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Multifunctional nanoparticles as somatostatin receptor-targeting delivery system of polyaniline and methotrexate for combined chemo–photothermal therapy

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ABSTRACT

Lanreotide (LT), a synthetic analog of somatostatin, has been demonstrated to specifically bind to somatostatin receptors (SSTRs), which are widely overexpressed in several types of cancer cells. In this study, we incorporated a chemotherapeutic agent, methotrexate (MTX), and a photosensitizer material, polyaniline (PANI), into hybrid polymer nanoparticles (NPs), which could target cancer cells after conjugation with LT (LT-MTX/PANI NPs). The successful preparation of LT–MTX/PANI NPs was confirmed by a small particle size (187.9 ± 3.2 nm), a polydispersity index of 0.232 ± 0.011, and a negative ζ potential of −14.6 ± 1.0 mV. Notably, LT-MTX/PANI NPs showed a greater uptake into SSTR-positive cancer cells and thereby better inhibited cell viability and induced higher levels of apoptosis than MTX, PANI NP, and MTX/PANI NP treatments did. In addition, the heat associated with the burst drug release induced by near-infrared (NIR) irradiation resulted in remarkably enhanced cell apoptosis, which was confirmed by an increase in the expression levels of apoptotic marker proteins. In agreement with the *in vitro* results, the administration of the SSTR-targeting NPs, followed by NIR exposure, to xenograft tumor-bearing mice resulted in an improved suppression of tumor development compared to that shown by MTX, PANI NPs, and MTX/PANI NPs, as well as by LT-MTX/PANI NPs without photothermal therapy. Thus, the SSTR-targeting NPs could be a promising delivery system for the effective treatment of SSTR-positive cancers.

Statement of significance

Somatostatin receptors are widely overexpressed in several types of cancer cells. In this study, we designed nanoparticles for targeted delivery of chemotherapeutic agents to tumor sites by conjugating hybrid polymers with a synthetic analog of somatostatin, specifically binding to somatostatin receptors. In addition, a photosensitizer material, polyaniline, was incorporated into the nanoparticles for combined chemo–photothermal therapy. The results demonstrated clear advantages of the newly designed targeted nanoparticles over their non-targeted counterparts or a free chemotherapeutic drug in inhibiting the viability of cancer cells *in vitro* and targeting/suppressing the tumor growth in an animal xenograft model. The study suggests that the designed nanoparticles are a promising delivery system for the effective treatment of somatostatin receptor-positive cancers.

1. Introduction

Somatostatin receptors (SSTRs) consisting of five subtypes (SSTR1–5) [1–3] are G protein-coupled seven-transmembrane receptors, which are overexpressed in various tumors such as

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breast cancer [1,4], pancreatic cancer [5], neuroendocrine tumors [6,7], and prostate cancer [8]. Somatostatin and its synthetic analogues belong to neuropeptide which have high binding affinity to SSTR subtypes and inhibit the cell proliferation by inducing the cell cycle arrest and apoptosis or by suppressing hormones [9,10]. However, the therapeutic applications of the natural neuropeptide compound is limited because it is quickly degenerated in the body (2–4 min) [11]. In order to overcome this disadvantage, various synthetic somatostatin analogues, such as lanreotide (LT), octreotide, and vapreotide are synthesized by shortening the sequence with eight amino acids and replacing natural L-amino acids by D-amino acids. In comparison to natural somatostatin, LT is more stable, shows a longer half-life, produces more prolonged effects and selectively binds mainly to SSTR2/5 [11,12]. The smaller structure of LT is also an important benefit to form a stable conjugation with several nanoparticles [13], which has motivated to use this peptide as a mean to enhance the therapeutic efficacy of anticancer drugs in SSTR-expressing tumors [1,6,13,14].

Methotrexate (MTX), a folate analog, acts by inhibiting the metabolism of folic acid via dihydrofolate reductase and by blocking the thymine and purine synthesis, which results in the impairment of tumor growth and induction of apoptosis [15–17]. However, the effectiveness of this compound is limited owing to its low solubility and fast clearance from the body, as well as to multidrug resistance of cancer cells [17,18]. More interestingly, Sun and Coy [10] reported in their review that the coupling of chemotherapeutics agents including MTX and SST analogs could specifically target SSTR-positive tumor, leading to enhance antitumor efficiency and reduce the multi-drug resistance.

Conventional chemotherapy has been showed its limitation due to the side effects and multi-drug resistance [19]. Therefore, the combination of photothermal therapy (PTT) and chemotherapy has been demonstrated to be a promising treatment strategy for resistant cancers [19–24]. Recent studies have focused on the incorporation of a therapeutic agent and inorganic or organic material responsive to near-infrared (NIR) laser irradiation to induce synergistic anticancer effects [20,25–27]. Among organic NIR-responsive materials, polyaniline (PANI) has emerged as an interested choice owning to its attractive properties such as chemical stability, simple synthesis, as well as advantageous optical, electrical, and electrochemical characterization [25,28–30]. PANI has been reported to exhibit a strong and stable hyperthermia effect owning to the extended π-electrons [31]. Moreover, the biodegradability and biocompatibility of this material could be a crucial benefit for biomedical and tissue engineering applications [31,32]. Although there have been mixed reports regarding the potential cytotoxicity of PANI owning to the polymerization reaction of intermediates, several previous studies have indicated the biocompatibility and biodegradable as well as the potential application of PANI grafted scaffolds such as PANI/PLGA [33], PANI/poly (L-lactide-co-e-caprolactone) [34] or PANI/porous silicon-based nanocomposite [35]. This promotes to develop a biodegradable and photothermal nanocarrier for the effective combined strategy in cancer treatment.

In this study, a multifunctional hybrid polymer system of PANI NPs and MTX was designed for combined photo–chemotherapy and to target SSTR-expressing tumor cells, as described in the schematic illustration. It was hypothesized that LT on the surface of NPs would contribute to selective binding to SSTR-positive cancer cells, leading to the enhancement of intracellular uptake and anticancer activity of the drug delivery system. A combination of PTT, mediated by PANI, which was incorporated in the core of hybrid polymeric NPs, and chemo-cytotoxicity of MTX was evaluated for synergistic efficiency in the treatment of an SSTR-expressing tumor to facilitate the potential application of this multifunctional drug delivery system.

2. Materials and methods

2.1. Materials

LT acetate was purchased from Santa Cruz Biotechnology (Dallas, TX, USA). Poly (lactic-co-glycolic acid) (PLGA) [50:50, molecular weight (MW) 17 kDa] was from Lakeshore Biomaterials (Birmingham, AL, USA). 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-*N*-[methoxy(polyethylene glycol)-2000] maleimide (DSPE-PEG_{2000}-mal) was obtained from Nanocs (New York, NY, USA). MTX, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), 2-iminothiolane hydrochloride, polyvinyl pyrrolidone (PVP, 30 kDa), dimethylformamide (DMF), AO, PI, and PANI [emeraldine base (EB), 5 kDa] were from Sigma–Aldrich (St. Louis, MO, USA). LysoTracker Green was purchased from Thermo Fisher Scientific (Waltham, MA, USA). SDS and dimethyl sulfoxide (DMSO) were purchased from Duksan Chemical Co. (Ansan, South Korea). All other chemicals were of reagent grade and were used without further purification.

2.2. Cell lines and animals

Human breast cancer cells (MDA-MB-231 and MCF-7) obtained from the Korean Cell Bank (Seoul, South Korea), were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum and a 1% penicillin/streptomycin solution. BALB/c nude mice (female, 6-week-old, 16–19 g) were individually maintained at 21–22 °C/55 ± 2% relative humidity for 1 week before the experiments. Animal care, handling, and experiments strictly followed the guidelines of the Institutional Animal Ethical Committee, Yeungnam University, South Korea.

2.3. Preparation of multifunctional hybrid polymer NPs

2.3.1. Preparation of PANI NPs

PANI NPs were self-doped in the EB state [36], with slight modifications. Briefly, a PANI EB solution in DMF (1 mg/mL, 1 mL) was added to 9 mL of an aqueous solution (pH 3.0 with 0.1 N hydrochloric acid) containing PVP (1%, w/w) as a stabilizer [28,37] and SDS (0.2%, w/w) as a surfactant [38], with probe sonication (80 W) for 10 min [39]. The solution was stirred for 4 h at room temperature, followed by centrifugation at 14,000 rpm for 15 min and freeze-drying.

2.3.2. Preparation of MTX/PANI-loaded hybrid polymer NPs

PANI NPs were incorporated into hybrid polymer NPs consisting of PLGA and DSPE-PEG_{2000}-mal (2:1, w/w) as described in previous reports [40,41]. First, the polymers were dissolved in 1 mL of a mixture of acetone and ethanol (3:1). Then, an MTX solution in DMSO (1 mg/mL) was added to the organic solution. Afterward, the organic solution was added dropwise to aqueous solutions containing PANI NPs at different ratios (1:5, 1:10, 1:15, and 1:20) relative to the total amount of PLGA and DSPE-PEG_{2000}-mal. The mixture was stirred for 6 h, followed by removal of the organic solvents and the free drug using a dialysis membrane (10 kDa). Finally, MTX/PANI-loaded hybrid polymer (MTX/PANI) NPs were purified using a Sephadex G-25 column (Sigma–Aldrich).

2.3.3. Conjugation of LT and hybrid polymer NPs for targeting SSTR

LT was conjugated with the hybrid polymer NPs by interaction between the maleimide group of DSPE-PEG_{2000}-mal and the thiolated peptide as previously reported [42,43]. Briefly, LT (1 mg/mL), modified by reacting with 2-iminothiolane hydrochloride (1:50 M ratio) in carbonate buffer pH 9.0, was added dropwise at a 1:1 M ratio into a suspension of hybrid polymer NPs, followed

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