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 patients[1,2]; 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 (T_m) of TSL and act as T_1-shortening MR contrast agent[3]. For optimal visualization an effective temperature induced relaxivity (r_1) change and stability in the presence of proteins is needed. Here, temperature induced r_1 change of 6 clinical approved Gd-based CAs (Gd-DTPA, Gd-BOPTA, Gd-DOTA, Gd-BT-DO3A, Gd-DTPA-BMA, and Gd-HP-DO3A) comprising diverse chemical structures and charges were investigated for encapsulation into TSL and the resulting T_1 relaxation properties.

Methods: TSL composed of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine / 1,2-distearoyl-*sn*-glycero-3-phosphocholine / 1,2-dipalmitoyl-*sn*-glycero-3-phosphodiglycerol 50/20/30 (mol/mol) ($DPPG_2$-TSL)[4] were prepared and characterized as described before[5]. Each CA was passively encapsulated with 323 mOs kg^{-1} to obtain stable formulations and to minimize osmotic stress on the vesicle membrane. As a consequence encapsulated CA concentration depended on their osmolality. T_1-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. T_1 relaxivity (r_1) and diffusive permeability to water (P_d) across the membrane were determined[6]. Shelf life at 4°C was investigated by determining lysolipid content up to 10 weeks after preparation.

Results: All CA could be formulated into $DPPG_2$-TSL. The preparations were monodisperse with comparable small vesicle sizes (Ø ~135 nm). Neither zeta potential nor T_m was affected by the CA. All samples showed temperature induced sigmoidal increase in r_1 in the range between 38 and 44°C. Change in r_1 ($\Delta r_1 = r_1(45.3°C) - r_1(37.6°C)$) and r_1 ($T < T_m$) depended on the encapsulated CA concentration. Above T_m, r_1 reached the value of non-encapsulated CA. Considering osmolality, encapsulated concentration was highest with Gd-DTPA-BMA (250mM) and Gd-HP-DO3A (256mM) resulting in high r_1 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 considerable higher P_d from 30 to 38°C (such as at 32°C: ionic 86-154*10^{-5}cms^{-1} and nonionic: 20-40*10^{-5}cms^{-1}) and consequently showed less effective r_1 change ($r_1(44°C)/r_1(37.6°C)$=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).

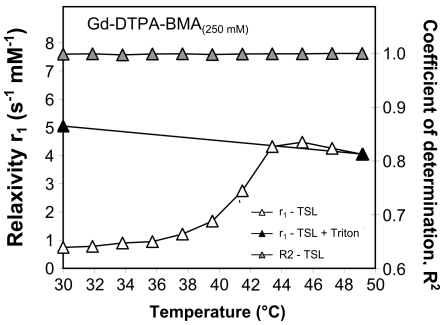

Fig.1 Example of temperature dependent r_1 profile of TSL with encapsulated Gd-DTPA-BMA in fetal calf serum/5% glucose 1/1 (vol/vol). (In parentheses CA concentration inside the liposomes) Δ: r_1 of liposome dispersions; ▲: Coefficient of determination (R^2) of linear regression; Gray triangles: r_1 of samples with completely released CA.

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 $DPPG_2$-TSL. Neither zeta-potential nor T_m was significantly different between the formulations, indicating no verifiable effect of the encapsulated CA type on the TSL membrane. A T_m 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 aggregation[7,8]. Therefore, our experimental approach allowed a fair comparison of the biophysical properties (e.g. temperature dependent r_1 profile, diffusive permeability to water) of the resultant formulations. As a consequence, r_1 at temperatures below T_m as well as Δr_1 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 Δr_1 during heat triggered CA release combined with a low r_1 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-DO3A.

Considering shelf life, $DPPG_2$-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:

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