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In vivo biocompatibility, clearance, and biodistribution of albumin vehicles for pulmonary drug delivery

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ABSTRACT

The development of clinically acceptable albumin-based nanoparticle formulations for use in pulmonary drug delivery has been hindered by concerns about the toxicity of nanomaterials in the lungs combined with a lack of information on albumin nanoparticle clearance kinetics and biodistribution. In this study, the *in vivo* biocompatibility of albumin nanoparticles was investigated following a single administration of 2, 20, and 390 μg/mouse, showing no inflammatory response (TNF-α and IL-6, cellular infiltration and protein concentration) compared to vehicle controls at the two lower doses, but elevated mononucleocytes and a mild inflammatory effect at the highest dose tested. The biodistribution and clearance of ^{111}In labelled albumin solution and nanoparticles over 48 h following a single pulmonary administration to mice was investigated by single photon emission computed tomography and X-ray computed tomography imaging and terminal biodistribution studies. ^{111}In labelled albumin nanoparticles were cleared more slowly from the mouse lung than ^{111}In albumin solution (64.1 ± 8.5% *vs* 40.6 ± 3.3% at t = 48 h, respectively), with significantly higher ($P < 0.001$) levels of albumin nanoparticle-associated radioactivity located within the lung tissue (23.3 ± 4.7%) compared to the lung fluid (16.1 ± 4.4%). Low amounts of ^{111}In activity were detected in the liver, kidneys, and intestine at time points >24 h indicating that small amounts of activity were cleared from the lungs both by translocation across the lung mucosal barrier, as well as mucociliary clearance. This study provides important information on the fate of albumin vehicles in the lungs, which may be used to direct future formulation design of inhaled nanomedicines.

1. Introduction

The potential for nanoparticles to provide controlled drug delivery to the lungs is well recognised [1,2]. The benefits of using nanoparticles as oppose to micron-scale carriers in the lungs include the ability to reduce unwanted macrophage uptake [3], modify dissolution rates particularly of poorly soluble drugs [4], and to improve deposition and retention profiles in the lungs [5,6]. However, there has been little translation of pulmonary nanomedicine from the research bench to the clinic, not least because of safety concerns associated with inhalation of nanoparticulate matter [7,8].

Chronic exposure to certain types of nanoparticles has been associated with inflammation, fibrosis and the release of pro-thrombotic mediators which can lead to induced vascular thrombosis and cardiac arrest [9]. In addition, inhaled nanoparticles have been observed to pass through the lung epithelium and enter the systemic circulation prompting fears concerning accumulation in secondary organs [10]. The type and extent of toxicity elicited by a particulate nanocarrier has been linked to a variety of factors including particle size, composite material, surface charge and reactivity, hydrophobicity, and aspect ratio [11–15]. In addition to acute inflammatory response, insoluble nanoparticles are associated with long retention times in the lungs making accumulation associated toxicity upon repeated dosing a concern [16]. Any candidate as a medical nanoparticle formulation for pulmonary administration must therefore be inert, biodegradable, and biocompatible both at the nanoscale and in terms of breakdown products [17,18].

Albumin, both in its native form and when fabricated into nanoparticles, is used with great success and versatility as a drug carrier in injected formulations [19,20]. An albumin nanoparticle based-formulation, Abraxane®, is used clinically to deliver the highly potent anti-cancer drug, paclitaxel, without the need for stabilizing agents [21]. With regard to its suitability for pulmonary drug delivery, albumin is an abundant component of lung lining fluid [22], with a concentration ~10% of that found in serum [23]. As albumin is naturally present within the lungs, endogenous transport and metabolic processes exist which could be utilised for drug release [24,25] and alleviate concerns about long term accumulation associated toxicity. Self-assembling

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human serum albumin nanoparticles have been recently developed as a novel vehicle for a combination formulation consisting of doxorubicin and tumour necrosis factor related apoptosis-inducing ligand (TRAIL). This formulation was shown to significantly reduce lung tumour size following pulmonary administration in a mouse model of lung cancer [26]. In addition, the ^{99m}Tc-labelled aggregated albumin complex ^{99m}Tc-Nanocoll® has been used extensively for diagnostic imaging in nuclear medicine and to determine pulmonary deposition patterns in human subjects following aerosol inhalation [27,28].

Despite the interest in developing albumin nanoparticles for pulmonary drug delivery, information on the safety, clearance kinetics, and biodistribution of the albumin vehicle is currently lacking. It is recognised that the highest achievable therapeutic dose of an inhaled nano-formulation may be limited by the highest tolerated dose of the nanomaterial rather than that of the therapeutic compound. Secondly, estimation of nano-formulation pharmacokinetics and administration frequency must include an understanding of the vehicle clearance kinetics as well as that of the therapeutic agent [29]. Therefore, the aim of this study was to evaluate the dose-dependent biocompatibility of two albumin vehicles (solution and nanoparticles) following a single pulmonary administration to mice *in vivo*. Clearance of ^{111}indium-diethylene triamine pentaacetic acid (^{111}In-DTPA) radiolabelled albumin vehicles from the lungs of mice was also measured longitudinally using combined single photon emission computed tomography and X-ray computed tomography (SPECT/CT) imaging over 48 h. Local distribution of albumin and albumin nanoparticles in the lung, as well as accumulation in secondary organs was also measured using terminal end-point organ harvest biodistribution studies. The provision of information on albumin vehicle biocompatibility, clearance kinetics and biodistribution will ultimately enable formulation scientists to decide whether the use of an albumin vehicle is an appropriate tool to achieve a desired pharmacokinetic profile for an intended therapy prior to engaging in lengthy formulation optimisation studies. Secondly, knowledge of the maximum tolerated dose of the albumin vehicle will enable estimations of the theoretical drug loading requirements, providing the basis for a go/no-go decision-making tool in nano-formulation design.

2. Methods

2.1. Materials and reagents

Bovine serum albumins (laboratory grade albumin, albumin tissue culture grade and low endotoxin, essentially IgG free albumin) were purchased from Sigma (Dorset, UK). S-2-(4-Isothiocyanatobenzyl)-diethylenetriamine pentaacetic acid (*p*-SCN-Bn-DTPA) was purchased from Macrocyclics (Dallas, USA). $^{111}InCl_3$ was purchased from Mallinckrodt Medical Inc. (Petten, Netherlands). Phosphate buffered saline (PBS) tablets were purchased from Oxoid (Basingstoke, UK) and prepared using ultrapure water according to the manufacturer's instructions. Foetal bovine serum was purchased from Life Technologies (USA). Diethylenetriamine pentaacetic acid (DTPA) and ammonium acetate were purchased from Fluka (UK). Sodium hydroxide (NaOH) and Tris(hydroxymethyl)aminomethane (Tris) were purchased from Fisher Scientific (Loughborough, UK). Glutaraldehyde (50% vol/vol in water) was purchased from Aldrich (UK). Ethanol was purchased from VWR (Radnor, USA).

2.2. Animals

All experiments were conducted in accordance with the United Kingdom Animal Scientific Procedures Act, 1986 and were approved by the ethics committee of Kings College, London. All *in vivo* experiments were performed with Male BALB/C mice (6–8 weeks of age; approx 20–26 g) which were purchased from Harlan, UK. Mice were housed in rooms maintained at a constant temperature (21 ± 2 °C) and humidity (55 ± 15%) with a 12 hour light–dark cycle. Animals had food (SDS, UK) and water available *ad libitum* and were allowed 1 week acclimatisation period before use.

2.3. Nanoparticle preparation and characterization

Nanoparticles were prepared using a desolvation method as widely reported in the literature [30,31]. In brief, bovine serum albumin (100 mg) was dissolved in 0.01 M Tris HCl buffer (1 mL) and the pH adjusted to 9 with 1 M NaOH. Ethanol was added drop-wise to the stirred protein (4.0 mL, 1 mL/min). Nanoparticles were cross-linked with 10% vol/vol glutaraldehyde/water (47.2 μL) and stirred overnight at room temperature, and purified into sterile PBS by four cycles of spin filtration (30 kDa molecular weight cut-off (MWCO) centrifugal filter units, Millipore, 2400 ×*g*, 10 min per cycle). Nanoparticle size and polydispersity were measured using a Zetasizer Nano Series ZS (Malvern Instruments, Malvern, UK). Particles were diluted 1:10 in PBS and measurements taken at 37 °C, scattering angle 173°, using viscosity value of 0.6885 cP for the dispersant. Zeta potential was measured in PBS at 25 °C at a concentration of 20 μg/mL. Particle concentration was determined gravimetrically.

2.4. In vitro macrophage activation assays

Albumin nanoparticles were prepared using three grades of bovine serum albumin: laboratory grade (>96% purity); tissue culture grade; low endotoxin grade (all Sigma, UK). J774.A1 cells, a macrophage cell line derived from BALB/C mice, were seeded in 24-well plates at a density of 1×10^6 cells per well and incubated overnight in cell culture medium (DMEM, 4.5 g/L glucose, 1% sodium pyruvate, 1% penicillin/streptomycin, 1% HEPES buffer and 1% L-glutamine) containing 10% (v/v) foetal bovine serum (FBS) in a humidified 5% CO_2: 95% air incubator at 37 °C. Following incubation, cell culture medium was replaced with 200 μL of fresh medium containing 4350 μg/mL of albumin nanoparticles prepared from each BSA grade. Positive control cells were primed for 18 h with interferon-gamma (IFN-γ) prior to incubation with 1 μg/mL lipopolysaccharide (from *Escherichia coli* 0111:B4; Sigma Aldrich, UK). Untreated cells were used as a negative control. Cells were incubated with nanoparticles for 4 h, following which 100 μL supernatant was removed and analysed to quantify levels of interleukin-6 (IL-6), tumour necrosis factor alpha (TNF-α) and nitric oxide. TNF-α and IL-6 release were measured using enzyme-linked immunosorbent assay (eBioscience, conducted according to manufacturer's instructions). Nitric oxide (NO) production was measured using the Griess reaction [32].

2.5. In vivo biocompatibility

Low endotoxin grade albumin nanoparticles were suspended in sterile phosphate buffered saline (PBS) and administered at three doses for biocompatibility studies, 2, 20, and 390 μg per mouse (~0.1, 1, 16 mg/kg). The doses fall within the range typically used for inhalation studies of this nature [15,33,34]. Sterile PBS was used as a vehicle control. Albumin nanoparticles were administered *via* oropharyngeal aspiration (o.a.; method adapted from Lakatos et al., 2006 [35]). Briefly, anaesthetized mice (3–5% isoflurane, O_2 flow rate of 1.0 mL/min) were administered a droplet of 25 μL of albumin nanoparticle suspension in sterile saline (2, 20 or 390 μg/mouse) applied to the posterior base of the pharynx, where it was readily aspirated. Mice were euthanized humanely after 24 h with an intraperitoneal (i.p.) injection of urethane (500 mg/mL in physiological saline, 0.3 mL/mouse). The lungs were lavaged with 3 × 0.5 mL sterile physiological saline. The total number of cells in the lavage fluid was counted using a Neubauer haemocytometer (Fisher Scientific, Loughborough, UK). Diffquick® (DADE Behring, Marburg, Germany) staining was used to perform differential cell counts in 100 cells per mouse. Eosinophils were not observed in the BAL fluid, so only macrophages and neutrophils have been reported. Total protein