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Cationised radiolabelled nanoparticles for perfusion imaging of the lungs

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ABSTRACT

Diagnostic imaging of the blood perfusion of the lungs is currently performed using particles of macro-aggregated albumin, which are mechanically arrested at limiting diameters of the capillary bed. While the proportion of blood flow obstructed is typically very low and temporary, it would seem more desirable to image lung perfusion in patients using a non-obstructive method, and using materials that avoid biological hazards. We have characterised the *in vitro* and *in vivo* properties of a colloid of a cationised radiolabelled nanocomposite. The nanoparticles comprise a Technetium-99m core encapsulated in graphitic carbon, and coated with low molecular weight poly-lysine to provide a strong charge-based affinity for the endothelial glycocalyx of the lung. Following intravenous injection in rabbits and cats, the nanoparticles rapidly distribute and localise in the lungs, thus enabling gamma camera imaging of lung perfusion. Repeat administration of this colloid in both species over several weeks indicates favourable biocompatibility.

1. Introduction

Macro-aggregated albumin (MAA) labelled with Tc99m has long been established as a routine clinical agent for gamma camera imaging of lung perfusion. These particles are produced with a median diameter of 30 microns, so that they lodge mechanically in microvessels of limiting diameter after intravenous injection [1]. There is necessarily some obstruction to blood flow, but this occurs in a seemingly insignificant proportion of the capillary bed, and is temporary in nature; the imaging agent dissipates within a few hours by disaggregation and/or proteolysis. Thus adverse events, even in patients with already compromised lung circulation, are quite rare.

However the use of albumin has some disadvantages, including the possibility of allergic hypersensitivity reactions [2], as well as potential biological hazards such as prions, HIV, HCV and HPV. Suggestions for the replacement of plasma-derived albumin with recombinant human albumin [3] have therefore been made, but this would substantially increase the cost of routine imaging. Recently other replacement strategies have been reported including particles made of plant-sourced starch [4], or tin fluoride [5].

Imaging of the lung circulation without the need for any mechanical obstruction of vessels and using a non-biological material can therefore be seen as desirable. To achieve this goal, it is first required to provide materials that can be used at very low concentration, but with sufficient specific activity of the radiolabel to enable imaging, and a special ability to rapidly localise in lung endothelium.

It has been recognised for some time that polycationic substances interact with vascular endothelium, so that polycation conjugates have actually been proposed for facilitating drug delivery to vessel walls [6]. Vascular permeability changes induced by polycations can be reversed by polyanions such as heparin or dextran sulfate [7], suggesting that a charge interaction with glycosminoglycans on the endothelial surface may be responsible. This has been especially studied in the lung where large bolus doses of polycations such as protamine [8] and poly-arginine [9] can produce oedema and infiltrates of eosinophils and neutrophils. Studies of lung capillary endothelium suggest it has a glycocalyx that functions as an anionic charge barrier [10]. On this basis, polycation conjugates of anti-cancer agents have been proposed as a strategy for targeting lung tumours [11].

In this report we take a colloid of a nanoparticle composite comprised of a core of Technetium-99m encapsulated in graphitic carbon [12], and bind polycations to the nanoparticles' surface. We then test the cationised nanoparticles for charge-interactions *in vitro* and their biodistribution *in vivo* in rabbits and cats, using SPECT imaging techniques.

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2. Materials and methods

2.1. Nanoparticle synthesis

An aqueous colloid of Technegas™ nanoparticles comprised of Technetium-99m cores encapsulated in graphitic carbon, (FibrinLite; FL) was synthesised as described [12]. Briefly, sodium Tc99m-pertechnetate was loaded into a graphite crucible and after heating to 2750 °C the reduced metallic isotope was plasma ablated into an argon gas stream leading to a Browitt sonicating precipitator [13], where the aerosol nanoparticles were collected as a stable colloidal suspension in water (6.0 mL). The radioactive colloid was filtered through a 450 nm hydrophilic membrane [mixed cellulose ester (MCE); Millipore] before use. A typical preparation contained approximately 5 μg/mL of graphitic carbon with a specific activity of 20 MBq/μg.

2.2. In vitro assays of protein binding to FL nanoparticles

Microwell Assays: The pH-dependant binding of FL to rabbit serum albumin (RSA, Sigma–Aldrich, Castle Hill, Australia), rabbit IgG (RIgG, Sigma–Aldrich) and protamine sulphate (PS, Sigma–Aldrich) was tested by microwell binding as follows. Triplicate microwells (LockWell MaxiSorp; Nunc, Denmark) were coated with 100 μg/mL of each protein in phosphate buffered saline (PBS; 100 μL) adjusted to each pH of interest (pH 3.0, 7.0 and 9.5). The proteins were allowed to bind for 1–2 h at 37 °C with shaking, and then rinsed three times with the corresponding buffer. Diluted FL (100 μL, 1 MBq) was then added to each well and after 1 h incubation at 20 °C with shaking, the wells were rinsed three times with water. The radioactivity of bound FL was determined as Tc99m activity by gamma counting of the separated individual wells in a Capintec well counter (Ramsey NJ, USA).

Microwell assays were also used to test FL binding to poly-D-lysines (PDL's) of molecular mass 4–15, 15–30 and 30–70 kDa (Sigma–Aldrich). Duplicate microwells were coated for 1 h at 37 °C with serial dilutions (4, 2, 1, 0.5, 0.25 and 0 μg/mL) of the three PDL's in 0.5 mM Tris–acetate–EDTA (TAE; pH 7.5). After rinsing three times with TAE, diluted FL (100 μL, 1 MBq) was added to the wells in the same buffer and incubated at 20 °C with shaking. After rinsing three times with water the microwell plate was imaged using a Siemens Diacam gamma camera, and regions of interest (ROI) were made with MedView software for each pair of duplicates so as to provide the counts bound.

2.3. Secondary interactions of FL after treatment with polyions

Membrane filtration experiments were used to assess secondary interactions of FL after treatment with polyions. FL was incubated with a set of serial dilutions (1.2–0 μg/mL) of protamine sulphate or dextran sulphate (500 kDa; Sigma–Aldrich) in 0.5 mM TAE (pH 7.5) for 1 h at 25 °C and then filtered through 450 nm MCE syringe filters.

In another experiment the filters were pretreated by first filtering 5% plasma in PBS (1 mL), and then rinsed twice with 5 mL of 0.5 mM TAE (pH 7.5). FL pretreated with protamine or dextran sulfate (both 0.5 and 1.0 μg/mL) was then passed through the pretreated MCE filters and control untreated filters. The Tc99m activity in each filtrate was then measured in a Capintec well counter.

In another filtration experiment, FL was incubated with PDL (15–30 kDa; 5 μg/mL), poly-L-arginine (PLA 4–15 kDa; 5 μg/mL) or protamine (20 μg/mL) in 0.5 mM TAE (pH 7.5), for 1 h at 20 °C. The pretreated FL was then filtered with or without addition of unfractionated heparin (Sigma–Aldrich; 100 μg/mL). The Tc99m activity in each filtrate was measured in a Capintec well-counter.

2.4. Scanning electron microscopy (SEM) of PDL-treated FL adherent on MCE filter membrane

FL was treated with PDL (15–30 kDa; 5.0 μg/mL) in 0.5 mM TAE (pH 7.5), for 1 h at 20 °C. The pretreated FL was then filtered through 450 nm MCE syringe filters, which were dried before disassembly to recover the membrane. SEM was then performed with platinum coating, using a Zeiss UltraPlus FESM instrument with a 3 kV beam.

2.5. SPECT imaging of FL biodistribution in rabbits and cats

All animal imaging procedures adhered to the National Health and Medical Research Council's Animal Welfare Code for the appropriate use of animals for scientific purposes (Australian Government, 7th Ed. 2004), and the experimental protocols were approved by the Australian National University (ANU) Animal Ethics Committee. Rabbit and cat biodistribution studies were done under ventilation anaesthesia with isoflurane according to a protocol approved by the Animal Ethics Experimentation Committee of the Australian National University. Imaging of the whole animal was performed with a Siemens Diacam gamma camera, set to automatically acquire dynamic images of FL Tc99m activity over a 14 min time course (4 × 30 s, then 4 × 60 s, then 4 × 120 s), beginning immediately after injection.

FL (2 mL, 150 MBq) was used either untreated or first coated with PDL's (3 μg/mL in 0.5 mM TAE buffer) for 1 h at 20 °C before intravenous (ear vein) injection with or without addition of unfractionated heparin (1 mg/kg subject). Accumulation of FL by the liver during the dynamic imaging was estimated using MedView software and an ROI drawn over the liver in each frame. Liver counts were expressed as a percentage of the total field counts in each frame.

In a modified experiment the rabbit was pre-injected intravenously with 15–30 kDa PDL (1 mg/kg rabbit) 10 min before intravenous injection of FL pretreated with the same PDL (5 μg/mL), and without addition of heparin.

3. Results

3.1. Binding of FL to proteins and PDL's

Technegas™ nanoparticles have previously been characterised as comprising a core of reduced metallic Tc-99m encapsulated in a plurality of carbon layers [14], so that only graphene is presented on the external surface. The present study employed an aqueous colloid of these nanoparticles, further characterised and formulated as FibrinLite (FL [12]). The interaction of FL with selected proteins at different pH's was tested by direct measurement of radioactivity binding to protein-coated microwells. RSA bound a similar amount of FL at pH 3.0, pH 7.5 and pH 9.5, whereas RIgG and protamine bound noticeably more FL at neutral/physiological pH (Fig. 1). All three of the different length PDL's tested could bind FL at neutral pH (Fig. 2A). Note that FL was easily obtainable at a specific activity that enabled imaging of the small amount of FL that bound in the PDL coated microwells (Fig. 2A). However, maximum binding occurred at different coating concentrations; PDL 4–15 kDa at 1 μg/mL, PDL 15–30 kDa at 0.5 μg/mL and PDL 30–70 kDa at 2 μg/mL (Fig. 2B). Thus binding was not a direct function of polycation polymer chain length.

3.2. Secondary interactions of polyion treated FL

Membrane filtration of FL through syringe filters after treatment of the FL with various polyions was found to provide a convenient model for assessing secondary interactions of the coated nanoparticle surface. Hydrophilic membranes made of mixed cellulose ester (MCE) and 450 nm pore size provided a large interaction

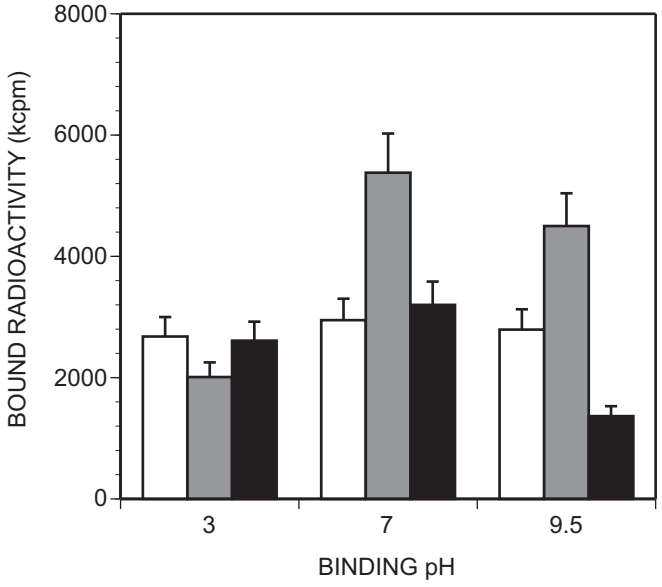

Fig. 1. pH dependant binding of FibrinLite (FL) to polypeptides. Microwells were coated in triplicate with rabbit serum albumin (RSA; open bars), rabbit IgG (RIgG; grey bars) and protamine sulphate (PS; black bars), all at 100 μg/mL in phosphate buffered saline adjusted to pH 3.0, 7.0 and 9.5. After rinsing with the respective buffers, FL diluted in each buffer (100 μL) was added to each well and after incubation for 1 h at 20 °C with shaking, the wells were rinsed again. The microwells were then separated and the radioactivity of bound FL measured in each.