Characterization of Gd-based contrast agents encapsulated in thermosensitive liposomes as potential tool for MRI assisted hyperthermia

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Target audience: Scientists and physicians interested in MRI assisted targeted tumor therapy

Purpose: Thermal dose is a key factor for the synergistic interaction of local hyperthermia in combination with chemotherapy in tumor patients1,2; for targeting and accumulation of liposomes in a target volume, for triggering fast and efficient content release from thermosensitive liposomes (TSL) in that target volume. Thus, for experimental investigation of TSL and for in vivo application non-invasive visualization techniques are expected to play a key role. TSL with encapsulated Gd(III) have been proposed for therapy monitoring using the thermotropic polymorphism of liposomes. The paramagnetic compounds are released around the gel to liquid-crystalline phase transition temperature (Tm) of TSL and act as T1-shortening MR contrast agent3. For optimal visualization an effective temperature induced relaxivity (r1) change and stability in the presence of proteins is needed. Here, temperature induced r1 change of 6 clinical approved Gd-based CAs (Gd-DTPA, Gd-BOPTA, Gd-DOTA, Gd-BT-DOTA3, Gd-DTPA-BMA, and Gd-HP-DOTA) comprising diverse chemical structures and charges were investigated for encapsulation into TSL and the resulting T1 relaxation properties.

Methods: TSL composed of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine / 1,2-distearoyl-sn-glycero-3-phosphoglycerol 50/20/30 (mol/mol) (DPPG2-TSL)4 were prepared and characterized as described before. Each CA was passively encapsulated with 323 mOs/kg1 to obtain stable formulations and to minimize osmotic stress on the vesicle membrane. As a consequence encapsulated CA concentration depended on their osmolality. T1 measurements were performed using an IR-technique on a 0.5T-NMR-analyzer (Minispec NMS120, Bruker, Germany). Samples in fetal calf serum / 5% glucose were characterized in steps of 2°C from 30 to 50°C. T1 relaxation (r1) and diffusive permeability to water (Pd) across the membrane were determined. Shelf life at 4°C was investigated by determining lysolipid content up to 10 weeks after preparation.

Results: All CA could be formulated into DPPG2-TSL. The preparations were monodisperse with comparable small vesicle sizes (Ø ~135 nm). Neither zeta potential nor Tm was affected by the CA. All samples showed temperature induced sigmoidal increase in r1 in the range between 38 and 44°C. Change in r1 (Δr1 = r1(Tm)-r1(37.6°C)) and r1 (T < Tm) depended on the encapsulated CA concentration. Above Tm, r1 reached the value of non-encapsulated CA. Considering osmolality, encapsulated concentration was highest with Gd-DTPA-BMA (250mM) and Gd-HP-DOTA (256mM) resulting in high r1 changes (r1(44°C)/r1(37.6°C)) by a factor of 3.7 and 7.2; respectively. Encapsulated ionic in comparison to nonionic CA showed considerably higher Pd from 30 to 38°C (such as at 32°C: ionic 86-154*10^-12 cm²/s and nonionic: 20-40*10^-12 cm²/s) and consequently showed less effective r1 change (r1(44°C)/r1(37.6°C)=1.5 to 1.8). All CA except Gd-DTPA-BMA induced phospholipid hydrolysis during storage at 4°C, which resulted in unwanted CA leakage. Shelf life of TSL was highest encapsulating Gd-DTPA-BMA (min. 10 weeks).

Discussion: Six clinically used Gd-based CA with differences in chelator structure (linear vs. macrocyclic) and overall charge (ionic vs. non-ionic) were successfully encapsulated into DPPG2-TSL. Neither zeta-potential nor Tm was significantly different between the formulations, indicating no verifiable effect of the encapsulated CA type on the TSL membrane. A Tm between 42.2 °C and 43.1 °C indicated the ability of the TSL to release the CA at mild hyperthermic conditions (39 – 41°C) (Fig. 1). For comparison of the CA-TSL osmotic stress on the vesicle membrane had to be minimized. Osmotic stress is known to destabilize the membrane of liposomes, which leads to unwanted leakage or vesicle aggregation7,8. Therefore, our experimental approach allowed a fair comparison of the biophysical properties (e.g. temperature dependent r1 profile, diffusive permeability to water) of the resultant formulations. As a consequence, r1 at temperatures below Tm as well as Δr1 varied by the difference in encapsulated CA concentration.

Conclusion: To our knowledge this is the first comprehensive study comparing all types of MRI-CA based on Gd in a specific TSL formulation. For that purpose we provide an experimental methodology that allows in vitro comparison of CA and thus minimized osmotic stress. A high concentration of encapsulated CA is a prerequisite to achieve a sufficiently high Δr1 during heat triggered CA release combined with a low r1 at 37°C. Hence, the optimal CA is characterized by a non ionic structure and a low contribution to osmolality such as Gd-DTPA-BMA and Gd-HP-DOTA.

Considering shelf life, DPPG2-TSL encapsulating Gd-DTPA-BMA was superior to the other investigated clinically used Gd-based CAs as a potential tool for MRI assisted interventional thermotherapy.

References:

1Issels, R. et al. Lancet Oncol., 2010;11(6):561-70