In vivo MRI-monitoring of Gadodiamide release from phosphatidylglyceroglycerol containing thermosensitive liposomes in heated and non-heated tumors

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Introduction*:* Thermal dose is the key factor for the synergistic interaction of hyperthermia (HT) in combination with chemotherapy in tumor therapy (1,2); targeting and accumulation of liposomes in a target volume, triggering fast and efficient content release from thermosensitive liposomes (TSL) in that target volume. Thus, for experimental investigation of TSL and for a potential clinical application non-invasive visualization techniques are expected to play a key role. TSL with either encapsulated Gd^{3+} or Mn^{2+} have been proposed for therapy monitoring using the thermotropic polymorphism of liposomes. The paramagnetic compounds are released at the gel to liquid-crystalline phase transition temperature (T_m) of TSL and act as T1-shortening MR contrast agent (3,4,5). Recently, a novel formulation for TSL has been successfully developed composed of 1.2-dipalmitoyl-*sn*-glycero-3-phosphoglyceroglycerol (DPPGOG) for prolonged circulation time and an increased content release at the phase transition temperature T_m of about 42°C, which is consistent with the therapeutical hyperthermia temperature level aimed at combined HT/chemotherapy tumor treatment concepts (6).

Purpose of this study was to investigate temperature induced contrast agent release from this TSL (6) with encapsulated Gd-DTPA-BMA (Gd) in the presence of tumor perfusion using a clinically approved contrast agent in a clinical MRI setting.

Material and Methods: Gd-TSL composed of the phospholipids 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC) and DPPGOG (5:2:3) with a T_m of 43.5 ± 0.4 °C as determined by differential scanning calorimetry were loaded with Gd (OMNISCAN™, GE-Healthcare, USA) (7,8).

Liposomes were characterized *in vitro* by MR at 0.5 T (Minispec NMS120, Bruker BioSpin GmbH, Rheinstetten, Germany) during heating at various temperature levels between 37 °C and 45 °C.

Six C57BL/6 mice (body weight: 31 ± 3g) bearing BFS-1 fibrosarcomas at the hind leg were studied 2 weeks after tumor inoculation. The mice were anesthetized intraperitoneally with a solution of Ketamine (Ketavet®, Pharmacia, Germany), 0.9% NaCl and Xylazin (Rompun® 2%, Bayer, Germany). The tumor bearing legs were immersed in a temperature controlled water bath (containing 0.5 g/l $MnCl_2$) that induced HT at 42.0 ± 0.4 °C for at least 40 min. Gd-TSL (250 µl saline solution containing Gd-LTSL at Gd-concentration 0.1 mmol/kg) were injected into the lateral tail vein during MRI at 42°C. Three out of six mice beared a second tumor on the contralateral leg, which remained unheated. Body temperature was monitored using a fiberoptic temperature probe placed in the rectum and was maintained by air flow at 36.1 ± 0.7°C.

In vivo studies were performed using a dedicated experimental setup (9) allowing simultaneous HT and MRI in a clinical 1.5 T MRI system (MAGNETOM Vision, Siemens Medical Solutions, Germany). An anatomical survey was performed with high resolution T1 and T2-weighted spin-echo sequences at 37°C. Contrast agent release was monitored by T1-weighted spin-echo (TR=300ms; TE=12ms; 0.6x0.3x3mm³) supplying high image quality considering the small animal.

Results: *In vitro* characterization at 0.5 T showed a strong increase in T1-relaxivity of Gd-TSL solutions from 0.4 $mM^{-1}s^{-1}$ (37.5 °C) to 4.2 $mM^{-1}s^{-1}$ (43.3 °C). A dedicated experimental setup was developed for standardized *in vivo* investigation of the rodent tumor model. In all animals signal intensity changes were detectable in the heated tumors (mean tumor volume: 284 ± 86 mm³). Contrast agent release was investigated in regions (ROIs) that were manually selected comprising the tumor volume except for the tumor rim, which may be prone to water diffusion from the tumor water bath. After i.v. injection, signal increased (+17 ± 7 % calculated relative to signal level before injection) homogeneously distributed within 1 min and persisted at high levels thereafter (+17 ± 4 % at 40 min again calculated relative to signal level before injection). In the subgroup of animals bearing a tumor in each hind leg a signal increase of 20 ± 2 % in the heated (333 ± 102 mm³) and 11 ± 3 % in the non-heated tumor (345 ± 60 mm³) was detectable within 1 min. There was a trend to further signal increase thereafter in the non-heated tumor (14 %; N=2). Data acquired after 15 min post injection for one unheated tumor had to be excluded because of motion. Signal of a reference phantom kept at constant temperature of (24.7 ± 0.4 %) stayed stable during the course of experiments (+ 2 ± 2 % at 1 min and +1 ± 3 % at 40 min post injection).

Conclusion: The temperature sensitivity of r_1-relaxivity the ability of DPPGOG-TSL containing Gd for MRI-signal enhancing was demonstrated.

The studied contrast agent release was visualized *in vivo* after i.v. injection of the Gd-TSL at a contrast agent dose commonly used in clinical diagnostics. The temperature induced release of contrast agent causes a fast and strong increase of T_1-weighted signal intensity even in the presence of tumor perfusion after i.v. injection of TSL. Gd-TSL showed a significant higher signal increase in heated tumors compared to non-heated tumors. The Gd-TSL appear to be suitable for MR-monitoring of HT tumor treatment in a clinical MRI setting.

Moreover, our results indicate that if liposomal encapsulated drugs are used, a potential higher drug release in the target region may be triggered by HT.

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