Biomaterials 35 (2014) 8635–8648

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

Anti-cancer activity of curcumin loaded nanoparticles in prostate cancer

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ARTICLE INFO

Article history:
Received 14 June 2014
Accepted 22 June 2014
Available online 12 July 2014

Keywords:
Nanoparticles
Curcumin
Chemoprevention
Prostate cancer
Targeted delivery

ABSTRACT

Prostate cancer is the most commonly diagnosed cancer disease in men in the Unites States and its management remains a challenge in everyday oncology practice. Thus, advanced therapeutic strategies are required to treat prostate cancer patients. Curcumin (CUR) is a promising anticancer agent for various cancer types. The objective of this study was to evaluate therapeutic potential of novel poly(lactic-*co*-glycolic acid)- CUR nanoparticles (PLGA-CUR NPs) for prostate cancer treatment. Our results indicate that PLGA-CUR NPs efficiently internalize in prostate cancer cells and release biologically active CUR in cytosolic compartment of cells for effective therapeutic activity. Cell proliferation (MTS), clonogenic, and Western blot analyses reveal that PLGA-CUR NPs can effectively inhibit proliferation and colony formation ability of prostate cancer cells than free CUR. PLGA-CUR NPs showed superior tumor regression compared to CUR in xenograft mice. Further investigations reveal that PLGA-CUR NPs inhibit nuclear β-catenin and AR expression in cells and in tumor xenograft tissues. It also suppresses STAT3 and AKT phosphorylation and leads to apoptosis *via* inhibition of key anti-apoptotic proteins, Mcl-1, Bcl-xL and caused induction of PARP cleavage. Additionally, significant downregulation of oncogenic miR21 and up-regulation of miR-205 was observed with PLGA-CUR NPs treatment as determined by RT-PCR and *in situ* hybridization analyses. A superior anti-cancer potential was attained with PSMA antibody conjugated PLGA-CUR NPs in prostate cancer cells and a significant tumor targeting of ^{131}I labeled PSMA antibody was achieved with PLGA-CUR NPs in prostate cancer xenograft mice model. In conclusion, PLGA-CUR NPs can significantly accumulate and exhibit superior anticancer activity in prostate cancer.

1. Introduction

Prostate cancer is the most commonly diagnosed cancer in men. Recent statistics estimated 233,000 new cases and 29,480 deaths will occur from prostate cancer in the United States in 2014 [1]. Prostate cancer mortality has been declining but therapy and surgery expenses are still very high [2,3]. Conventional chemotherapy results in off-target effects; anti-cancer drug(s) cause damage to healthy rapidly dividing cells and thus patients often experience normal organ toxicity [4]. Epidemiological reports indicate that natural polyphenol compounds used in daily diet reduce the risk and incidence of various types of cancers [5,6]. These compounds benefit overall health and longevity. Use of anti-cancer natural compounds is highly suitable for prostate cancer due to its high incidence rate as well as very long latency [7]. Natural polyphenol compounds may also act as an adjunct to surgery, chemotherapy and radiation [8]. Among many natural anti-cancer agents, curcumin is a favorable phytochemical that has demonstrated remarkable therapeutic potential for prostate cancer [9]. Research studies have proven that curcumin efficiently induces apoptosis through a number of different molecular targets and inhibits metastasis, invasion and angiogenesis [10]. At this stage, there are about 40 clinical trials studying the therapeutic effects of CUR in curing cancer(s). Based on these data, we expect that curcumin formulated in the form of pills or gel capsules has the potential to either delay the onset or prevent cancer progression.

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http://dx.doi.org/10.1016/j.biomaterials.2014.06.040
0142-9612/

Despite curcumin's significant therapeutic value, its translation from basic research to clinical trials and human application is hindered due to its extreme degradation and metabolization which are responsible for poor pharmacokinetics, low bioavailability and pharmacological activity [11]. It is important to note that a trace amount of curcumin was detected in the serum of human when up to 4–12 g/day of CUR was administered [12]. Such a low bioavailability profile significantly hampers its clinical utility [13]. Additionally, it is a prerequisite that curcumin molecules reach the tumor site for its effective pharmacological actions. To achieve this, curcumin must be formulated using nanotechnology approaches for better medical application. To-date a number of drug delivery strategies have been adopted to incorporate or encapsulate curcumin in polymer nanoparticles (NPs), liposomes, inclusion complex, and self-assemblies utilizing their complex molecular structures. This resulted in active binding and enhanced uptake by the cancer cells and thus exhibited enhanced anti-cancer activity [14–16]. Poly(lactic-*co*-glycolic acid) (PLGA) based nanoformulations are non-toxic, biodegradable and non-immunogenic and are thus valuable in medical applications [17]. Abraxane®, a paclitaxel albumin-bound nanoparticle formulation, has been approved by the FDA and is in the market for metastasis cancer treatment(s) [18]. Another successful formulation of modified PLGA based docetaxel has successfully completed Phase I clinical trials for prostate cancer [19]. The clinical proof-of-success has inspired us to encapsulate curcumin in PLGA NPs [20] that can be a suitable/better choice for prostate cancer therapeutics. Furthermore, it has been demonstrated that curcumin bioavailability can be improved by formulating with PLGA polymer or PLGA with other polymers/copolymers.

Targeted nanotherapies may improve therapeutic outcome of prostate cancer treatment through targeting, accumulation and sustained release of therapeutics. Targeted nanoformulations can target the antigens specifically present and/or overexpressed in cancer tissues such as prostate stem cell antigen (PSCA), prostate specific membrane antigen (PSMA), six transmembrane epithelial antigen of the prostate 1 (STEAP1), prostatic acid phosphatase (PAP), T cell receptor gamma alternate reading frame protein (TARP), transient receptor potential (trp)-p8 (Trp-p8), etc. PSMA is a 100 kDa prostate epithelial cell type II transmembrane glycoprotein [21,22]. PSMA is highly expressed in almost all prostate tumors and its expression progressively increases in higher Gleason grade and hormone refractory prostate cancer (PrCa) [21,22]. PSMA is a highly validated target. This information suggests its applicability for targeting advanced stage PrCa; therefore, we propose to develop an anti-PSMA antibody coupled PLGA-CUR nanoparticle for prostate cancer.

Inhibition of androgen receptor (AR) and β-catenin nuclear localization play important roles in prostate tumorigenesis [23]. AR binds to β-catenin directly to stimulate AR-mediated gene transcription [24] and importantly, the AR gene itself is a transcriptional target of β-catenin [25]. Furthermore, enhanced crosstalk between AR and β-catenin has been observed in *in vivo* models. Therefore, inhibitors of nuclear AR and β-catenin, which act as oncogenes in prostate cancer, are highly desired. In the present study, we have generated an antibody conjugation compatible curcumin loaded PLGA nanoparticle formulation for improved targeting AR/β-catenin to induce anti-cancer activity in prostate cancer.

2. Experimental design

2.1. Materials

All reagents, solvents, chemicals and cell culture plastics were purchased from Sigma–Aldrich Co. (St. Louis, MO) or Fisher (Pittsburgh, PA) unless otherwise mentioned. All chemicals were used as received without further purification. PLGA-CUR was prepared following our previously published protocol using nanoprecipitation method [20]. Blank PLGA NPs were also prepared to use as control for all our studies. PLGA-CUR NPs and PLGA NPs were also termed as PLGA-CUR and PLGA, respectively in the manuscript.

2.2. Cell culture

LNCaP sublines have been generated to provide an androgen dependent (AD) state and the most clinically relevant phenomenon. C4-2, a subline of LNCaP cells (metastatic lesion of human prostatic adenocarcinoma), were procured from Dr. Jaggi's lab. Human prostate cancer cell lines (DU-145 and PC-3 cells, androgen independent (AI) characteristic) were purchased from the American Type Culture Collection (ATCC), have the propensity of prostate cancer to metastasize to bone but these cells in bone do not fully mimic clinical human disease. These cell lines were cultured in Roswell Park Memorial Institute (RPMI)-1640/Dulbecco's Modified Eagle Medium (DMEM)-high medium (FBS, Invitrogen) supplemented with fetal bovine serum (10%, volume percentage) and 1% antibiotics (penicillin, 100 units/mL plus streptomycin) at 37 °C in a humidified incubator containing 5% CO_2.

2.3. Cellular internalization, accumulation and retention of PLGA-CUR NPs

Transmission electron microscopy (TEM) and flow cytometry (FCM) methods were employed to elucidate the internalization of PLGA-CUR NPs in C4-2, DU-145 and PC-3 cells. For the TEM internalization study, cells (1×10^7 cells in 20 mL) were seeded in 150 mm dish and were allowed to attach overnight. To observe internalization and trafficking, cells were incubated with 10 μM CUR equivalent PLGA-CUR NPs for 0–18 h. Cells were then washed with cold phosphate buffer saline (PBS) solution, trypsinized, and centrifuged at 3000 rpm to obtain a cell pellet. Cells were fixed with standard ice-cold formaldehyde (4%)-glutaraldehyde (1%) fixative solution followed by osmium tetroxide fixative solution, thin sectioned, and imaged under TEM according to our previously published method [20]. PLGA-CUR NPs in cancer cells were distinguished with high electron density (due to uranyl acetate staining). For flow cytometry, cells (2×10^5 cells per well) were seeded in 6-well plates, allowed to attach and then treated with 5 μM CUR or PLGA-CUR NPs. After treatment, the cells were washed with PBS, trypsinized, and centrifuged at 3000 rpm and the cell pellet was resuspended in PBS containing 5% FBS. The internalization of CUR or PLGA-CUR was assessed by cellular fluorescence due to curcumin using an Accuri C6 flow cytometer (BD Accuri Cytometers, Inc., Ann Arbor, MI) with FL1 channel (488 nm excitation, Blue laser, 530 ± 15 nm, FITC/GFP) [20]. The flow cytometer acquisition was performed within 90 min of cell collection, thus there was no significant leach of CUR or PLGA-CUR NPs from the cancer cells. Similarly, the uptake of PLGA-CUR NPs were tested in the presence of endocytosis inhibitors such as genistein, chlorpromazine, nocodazole, methyl-β-cyclodextrin, or at 4 °C (energy deprivation) to evaluate the internalization mechanisms in C4-2 and PC-3 (1×10^5 in 6 well-plates) cancer cells. The treatment concentrations were 10 μM or 15 μM PLGA-CUR NPs for C4-2 and PC-3 cells, respectively. The higher concentration was chosen in the case of PC-3 cells because this cell line exhibits lower cellular uptake compared to C4-2 cells. The internalization acquisition of PLGA-CUR NPs in the presence of endocytosis inhibitors or energy deprivation method was assessed using a flow cytometer as mentioned above. To examine the cellular accumulation and retention of PLGA-CUR NPs, cells (2×10^5) were grown in 6-well plates and incubated with CUR or PLGA-CUR NPs for 1, 2 and 4 days. After treatment, cells were washed with ice-cold PBS and the media was replaced with fresh media. Cells were then collected and processed to estimate curcumin levels by flow cytometer as described above.

2.4. Cell proliferation assay

The cytotoxicity of the PLGA-CUR nanoformulation was performed in prostate cancer cells using CellTiter 96® AQ_{ueous} One Solution Cell Proliferation solution (Promega Corporation, Madison, WI). For this assay, cells (5000 cells) were plated in 96-well plates and allowed to attach overnight. The cells were treated with 2.5–40 μM CUR or PLGA-CUR NPs for 2 days. Appropriate equivalent amounts of dimethyl sulfoxide (DMSO) or PLGA in PBS were used as controls. After completion of the treatment, the media was replaced with 100 μL fresh media containing 20 μL CellTiter 96® AQ_{ueous} One Solution for 2–3 h. The color intensity developed by intracellular formazan was measured at 492 nm using a microplate reader (BioMate 3 UV–Vis spectrophotometer, Thermo Scientific, Waltham, MA). The percentage of cell growth was calculated as the percentage of the absorption of treated cells to the absorption of non-treated cells. Each treatment condition was replicated six times.

2.5. Clonogenic assay

Cancer cells (500 cells/well in 2 mL medium) were seeded in 6-well plates. After confirming that cells initiated colonies (day 3), cells were treated with 2–6 μM CUR or PLGA-CUR for a week, then media was replaced with fresh media without drugs and maintained until day 14. At the end of the treatment, cells were rinsed with PBS, fixed with cold methanol and stained with hematoxylin. The colonies were photographed using a Multimage™ light cabinet (Alpha Innotech Corporation, San Leandro, CA) with the help of AlphaEaseFC™ (Alpha ImagerHP AIC) software. The number of colonies was counted and quantified using the analysis tool for the auto count method at a constant density threshold as described in our previous