Abstract

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Title of the thesis: Development of aptamer-functionalized biodegradable polymeric nanoparticles for targeted prostate cancer therapy: *in vitro* and *in vivo* study

Prostate cancer is a common type of cancer that develops in the prostate gland, a small, walnut-sized organ in males found directly below the bladder. It grows slowly and may not cause symptoms in the beginning. However, when it progresses, it can cause symptoms such as urinatingtrouble, frequent urination, blood in the urine or sperm, and pelvic or lower back pain. A digital rectal exam (DRE) and a prostate-specific antigen (PSA) blood test are frequently used to diagnose prostate cancer. Because of off-target adverse effects on healthy organs and the development of drug-resistance qualities after numerous doses of treatment regimen, conventional therapies like as radiation, chemotherapy, and hormone therapy cannot deliver a long-term satisfactory outcome.

Abiraterone, an active metabolite of abiraterone acetate, is a potent specific inhibitor of 17-hydroxylase/C17, 20-lyase (CYP17), a crucial enzyme in the production of testosterone and an inhibitor of androgen production in testicular, adrenal, and prostatic tumor tissues. The drug, BCS (Biopharmaceutics Classification System) class IV medication, is a current potential treatment option for prostate cancer. However, some inevitable limitations, such as poor solubility, permeability, and low biodistribution, make the drug difficult for its successful clinical use. To overcome the adverse side effects and minimize the disease burden, targeted nanocarrier-mediated therapy can deliver the drug to the organ of interest to manage prostate cancer more efficiently.

The utilization of nanoparticles (NPs) in the field of anticancer medication delivery has garnered considerable interest. Nanoparticles have the potential to facilitate the delivery of a diverse array of anticancer medicines that exhibit suboptimal pharmacokinetic characteristics. In our investigation, we used nanoparticles (NP), which are gaining interest in anticancer drugs delivery. Nanoparticles can be used to deliver a broad range of anticancer medicines with poor pharmacokinetic qualities. The site-specific delivery of drug molecules via ligand attachment to nanoparticles can sustainably maintain drug levels at an optimum therapeutic rate at the target organ. Poly(lactic-co-glycolic acid) (PLGA) is one of the most extensively used biodegradable polymers approved by the United States Food and Drug Administration (US-FDA) for systemic use. Upon hydrolysis, the polymer breaks down into two monomers, lactic acid and glycolic acid. These two monomers are endogenous and swiftly metabolized by the body via the Krebs cycle; employing PLGA to deliver drugs or biomaterial applications with no considerable systemic toxicity.

One of the most promising treatment techniques for cancer cell targeting is targeting biomarkers overexpressed on the surface of cancer cells. Aptamers are synthetic single-stranded RNA or DNA oligonucleotides (typically 25-90 nucleotide bases) with the ability to fold into complex three-dimensional structures via intramolecular interactions, with target specificity, low immunogenicity, and high tissue penetration ability, making them superior to antibodies and other targeting molecules. Aptamers have been identified as interesting candidates for the development of a wide range of smart devices, including drug administration, treatment, diagnostics, and bio-imaging. In comparison to antibodies, aptamers have good stability in a wide pH range, temperatures, organic solvents, low cost, simple synthesis, sensitivity, and high affinity to binding pockets of diverse target antigens.

We developed ΔPSap4#5 aptamer-conjugated abiraterone (ABR)-loaded PLGA nanoparticle (Apt-ABR-NP) and it was further characterized physicochemically. The conjugation was confirmed by agarose gel electrophoresis and X-ray photoelectron spectroscopy. In agarose gel electrophoresis study, the DNA aptamer moved through the gel and landed parallel to the conventional DNA ladder of the 50bp marker. The aptamer-coupled nanoparticles (Apt-ABR-NP), on the other hand, stayed inside the loading well, as evidenced by fluorescence. Interaction between the drug and the chosen excipients were analyzed by using Fourier-transform infrared (FTIR) spectroscopy. The FTIR study indicates no chemical interaction between the drug and the excipients used in this study. However, a few slight shifts in the peak were solely responsible for the physical interactions that might provide the structure of the formulation. Further, the average nanoparticle size and zeta potential were measured using the Dynamic light scattering method.The average values of the hydrodynamic diameter of ABR-NP and Apt-ABR-NP were found to be 130.6 nm and 149.30 nm, respectively and zeta potential values for ABR-NP and Apt-ABR-NP were -10.1 mV and -18.5 mV, respectively. For the morphological analysis of the nanoparticles, FESEM, HR-TEM and AFM were used. FESEM images showed that nanoparticles were round in shape and had smooth surfaces with no apparent

porosity or fractures. HR-TEM images showed a dark structure, indicating that the nanoformulation had a homogeneous drug distribution throughout the particles and the nanoparticles had a spherical structure with a smooth surface, as revealed by AFM images. Further drug loading and drug encapsulation efficiencies were measured using the experimental nanoparticles. Results suggest that ABR-NP and Apt-ABR-NP had drug loading 8.5% and 8.02%, respectively. The encapsulation efficiency of ABR-NP was 93±3.30% and the value was 88.2% in the case of Apt-ABR-NP. In vitro drug release was conducted in phosphate buffer saline (PBS) (pH 7.4), PBS with 1% β-hydroxycyclodextrin (pH 7.4), citrate buffer (pH 3), acetate buffer (pH 5), and bicarbonate buffer (pH 10). The cumulative percentage of drug release after the mentioned time period was found to be 73.12%, 81.45%, 92.76%, 92.45%, and 43.30%, respectively, in 672 h of study in the five different release media and R^2 values suggest drug releases, according to Korsmeyer-Peppas, in all five release media mentioned earlier.
Among the cellular studies, the cytotoxic effects of ABR, ABR-NP, and Apt-ABR-NP on LNCaP, 22Rv1, and PC3 cells were analyzed using an MTT assay. Apt-ABR-NP had the highest cytotoxicity on LNCaP cells (lowest IC_{50} value 14.5±1.2 μM) followed by 22Rv1 (IC_{50} value 25.3±1.1 μM) compared to ABR and ABR-NP. The IC_{50} values on PC3 cells were found to be 35 ± 2.0 μM for Apt-ABR-NP treatment. Uptake of the nanoparticles in the LNCaP, 22Rv1, and PC3 cells were analyzed by confocal microscopy and quantitatively by flow cytometry (FACS) analysis. Flow cytometry analysis revealed a time-dependent gradual accumulation of Apt-ABR-NP in PC3, LNCaP and 22Rv1 cells. We further compared the cellular internalization of ABR-NP and Apt-ABR-NP nanoparticles at 12 h confocal microscopy for PC3 and LNCaP cells and the corresponding images reflected nanoparticles accumulation inside the cytoplasm of PC3 and LNCaP cells with the progression over time. Induction of apoptosis by ABR, ABR-NP, and Apt-ABR-NP was assessed in LNCaP cells following the Annexin V-FITC, propidium iodide (P.I.) dual staining method.The total apoptotic population in 22Rv1 cells after treatment with ABR for 48 h was 12%, ABR-NP treatment increased apoptosis to 43.8% and Apt-ABR-NP treatment showed the value 60.4%. For LNCaP cells, the total apoptotic population after treatment with ABR for 48 h was 17%, which was increased up to 66.8% and 98.4% in the cases of ABR-NP and Apt-ABR-NP treatments, respectively

In vivo pharmacokinetic studies of ABR and its nanoformulations were conducted in Swiss albino mice; having an average body weight of 25 g. Data suggest an increased bioavailability of the active drug while delivered through nanoparticle formulations. *In vivo* biodistribution and gamma scintigraphy of ABR-NP and Apt-ABR-NP were studied in a Swiss albino prostate-cancer mice model using technetium-99m radiolabeled nanoparticles. Results suggest that nanoparticles remained in the blood for a longer time in blood circulation. In contrast, the free drug (ABR) was eliminated rapidly. The majority of the radiolabeled nanoparticles was eliminated through urine. In the case of gamma scintigraphy imaging, radiolabeled PSMA-targeted Apt-ABR-NP signaling was observed to increase the prostate and surrounding area in experimental mice with prostate cancer in a time-dependent manner, ensuring the target specificity of conjugated aptamer toward prostate cancer. Successful aptamer-prostate cancer antigen binding ability was tested by molecular docking.

A recently discovered aptamer conjugated with ABR-NPs (Apt-ABR-NPs) predominantly accumulated to PSMA overexpressed prostate cancer. Apt-ABR-NPs showed better cellular internalization. Abiraterone-loaded PLGA nanoparticles with aptamer conjugation on the surface which ensured successful targeted drug delivery to PSMA overexpressed prostate cancer cells, with a sustained drug release profile. Apt-ABR-NPs exhibited prolonged blood retention, and tumor tissue-specific accumulation. We avoided utilizing any hazardous chemicals and instead employed biocompatible, biodegradable FDA-approved polymer and negligibly immunogenic short nucleotide sequence aptamers to effectively deploy the possible targeted drug delivery *in vivo* that could lessen its cytotoxicity to healthy tissues by their preferential accumulation in PSMA overexpressed prostate cancer tissue.The findings presented the aptamer-coupled nanoparticulated therapeutic technique, demonstrating the maximum in vitro and in vivo therapeutic effectiveness of the experimental formulations. All our data support the translation of the promising aptamer functionalized nanoparticle toward achieving successful human clinical trials as a possible targeted treatment for prostate cancer. However more studies are required.

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