Journal of Pharmaceutical Sciences 105 (2016) 1891–1900

Contents lists available at ScienceDirect

Journal of Pharmaceutical Sciences

journal homepage: www.jpharmsci.org

Pharmaceutics, Drug Delivery and Pharmaceutical Technology

Hyaluronan-Lysine Cisplatin Drug Carrier for Treatment of Localized Cancers: Pharmacokinetics, Tolerability, and Efficacy in Rodents and Canines

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ARTICLE INFO

Article history:
Received 28 October 2015
Revised 9 February 2016
Accepted 15 March 2016
Available online 4 May 2016

Keywords:
cancer chemotherapy
polymeric drug delivery systems
controlled release
pharmacokinetics
synthesis
biodegradable polymers

ABSTRACT

The purpose of this study was to develop a safe and efficacious drug delivery platform for sustained release of cisplatin after locoregional administration. We successfully synthesized hyaluronan-cisplatin nanoconjugates (HA-Lys-Pt) using an N-Ac-lysine linker, which formed a thermodynamically stable five-membered ring with the platinum. The conjugate was characterized for release kinetics, *in vitro* antiproliferative activity, degradability, impurity content, formation of Pt-DNA adducts, pharmacokinetics, tolerability in rodents and canines, and for efficacy in rodents. The 75 kD HA-Lys-Pt (75HA-Lys-Pt) sustained release of platinum with a 69 h half-life in phosphate buffered saline without substantial burst release. Compared to intravenous cisplatin, subcutaneously injected 75HA-Lys-Pt formed 3.2-fold more Pt-DNA adducts in rat peripheral blood mononuclear cells compared to intravenous cisplatin over 96 h. Subcutaneous 75HA-Lys-Pt was tolerable in rats at 40 mg/kg (4 × LD50 of conventional cisplatin) and resulted in 62.5% partial response and 37.5% stable disease in murine xenografts of head and neck squamous cell cancer (20 mg/kg/wk × 3 weeks). 75HA-Lys-Pt demonstrated extended t_{max} and improved area-under-the-curve compared to cisplatin in rats and canines. Canine safety was demonstrated by liver enzyme and electrolyte levels, complete blood count, and urinalysis.

Introduction

Cisplatin and its analogs have been among the most widely utilized anticancer drugs in the world for over 3 decades. Its success has led to the development of over 1000 analogs since the 1960s to improve tolerability, broaden activity, and to overcome drug resistance. Yet, less than 20 platinum drugs have made it into human clinical trials, and only 3 have won regulatory approvals in the USA. They are the original cisplatin initially approved for ovarian and testicular cancers in 1978, carboplatin approved for palliative treatment of recurrent ovarian cancer in 1988, and oxaliplatin approved for second-line treatment of metastatic colorectal cancer in 2002. Cisplatin remains the most potent in most indications, but these analogs generally have improved tolerability and equivalent overall survival and/or remission rates in the approved indications.[1-5] There remains a need for platinum-based anticancer drugs with improved tolerability and efficacy.

Despite different chemical structures, cisplatin and its analogs undergo similar ligand hydrolysis reactions *in vivo* that form the active platinum species *cis*-$[Pt(X)_2(OH_2)(Y)]^+$, (Pt-monoaqua) and *cis*-$[Pt(X)_2(OH_2)_2]^{2+}$, (Pt-diaqua). The aqua ligands on the Pt-monoaqua and Pt-diaqua are easily displaced by DNA bases, preferentially adenine and guanine, forming $[Pt(X)_2(Y)$ (adenine-DNA)$]^+$ and

Conflict of interest: M.L.F., D.A., W.C.F., S.C., C.G., T.Z., and E.M. are employees and/or have ownership interest in HylaPharm. No potential conflicts of interest were disclosed by the other authors.
This article contains supplementary material available from the authors by request or via the Internet at http://dx.doi.org/10.1016/j.xphs.2016.03.018.

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http://dx.doi.org/10.1016/j.xphs.2016.03.018
0022-3549/

$[Pt(X)_2(Y)(guanine\text{-}DNA)]^+$, subsequently $[Pt(X)_2(adenine\text{-}DNA)_2]^{2+}$ and $[Pt(Y)_2(guanine\text{-}DNA)_2]^{2+}$ via the displacement of the other ligands (Supporting Information Fig. 4). The formation of intra- and interstrand DNA crosslinks in cancer cells interfere with cell division and DNA repair, and in turn trigger cell death.[6] The ligands function primarily to alter the reactivity to the DNA bases and lipophilicity and hence alter tissue distribution and pharmacokinetics. The mechanisms of cisplatin activation and cell death are a complex process and may involve multiple possible pathways, which have been reviewed elsewhere.[7]

Platinum chemotherapy is also highly damaging to normal cells that divide rapidly. Approximately 30% of patients who received 50-100 mg/m^2 cisplatin as an initial dose developed severe nephrotoxicity, due to the damaged pars recta of the proximal tubules of the kidney,[8] and over half of the patients develop hypomagnesemia and hypocalcemia.[9] In contrast, nephrotoxicity is greatly reduced in the carboplatin regimen, but thrombocytopenia, which is uncommon for cisplatin, is dose limiting in 25% of carboplatin patients.[10]

The distinct toxicity profiles of cisplatin and carboplatin are likely attributed to the differences in the chemical reactivity, pharmacology, and toxicology of the 2 platinum compounds. The cyclobutane-dicarboxylate ligands of carboplatin are slowly hydrolyzed to form the same membrane impregnable aquated species as cisplatin, but the hydrolysis rate of carboplatin is approximately 10-fold slower than cisplatin,[11] which causes significant changes in the pharmacokinetics, distribution, and tolerability. In terms of excretion, 25% of cisplatin is excreted unchanged in the urine, while 90% of carboplatin is excreted unchanged in the urine. In regards to metabolism, cisplatin undergoes inactivation by sulfhydryl groups *in vivo*, while carboplatin is not significantly metabolized. These differences in pharmacokinetics may contribute to the longer elimination half-life and improved toxicity profile of carboplatin.[12]

In our previous studies, we developed a first-generation cisplatin-based polymeric conjugate, HylaPlat (HA-Pt), using the biocompatible and nonimmunogenic polysaccharide hyaluronan (HA) for locoregional delivery of platinum chemotherapy to treat spontaneous canine cancers.[13] This conjugate overcame the lack of targeting inherent to most polymeric cisplatin delivery vehicles, such as N-(2-Hydroxypropyl)methacrylamide (HPMA), polyethylene glycol (PEG), and polyamino acid conjugates,[14] which relied on enhanced permeability and retention effects[15] found in some tumors, but these conjugates lacked tumor-receptor-specific targeting. The first-generation HA carrier was based on a direct attachment of cisplatin to the polymer, and it released active aquated cisplatin over several hours ($t_{1/2}$ 10 h). It demonstrated superior pharmacokinetics in rodents and canines compared to cisplatin, and showed a 23% cure in heterogeneous oral squamous cell carcinomas in dogs (compared to 7% for cisplatin[16]). The conjugate did not demonstrate renal toxicity in either rodents or dogs, which is the dose-limiting side effect of cisplatin chemotherapy. However, it resulted in myelosuppression, hepatic, and cardiac toxicities in some patients in a phase I/II canine clinical trial. Platinum chemotherapeutics rarely cause hepatic and cardiac toxicities in clinical practice.[13] We believe that the unexpected toxicity was due to rapid release of the Pt-diaqua from the conjugate, altered deposition of the HA bound platinum, and prolonged retention in the liver during HA metabolism. To address potential hepatic and cardiac toxicities, we developed a second-generation stabilized ring conjugate, HA-Lys-Pt, HA-*cis*-$[Pt(NH_3)_2(N^{\alpha}$-Acetyl-L-Lysine)], via a linker chemistry, with extended release half-life and improved safety as detailed in this work. Our hypothesis was that slower release would decrease burst release after injection, allow equilibrium to the nonaquated forms upon release from the polymer, prolong release into tissues, and alleviate toxicities.

Materials and Methods

Materials

Unless noted, all reagents were of highest grade available from Fisher Scientific (Pittsburgh, PA). The MDA-1986 human oral squamous carcinoma and the 4T1.2-neu murine breast cancer cell lines were kindly provided by Dr Jeffery Myers (M.D. Anderson Cancer Center, Houston, TX) and Dr Zhaoyang You (The University of Pittsburgh, Pittsburgh, PA), respectively. The human melanoma cell line was purchased from ATCC (Manassas, VA). Water for all aqueous solutions was ASTM Type 1. All reactions were conducted at ambient temperature (ca. 20°C) unless noted otherwise.

Synthesis of Hyaluronic Acid-Tetrabutylammonium Salt

Sodium hyaluronate (33 or 75 kDa; Lifecore Biomedical, Chaska, MN) was dissolved in water (1 g in approximately 300 mL) and then stirred overnight with 10 g of cation exchange resin (Dowex 50W-X8; Bio-Rad, Hercules, CA). After filtration, the filtrate was neutralized with tetrabutylammonium hydroxide solution (55%-60% wt/vol in water), and then freeze dried to obtain an orange to pink powder. The products were named 33HA-TBA and 75HA-TBA (hyaluronic acid-tetrabutylammonium) to reflect the molecular weights of the HA used.

Synthesis of HA-N^{α}-Acetyl-L-Lys

Two hundred milligrams of HA-TBA was dissolved in 10 mL of dimethyl sulfoxide (DMSO) and after 10 min the solution turned clear and homogenous. The polymer was activated with 123.6 mg of *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC·HCl) and 140 mg of *N*-hydroxysulfosuccinimide (sulfo-NHS), and after 2 h of stirring, 121.3 mg of N^{α}-Acetyl-L-lysine (Chem-Impex International, Inc., Wood Dale, IL) in 4 mL of DMSO was added and stirred for another 22-24 h. The product was dialyzed (10,000 MWCO; Fisher Scientific) against water for 4 h, 100-mM sodium chloride for 2 h, followed by 2 water changes over 18 h. The first dialysis in water was to remove DMSO; the second dialysis in NaCl solution was to remove TBA, EDC, sulfo-NHS, and excess N-Ac-lysine; and the third and fourth dialyses in water were to remove any small molecule residues and NaCl. The product was filtered (0.22-μm polyethersulfone syringe filter) and concentrated using a centricon filter (10,000 MWCO; Fisher Scientific). The products were named 33HA-Lys and 75HA-Lys (HA-N^{α}-Acetyl-L-Lys) to reflect the molecular weights of the HA used. A smaller molecular weight HA-Lys was also made using 6.4 kD HA. Synthesis of the 6.4HA-Lys is in the Supporting Information.

Synthesis of HA-cis-[$Pt(NH_3)_2(N^{\alpha}$-Acetyl-L-Lysine)]

Cisplatin (60 mg, 200 μmol; Qilu Pharmaceutical, Inc., Shandong, China) was suspended in 3 mL of approximately 35°C water and then stirred with $AgNO_3$ (68 mg, 400 μmol) for 1 day at approximately 20°C in the dark. The AgCl precipitate was removed by centrifugation (4000 rpm, 10 min), and the supernatant was filtered (0.22-μm nylon syringe filter) to obtain *cis*-$[Pt(NH_3)_2(OH)_2]$.

The HA-lys (33HA-Lys or 75HA-Lys, 125 mg) was dissolved in 15 mL of water, to which 815 μL of *cis*-$[Pt(NH_3)_2(OH)_2]$ (27 mg/mL on cisplatin basis) was added, and the pH of the mixture periodically adjusted to ~5.0 using 1-N NaOH. The reaction proceeded at 40°C for 1 day and 50°C for 2 days in the dark (Fig. 1). The resulting solution was filtered (0.22-μm nylon syringe filter) and then dialyzed (10,000 MWCO) against water for 4 h, sodium phosphate solution (100-mM NaCl, 10-mM NaH_2PO_4, pH 7.4) for 2 h, and 2