A Novel Nanopolymer Based Tumor Targeted Delivery System for Paclitaxel

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ABSTRACT 11095 Background : We have developed a novel Cremophor free Paclitaxel formulation –Nanoxel™ (DO/NDR/02) in which the drug is delivered as nanomicelles using a polymeric carrier. Our Phase I data has previously shown that DO/NDR/02 has linear pharmacokinetics and can be administered without premedication. The Phase II data in metastatic breast cancers showed comparable efficacy and better safety profile than Cremophor Paclitaxel. We report here the putative mechanism of action of DO/NDR/02. **Methods :** We have designed and synthesized a pH sensitive co-polymer of N–isopropyl acrylamide (NIPAM) and Vinylpyrrolidone (VP) monomers. The nanomicelles were characterized for size and morphology by scanning electron microscopy (SEM). The journey of nanomicelles after systemic administration was tracked using chromatographic methods and transmission electron microscopy (TEM). The cellular uptake studies were carried out in target cancers and the tumor uptake was assessed in xenograft models. Release of intracellular Paclitaxel from the nanomicelles in the lysosomal compartment was correlated with the *in-vitro* release of the drug at varying pH conditions. Tubulin polymerization and apoptosis were assessed by standard techniques. **Results :** Paclitaxel was found to retain the *in-vitro* cytotoxicity in nanomicellar particles. Smooth spherical particles of 80-100 nm were observed by SEM. DO/NDR/02 showed upto three-fold higher uptake in target cancer cells as compared to Cremophor Paclitaxel. TEM demonstrated that the nanomicelles were endocytosed and entrapped in the acidic endolysosomal compartment. *In-vitro* release of Paclitaxel was faster at lower pH conditions. This resulted in an increase in tubulin stabilization & induction of apoptosis. **Conclusion :** Our data delineates the putative mechanism of action of DO/NDR/02, which may explain its clinical efficacy & improved safety. It also suggests that this nanopolymer is a novel pH sensitive tumor targeting delivery system with potential for application to other hydrophobic drugs.

DABUR RESEARCH FOUNDATION

I. Introduction

- We have developed a novel amphiphilic copolymer based Nanomicellar delivery system for Paclitaxel code named-DO/NDR/02.
- Paclitaxel is microtubule-stabilizing taxane currently approved for the treatment of breast and ovarian cancer, NSCLC and Kaposi's sarcoma.
- The currently marketed Cremophor based formulations of Paclitaxel are associated with severe & unpredictable hypersensitivity reactions.
- DO/NDR/02 is a novel Cremophor free Polymeric Nanomicellar delivery system for Paclitaxel.
- DO/NDR/02 comprises of a co-polymer of N–isopropyl acrylamide (NIPAM) and Vinylpyrrolidone (VP) monomers, which encapsulate Paclitaxel in its hydrophobic core.
- This Nanomicellar delivery system leverages its unique pH sensitivity to deliver Paclitaxel selectively in the endo-lysosomal compartment of the tumor cells.
- DO/NDR/02 showed good efficacy & minimal toxicities in Phase II clinical trials for metastatic breast cancer.
- We report here the putative mechanism of action of the delivery system. Data suggests that it is a novel pH sensitive tumor targeting delivery system for Paclitaxel with potential for application to other hydrophobic drugs.

II. Preclinical Efficacy

- ***In-vitro* anticancer activity**
  Broad spectrum anticancer activity comparable to Cremophor Paclitaxel formulation in human cancer cell lines representing breast, ovary, NSCLC and prostate.
- ***In-vivo* efficacy in tumor xenografts**
  Complete regression of oral squamous carcinoma (KB) tumor xenografts grown in Balb/c athymic mice. Efficacy was comparable to conventional Cremophor Paclitaxel.

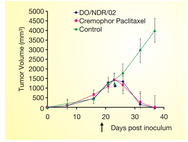


Figure 1. *In-vivo* antitumor activity of DO/NDR/02 and Cremophor Paclitaxel at dose of 8 mg/kg in KB tumor xenografts

III. Pharmacokinetics

Rodent Pharmacokinetics

- DO/NDR/02 has a more linear PK profile as compared to Cremophor Paclitaxel.

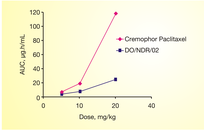


Figure 2. Pharmacokinetic profiles of DO/NDR/02 and Cremophor Paclitaxel in Swiss Albino mice at dose levels of 5, 10 and 20 mg/kg

IV. Formulation

Preparation & Characterization

- The micelles of DO/NDR/02 are prepared *in-situ* by the addition of polymer into the aqueous solution using proprietary technology.
- Encapsulates Paclitaxel with an efficiency of > 95%.

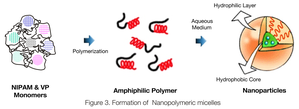


Figure 3. Formation of Nanopolymeric micelles

Size analysis

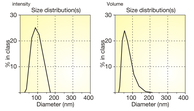


Figure 4. Representative size distribution profile of DO/NDR/02 using Photon Correlation Spectrometer

- Mean particle size of nanoparticles is less than 100 nm.
- Size distribution pattern was unimodal.
- Polydispersity was in the range of 0.12 to 0.2 indicating uniform particle size range.

Zeta Potential

- Zeta potential was in the range of –13.3 mV to –20.1 mV.

Surface Morphology

Figure 5. Scanning Electron Micrographs of DO/NDR/02 at 100 kx

- Nanoparticles encapsulating Paclitaxel are smooth and spherical as visualized by Scanning Electron Microscopy.

pH dependent Least Critical Solution Temperature(LCST)

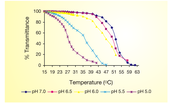


Figure 6. Plot of % transmittance of DO/NDR/02 at varying pH conditions

- At physiological conditions (pH 7.0), LCST of DO/NDR/02 is 52°C enabling the nanoparticles to be well dispersed & stable.
- Under acidic pH conditions (pH 5.0), LCST drops to 27°C leading to a loss of nanoparticle integrity & subsequent Paclitaxel release.
- This pH dependent polymeric switch enables release of Paclitaxel selectively in the endo-lysosomal compartment of the tumor cells.

Paclitaxel release *in-vitro*

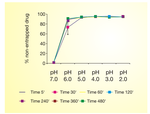


Figure 7. Release of Paclitaxel from DO/NDR/02 under varying pH conditions

- Paclitaxel release from DO/NDR/02 was found to be pH sensitive. > 90% release was observed in the acidic range (pH 2.0 to 5.0).

V. Uptake in target cancer cells

Method

- Cellular Uptake of DO/NDR/02 and Cremophor Paclitaxel was evaluated in human breast & ovarian cancer cells.
- The cancer cells were treated with formulations for 3 hours prior to analysis by chromatography based methods.

Results

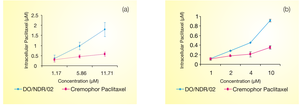
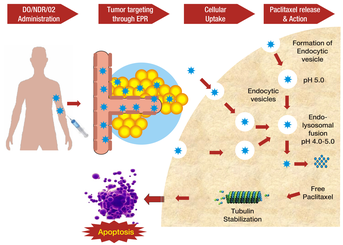


Figure 8. Intracellular accumulation of Paclitaxel in MDA-MB-453 (a) & PA-1 (b)

- DO/NDR/02 showed 2.5-3 fold higher accumulation in target cancer cells as compared to Cremophor Paclitaxel at concentration of 10µM.

Proposed Mechanism of Selective Paclitaxel Delivery by DO/NDR/02



VI. Intracellular trafficking

Method

- Cellular uptake & Intracellular trafficking of DO/NDR/02 was visualized in breast cancer cells by Transmission Electron Microscopy.

Results

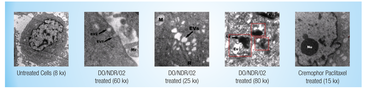


Figure 9. Transmission electron micrographs of MDA-MB-453 cells treated with 10µM DO/NDR/02 & Cremophor Paclitaxel for 30 minutes.
Nu: Nucleus; M: Mitochondria; EV: Endocytic Vesicle; Ly: Lysosome

- DO/NDR/02 treated cells showed numerous clusters of EVs in the cytoplasm.
- Uptake of nanoparticles is mediated by process of endocytosis as suggested by invagination of plasma membrane, which may lead to higher paclitaxel uptake.
- Internalized nanoparticles captured in EVs were transported to lysosomes (Ly) by endo-lysosomal fusion.
- Cremophor Paclitaxel treated cells showed numerous swollen mitochondria with absence of EVs.

VII. Microtubule stabilization

Method

- Effect of DO/NDR/02 & Cremophor Paclitaxel on microtubule stabilization in breast cancer cells was determined by tubulin polymerization *in situ*.

Results

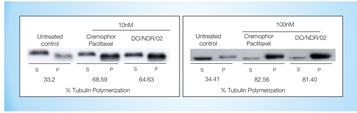


Figure 10a. Western Blots showing tubulin polymerization potential of DO/NDR/02 & Cremophor Paclitaxel in MDA-MB-453 cells after 16 hours of treatment

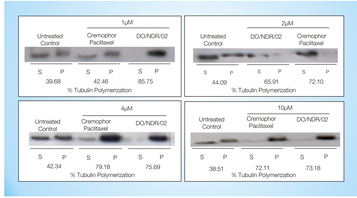


Figure 10b. Western Blots showing tubulin polymerization potential of DO/NDR/02 & Cremophor Paclitaxel in MDA-MB-453 cells after 3 hours of treatment

- Tubulin polymerization potential of DO/NDR/02 was comparable with Cremophor Paclitaxel in the dose range of 10nM to 10µM.
- Paclitaxel entrapped in DO/NDR/02 retains the potential of tubulin binding and stabilization of microtubules.

VIII. Induction of apoptosis

Method

- Apoptotic potential of DO/NDR/02 & Cremophor Paclitaxel was determined in breast cancer cells by visualization of micronuclei using DAPI based method.

Results

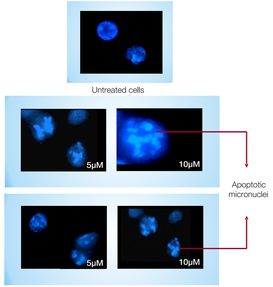


Figure 11. Visualization of micronuclei in MDA-MB-453 cells treated with DO/NDR/02 & Cremophor Paclitaxel at 5µM & 10µM for 7 hours

- Apoptotic potential of DO/NDR/02 & Cremophor Paclitaxel was found to be comparable.

IX. Safety

Polymer Safety

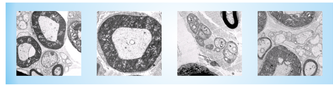


Figure 12a. Electron micrographs of Sciatic nerve in Wistar rats treated with polymer

- Does not cause peripheral nerve damage.

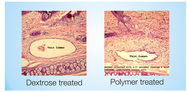
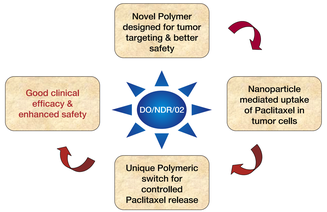


Figure 12b. H & E stained sections of rabbit ears treated with dextrose & polymer

- Does not accumulate in organs with high vasculature.
- No local toxicity at site of injection by intravenous or subcutaneous routes.
- No adverse effects on histaminic and cholinergic responses.
- No undesirable effects on cardiac and renal functions.

X. Conclusions

- DO/NDR/02 is a novel drug delivery system that exploits a unique polymer designed to deliver the entrapped Paclitaxel in its target cancers.
- DO/NDR/02 shows the potential to overcome the pharmacological shortcomings associated with Cremophor based therapy such as solubility, pharmacokinetics and toxicity.
- Mechanistic profiling revealed that the polymeric nanomicelles leverage their pH sensitivity for preferential targeted delivery of Paclitaxel into the tumor cells.
- DO/NDR/02 mediated higher cellular uptake & selective delivery of Paclitaxel manifests in good clinical efficacy & better safety profile compared to the Cremophor formulation.
- DO/NDR/02 may serve as a promising and efficient drug delivery system with the potential to enhance the clinical utility of Paclitaxel.



XI. Acknowledgments

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