Design and Development of Ligand Conjugated Biodegradable Nanoparticles Containing Paclitaxel to Target Prostate Cancer Cells

Abstract: Prostate cancer is a common and recurrent cancer type in males globally, with a growing incidence of mortality. Research shows that the risk of developing prostate cancer increases with age. Prostate cancer is often asymptomatic in the early stages; hence, detecting and treating it in the initial stages becomes difficult. The initial symptoms of prostate cancer are similar to problems observed with an enlarged prostate. Once the disease becomes malignant, the prognosis is poor in most cases, increasing the number of fatalities. Metastasis in later stages often becomes incurable. Several treatments are being studied in conjunction with surgery or radiotherapy to enhance overall survival in newly diagnosed prostate cancer as well as early metastatic prostate cancer. Conventional chemotherapies target cancer cells in a non-specific manner.

Nanotechnology is rapidly expanding, with far-reaching implications for the future of science and medicine. By using active drug delivery vehicles such as ligand attachment, significant efforts are being undertaken to target cancer-specific cells more efficiently with nanoformulations because of the use of lower therapeutic agent doses, increased efficacy while minimizing toxicity, and increased residence duration in the body while reducing harmful effects on healthy tissues. Active targeting ligands such as antibody are commonly employed for cancer cell site-specific targeting, resulting in higher biocompatibility, bioavailability, and active targeting at the cancer site. In recent years, there has been a significant surge in the manufacture and development of nanoparticles to treat prostate cancer.

In the present study, paclitaxel-loaded poly (d,l)-lactic-*co*-glycolic acid (PLGA) nanoparticles (PTX-NP) were prepared, and conjugated with the J591 antibody (Ab-PTX-NP) in order to bind specifically to the specific prostate cancer cell receptor, prostate-specific membrane antigen (PSMA). PSMA expression is elevated in prostate cancer cells. The conjugated antibody to paclitaxel nanoparticles would bind with the highly expressed PSMA on prostate cancer cells, delivering the encapsulated drug paclitaxel (PTX) to the cells. Paclitaxel is an anticancer drug with a broad anti-tumor activity spectrum. PTX promotes and stabilizes microtubules while inhibiting the late G2 or M phases of the cell cycle, thus further causing apoptosis. However, in our study, we tried to use its therapeutic application in cancer therapy, which was limited due to its low water solubility and associated toxicity. PLGA is a United

States Food and Drug Administration (USFDA) approved polymer that has several advantages over other polymers, such as the fact that it is biodegradable, drug release can be manipulated, and it produces stable, desired nanoparticles.

Both PTX-NP and Ab-PTX-NP were made to obtain the optimal parameters as per the needs of the study. SDS-PAGE gel electrophoresis confirmed the conjugation of the antibody to the surface of PTX-NP. The experimental formulations were evaluated on various parameters; the desirable size range was obtained with a negative zeta charge. Morphological evaluations were done with the help of scanning electron microscopy, atomic force microscopy, and transmission electron microscopy, which revealed the particles to be distinct and homogenous with a smooth surface with a uniform distribution of the drug throughout the nanoparticles. The encapsulation efficiency was found to be satisfactory. The experimental data on drug release elucidated that both PTX-NP and Ab-PTX-NP had an efficient sustained drug release, which could result in optimal bioavailability of PTX within the therapeutic window over a long period of time. Further, the release pattern of paclitaxel from Ab-PTX-NP was evaluated in various release media. The release kinetic study revealed that the PTX release pattern from PTX-NP and Ab-PTX-NP in the respective medium followed the Korsmeyer—Peppas kinetic model. The PTX-NP and Ab-PTX-NP were found to be stable when stored in the refrigerator (at 4–8°C).

The in vitro cellular investigations of the experimental formulations were done on LNCaP and PC3 cells. PSMA is overexpressed in LNCaP cells while absent in PC3 cells, this data was supported by Western blot analysis. The cytotoxicity assay was done by MTT analysis, which displayed that the lower dose of Ab-PTX-NP produced an elevated cytotoxic effect when compared with PTX-NP and free drug in PSMA-overexpressing LNCaP cells. Cellular internalization of the formulation in LNCaP cells ensured the encapsulated drug was delivered to the cytosol. The in vitro cellular uptake study of the prepared formulations wascarried out both quantitatively and qualitatively in prostate cancer cells, revealing superior cellular internalizations of antibody-conjugated nanoparticles in LNCaP cells. The apoptosis was induced by Ab-PTX-NP, which was demonstrated by the colour shift of apoptotic cells as observed by AO/EB staining. Further, the hallmarks of apoptosis were observed in LNCaP cells by Ab-PTX-NP, such as loss of membrane integrity, nuclear fragmentation, chromatin condensation, and morphological alterations owing to apoptotic body formationspaving the wayfor the identification of theprogression of cancerous cell death

by apoptosis. Variations in mitochondrial transmembrane potential (MMP) lead to depolarization of the mitochondrial membrane. Apoptosis is characterized by mitochondrial depolarization and rupturing, which disrupts Ca^{2+} equilibrium between the mitochondria and the endoplasmic reticulumand shows that cells undergoing apoptosis have potentially lower MMP levels. This study confirmed that increased cellular uptake of Ab-PTX-NP correlates with effective depolarization of the mitochondrial membrane in LNCaP cells.

As a result, Ab-PTX-NP had the maximum potency and speed in inducing apoptosis in PSMA-expressing prostate cancer cells. This work acknowledges the efficacy of Ab-PTX-NP in vitro, demonstrating increased cellular cytotoxicity and internalization, as well as maximal apoptosis (74.1%) in PSMA-abundant LNCaP cells against PSMA-negative PC3 cells. Ab-PTX-NP can be rendered safe for intravenous administration as the hemolysis rate was within the permissible range. Pharmacokinetics data (by LC-MS/MS) for the experimental nanoparticles and free drug at various predefined time points revealed increased bioavailability and prolonged drug release from Ab-PTX-NP and PTX-NP during the study period upon i.v. administration into the systemic circulation of male Balb/c mice.

Targeted drug delivery for prostate cancer was focused on developing our formulation Ab-PTX-NP as an overarching treatment for patients with prostate cancer in order to prevent further metastases, maintain quality of life, and prolong overall survival.