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Self-assembled nanoscale coordination polymers carrying oxaliplatin and gemcitabine for synergistic combination therapy of pancreatic cancer

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ABSTRACT

Gemcitabine has long been the standard of care for treating pancreatic ductal adenocarcinoma (PDAC), despite its poor pharmacokinetics/dynamics and rapid development of drug resistance. In this study, we have developed a novel nanoparticle platform based on nanoscale coordination polymer-1 (NCP-1) for simultaneous delivery of two chemotherapeutics, oxaliplatin and gemcitabine monophosphate (GMP), at 30 wt.% and 12 wt.% drug loadings, respectively. A strong synergistic therapeutic effect of oxaliplatin and GMP was observed in vitro against AsPc-1 and BxPc-3 pancreatic cancer cells. NCP-1 particles effectively avoid uptake by the mononuclear phagocyte system (MPS) in vivo with a long blood circulation half-life of 10.1 ± 3.3 h, and potently inhibit tumor growth when compared to NCP particles carrying oxaliplatin or GMP alone. Our findings demonstrate NCP-1 as a novel nanocarrier for the co-delivery of two chemotherapeutics that have distinctive mechanisms of action to simultaneously disrupt multiple anticancer pathways with maximal therapeutic efficacy and minimal side effects.

1. Introduction

Pancreatic cancer has one of the poorest prognoses of all cancer types, with a five-year survival rate of less than 6% [1,2]. Pancreatic ductal adenocarcinoma (PDAC) is the most common type of pancreatic cancer, and accounts for 95% of all cases of these tumors. Upon diagnosis, 80% of pancreatic cancer cases are deemed inoperable due to the high risk of surgically resecting tumors connected to surrounding blood vessels and digestive ducts [3,4]. Developing effective chemotherapy is thus of great importance in treating this deadly cancer.

Gemcitabine (gem) alone has long been the standard care for PDAC in the clinic [5,6]. As a nucleotide analog [7], gem enters the cells through nucleotide transporters [8] and is then phosphorylated to gemcitabine monophosphate (GMP) by deoxycytidine kinase [9,10]. GMP is further phosphorylated by uridine/cytidine monophosphate (UMP/CMP) kinase and nucleoside diphosphate kinase (NDK) to generate pharmacologically active gemcitabine diphosphate (GDP) and gemcitabine triphosphate (GTP) [11]. Although gem is the standard of care for PDAC, the gem treatment has many drawbacks. First, free gem lacks tumor specificity, and enters cancerous and healthy cells indiscriminately, leading to high general toxicity and narrow therapeutic windows [12]. Second, about 90% of gem is rapidly deactivated with a short half-life of 32 min in blood circulation due to deamination to the inactive 2′,2′-difluorodeoxyuridine (dFdU). Third, many pancreatic cancer cells develop resistance to gem, making repeat treatments with gem ineffective.

Combination therapy with multiple chemotherapeutics is a successful strategy for treating many cancers [13–16]. In particular, several different multidrug combination regiments have emerged for the treatment of pancreatic cancer, such as FOLFIRNOX and the combination of gemcitabine and nab-paclitaxel [17,18]. Compared with conventional single-agent treatment, multi-agent therapy can promote synergism of different drugs, increase therapeutic target selectivity, and overcome drug resistance through distinct mechanisms of action [19–21]. However, combination therapy has its own drawbacks as the drugs typically have different pharmacokinetic properties, which often makes it difficult to obtain the optimal dose and increases the chances of adverse side effects [22,23]. As a result, there is a great need in developing a combination drug delivery system that would specifically and selectively deliver multiple chemotherapeutics to tumor sites.

Nanoparticle drug delivery has been shown to promote therapeutic effectiveness and reduce side effects by improving pharmacokinetics [24–27]. It is even more advantageous to develop nanocarriers that are able to deliver multiple chemotherapeutics with controlled release characteristics and optimal pharmacokinetic profiles. Oxaliplatin has been used in combination with gem to treat metastatic pancreatic cancer patients with significantly enhanced response rates and tumor growth inhibition than their monotherapy counterparts [28–31]. However, this combination is not safe in patients with advanced solid tumors

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due to serious adverse side effects [32]. In view of this clinical need, we sought to develop a novel nanoparticle system for simultaneous delivery of oxaliplatin and gem for synergistic combination therapy of PDAC.

We recently reported the development of nanoscale coordination polymers (NCPs) as versatile nanocarriers for both cisplatin and oxaliplatin prodrugs [33]. NCP nanoparticles are constructed from polydentate bridging ligands and metal ions or clusters through self-assembly processes [34,35]. NCPs possess many beneficial characteristics as drug delivery vehicles, including chemical diversity, high loading capacity, and intrinsic biodegradability [36–38]. NCPs showed long blood circulation half-lives and significantly enhanced tumor growth inhibition over their free drug counterparts [33]. We hypothesized that NCPs could incorporate multiple chemotherapeutics, in particular platinum drugs and gem, for their selective delivery to PDAC cells to elicit synergistic therapeutic effects. In this work, NCP-1 particles carrying both oxaliplatin (30 wt.%) and GMP (12 wt.%) were synthesized in reverse microemulsions, and extensively characterized by dynamic light scattering (DLS), transmission electron microscopy (TEM), and in vitro release profiles. Synergistic effects of oxaliplatin and gem of NCP-1 on pancreatic cancer cells were demonstrated in vitro by cytotoxicity assays, flow cytometry analysis, and confocal scanning laser microscopic (CLSM) imaging. Pharmacokinetics and biodistributions of intravenously injected particles of NCP-1 were evaluated in subcutaneous xenograft mouse model of colon cancer CT26, whereas in vivo efficacy studies were carried out on subcutaneous xenograft mouse models of human PDACs including BxPc-3 and AsPc-1. The low general toxicity of NCP-1 was indicated by histology analysis and lack of immunogenic responses. Our results indicate that the co-delivery of oxaliplatin and GMP in NCP-1 leads to synergistic therapeutic effects and much enhanced antitumor efficacy as compared to their single drug counterparts in human pancreatic cancer xenograft mouse models.

2. Materials and methods

2.1. Materials, cell lines, and animals

Please see Supplementary materials for details.

2.2. Preparation of NCP particles

A 5 mL mixture of 0.3 M Triton X-100/1.5 M 1-hexanol in cyclohexane consisting of 0.2 mL of an aqueous 25 mg/mL (dach)Pt(BP) sodium salt solution (7.6 μmol) and 0.030 mL of an aqueous 15 mg/mL GMP sodium salt solution (1.3 μmol) was stirred vigorously at room temperature. Another 5 mL of 0.3 M Triton X-100/1.5 M 1-hexanol in cyclohexane containing 0.2 mL of an aqueous 100 mg/mL $Zn(NO_3)_2$ solution (67 μmol) was stirred in a similar manner. Twenty microliters of dioleoyl-sn-glycero-3-phosphate sodium salt (DOPA, 11 μmol in $CHCl_3$) was added to the solution containing (dach)Pt(BP) and GMP. The two microemulsions were stirred continuously for 15 min until clear solutions were formed. The resulting $W = 7.4$ microemulsions were combined and stirred for an additional 30 min. NCP-1 particles were obtained by the addition of 20 mL ethanol, and washed once with ethanol, once with 50% (v/v) ethanol/cyclohexane and once with 50% (v/v) ethanol/tetrahydrofuran (THF), and redispersed in THF. The nanoparticles were purified by filtration through a 200 nm syringe filter.

NCP-1 was synthesized at a 20× scale, which shows similar prodrug loading, morphology, and size as those obtained at 1× scale. NCP-2, the nanoparticle carrying oxaliplatin, and Zn Control nanoparticles were synthesized according to our previous report [33]. NCP-3 particles carrying GMP monotherapy were prepared similarly as NCP-1.

The lipid-coated and PEGylated particles were obtained by adding a THF solution of 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), cholesterol (1:1 molar ratio), and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] ($DSPE\text{-}PEG_{2k}$, 20 mol%) to the DOPA-capped NCP nanoparticles. The resulting mixture was added to 500 μL of 30% (v/v) ethanol/H_2O at 50 °C. THF was evaporated, and the dispersion was allowed to cool to room temperature before use.

2.3. Characterization of NCP particles

Please see Supplementary materials for details.

2.4. In vitro stability studies

Please see Supplementary materials for details.

2.5. In vitro cytotoxicity assays and synergistic effects of drug combinations

In vitro cytotoxicity assays were performed on AsPc-1 and BxPc-3 cancer cell lines. Confluent AsPc-1 and BxPc-3 cells were trypsinized and counted with a hemocytometer. Cells were plated in 96-well plates at a cell density of 2000 cells/well and a total of 100 μL fresh culture media, followed by further incubating at 37 °C and 5% CO_2 for 24 h. The culture medium was replaced by 100 μL of fresh RPMI 1640 containing 10% FBS, and different concentrations of oxaliplatin, GMP, free oxaliplatin/GMP mixture (at the same NCP-1 drug dose), Zn Control, NCP-1, NCP-2, and NCP-3 were added. The cells were incubated at 37 °C and 5% CO_2 for 48 h, and cell viability was determined by MTS assay (Promega, USA) according to the manufacturer's instructions. The concentrations of oxaliplatin or GMP required to inhibit cell growth by 50% (IC_{50} values) were calculated.

The combination index (CI) was calculated using the following equation [39,40]

$$CI = \frac{D_1}{D_{m1}} + \frac{D_2}{D_{m2}}$$

where D_1 and D_2 are the concentrations of drug 1 and drug 2, respectively, in combination at a specific drug effect level (e.g. 50% inhibition concentration), while D_{m1} and D_{mB} are the concentrations of the drugs dosed individually to achieve that same drug effect level. CI values were plotted against drug effect level (IC_x values), with CI values lower than, equal to, and greater than 1 indicating synergism, additivity, and antagonism, respectively.

2.6. Cell apoptosis by Annexin V staining

Please see Supplementary materials for details.

2.7. Flow cytometry

Please see Supplementary materials for details.

2.8. Pharmacokinetics of NCP-1

Nude mice bearing C26 tumors were intravenously injected with NCP-1 at an oxaliplatin dose of 3 mg/kg. The mice were randomly distributed into different time period groups ($n = 3$ for each time point). At 5 min, 1 h, 3 h, 8 h, 24 h, and 48 h post-injection time point, the mice were sacrificed, and the liver, lung, spleen, kidney, bladder, tumor, and blood were collected. Organs were digested in concentrated nitric acid overnight and then diluted with water and filtered for ICP-MS measurements of the Pt. Blood circulation half-life was fitted by an one-compartment model with nonlinear elimination using PKSolver [41].

Pharmacokinetics of GMP was analyzed using high-performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS, Agilent 6460 QQQ MS–MS) following a literature procedure [42]. The initial sample was prepared on ice to minimize enzyme-mediated degradation. To 50 μL of blood, 200 μL of ice-cold acetonitrile was added, vortex mixed, and centrifuged. The resulting supernatant was