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Doxorubicin Conjugation and Drug Linker Chemistry Alter the Intravenous and Pulmonary Pharmacokinetics of a PEGylated Generation 4 Polylysine Dendrimer in Rats

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ABSTRACT

PEGylated polylysine dendrimers have demonstrated potential as inhalable drug delivery systems that can improve the treatment of lung cancers. Their treatment potential may be enhanced by developing constructs that display prolonged lung retention, together with good systemic absorption, the capacity to passively target lung tumors from the blood and highly selective, yet rapid liberation in the tumor microenvironment. This study sought to characterize how the nature of cathepsin B–cleavable peptide linkers, used to conjugate doxorubicin (Dox) to a PEGylated (PEG570) G4 polylysine dendrimer, affects drug liberation kinetics and intravenous and pulmonary pharmacokinetics in rats. The construct bearing a self-emolative diglycolic acid-V-Citrulline linker exhibited faster Dox release kinetics compared to constructs bearing self-emolative diglycolic acid-glycine-leucine-phenylalanine-glycine (GLFG), or non-self-emolative glutaric acid-GLFG linkers. The V-Citrulline construct exhibited slower plasma clearance, but faster absorption from the lungs than a GLFG construct, although mucociliary clearance and urinary elimination were unchanged. Dox-conjugation enhanced localization in the bronchoalveolar lavage fluid compared to lung tissue, suggesting that projection of Dox from the dendrimer surface reduced tissue uptake. These data show that the linker chemistry employed to conjugate drugs to PEGylated carriers can affect drug release profiles and systemic and lung disposition.

Introduction

Previous work has shown that inhaled PEGylated polylysine dendrimers provide an excellent platform for the delivery of drugs to the lungs and for subsequent systemic exposure.[1,2] They exhibit tunable pharmacokinetic properties that can be tailored to promote prolonged lung retention, systemic access, or both.[2] For example, pulmonary administration of a 56 kDa PEGylated G5 dendrimer conjugated with the chemotherapeutic drug doxorubicin (Dox) via an acid-labile hydrazone linker, dramatically improved the activity of Dox against lung metastases in rats when compared to the intravenous administration of drug alone.[1] This construct was also well tolerated by rats after administration of up to 80 mg of dendrimer to the lungs. In contrast, the pulmonary administration of Dox alone induced significant lung-related toxicity after a single dose. In spite of these encouraging results, some evidence of lung necrosis was apparent in ~50% of animals administered the Dox-dendrimer as a result of nonspecific Dox liberation.

To enhance the therapeutic utility of this system, we sought here to explore the potential of different conjugation chemistries to

Abbreviations used: BALF, bronchoalveolar lavage fluid; DGA, diglycolic acid; VCit, Valine-Citrulline; Dox, doxorubicin; Glu-GLFG, glutaric acid-glycine-leucine-phenylalanine-glycine; GFLG, glycine-leucine-phenylalanine-glycine; PAB, para-amino benzoic acid; G, generation.

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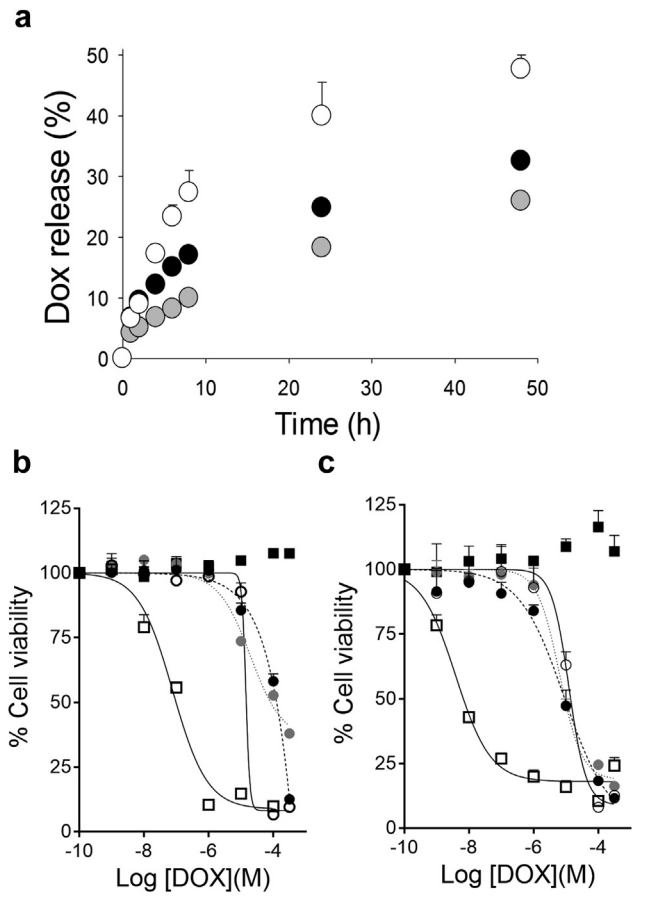

Figure 1. Dox release kinetics and *in vitro* cytotoxicity. (a) Kinetics of Dox release from each dendrimer in the presence of cathepsin. (b) Cytotoxicity of the 3 dendrimers against human lung carcinoma A549 cells and (c) human mammary MCF7 cells. Symbols represent: (○) $G4_{PEG570}$-DGA-VCit; (●) $G4_{PEG570}$-Glu-GLFG; (●) $G4_{PEG570}$-DGA-GLFG; (□) Dox; (■) blank dendrimer. Data represent mean ± SD (n = 3).

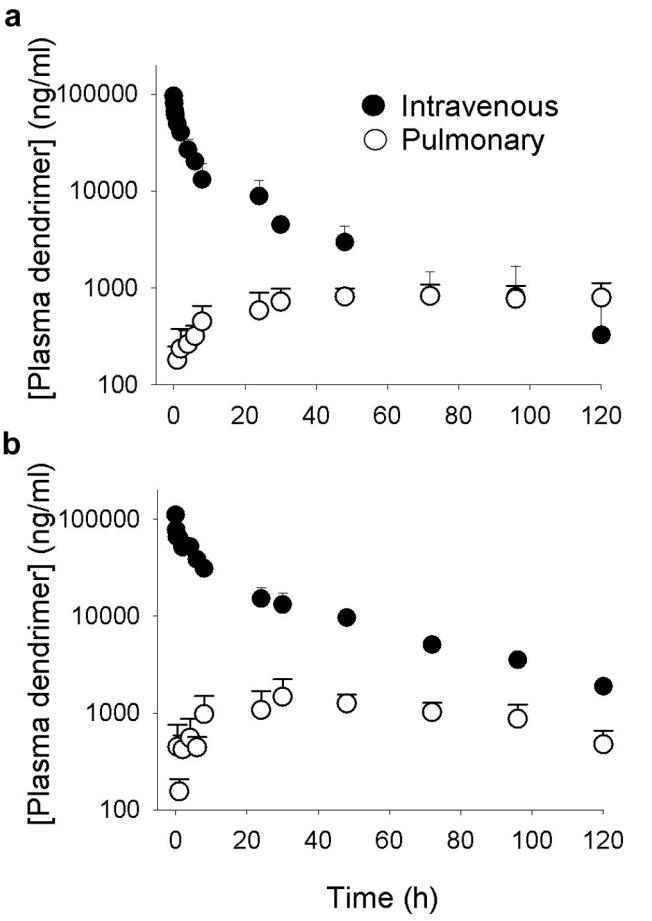

Figure 2. Plasma concentration-time profiles for (a) $G4_{PEG570}$-DGA-GLFG and (b) $G4_{PEG570}$-DGA-VCit after intravenous and pulmonary administration to rats at 5 mg/kg. Mean ± SD (n = 3-4).

reduce the degree of nontumor specific drug release, yet allow rapid drug liberation in tumors and also to try to identify constructs that exhibit prolonged lung exposure, good systemic absorption, and enhanced plasma exposure. Thus, we aimed to provide an inhalable delivery system with the potential to be retained in the lungs, resulting in specific drug liberation on the "air side" of lung tumors, together with absorption from the lungs and passive targeting from the "blood side" of tumors via enhanced permeation and retention to enhance drug exposure to the whole tumour. In this way, pulmonary and systemic side effects are expected to be reduced (compared to drug alone) by minimizing the exposure of noncancerous tissues to free drug.

A series of G4 dendrimers conjugated with PEG570 and Dox (20-30 kDa in size) were synthesized based on previous data showing that this scaffold construction offers both prolonged systemic exposure and good lung absorption[2,3] (see Figure in supporting information). Three peptide-based drug linkers that are specifically cleavable by cathepsin B were used to conjugate Dox to this dendrimer scaffold: a pentapeptide (glutaric acid-glycine-leucine-phenylalanine-glycine [Glu-GLFG[4]]) that results in the liberation of both peptide-modified and unmodified Dox and 2 self-emolative systems (diglycolic acid [DGA]-GLFG-para-amino benzoic acid[4] and DGA-Valine-Citrulline [VCit]-para-amino benzoic acid[5]) that facilitate the liberation of unmodified Dox. Cathepsin B-cleavable peptides were used since the extracellular and lysosomal expression of this enzyme is highly upregulated by cancer cells, particularly those at the invasive front of a tumor, but is constitutively expressed at low levels in normal tissue.[6-8]

Methods

Materials and Dendrimers

Scintillation vials, Soluene tissue solubilizer and IRGASafe scintillant were from Perkin Elmer (Victoria, Australia). Cell culture media and supplements were from Invitrogen (Victoria, Australia).

Dendrimers were synthesized and characterized as described previously,[9] with modification (see Supplementary Information for synthetic details). ^{3}H-lysine was incorporated into the penultimate generation to enable pharmacokinetic characterization of the dendrimer construct. The specific activity of the dendrimers was determined via scintillation counting.[10]

Dox Release Kinetics and In Vitro *Cytotoxicity*

The kinetics of Dox release in the presence of cathepsin B was initially investigated by incubating 1 μg/mL Dox equivalents (approximately 5 μg/mL dendrimer) with 1 IU/mL cathepsin B (Sigma, St. Louis, MO) in 0.1 M acetate buffer containing EDTA (0.01 M) and glutathione (0.05 M) at 37°C for 2 days. Dox release from $G4_{PEG570}$-DGA-VCit was also examined in the absence of cathepsin to confirm enzyme-specific liberation. Aliquots of the reaction mixture were collected at various time points and assayed via fluorescence high-performance liquid chromatography for free Dox.[11]