In vitro and *in vivo* characterization of (albumin-binding) dendritic MRI contrast agents for dynamic contrast-enhanced MRI

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Purpose

Dynamic Contrast-Enhanced Magnetic Resonance Imaging (DCE-MRI) is a widely used tool for evaluation of tumor treatment efficacy. Macromolecular contrast agents may be preferred over low molecular weight contrast agents for determination of specific pharmacokinetic parameters and changes in these parameters after treatment [1]. Dendritic contrast agents are especially suitable for assessment of the dependence of pharmacokinetic parameters on contrast agent size, because of their tunable dimensions. In this study dendritic contrast agents of various molecular weights, including albumin-binding agents that can be used for MR angiography, were characterized *in vitro* and *in vivo*. Eventually a set of contrast agents can be selected that is optimal for characterization of tumor vasculature.

Methods

Contrast agents: Eight Gd-DOTA based poly(propylene imine) (PPI) dendrimers of generation G0-G5 were synthesized by SyMO-Chem BV; G0-PPI-$GdDOTA_1$-$Palmitoyl_1$ (G0-Palm), G1-PPI-$GdDOTA_3$-$Palmitoyl_1$ (G1-Palm), G2-PPI-$GdDOTA_7$-$Palmitoyl_1$ (G2-Palm), G2-PPI-$RhoB_1$-$GdDOTA_6$-$Palmitoyl_1$ (G2-Palm-Rho), G2-PPI-$RhoB_1$-$GdDOTA_4$-PEG_3 (G2), G3-PPI-$RhoB_1$-$GdDOTA_8$-PEG_7 (G3), G4-PPI-$RhoB_1$-$GdDOTA_{16}$-PEG_{15} (G4), G5-PPI-$RhoB_3$-$GdDOTA_{31}$-PEG_{30} (G5). The palmitoyl moiety facilitates murine serum albumin (MSA) binding and Rhodamine B (RhoB) allows *ex vivo* fluorescence microscopy.

***In vitro* characterization**: Gadolinium (Gd^{3+}) content of the dendrimers was determined by means of inductively coupled plasma atomic emission spectroscopy (ICP-AES). Longitudinal and transversal relaxivity (r_1 and r_2, respectively) were determined at 37 °C and 60 MHz (1.41 T) on a Minispec MQ60 (Bruker). These measurements were also used for the determination of the Critical Aggregation Concentration (CAC) of the palmitoyl-carrying agents. Further information on aggregation behavior was obtained from Nuclear Magnetic Resonance Dispersion (NMRD) measurements. NMRD profiles were measured over proton Larmor frequencies from 0.01-20 MHz on a Stelar field-cycling relaxometer and from 20-70 MHz on a Stelar Spinmaster spectrometer. Albumin binding was investigated using proton relaxation enhancement (PRE) measurements. With this method the enhancement in R_1 (s^{-1}) at 37 °C and 20 MHz is measured as a function of MSA concentration (0-1.5 mM MSA) at fixed [Gd^{3+}] (below and above CAC) to quantify the maximum relaxivity enhancement ε_b (-) and binding strength nK_A (10^4 M^{-1}, with n the number of binding sites and K_A the association constant). The clinically used albumin-binding agent Gadofosveset was included as a reference.

***In vivo* characterization:** CT26 colon carcinoma bearing Balb/c mice (n=3/agent) were injected with one of the dendrimers or Gd-DOTA (0.1 mmol Gd/kg) and blood samples were taken until 24h after injection. R_1 (s^{-1}) relaxation rates were determined with a 6.3T Bruker Biospec and $\Delta R1$ (s^{-1}) values were calculated as R_1 post - R_1 pre injection. Distribution half-lives ($t_{½,\alpha}$) and elimination half-lives ($t_{½,\beta}$) were determined by fitting the data to a bi-exponential decay model. Biodistribution was assessed by ICP analysis of Gd content of tumor, muscle (thigh), blood, kidneys, spleen, liver, lungs and heart excised 24h after injection.

Results

Dendrimer relaxivity generally increased with molecular weight (r_1 of G2 and G5: 10.6±0.1 $mM^{-1}s^{-1}$ and 15.5±0.1 $mM^{-1}s^{-1}$ respectively). Additionally, for the palmitoyl-containing agents a CAC was observed above which the relaxivities were higher, with the strongest increase in relaxivity for G0-Palm (r_1 from 3.5±0.1 $mM^{-1}s^{-1}$ below CAC to 13.6±0.4 $mM^{-1}s^{-1}$ above CAC). Macromolecular peaks were most notably observed in the NMRD profiles of G0-Palm above CAC and of G5 (Figure 1). With PRE measurements the highest binding strength (nK_A=22.0±10.29 · 10^4 M^{-1}, ε_b=6.31±0.17) was observed for G0-Palm below the CAC. Gadofosveset had the lowest binding strength (nK_A=0.13±0.01 · 10^4 M^{-1}, ε_b=6.81±0.24) in comparison with all palmitoyl-containing dendrimers (Figure 2). Relatively long elimination half-lives of the palmitoyl-containing dendrimers were observed compared to other dendrimers (Table 1), indicative of albumin binding. In general elimination half-lives were dependent on molecular weight for the non-palmitoyl-containing dendrimers and longer compared to Gd-DOTA. Tumor/muscle uptake ratios determined by ICP were generally higher for the dendrimers (G1-Palm: 4.50±1.07, G2-Palm: 4.05±1.08, G2-Palm-Rho: 3.33±1.33, G2: 2.35±0.74, G3: 4.61±0.63, G4: 3.46±0.97, G5: 3.46±1.37) than Gd-DOTA (2.51±1.97), with the highest ratio for G3. Dendrimer accumulation in spleen, kidney and liver generally increased with molecular weight (Figure 3). Mice injected with G0-Palm were sacrificed prematurely due to agent toxicity, likely related to albumin-binding induced aggregation.

Agent	$t_{1/2,\alpha}$ (min)	$t_{1/2,\beta}$ (hours)
Gd-DOTA	0.28 ± 0.10	0.31 ± 0.11
G1-Palm	3.66 ± 1.58	9.50 ± 1.99
G2-Palm	3.34 ± 0.69	5.64 ± 0.45
G2-Palm-Rho	0.74 ± 0.27	1.61 ± 0.41
G2	0.67 ± 0.07	0.74 ± 0.18
G3	2.84 ± 0.92	0.92 ± 1.07
G4	1.53 ± 0.37	0.31 ± 0.08
G5	12.18 ± 2.65	1.59 ± 0.38

Table 1: $t_{½,\alpha}$ (min) and $t_{½,\beta}$ (h) for all dendrimers and Gd-DOTA. Mice injected with G0-Palm were prematurely sacrificed due to agent toxicity.

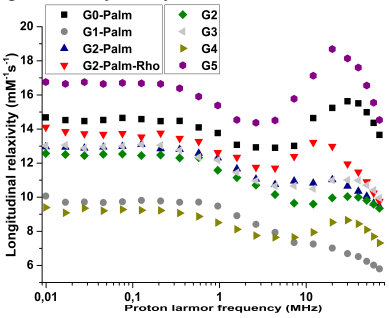

Figure 1: NMRD profiles of the dendrimers above CAC at 37°C.

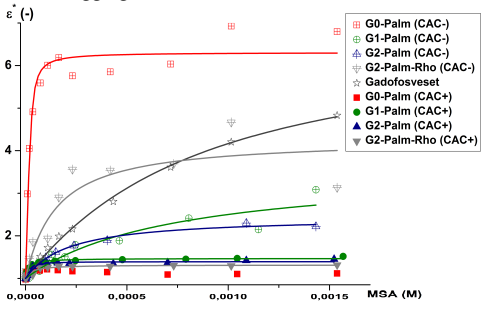

Figure 2: PRE curves of the palmitoyl-containing dendrimers and Gadofosveset, below and above the CAC at 37°C.

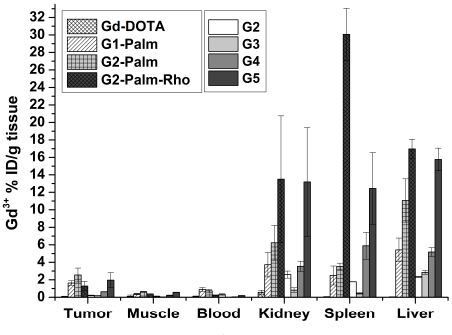

Figure 3: Biodistribution (Gd^{3+}, %ID/g tissue) of the different dendrimers and Gd-DOTA. Heart and lungs not shown.

Discussion and Conclusion

Dendrimer relaxivities and aggregation behavior were extensively characterized *in vitro*. Murine serum albumin binding was observed for the palmitoyl-containing dendrimers. This led to long blood circulation half-lives, making them suitable for MR angiography. A size-dependent range of circulation half-lives was observed for the other dendrimers. Tumor uptake ratios of the dendrimers were higher than those of Gd-DOTA. These findings imply that these agents likely possess a range of tumor wash-in and wash-out rates, making them suitable for investigation of the dependence of pharmacokinetic parameters on contrast agent size. In a next step, multi-bolus DCE-MRI with a selection of agents will be performed to characterize tumor vasculature and vascular changes in response to treatment.

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References

[1] de Lussanet et al., Radiology 2005; 235:65–72