Improved Safety and Efficacy of Surface Engineered Docetaxel Nanoparticles for Targeting in Prostate Cancer

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ABSTRACT SUMMARY
Prostate cancer globally is the sixth leading cause of cancer-related death in men. Docetaxel is the drug-of-choice for prostate cancer treatment. The present work aimed to determine the safety and efficacy of developed surface engineered Docetaxel modified nanolipid carrier in Prostate cancer and compare with marketed Taxotere injection (DTX-MKT) which has been associated with severe side effects due to presence of high amount of Polysorbate 80 as solubiliser.

INTRODUCTION
Cancer remains the most devastating disease and the in-vivo fate of the drug treatment is determined by the carrier system which permits a controlled and localized release of drug. This has urged the need for developing specialized lipid based drug delivery systems. Nanotechnology offers unprecedented and paradigm-changing opportunity to study and interact with normal and cancer cells at molecular level. The present research work explores modified nanolipid carrier for encapsulating docetaxel (DTX-MNLC). Further Linoleic acid grafted MNLC (DTX-MNLC-LA) were investigated for its physico-chemical properties, in-vitro cytotoxicity and cellular uptake in PC3 cell lines and in-vivo targeting in orthotropic prostate mice model

EXPERIMENTAL METHODS
DTX-MNLC was prepared by o/w melt emulsification. The hot emulsion was then subjected to homogenization at different pressure and number of cycles to obtain desired particle size. DTX-MNLC was suitably surface modified with linoleic acid to give DTX-MNLC-LA.

A. Characterization of DTX-MNLC and DTX-MNLC-LA:
(i) Particle size, size distribution and Zeta potential: The particle size, distribution and zeta potential were determine by photon correlation spectroscopy.
(ii) DSC, NMR, FTIR, TEM and AFM analysis: The surface morphology of the bare and surface modified nanoparticles was examined by TEM and AFM and the surface modification of DTX-MNLC-LA was confirmed by DSC, FTIR and NMR analysis
(iii) In-vitro drug release study: USP dissolution apparatus method II was employed. For determining the release pattern of the drug, the nanoparticles were enclosed within cellulose acetate dialysis bags.

B. In-vitro Safety assessment by hemolysis assay
Hemolytic activity of docetaxel loaded nanoparticles was investigated to find out the percent hemolysis of formulation.

C. In-vitro cytotoxicity assay in PC3 Human Prostate cancer cell line. The cells were seeded evenly into 96-well flat bottomed tissue culture plate at 5 x 10⁴ cell/well concentration and incubated for 24h in a humidified atmosphere of 5% CO₂ at 37°C and treated with the formulations at different concentrations.

D. In-vitro cell uptake study was carried out using PC3 cell lines by confocal laser microscopy at 25 and 50μg/ml concentration with respect to time.

F. In-vivo Tumor targeting was determined by gamma scintigraphy in orthotopic prostate tumor model.

G. In-vivo efficacy study was carried out in tumor developed in C57B16 mice by injecting B16F10 melanoma cell lines in prostate region

RESULTS AND DISCUSSION
The developed modified lipid nanocarrier could incorporate 1.0% drug with around 95% entrapment efficiency with mean particle size below 70nm with P.I less than 0.3 and zeta potential -15.8mv. The DSC of pure DTX, DTX-MNLC and Blank MNLC shown in fig.2 showed no presence of crystalline drug in MNLC confirming the encapsulation of drug inside the
nanocarrier. TEM and AFM images (Fig 3 and 4) confirm the size of NPs in nanometric range. The drug loaded nanocarrier further surface modified by chemical bonding using EDC/NHS with Linoleic acid and confirmed by change in particle size 87.7nm P.I 0.434, zeta potential -17.7mv, FTIR and NMR analysis. The formulation was evaluated for % haemolysis with respect to the marketed formulation, nanocarrier has shown reduced hemolysis within the acceptable range for I.V administration while the marketed formulation showed 80% hemolysis. In MTT assay IC50 for DTX-MKT was found to be more than 75µg/ml. similar treatment with DTX-MNLC resulted in IC50 75µg/ml and DTX-MNLC-LA gave an IC50 value less than 25µg/ml. Confocal microscopy showed that in first hour the formulations were not yet taken up by the cells. At 4h DTX-MKT and DTX-MNLC at 50ug/ml concentration had started approaching the cytoplasmic region of cells in while the DTX-MNLC-LA had reached the nucleus region and started destroying the cells with cell count reduced significantly. In 6th hour the DTX-MNLC-LA had reached the nuclear region and showed maximum cell death while DTX-MKT and DTX-MNLC were found to be still in the cytoplasmic region and had not reached the nucleus. The cell count was found to be significantly reduced showing maximum cell death in DTX-MNLC-LA > DTX-MNLC > DTX-MKT.

From gamma scintigraphic images it can be seen that DTX-MNLC-LA showed better localization in prostate tumor region than marketed and even DTX-MNLC. In-vivo efficacy study demonstrated that the tumor treated with DTX-MNLC-LA showed almost complete reduction in tumor volume in comparison to DTX-MKT, DTX-MNLC. From Hematological studies it was observed that the normal leucocyte count of mice was reduced to 50% of normal with DTX-MKT while DTX-MNLC and DTX-MNLC-LA the leucocytes count was reduced by 25%.

CONCLUSION:
The surface modified docetaxel loaded lipid nanoparticles were optimized and prepared successfully by hot high pressure homogenization technique with good entrapment efficiency and stability. The surface modification was confirmed by particle size, zeta potential, FTIR and NMR analysis. The nanocarrier system was found to be more effective and less toxic in comparison to marketed formulations as observed by in-vitro hemolysis. The surface modified lipid nanocarrier system evaluated in PC3 prostate cancer cell lines was found to show better efficacy and demonstrated potential for targeting in orthotopic tumors in prostate region.

REFERENCES:

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