

# Hyperthermia Induced Gadodiamide Release from Thermosensitive Liposomes in Solid Tumors and Muscle Tissue

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**Introduction:** Thermal dose is the key factor for the synergistic interaction of local hyperthermia (HT) in combination with chemotherapy in tumor therapy (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 a potential clinical application non-invasive visualization techniques are expected to play a key role. TSL with either encapsulated Gd<sup>3+</sup> or Mn<sup>2+</sup> 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).

Recently, we reported MR-characterization of Gd-DTPA-BMA loaded phosphatidylglyceroglycerol-TSL (Gd-TSL) at mild hyperthermia conditions in tumor tissue (7,8). Thereafter a retrospective study comparing results of heated and unheated tumors in relation to normal unheated muscle tissue was performed in order to assess signal response in normal tissue as a reference.

**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 molar ratio) with a  $T_m$  of  $43.5 \pm 0.4$  °C as determined by differential scanning calorimetry were loaded with Gd (OMNISCAN™, GE-Healthcare, USA) (9,10).

12 C57BL/6 mice bearing BFS-1 fibrosarcomas one at each hind leg were studied 2 weeks after tumor inoculation. The mice were anesthetized intraperitoneally with a solution of Ketamine (Ketavel®, Pharmacia, Germany), 0.9% NaCl and Xylazin (Rompun® 2%, Bayer, Germany). One tumor bearing leg was immersed in a temperature controlled water bath (containing 0.5 g/l MnCl<sub>2</sub>) that induced HT. The second tumor on the contralateral leg remained unheated. Body temperature was monitored using a fiberoptic temperature probe placed in the rectum and was maintained by air flow at  $36.1 \pm 0.7$ °C. Gd-TSL (250 µl saline solution containing Gd-TSL at Gd-concentration 0.1 mmol/kg) were injected into the lateral tail vein either at a bath temperature of  $T = 37.3 \pm 0.1$  °C or  $42.5 \pm 0.3$  °C. As a control, non-liposomal Gd-DTPA-BMA was injected at  $43.1 \pm 0.3$  °C. For all three groups data of heated and unheated tumor was evaluated (7,8). Additionally, unheated muscle tissue located in the animals back was selected for comparison.

*In vivo* studies were performed using a dedicated experimental setup (7,8) 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; 0.6x0.3x3mm<sup>3</sup>) supplying high image quality considering the small animal.

**Results:** Tumor and muscle signal intensity changes were detectable in all animals. After i.v. injection of Gd-TSL the signal increased homogeneously in heated tumor tissue ( $+ 19 \pm 3\%$ ) within 2 min persisting thereafter. The unheated control tumors on the contra-lateral legs showed a  $10 \pm 3\%$  and the muscle  $11 \pm 6\%$  signal increase within 2 min after injection. Injection at 37 °C showed a continuous signal increase in both tumors up to 8-10%. Muscle tissue showed comparable signal enhancement as unheated tumor tissue immediately after CA injection. In the course of experiments the signal in muscle stayed below that of unheated tumor tissue.

Non-liposomal CA injection demonstrated that tumors were well perfused during hyperthermia.

**Conclusion:** Hyperthermia induced CA release from Gd-TSL was monitored and characterized by MRI after i.v. injection in tumor bearing mice. Higher temperatures resulted in higher signal changes. Immediately after i.v. injection heated tumor tissue was distinguishable from unheated tumor and muscle tissue. Unheated muscle tissue may thus be less affected by a potential anti tumor therapy based on TSL.

**References:** (1) Issels, R. *Curr. Opin. Oncol.*; 20:438-443, 2008. (2) Falk, M. et al *Int. J. Hyperthermia*, 17(1), 1, 2001. (3) Fosheim, SL. et al *Acad. Radiol.*, 7(12), 1107, 2000. (4) Viglianti BL, et al. *Magn. Reson. Med.* 51, 1153, 2004 (5) Lindner, LH. et al *Int. J. Hyp.*, 21(6), 575, 2005. (6) Lindner, LH, et al. *Clin. Cancer. Res.* 10, 2168, 2004. (7) Peller, M. et al. *ISMRM* 16, 69, 2008 (8) Peller, M. et al. *Invest Radiol* 43, 877, 2008 (9) Reinl, HM. *ISMRM* 11, 1209, 2003. (10) Wang, T. et al. *ISMRM* 14, 1828, 2006