Chelator charge and structure and their effects on polymeric agent conformation and imaging efficacy

E. E. Uzgiris[1], B. Moasser[1], M. Amaratunga[1], D. E. Meyer[1], A. Narendran[2], J. F. Smith[1]

[1]GE Global Research Center, Niskayuna, NY, United States, [2]GE Global Research Center, Niskayuna, Ny, United States

Synopsis

The imaging effectiveness of Gd-polymeric agents relies on polymer conformation. We test the dependence of polymer conformation on Gd-chelator charge by measuring polymer hydrodynamic properties that include gel permeation chromatography, gel electrophoresis, and T_1 relaxivity and correlate these to tumor imaging efficacy in a rat mammary tumor model. As predicted by molecular modeling, charge interactions between neighboring residues are decisive factors in whether polymer is extended or collapsed. However, charged DOTA-isothyocyanate derivatives, although producing a correct polymer conformation, appear to cause the polymer agent not to be transported across the tumor endothelium.

Introduction

Extended linear polymeric agents have been shown to give high signal enhancement in comprised neovasculature that is associated with tumor angiogenesis. Compared to other macromolecular and nanoparticle agents, these signals are some 10 times larger indicating a higher degree of transport across the tumor endothelium.[1] The imaging efficacy of the agent Gd-DTPA-polylysine is dependent on conformation: If the DTPA conjugation is 90% or higher the conformation is of extended form and the imaging efficacy is high; If the conjugation is lower the polymer is coiled. This critical conformation change is driven by electrostatic and steric interactions between the adjacent charged DTPA groups as indicated by a molecular modeling calculation. To validate this view we replaced DTPA with DOTA (lys-Gd-DOTA is neutral) and eliminated charge interactions in the polymer conformation. To recover the charge interactions between neighboring residues, we conjugated polylysine with an isothiocyanate -DOTA derivative. The central question of this investigation is whether conformation recovery is sufficient to regain imaging efficacy.

Materials and Methods

Synthesis of highly conjugated DTPA or DOTA -polylysine was done by a modified mixed anhydride method.[2] The modifications enabled conjugation levels in the 95% range. Detailed synthesis procedures will be published elsewhere. Purified constructs gave a single peak on gel permeation chromatography or in gel electrophoresis. The polylysine backbone had a mean polymerization monomer number of 402. A DOTA-isothyocyanate derivative was coupled to the polylysine backbone by a standard coupling reaction. Female Fisher 344 rats where implanted with 10^6 MatB cells (ATCC 13762) subcutaneously and tumors grew up in about 8 days to a 5mm to 10mm size. These tumors were well vascularized on the periphery but not excessively leaky, i.e., albumin Gd-DTPA gave small signal enhancements. Imaging efficacy was thus related to a well studied macromolecular agent, albumin-Gd-DTPA. Polymeric agents and the control agent were injected at 0.025mmole Gd/kG, and signals changes at 24 hours were obtained (the clearance rate from the blood was such that at 24 hours the blood plasma levels were only ~10% of initial values). T_1 weighted tumor signal changes were noted in the periphery of the tumors on a GE Signa scanner at 1.5T utilizing a solenoid receive coil. Gel permeation chromatography was done on a TSK-GEL G-DNA –PW (Tosho Biosep) column using a Dionex HPLC sytem. The pattern of elution times is dependent on hydrodynamic size and the extended polymers were well displaced (1.4minutes longer) from the pattern of globular protein standards. The full displacement for a particular monomer number gives and index of conformation change. As the polymer is made to collapse the displacement is smaller and smaller and in the fully collapsed state (conjugation <80%) it falls on the elution pattern of globular proteins. Relaxivity is also dependent on conformation: In an extended conformation, the relaxivity is lower than in the collapsed state. Relaxivity was measured at 1.5 T using a multi-well plate and an inversion recovery sequence on a GE Signa scanner and an internally developed analysis program for multi-well data sets.

Results

Table 1. Comparison of DTPA and DOTA polylysine constructs

Constructs (polylysine,PL)	HPLC elution Index*	R_1**	Imaging efficacy#	Free lysines,%
Gd-DTPA-PL	100	7-8	120 +/- 22	4-9
Gd-DOTA-PL	14	12.0	18 +/- 5	9
Gd-DOTA*ITC-PL	84	8.9	0 +/-5	6
Gd-DTPA-albumin	0	13	17 +/- 5	--

* Gel permeation elution time: 100 index =maximum time displacement from the globular protein elution profile, i.e., to that of linear extended polymers such as DNA or dextran; 0 index= no displacement from protein profile.

** T_1 relaxivity, $mM^{-1}sec^{-1}$ at 23ºC and 1.5T. Low relaxivity corresponds to extended conformation and high relaxivity to coiled, collapsed conformation.

#Tumor signal enhancement in % after 24 hours for identical doses of 0.025 mmoles Gd/kg.

Conclusions

DOTA when coupled to polylysine through the carboxy moiety is neutral when labeled with Gd. Because of the lack of charge repulsion between residues, it can be expected that the polymer is not in an extended conformation as evidenced by gel permeation chromatography and relaxivity values. The consequence is minimal transport of the agent into the tumor interstitium(signal at 24 hours) compared to extended Gd-DTPA-PL. DOTA*ITC-PL retains a negative charge at each residue and the extended conformation is regained (Table 1) as predicted by molecular modeling calculations. Surprisingly, the imaging efficacy is not regained. In fact, the signal change at 24 hours is consistent with zero transport and retention and, thus, below that observed for control macromolecular agents. This unexpected result suggests that the configuration of the DOTA moieties on the chain by the isothyocyanate linkage is such as to prevent passage of the polymer through endothelium junctions or channels. Kinetic MR signal measurements over the first 30 minutes indicate that this is the case. That relatively small changes in polymer monomer structures can prevent transport across tumor endothelium is unexpected. In another case, we have capped the residual free lysines of Gd-DTPA-PL (free lysine content of 9%) with trinitrobenzylsulfate, thus eliminating the lysine positive charges. In this case, the conformation remained extended as expected, but the tumor signal changes at 24 hours were eliminated also. The apparent elimination of transport across the tumor endothelium by relatively small molecular alterations in a long polymer is unexpected and worthy of further investigation as it may lead to new insights regarding the tumor endothelium.

References

1) E.E.Uzgiris and A.Bogdanov,Jr., Proc. ISMRM, Denver, 1052 (2000).

2) P. Sieving et al., Bioconjugate Chem 1:65 (1990).