# A new model for visualization of contrast agent release from thermosensitive liposomes induced by laser based hyperthermia

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**Target audience:** Physicists, radiologists, clinicians.

**Introduction:** Efficacy of systemically applied anti-cancer drugs is limited by insufficient selectivity and thus dose is limited by side effects. Effectiveness can be further improved by triggering and targeting of the cytostatic drugs to the tumour. Using thermosensitive liposomes (TSL) as drug carrier, such targeting and triggering is achieved by control of temperature in the target volume [1]. An improved TSL-formulation has been successfully developed with prolonged circulation time and an increased content release rate at a temperature $T_m$ above 41°C, which is consistent with the therapeutical hyperthermia (HT) temperature level aimed at combined HT/chemotherapy tumour treatment concepts [2]. Visualization of content release is achieved by loading the TSL with a MRI contrast agent (CA). Such visualization is considered a prerequisite for further improvement of HT/chemotherapy concepts based on TSL and which may allow temperature and dose monitoring during HT [3]. Feasibility was shown in mice before [4]. In this study, we present a newly developed setup for MR-monitoring of laser-based hyperthermia in a rat model with CA-TSL for optimized visualisation of content release.

**Methods:** In vivo experiments were performed in 2 male Brown Norway rats (279g / 293g) with syngenic fibrosarcomas (BN175) located on both hind legs. In each animal, one tumour (Tu1; 0.5/0.8mm³) was selectively heated to 41°-42°C by a 940 nm diode laser and a new MRI-compatible fiberoptic device while the second tumour (Tu2; 0.1/0.1mm³) remained unheated for reference purposes. Temperature was monitored with an intratumoural fiberoptic temperature probe (IRE Polus FT-707). After the intended intratumoural temperature was reached, a bolus of CA-TSL (Gd-DTPA-BMA 0.6 mmol/kg; DPPC/DSPC/DPPG2 50/20/30 (mol/mol) [2] was injected intravenously. Throughout the experiment, imaging was performed on a 3T clinical MRI scanner (Magnetom Verio, Siemens Healthcare) with a View-Sharing-Sequence (TE = 2.1ms; TR = 6.3ms; α = 40°; FoV = 78*63mm²; spat.res. = 0.4*0.4*2.5mm³; dt = 7.49s) from 1 min prior to injection to 60min after injection. Before and after the treatment T1-mapping using a variable flip angle method was performed (α = [2.5°; 5°; 10°; 19°], TR = 9.7ms, FoV = 78*63mm²; spat.res = 0.5*0.5*2.5mm³), contrast agent concentration changes were calculated as c(t)=(1/T1post – 1/T1pre)/r1, with a value of the relaxivity r1=4.3[L/(mmol*s)] [5].

**Results**: For both animals, dynamic signal enhancement during HT showed a continuous signal increase of up to 84/87% in the heated tumours Tu1 (Fig. 1). In the non heated tumours Tu2 signal increased within one minute after iv injection by 13/16% and was constant (± 5%) thereafter (Fig. 1). Signal from a blood vessel showed peak enhancement during first pass of CA-TSL and an increased signal level of ~18-20% thereafter. The CA-concentration post 60 min HT calculated from $T_1$-relaxation change was in the range of 0.22/0.30 mmol/L in Tu1 and 0.02/0.04 mmol/L in Tu2.

**Discussion:** The strong signal increase selectively in the heated tumour compared to the minor increase in the non heated tumour demonstrated HT induced release of the CA and the effectiveness of the new heating device. This is supported by the small signal changes in the unheated tumour and in the blood vessel. Visualization compared to HT in mice induced by water bath [4] was significantly improved. The new rat model takes also into account that new results showed species-specific release kinetics of PG2-based TSL for doxorubicin in mice compared to human and rat plasma [6]. This seems important for further experiments using CA-TSL to characterize drugs release from TSL. Local differences in signal enhancement as seen in tumour Tu1 (figure 1 left) demonstrates the need for visualization of drug release from TSL for improved targeting of HT to a specific tissue volume

**Outlook:** The preliminary results show that an effective visualization of the CA-release in the new rat model using laser heating is feasible. The significant difference in CA concentrations found in the heated and not heated tumour after HT may be correlated to drug release in future studies allowing chemodosimetry in combined hyperthermia and chemotherapy.

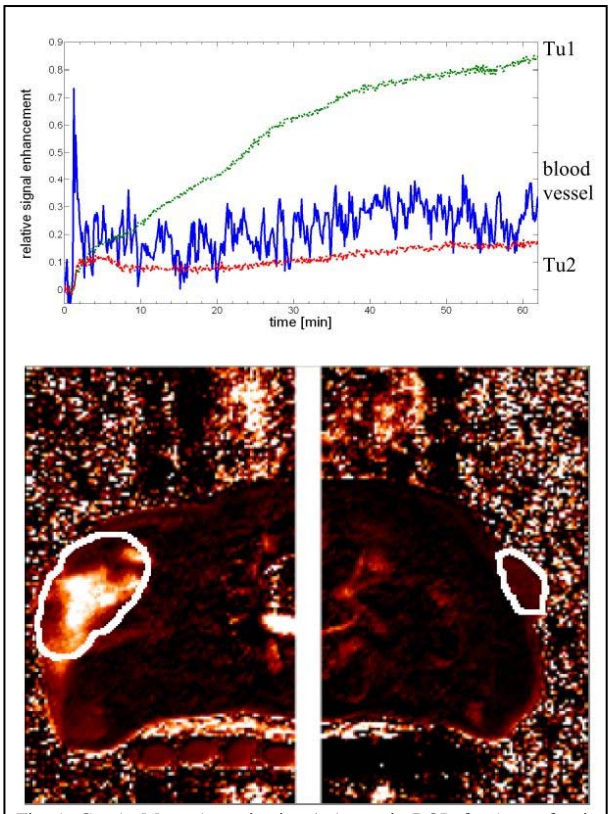


Fig. 1: Graph: Mean dynamic signal change in ROIs for 1 rat after iv injection of CA-TSL during HT in heated tumour Tu1 (green dots), non heated tumour Tu2 (red dots) and blood vessel (blue line). Left image shows relative signal enhancement after 60 min post injection in the heated tumour while the right image the signal enhancement of the non heated tumours Tu2. ROIs used for the graph are depicted for Tu1 and Tu2.

**References:** [1] Koning et al. (2010), Pharm. Res. 27 (8), p. 1750-4 [2] Lindner, et al. (2004) Clin. Cancer. Res. 10 (6), p. 2168-78 [3] Viglianti et al. (2006) Magn. Reson. Med 56 (5), p. 1011-1018 [4] Peller, et al. (2008), Invest. Radiol. 43(12), p. 877-892 [5] Rohrer et al. (2005) Invest. Radiol. 40(11) p. 715-724 [6] Hossann, et al. (2012), J. of Contr. Release 162(2), p. 400-406