Cancer Letters 334 (2013) 346–353

Contents lists available at SciVerse ScienceDirect

Cancer Letters

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

Original Research Article

Polyisoprenoyl gemcitabine conjugates self assemble as nanoparticles, useful for cancer therapy

Andrei Maksimenko, Julie Mougin, Simona Mura, Eric Sliwinski, Elise Lepeltier, Claudie Bourgaux, Sinda Lepêtre, Fatima Zouhiri, Didier Desmaële, Patrick Couvreur *

Univ Paris-Sud, Faculté de Pharmacie, 5, rue J.B. Clément, 92296 Châtenay-Malabry Cedex, France
CNRS UMR 8612, Institut Galien Paris-Sud, 5, rue J.B. Clément, 92296 Châtenay-Malabry Cedex, France

ARTICLE INFO

Keywords:
Nanomedicine
Pancreatic cancer
Prodrug
Gemcitabine
Squalene
Terpene

ABSTRACT

A series of new polyisoprenoyl prodrugs of gemcitabine, which can be formulated as nanoassemblies are described. These prodrugs were designed to improve gemcitabine efficacy and to overcome the limitations due to the systemic toxicity of this anticancer compound. *In vitro* biological assessment showed that these polyisoprenoyl gemcitabine nanoassemblies displayed notable cytotoxicity on several cancer cell lines, including murine melanoma cell line B16F10, human pancreatic carcinoma cell line MiaPaCa-2, human lung carcinoma cell line A549 and human breast adenocarcinoma cell line MCF7. Interestingly, it was observed that the anticancer efficacy of these nanoassemblies was dependant on the size of polyisoprenoyl moiety. The polyisoprenoyl prodrug of gemcitabine containing three isoprene units (**2d**) was the more active on all the cancer cell lines tested. The antitumor efficacy of the nanoassemblies (NAs) constructed with the most active prodrug **2d** was further evaluated on a human pancreatic (MiaPaCa-2) carcinoma xenograft model in mice. The prodrug **2d** NAs showed an increased antitumor efficacy as compared to free gemcitabine or to squalene-gemcitabine (SQ-gem, **2a**) nanoassemblies. Interestingly, MiaPaCa-2 tumors that did not respond to gemcitabine were inhibited by 76% after treatment with prodrug **2d** NAs, whereas SQ-gem-treated MiaPaCa-2 tumor xenografts decreased only by 41% compared to saline or to gemcitabine-treated mice. Together, these findings demonstrated that the modulation of the length of nanoassemblies polyisoprenoyl moiety made tumor cells more sensitive to gemcitabine treatment without flagrant toxicity, thus providing a significant improvement in the drug therapeutic index.
© 2012 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

Cancer is currently the second leading cause of death in Europe, while it shows probably the highest clinical complexity [1]. Nanomedicine bears the potential to provide an effective answer to the complexity of the disease as it offers additional therapeutic options, compared to present conventional therapy [2]. New tailor-made nanodevices have the potential to revolutionize medicine because of their demonstrated ability to target the diseased organs and tissues at the molecular and cellular levels in a controlled manner [3–6]. Nanotechnology now plays an important part in developing drug delivery methods, allowing the emergence of new treatments with an improved efficacy and/or reduced toxicity [7]. These new nanodevices can be tailor-made with particular functionalities and applications as a result of progresses in material sciences, as well as in the preparation of colloidal systems with defined properties [8,9]. Nanomedical users and developers are, however, faced with the challenge of balancing the medical and societal benefits and risks associated with nanotechnology [10]. Up to now, the adequacy of available tools, such as physiologically-based pharmacokinetic modeling or predictive structure–activity relationships, in assessing the toxicity and risk associated with specific nanomaterials is unknown [10]. Successful development of future nanomedicines requires a consolidated information base to select the optimal nanomaterial, understanding the toxicology and potential side effects associated with candidate materials for medical applications [10–12].

In this context, we have discovered the "squalenoylation" technology, consisting in the linkage of squalene, a natural and well tolerated triterpene, with anticancer and antiviral nucleoside analogues [4,13]. Remarkably, the resulting bioconjugates were found to be able to self-organize spontaneously as nanoparticles in water whatever the nucleoside analogue used. This generic approach has advantages of drug potentiation and minimal drug resistance, as the loading of the drug (approximately 50%) is radically improved compared to all the other currently available

* Corresponding author. Address: Univ Paris-Sud, Faculté de Pharmacie, CNRS UMR 8612, Institut Galien Paris-Sud, 5, rue J.B. Clément, 92296 Châtenay-Malabry Cedex, France. Tel.: +33 (0) 1 46 83 53 96; fax: +33 (0) 1 46 83 59 46.
E-mail addresses: andrey.maksimenko@u-psud.fr (A. Maksimenko), patrick.couvreur@u-psud.fr (P. Couvreur).

0304-3835/$ - see front matter © 2012 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.canlet.2012.08.023

nanomedicines. This concept has been applied to gemcitabine, an anticancer compound, active against various solid tumors. Gemcitabine has, however, some major limitations, such as a short biological half-life, a poor diffusion into cells and the induction of resistances, which all restrict its therapeutic potential [14,15]. To overcome these drawbacks, we made a covalent linkage between gemcitabine and squalene to obtain 4-(N)-Tris-nor-squalenoyl-gemcitabine (SQ-gem). The resulting nanoassemblies (100 nm diameter) were formed by the hexagonal molecular packing of the molecules as shown by X-ray diffraction and cryo-TEM experiments [13]. Moreover, these SQ-gem nanoassemblies with a drug loading of almost 50% (w/w) exhibited impressively greater anticancer activity than gemcitabine itself against either solid subcutaneously grafted tumours (Panc-1, L1210 and P388) or aggressive metastatic leukemia (leukemia L1210 wt, P388 and RNK-16 LGL) [4,16,17]. It also appeared that only SQ-gem nanoassemblies treatment resulted in long-term survivals at both equivalent and equitoxic doses. Additional *in vitro* and *in vivo* studies have attributed the impressive anticancer activity of this nanomedicine to: (i) the significantly higher deposition of SQ-gem nanoassemblies in spleen, liver and lungs, the major metastatic organs, (ii) the better intracellular diffusion of SQ-gem, (iii) the selective activation of the prodrug by cathepsin B, a major enzyme in cancer cells and (iv) the increased phosphorylation of gemcitabine when delivered as SQ-gem nanoassemblies. In the light of these results, the concept was also applied to other non nucleoside bioactive molecules, such as siRNA, paclitaxel and penicillin [18–20].

The aim of the current study was to enlarge this new and ground-breaking concept by varying the nature of the polyterpene used for the drug conjugation. It was intended first to identify the influence of the isoprene length on the ability (or disability) of the resulting bioconjugates to form nanoassemblies and second to emphasize the impact of terpene's nature on the *in vitro* and *in vivo* pharmacological activity, using the gemcitabine as a drug model. Thus, we focused on identifying the requisite chemical functions in 1,1′,2-tris-nor-squalenic acid which are really responsible for nanoassemblies formation and, we concentrated on the modulation of the length of the terpenoyl side chain, by synthesis of various polyisoprenic acids that will be coupled to gemcitabine. Each conjugate was submitted to nanoprecipitation conditions in order to assess the minimal requisite size of the side chain. Based on the biological activity of studied compounds *in vitro* and on their optimal structure for nanoassemblies formation, we selected the lead molecule which was evaluated on human pancreatic (MiaPaCa-2) carcinoma xenograft model in mice.

2. Materials and methods

2.1. Reagents

Gemcitabine was purchased from Sequoia Research Product Ltd. (UK). D-(+)-Trehalose dihydrate, squalene, 3-[4,5-dimethylthiazol-2-yl]-3,5-diphenyl tetrazolium bromide (MTT), ethylchloroformate, tetra-butylammonium fluoride, *tert*-butyl dimethylchlorosilane, methanesulfonyl chloride, 2′-deoxycitidine, sodium acetate and sodium hydride were purchased from Sigma–Aldrich Chemical Co. (St. Quentin Fallavier, France). Poly(ethylene glycol) monomethyl ether (Mn, 2000 g mol^{-1}) was purchase from Fluka (France). Tetrahydrouridine was purchased from Merck Chemicals Ltd. (Nottingham, UK). All solvents were purchased from Carlo Erba (Val-de-Reuil, France). Annexin V-FITC apoptosis assay kit and cell culture reagents were purchased from Invitrogen-Life Technologies (Cergy Pontoise, France).

2.2. Synthesis, purification, and characterization of the polyisoprenoyl gemcitabine prodrugs

The synthesis of squalenoyl-gemcitabine **2a** or 1,1′,2-tris-nor-squalenic acid **3a** has been previously reported (Fig. 1) [4]. Bioconjugates **2b**–**2g** were obtained by reaction of the corresponding polyisoprenic acids **3b**–**3g** with 3′,5′-bis TBDMS-protected gemcitabine, followed by conventional tetrabutylammonium fluoride (TBAF) deprotection. Accordingly, the requisite acid was reacted with ethyl chloroformate in THF at 0 °C to give the intermediate anhydride which was directly reacted with the TBDMS-protected gemcitabine in THF for 12 h using DMAP as catalyst, followed by TBAF deprotection. The crude products were purified by column chromatography on silica gel providing the expected conjugate in fair yields (Table 1) (full synthetic details and characterization of the polyisoprenic acids **3b**–**3g** and prodrugs **2b**–**2g** can be found in Supplementary material).

The synthesis of squalene coupled to polyethyleneglycol (SQ-PEG, Trisnorsqualene-MePEG) has been achieved in 60% yield as previously reported [21] by alkylation of the sodium alkoxide of MePEG with 1,1′,2-trisnorsqualenyl methanesulfonate.

2.3. Preparation and characterization of the squalenoyl (**2a**) and polyisoprenoyl (**2b**–**2g**) gemcitabine nanoassemblies (NAs)

Two milligram of compounds **2a**, **2b**, **2c**, **2e**, **2f** and **2g** or 2 mg of compound **2d** completed with 400 μg of SQ-PEG (20%, w/w) were dissolved in THF (500 μL, compounds **2a**, **2c**–**2g**) or ethanol (1 mL, compound **2b**). The resulting solutions of compounds **2b** and **2c** were added drop-wise under stirring (1000 rpm) into 1 mL of an aqueous solution of trehalose (10%). Other solutions (i.e. **2a**, **2d**, **2e**, **2f** and **2g**) were added drop-wise under stirring (1000 rpm) into 1 mL of distilled water. The formation of the polyisoprenoyl gemcitabine nanoassemblies occurred spontaneously. THF or ethanol were then completely evaporated using a Rotavapor® at 37 °C under vacuum to obtain aqueous suspensions of pure polyisoprenoyl gemcitabine nanoassemblies (final concentration 2 mg/mL). The samples of nanoassemblies **2a**, **2b**, **2e**–**2g** were stored at 5 °C whereas **2c** and **2d** NAs were maintained at 37 °C before *in vitro* and *in vivo* experiments. Nanoassemblies made of 1,1′,2-tris-nor-squalenic (**3a**) or other (**3b**–**3g**) corresponding isoprenic acids alone were prepared in a similar manner. The final concentration of the aqueous suspension of squalenic or farnesylacetic acids was 2 mg/mL.

The size of the nanoassemblies was determined at 20 °C (for compounds **2a**, **2b**, **2e**–**2g**) or at 37 °C (for compounds **2c** and **2d**) by quasi-elastic light scattering (QELS) with a nanosizer (Zêtasizer Nano ZS Malvern; Malvern Instruments SA, Orsay, France) after 1:10 dilution in water. All the polyisoprenoyl gemcitabine nanoassemblies prepared in conditions as described above gave stable nanoassemblies of 112–270 nm (PDI 0.2).

The morphology of the different polyisoprenoyl gemcitabine nanoassemblies was examined by cryomicroscopy (cryo-TEM). Briefly, one drop (5 μL) of the polyisoprenoyl gemcitabine nanoassemblies suspension (2 mg/mL) was deposited onto a perforated carbon film mounted on a 200-mesh electron microscopy grid. Most of the drop was removed with a blotting filter paper and the residual thin films remaining within the holes were vitrified after immersion in liquid ethane. The specimen was then transferred using liquid nitrogen to a cryo-specimen holder and observed using a JEOL FEG-2010 electron microscope.

2.4. Drug release

To determine the kinetics of gemcitabine release from polyisoprenoyl gemcitabine nanoassemblies, 100 μL of nanoassemblies **2a**–**2d**, **2f** and **2g** (1 mg/mL) were added to 900 μL of heat-inactivated fetal bovine serum (FBS) (56 °C, 30 min) supplemented with 200 μg/mL tetrahydrouridine (THU). Individual vials were used for each time point. The reaction mixture was incubated at 37 °C, and aliquots (100 μL) of incubation medium were removed at predetermined time points (0, 2, 4, 8 and 24 h), spiked with 10 μL of 200 μM 2′-deoxycytidine solution (Internal Standard, IS) before addition of 1 mL of a mixture of acetonitrile/methanol (90/10, v/v) and ultracentrifugated (15000 g, 20 min, 4 °C). Supernatant was then evaporated to dryness under a nitrogen flow at 30 °C. The released drug was quantified by reverse-phase HPLC (Waters, Milford, MA 01757, USA) with a C18 column as described previously [22,23]. Briefly, the chromatographic system consisted of a Waters 1525 Binary LC pump, a Waters 2707 Autosampler, a C18 Uptisphere column (3 μm, 150 × 4.6 mm; Interchim), HPLC column temperature controllers (model 7950 column heater and chiller; Jones Chromatography, Lakewood, CO), and a Waters 2998 programmable photodiode-array detector. The HPLC column was maintained at 30 °C. Detection was monitored at 270 nm. The HPLC mobile phase was methanol:water (5:95, by vol, with 0.05 M sodium acetate in water, pH 5, solvent A) and methanol:water (97:3, by vol, with 0.05 M sodium acetate in water, pH 5, solvent B). The residues were dissolved in 100 μL of solvent A and elution was carried out at a flow rate of 0.8 mL/min isocratically for 8 min with solvent A followed by a 1-min linear gradient to 100% solvent B. This was followed by a 16-min hold at solvent B, and a 1-min linear gradient back to 100% solvent A. The system was held at 100% solvent A for 14 min for equilibration back to initial conditions.

2.5. Cell culture

Murine melanoma cell line B16F10, human pancreatic carcinoma cell line MiaPaCa-2, lung carcinoma cell line A549 and breast adenocarcinoma cell line MCF7 were obtained from the American Type Culture Collection and maintained as recommended. Briefly, A549 cells were maintained in F12-K medium. MiaPaCa-2 and B16F10 cells were grown in Dulbecco's minimal essential medium (DMEM) – Glutamine medium. MCF7 cells were cultured in a mixture of DMEM/Ham's F12 (1:1). All

Download English Version:

https://daneshyari.com/en/article/2116310

Download Persian Version:

https://daneshyari.com/article/2116310

Daneshyari.com