Contrast agent for blood pool imaging and targeted contrast delivery using rHA and Gd-DTPA

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Introduction Blood pool agents have been developed in recent years as a means of prolonging the lifetime of a paramagnetic contrast agent in the intra-vascular space and increasing blood relaxivity [1-3]. Many of these agents use a chelate of Gadolinium [3], which attaches to native albumin in the blood. In this work significant quantities of **Gd-DTPA** were attached to the recombinant human albumin (**rHA**) molecule in the laboratory. The molar relaxivity of the new agent was tested in-vitro in its micro-particulate and soluble forms, and in-vivo in rats. Comparisons with Gd-DTPA (Magnevist, Schering) and the polymeric blood-pool agent Gadomer (Schering) were made and the clearance kinetics of the new agents were examined. With free sites on the Gd loaded rHA molecule, there are possibilities for binding the agent to antibodies in the laboratory, this was demonstrated, and thus there exist potential applications for in–vivo molecular imaging with this agent.

Methods. Contrast agent synthesis The work was performed using recombinant human albumin (rHA), (Delta Biotech, UK). The DTPA was first linked to the rHA molecule by using the anhydride form of DTPA. The anhydride reacts with the free amino groups on the albumin molecule. Once the rHA has been labelled with DTPA, gadolinium chloride was added and immediately chelated by the DTPA molecule to form a stable compound. Analysis of the rHA sequence reveals that there are no fewer than 60 free amino groups available on each albumin molecule. Complexometric titration shows a loading of up to 53 molecules of Gd-DTPA per rHA molecule, with some residual open binding sites for cross-linking to antibodies. Cross linking involved a bifunctional linker that binds to the free terminal sulphydryl on the Gd-DTPA labelled rHA and carbohydrate groups on the constant region of the antibody. A monoclonal antibody (Mouse anti Rat CD 45) was used (Serotec, UK) to test the chemistry. Results confirmed that the Gd-DTPA labelled rHA will react with the bi-functional crosslinker to form a reactive species that can then crosslink to the antibody. The antibody has also been shown to react with the crosslinker (following activation) in a highly specific way. The result is an antibody that retains binding activity and has at least 2 Gd-DTPA labelled rHA molecules (approximately 100 Gd-DTPA molecules) bound to the constant region of the antibody.

MR characterisation of the agents - In vitro: Data was collected from test tubes containing aqueous solutions of varying concentration of the soluble rHA agent and from aqueous Gd-DTPA solution (Magnevist, Schering) as a comparison. Relaxometry was performed at 1.5T using a whole body system (Philips, Eclipse). 2D spin echoes were collected at a range of TR's (100 ms –7000 ms) and the longitudinal relaxivity (R_1) was calculated from the saturation recovery curve. The transverse relaxivity (R_2) was measured from a mono-exponential fit to the amplitudes of spin echoes acquired at TE = 30ms, 60ms, 90ms and 120ms with TR= 3000 ms.

In-vivo work was performed at 1.5T using n=3 rats who were i.v. injected with 0.05 mmol Gd/kg doses of ; (i) soluble rHA agent (ii) micro-particular rHA agent (iii) blood pool agent (Gadomer, Schering, AG) and (iv) Gd-DTPA (Magnevist, Schering, AG) as a comparison. Imaging was performed at baseline (prior to injection) and at subsequent intervals following injection with a 3D gradient echo imaging sequence (TR/TE =27/3.5 ms, 4 aves, 40° flip angle, 64 slices, 0.5 mm thick, Tacq=7.24 min).

Pharmacokinetics: Whole body retention: For the biodistribution study the same animals from the imaging study were used. Residual whole body gadolinium was determined by inductively coupled plasma atomic emission spectrometry (ICP-AES) at 14d post injection. Blood/Plasma kinetics: Blood samples were taken at 1, 3, 5, 10, 15, 30, 60, 90 min, 2, 4, 6 and 24h post injection and the Gd content was assayed. A two-compartment distribution model was used and pharmacokinetic parameters such as initial and terminal half-lives, volumes of distribution, and total clearance were calculated. The parameters were converted to plasma values by assuming a hematocrit value of 0.625 (blood volume: 64 ml/kg, plasma volume: 40 ml/kg).

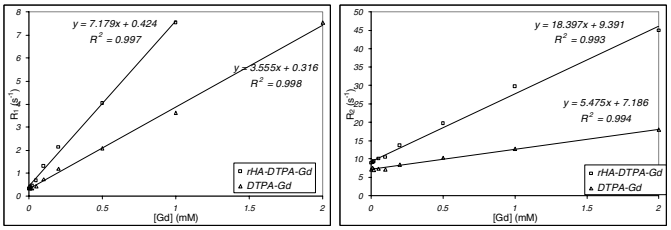


Fig 1 R1 (l) and R2 (r) versus [Gd] for soluble rHA agent and Gd-DTPA

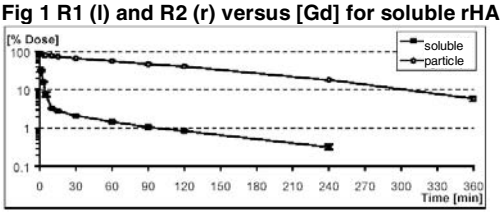
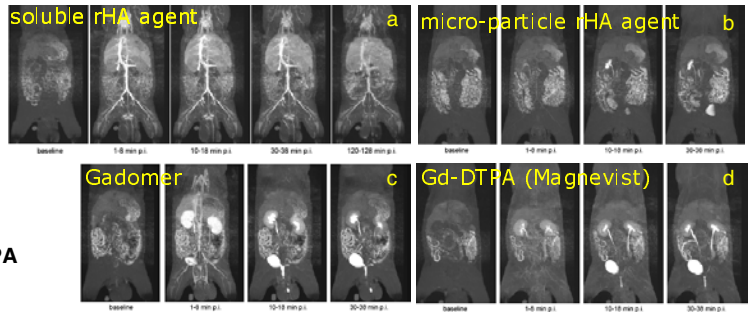


Fig. 3 Pharmacokinteics: Plasma levels after iv injection of 0.05 mmol Gd/kg. The whole body retention at 14 days after iv injection of 0.05 mmol Gd/kg was 21.6% of original dose for the micro-particulate form and 29.2% for the soluble form.



Results

The resulting soluble rHA-Gd-DTPA agent has a higher molar longitudinal relaxivity and molar transverse relaxivity in water (R1 = 7.2 $s^{-1}mM^{-1}$, R2 = 18.4 s^{-1} mM^{-1}) than those measured for Gd-DTPA solution (R1 3.5 s^{-1} mM^{-1}, R2 = 5.5 s^{-1} mM^{-1}).–see Fig 1. The **in vivo** results are shown in **Fig. 2** (above right). These are MIPs from the 3D images, the first one was taken at baseline (left). The Gd-DTPA injection (d) is cleared from the blood pool within 10-18 minutes with hyperintensity visible quickly in the urinary tract. The first image post injection of soluble rHA –Gd DTPA (a) (1-8 mins) shows good enhancement in all of the vessels. The residual blood pool hyperintensity 120-128 mins post injection indicates the longevity of the agent in the blood pool with significant residual enhancement of the vasculature. In contrast, the microparticles of rHA-Gd-DTPA (b) showed only a very slight enhancement of the aorta and the femoral arteries on the first angiograms (1 min p.i.). Gadomer (c) also showed strong enhancement of the major vessels at the early time point. However, due to the long acquisition time of 7:24 min and the rapid renal elimination of this polymeric compound, the urinary tract was already visible in the early images.

Discussion We have confirmed that the Gd-DTPA labelled recombinant albumin molecule (rHA) can generate a much stronger T1 (and T2) contrast effect than seen using Gd-DTPA alone. This is because the larger molecular structure slows down the rate of rotational tumbling of proximal water molecules in the tissue and of the attached paramagnetic agent. Gd-DTPA:rHA molar ratios of greater than 50:1 have been achieved, which enhance the images of the blood pool for several hours. Due to the incomplete elimination within 14 days post injection, both rHA labelled compounds are probably not suitable for development as routine blood pool contrast media despite the soluble RHA agent being a highly effective blood pool agent - Fig 2a. However the lifetime has potential advantages for targeted imaging applications in that the long blood pool persistence of the soluble rHA complex allows plenty of time for successful targeting interactions to take place post administration. Furthermore we have demonstrated the Gd loaded rHA molecule can be linked to antibodies, meaning the agent has potential uses in targeted contrast delivery for molecular imaging in cancer, which is the subject of further work. rHA (purified from Saccharomyces cerevisiae), has a number of properties that make it potentially useful in *in-vivo* MR. It is biocompatible, is free of any of the potential viral/prion contamination associated with blood-derived albumin, and is safe and well tolerated with doses as high as 50g administered intravenously to human subjects without adverse effect [4].

References [1] Schneider et al JMRI, 14, 525-539, 2001. [2] Misselwitz B et al. MAGMA. 2001 ;12:128-34. [3] Perreault et al. Radiology 2003, 229, 811-820. [4] Schindel F, et al J Clinical Pharmacology 2003;43:1032.