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# Pulmonary administration of a doxorubicin-conjugated dendrimer enhances drug exposure to lung metastases and improves cancer therapy

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ABSTRACT

Direct administration of chemotherapeutic drugs to the lungs significantly enhances drug exposure to lung resident cancers and may improve chemotherapy when compared to intravenous administration. Direct inhalation of uncomplexed or unencapsulated cytotoxic drugs, however, leads to bolus release and unacceptable lung toxicity. Here, we explored the utility of a 56 kDa PEGylated polylysine dendrimer, conjugated to doxorubicin, to promote the controlled and prolonged exposure of lung-resident cancers to cytotoxic drug. After intratracheal instillation to rats, approximately 60% of the dendrimer was rapidly removed from the lungs (within 24 h) *via* mucociliary clearance and absorption into the blood. This was followed by a slower clearance phase that reflected both absorption from the lungs (bioavailability 10–13%) and biodegradation of the dendrimer scaffold. After 7 days, approximately 15% of the dose remained in the lungs. A syngeneic rat model of lung metastasised breast cancer was subsequently employed to compare the anticancer activity of the dendrimer with a doxorubicin solution formulation after intravenous and pulmonary administration. Twice weekly intratracheal instillation of the dendrimer led to a >95% reduction in lung tumour burden after 2 weeks in comparison to IV administration of doxorubicin solution which reduced lung tumour burden by only 30–50%. Intratracheal instillation of an equivalent dose of doxorubicin solution led to extensive lung-related toxicity and death within several days of a single dose. The data suggest that PEGylated dendrimers have potential as inhalable drug delivery systems to promote the prolonged exposure of lung-resident cancers to chemotherapeutic drugs and to improve anti-cancer activity.


## 1. Introduction

Primary lung cancer is one of the leading causes of death in developed countries and is responsible for 23% of all cancer-related deaths [1]. Primary lung cancers are mainly of epithelial origin and most commonly result from inhaled exposure to cigarette smoke (the main cause of primary lung cancer) and environmental contaminants such as asbestos and radon. The lungs are also a major site of metastasis for other cancers including those of the breast, prostate and colon. These cancers typically metastasise towards the lungs by invading into the blood stream, from where they lodge within the narrow pulmonary capillaries, invade into the surrounding tissue and establish solid secondary tumours. From here, they may metastasise further to thoracic lymph nodes or elsewhere in the body. Despite their prevalence, lung-resident cancers are difficult to treat, and mortality rates with conventional intravenous chemotherapy are high (approximately 85% within 5 years) [2]. The lack of efficacy of intravenous chemotherapy against lung tumours stems from a combination of (typically) late diagnosis, and poor drug access to lung tissue after intravenous administration [2–5]. Inhaled drug delivery results in improved access to lung tissue and has shown superior activity when compared to intravenous administration in both animals and humans [6–10]. Clinical studies, however, suggest that inhaled delivery of cytotoxic drug alone, leads to high local drug concentrations and unacceptable lung-related toxicity [9,11,12].

The realisation that pulmonary delivery of cytotoxic drug may enhance therapy, but is associated with unacceptable toxicity, has stimulated increasing interest in the development of inhaled delivery systems that improve drug exposure to lung-resident tumours, but limit the toxic effects of the drug. Thus, a number of nanoparticulate and liposomal formulations of doxorubicin have been evaluated as

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potential delivery systems for the improved treatment of lung-resident cancers after inhaled administration [2,10,13,14]. These studies provide promising indications of pre-clinical utility, with reduced lung-related side effects compared to the pulmonary administration of drug alone, and reduced systemic toxicity compared to intravenous drug delivery [2,10,13,14].

The nanoparticulate carriers evaluated to this point, however, have been relatively large (54 to >600 nm) [10,15–20]. Absorption across the pulmonary epithelia or diffusion into solid lung tumours is therefore expected to be limited [21–24]. For colloidal carriers, such as liposomes (reviewed in [25]), drug release is also usually non-specific, providing limited control over the kinetics of drug release [2,26]. Finally, for non-biodegradable particles that are retained in the lungs for extended periods of time, there are increasing risks of toxicity via the promotion of localised infiltration of alveolar macrophages and inflammation in response to the presence of the particles [27,28].

In contrast, dendritic polymers (dendrimers) [29,30] are approximately one order of magnitude smaller (approx 4–20 nm) than the majority of nanoparticles and liposomes, and therefore provide possible advantage with respect to the efficiency of interstitial diffusion, the extent of absorption and the degree of tumour penetration [31]. Drug conjugation to the dendrimer scaffold *via* linkers that are cleaved selectively in the environment of a tumour also provides a greater level of control over the location and kinetics of drug release [32]. Dendrimers based on poly-amino acid structures are also biodegradable, reducing the potential for immune stimulation. The kinetics of dendrimer clearance from the lungs of rats were therefore recently evaluated using PEGylated systems based on a poly-L-lysine scaffold. Small PEGylated dendrimers (<20 kDa) were absorbed after pulmonary instillation as low molecular weight fragments following initial degradation in the lungs. In contrast, increasing the molecular weight of the PEGylated dendrimers led to increased metabolic stability and significant lung retention [33]. PEGylated dendrimer based delivery systems therefore have the potential to provide controlled and site specific drug delivery to lung resident tumours. For these systems, programmed drug liberation followed by metabolic breakdown is expected to provide advantage over other colloidal or particulate vehicles as inhalable drug delivery systems for chemotherapeutic drugs.

In the current study, we have explored whether a 56 kDa biodegradable PEGylated polylysine dendrimer, conjugated with doxorubicin *via* an acid labile linker (Fig. 1, described previously [34]) provides therapeutic benefit against lung-resident cancers when compared to the inhaled or intravenous administration of solution formulations of drug alone. Since a lack of understanding of the mechanisms by which nanomedicines are cleared from the lungs is also a significant current limitation to the translation of inhalable nanomedicine technologies into human clinical trials, the mechanisms and time course of dendrimer (and associated doxorubicin) clearance from the lungs have also been evaluated in detail.

## 2. Methods

### 2.1. Materials and reagents

Doxorubicin, thiazolyl blue formazan (MTT) and heptanesulphonic acid were purchased from Sigma-Aldrich (Sydney, Australia). RPMI media, hanks balanced salt solution (HBSS), penicillin/streptomycin, fetal bovine serum and glutamax were obtained from Gibco (NY, USA). Soluene-350 and IRGASafe scintillant were from Packard Biosciences (Meriden, CT). Cell culture flasks and microplates were from Corning (NY, USA). Polyethylene tubing (0.96 × 0.58 mm external and internal diameter) was purchased from Microtube Extrusions (NSW, Australia). HPLC grade acetonitrile was from Merck (VIC, Australia). All other reagents were AR grade.

### 2.2. Dendrimer

The doxorubicin-conjugated dendrimer (D-DOX, Fig. 1) contained PEG1100 conjugated to ε-amino groups on the surface of a G5 polylysine dendrimer and doxorubicin conjugated to surface α-amino groups *via* a 4-(hydrazinosulphonyl) benzoic acid linker [35]. The synthesis, characterisation, intravenous pharmacokinetics and anti-tumour efficacy of this construct against solid mammary carcinomas have previously been reported [34–36]. D-DOX was reconstituted in pH 7.4 PBS to 10 mg/ml for pharmacokinetic studies as previously described [35] (1.5 mg/ml doxorubicin equivalents) and 30 mg/ml (4.5 mg/ml doxorubicin equivalents) for anticancer activity studies immediately prior to dosing. The drug-free dendrimer control (D) employed the same dendrimer scaffold but without doxorubicin. The synthesis of the control dendrimer has been described previously [35].

### 2.3. Animals

Male Sprague Dawley rats (270–320 g) were obtained from Monash Animal Services (VIC, Australia). Female F344 rats (100–150 g) were supplied by Animal Resources Centre (Perth, Australia). Rats were maintained on a 12 hour light dark cycle and were supplied water at all times. Food was withheld only after cannulation surgery and for 8 h after intravenous or pulmonary dosing for pharmacokinetic experiments. All experimentation involving animals was approved by the institutional Animal Ethics Committee.

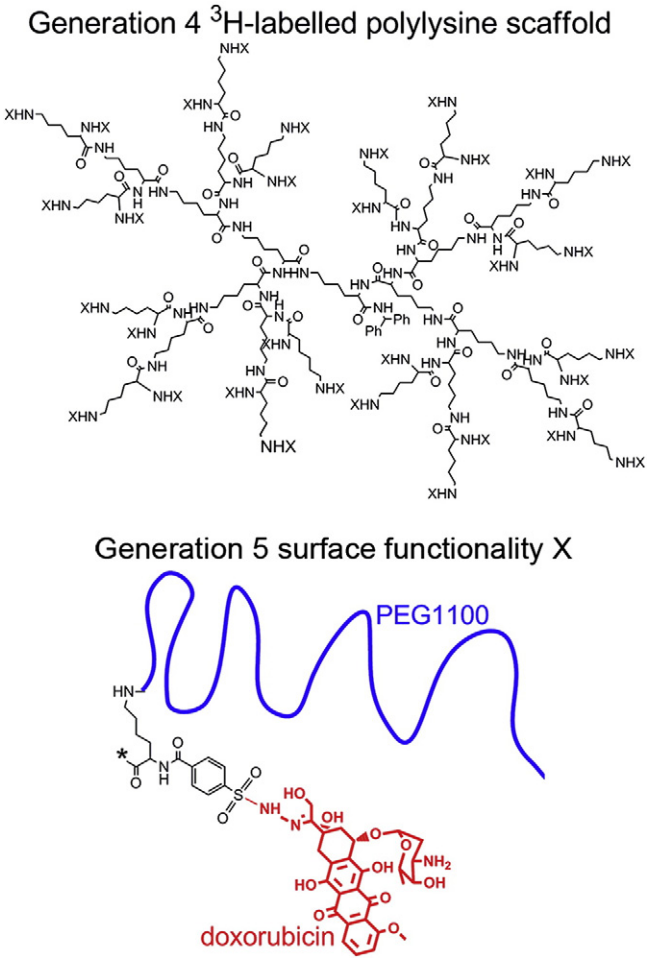


**Fig. 1.** Structure of a generation 5 doxorubicin conjugated dendrimer (D-DOX, 56 kDa), based on a generation 4 polylysine scaffold.