Journal of Controlled Release 247 (2017) 73–85

Contents lists available at ScienceDirect

Journal of Controlled Release

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

Tumour regression and improved gastrointestinal tolerability from controlled release of SN-38 from novel polyoxazoline-modified dendrimers

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ARTICLE INFO

Article history:
Received 28 September 2016
Received in revised form 20 December 2016
Accepted 29 December 2016
Available online 30 December 2016

Keywords:
Nanomedicine
Irinotecan
SN-38
Dendrimer
Colorectal cancer
Drug delivery
Therapeutic index

ABSTRACT

Irinotecan is used clinically for the treatment of colorectal cancer; however, its utility is limited by its narrow therapeutic index. We describe the use of a generation 5 L-lysine dendrimer that has been part-modified with a polyoxazoline as a drug delivery vehicle for improving the therapeutic index of SN-38, the active metabolite of irinotecan. By conjugating SN-38 to the dendrimer *via* different linker technologies we sought to vary the release rate of the drug to generate diverse pharmacokinetic profiles. Three conjugates with plasma release half-lives of 2.5 h, 21 h, and 72 h were tested for efficacy and toxicity using a mouse SW620 xenograft model. In this model, the linker with a plasma release half-life of 21 h achieved sustained SN-38 exposure in blood, above the target concentration. Control over the release rate of the drug from the linker, combined with prolonged circulation of the dendrimer, enabled administration of an efficacious dose of SN-38, achieving significant regression of the SW620 tumours. The conjugates with 2.5 and 72 h release half-lives did not achieve an anti-tumour effect. Intraperitoneal dosing of the clinically used prodrug irinotecan produces high initial and local concentrations of SN-38, which are associated with gastrointestinal toxicity. Administration of the 21 h release dendrimer conjugate did not produce a high initial C_{max} of SN-38. Consequently, a marked reduction in gastrointestinal toxicity was observed relative to irinotecan treatment. Additional studies investigating the dose concentrations and dose scheduling showed that a weekly dosing schedule of 4 mg SN-38/kg was the most efficacious regimen. After 4 doses at weekly intervals, the survival period of the mice extended beyond 70 days following the final dose. These extensive studies have allowed us to identify a linker, dose and dosing regimen for SN-38 conjugated to polyoxazoline-modified dendrimer that maximised efficacy and minimised adverse side effects.

1. Introduction

One of the key considerations for cancer medicines remains establishing a therapeutic index [1], since targeting key cellular processes can induce significant toxicity, and therapeutic margins are typically narrow, or almost non-existent for some specific potent cytotoxic therapies.

Topoisomerases are ubiquitous enzymes that control DNA supercoiling and entanglements. They cleave the DNA backbone, releasing DNA supercoils and re-ligating the cleaved DNA. Topoisomerases are essential during the transcription and replication process, and topoisomerase inhibitors that cause single or double strand cleavage (type I or II, respectively) are among the most effective and most commonly used anticancer and antibacterial drugs [2]. Topotecan and irinotecan are topoisomerase I inhibitors routinely used in the clinic in a range of different cancers. They are water soluble semi-synthetic derivatives of

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http://dx.doi.org/10.1016/j.jconrel.2016.12.034
0168-3659/

the potent cytotoxic plant alkaloid camptothecin. However, serious adverse effects, including myelosuppression and diarrhoea, have limited their efficacy in the clinic.

In addition, irinotecan is a prodrug, which is hydrolysed to its more potent active metabolite, SN-38, by carboxylesterases in the liver and tumour cells. This conversion is often inefficient in humans, and leads to variability in SN-38 exposure [3]. Although SN-38 is ~100- to 1000-fold more potent than irinotecan [3], its clinical use is limited by its poor water solubility and chemical instability of its pharmacologically active lactone ring at pH > 6. Alongside the narrow therapeutic index of irinotecan and SN-38, these issues present further challenges for their use as anti-cancer therapeutics in the clinic.

In principle, one way to improve therapeutic index is to increase drug exposure in diseased tissue, without accumulation in other healthy tissues. Nanotechnology offers the possibility to achieve greater site specificity by changing the biodistribution of a drug. These drug delivery systems have been shown to increase drug concentration at tumour sites, relative to off-target tissues. This is often achieved by minimising the high initial drug concentrations in the plasma (C_{max}) and allowing tissue accumulation and retention *via* the enhanced permeability and retention (EPR) effect [4,5]. The rapidly developing field of nanomedicines covers a range of nano-scale constructs, such as liposomes, polymeric nanoparticles, polymeric micelles, various polymer conjugates and inorganic particles.

Several nanomedicine delivery strategies have been investigated to improve the therapeutic index of SN-38 and camptothecin analogues, by reducing accumulation in normal tissues while increasing delivery to tumours. To date, SN-38 and camptothecin analogues have been delivered using liposomes, polymeric nanoparticles, polymeric micelles and various polymer conjugates and antibody drug conjugates [6–12].

Onivyde™ (Merrimack Pharmaceuticals), a liposomal irinotecan, has recently been approved for use in metastatic pancreatic cancer and is currently being used in Phase II trials in gastric cancer. Etirinotecan pegol (ONZEALD™, formerly NKTR-102; Nektar Therapeutics) is in advanced clinical development for ovarian, breast, colon, and lung cancers [13,14]. Both of these nano-formulations provide greater area under the curve (AUC) for SN-38 than does irinotecan treatment in pre-clinical tumours [14]. A camptothecin nanoparticle polymer conjugate, CRLX101 (Cerulean Pharma Inc.) is currently in clinical trials in a number of cancers. CRLX101 has demonstrated an extended circulation half-life and prolonged release of camptothecin in rat, dog, and human plasma [15], sustained tumour pharmacokinetics and inhibition of target [16], and evidence of localisation in tumour rather than healthy tissues in gastric cancer patients [17].

All of these systems have demonstrated positive results in pre-clinical models and are progressing in the clinic with promising activity. However, the ability to control the release of the payload from the nano-carrier has not been explored, precluding the possibility of fine-tuning the release rate to maximise the therapeutic index. The ability to explore the relationship between efficacy and toxicity across a range of release rates generates insight that drives biology-focussed nanomedicine design [18].

Dendrimer drug conjugates offer many advantages over other drug delivery systems, including tuneable drug release. Additional benefits of the dendrimer platform include: control of size during synthesis, near-monomodal molecular weight distributions, and reproducible synthetic sequences. Dendrimers are significantly smaller (~7–15 nm) than polymeric nanoparticles prepared *via* other routes. Nanomedicine systems of similar sizes have been shown to extravasate to a greater extent and/or penetrate farther from the vasculature than do larger systems, which has been associated with improved efficacy [19–21]. In addition, dendrimers have large numbers of surface groups available for conjugation. These surface groups can be used for drug conjugation *via* different linker chemistries, without significantly impacting solubility, or to modify the outer layer of the dendrimer. This modification controls surface charge and provides a corona to prevent protein adsorption, thereby extending the circulation time of the dendrimer conjugate. PEGylation has been widely employed with polyamidoamine (PAMAM) [26–29] and poly(L-lysine) dendrimers [30–33] for surface modification. There is now significant diversity in the dendrimer-based nanomedicines being explored pre-clinically, including amphiphilic dendrimers forming nanomicelles [22], dendrimer-assisted metal nanocomposite particles for CT/MR imaging [23], lactoferrin-containing dendriplexes [24], and doxorubicin-conjugated dendrimers for pulmonary delivery [25].

We have built on this strategy using a biodegradable fifth generation L-lysine dendrimer, part-modified with a polyoxazoline [34], to deliver the potent topoisomerase I inhibitor SN-38. Recognising the importance of controlled drug release in designing nanomedicines and optimising therapeutic index [35–40], we designed a range of linker chemistries to tune the release rate of SN-38, and explored the synthesis, characterisation and therapeutic application of three dendrimer-SN38 (Dend-SN38) conjugates *in vitro* and *in vivo*.

2. Materials and methods

2.1. Materials

All reagents and solvents were purchased from Sigma-Aldrich and used as received with exception of SN-38 (TCI Europe N.V.), *N*,*N*′-disuccinimidyl carbonate & 2-chloro-4,6-dimethoxy-1,3,5-triazine (Chem-Impex International). Irinotecan was purchased from CarboSynth (UK).

2.2. Methods

HPLC-UV was performed on an Agilent 1100 fitted with an Atlantis dC18 5 μm 4.6 × 150 mm column with an acetonitrile/water gradient + 1% TFA modifier. See Supporting information Table S1 for HPLC-UV run parameters. The data were analysed using Thermo Scientific™ Atlas Chromatography Data System (CDS) software. GPC was performed on a Malvern TDA302 using a Tosoh Bioscience TSKgel GMPWxl column and an eluent of a 40: 10 mM $NaNO_3$: NaH_2PO_4 + 10% MeOH in water. Samples were made to 2 mg/mL in the eluent. The system calibration was performed using a single poly(ethylene oxide) standard (16,100 g mol^{-1}) and absolute molecular weights determined using the refractive index and light scattering signals. Dynamic light scattering was performed on a Malvern Zetasizer® Nano ZS instrument with back scattering detector (173°, 633 nm laser wavelength). The dispersant RI and viscosity were assumed to be that of water (n = 1.59 and η = 0.888 mPa·s). The sample RI was 1.59 and the temperature was set at 25 °C. The hydrodynamic diameter (DH) was reported as the volume-weighted average after a minimum of twelve measurements per sample and was calculated by the software. Samples were made to concentrations of 5 mg/mL in PBS at pH 7.4 and were filtered using a 0.2 μm syringe filter prior to measurement. Data was obtained using Malvern Zetasizer software version 6.21J.

4-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium tetrafluoroborate (DMTMM.BF_4) was synthesised according to methods reported previously [41].

G5-PLL[PMOx]$_x$[NH_2]$_y$. This product was synthesised according to methods previously described [34]. M_n = 75.7 kg/mol, PDI = 1.17 from triple detection size exclusion chromatography.

G5-PLL[PMOx]$_x$[Azide]$_y$. G5-PLL[PMOx][NH_2] (750 mg, 9.78 μmol) was dissolved in anhydrous DMF (5 mL) at 45 °C. The solution was cooled to room temperature. A pre-mixed solution of 2-azidoacetic acid (93 μL, 1.25 mmol), *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC) (240 mg, 1.25 mmol) and 4-(dimethylamino)pyridine (DMAP) (15 mg, 0.12 mmol) in anhydrous DMF (5 mL) was added to the polymer solution and stirred overnight