Cetuximab Conjugated Docetaxel Loaded γ-Poly (glutamic acid) Based Targeted Nanomedicines for Epidermal Growth Factor Receptor Overexpressing Cancers

Abstract

Molecular targeted therapy or active targeting specifically promotes binding of nanoparticles to cancer cells via its up-regulated receptors and rapid intracellular uptake of nanoformulations. Epidermal growth factor receptor (EGFR) has been upregulated in many human malignancies like glioma, gastric, colorectal, head, neck, Non-small cell lung, ovarian, breast and prostate cancers. Tumor specific delivery of therapeutic payload thereby improved therapeutic efficacy can be achieved by conjugating nanocarriers with EGFR specific ligands and hence EGFR serve as a promising target for actively targeted cancer drug delivery. Anti-EGFR targeted therapies based on monoclonal antibodies have improved the efficacy of conventional chemotherapy in both preclinical and clinical studies. Cetuximab (Erbitux) is an immunoglobulin G1 mouse-human chimeric monoclonal antibody that targets human EGFR with high affinity and abrogates ligand-induced EGFR phosphorylation, thereby blocking EGFR signalling cascades. Nanotechnology inculcates innovative strategies to overcome the limitations of conventional chemotherapeutics by entrapping the anticancer agents in biocompatible and biodegradable nanocarrier systems with varying architecture resulting in its controlled and specific release to the target cancer tissue. γ-Poly (glutamic acid) (γ-PGA) naturally occurring polyamides has been exploited as a good sustained release material or drug carrier owing to its biodegradability and non-toxicity.

This work has attempted to develop an actively targeted nanoformulation based on γ-PGA carrier for delivering hydrophobic taxane Docetaxel (DTXL) as a model anticancer drug. Therapeutic potential of DTXL-γ-PGA Nps can be augmented by tagging them with Cetuximab (CET) that selectively interact with EGFR over expressed on colon cancer (HT-29), Non small cell lung cancer (A549) and gastric carcinoma (MKN-28) cells. To initiate the study the therapeutic moiety; DTXL was
loaded into a nanomatrix based on γ-PGA and chitosan following polyionic complexation technique which was further decorated with targeting moiety; CET antibody through simple EDC-NHS conjugation chemistry and characterised. The developed non-targeted (DTXL-γ-PGA Nps; NT Nps) and targeted (CET-DTXL-γ-PGA Nps; T Nps) nanoformulation had uniformly distributed nanoparticles with average size of 131.3±17.5 nm (PDI: 0.31±0.13) and 182.58±16.47 nm (PDI: 0.182±0.041) respectively. The DTXL encapsulation and loading efficiency for the DTXL-γ-PGA Nps and CET-DTXL-γ-PGA Nps was found to be 49±6% & 16±6% and 32±8% & 12±2% respectively along with 42±10% of CET conjugation efficiency from Bicinchoninic acid (BCA) assay.

The biological activity of these nanoparticles were evaluated in EGFR +ve cancer cell lines (HT-29, A549 and MKN-28) and EGFR -ve fibroblast cell line (NIH3T3). Cellular interactions of these nanoparticles was analysed by flow cytometry and confocal microscopy for cellular uptake and internalization revealed EGFR specific enhanced uptake by targeted Nps in EGFR +ve HT-29, A549 and MKN-28 cancer cells compared to that of non-targeted Nps and EGFR -ve NIH3T3. The cytotoxicity induced by these targeted Nps was evaluated primarily by MTT assay and later confirmed with cell cycle analysis, mitochondrial membrane potential and apoptotic assays. It was observed that targeted Nps showed superior antiproliferative potential to kill the EGFR +ve cancer cells compared to that of non-targeted Nps. The in vitro analysis thus indicated CET mediated EGFR targeted uptake of nanomedicines which enhanced the availability of DTXL within the cancer cells thereby enhancing the therapeutic effect.

The results obtained from the in vitro interactions of the engineered nanomedicines prompted the study on in vivo models. In vivo pharmacokinetics and organ distribution analysis in Swiss albino mice confirmed the slow and controlled release of DTXL in plasma in turn indicating the prolonged circulation of the nanoformulations enhancing the availability of DTXL compared to that of free DTXL. However no significant difference was observed between targeted and non-targeted Nps. The potential of these formulations as cancer nanomedicines were further evaluated in EGFR +ve MKN-28 based gastric cancer xenograft models in
Balb/c nude mice. Imaging of IR780 labelled targeted Nps revealed enhanced *in vivo* retention and tumour accumulation. Higher amount of DTXL was observed in tumour tissues excised from mice treated with CET conjugated Nps compared on day 1 and day 4 to that of non-targeted Nps indicating the prolonged circulation of these Nps and targeted accumulation in tumors. Time dependant tumour volume measurement showed that targeted Nps significantly arrested the growth of tumors compared to that of non-targeted and free DTXL formulations. Tumor mass was very low for the mice treated with CET-DTXL-γ-PGA Nps post 20 day treatment with 3 doses (one per week) which was statistically significant.

Overall, the present thesis work highlighted the potential of CET conjugated DTXL loaded γ-PGA Nps as an EGFR targeted drug delivery system and also provides thrust to the therapeutic potential of these targeted nanomedicine towards EGFR overexpressing malignancies.