect with Taxotere by single injection (Fig.3A). At Day 29 after multiple treatment, tumor volume was increased about 2.4 fold in Nufs-DTX treated group while increased 8.1 and 4.2 fold in untreated and Taxotere treated group. Hence, Nufs-DTX was confirmed to have enhanced anticancer effect than Taxotere by multiple injection (Fig.3B).

CONCLUSION
Nufs-DTX showed higher anticancer activity with less toxicity in NSCLC xenograft model. The high effectiveness and low toxicity of Nufs-DTX might be an attractive new formulation and an appropriate choice for the clinical administration of Taxotere.

REFERENCES

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