T1 based surrogate MRI marker for hyperthermia-induced release of doxorubicin from thermosensitive liposomes in solid tumors

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Target audience: Researchers interested in MRI-based quantification of targeted tumor therapy.

Purpose: Efficacy of systemically applied anti-cancer drugs is limited by insufficient selectivity and applicable dose is limited by side effects. Effectiveness can be further improved by triggering and targeting of the cytostatic drugs to the tumor. 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 above 40°C, which is consistent with the therapeutical hyperthermia (HT) temperature level aimed at combined HT/chemotherapy tumor treatment concepts[2]. Visualization of content release is achieved by loading the TSL with a MRI contrast agent (CA)[3]. Such visualization is considered a prerequisite for further improvement of HT/chemotherapy concepts based on TSL and may allow chemodosimetry during HT[4]. Other groups have reported correlation of MRI markers with DOX based on paramagnetic Mn-DOX complex[4] or loading of Gadolinium based CA and DOX in the same TSL[5]. To allow higher loading of the TSL a third approach using a mixture of TSL loaded with DOX and TSL loaded with CA is applied here. Purpose of this feasibility study was to test T1-based quantification of hyperthermia induced doxorubicin (DOX) release in tumors after injecting a mixture of TSL loaded either with CA or DOX.

Methods: In vivo experiments were performed in 5 male Brown Norway rats (312 ± 57 g) with a syngenic fibrosarcoma (BN175) located on each hind leg. Tumor temperature was monitored using an intratumoral fiberoptic temperature probe (ReflexTM Signal Conditioner, Neoptic Inc, Ca). In group A (N=3) one tumor (Tu1 1.2 ± 0.7cm³) was selectively heated above 40°C by a 940 nm diode laser and a new MRI-compatible fiberoptic device[6]. The second tumor on the other leg (Tu2; 1.0 ± 1.3cm³) remained unheated for reference purposes. After the intended minimum intratumoral temperature of 40°C was achieved, a bolus of mixed TSL (DPPC/DSPC/DPPG2 50/20/30 (mol/mol)[2]) loaded with CA (Gd-DTPA-BMA 0.1 mmol/kg) or DOX (2 mg/kg) was injected intravenously and hyperthermia continued for 1 h. Thereafter the tumor was allowed to cool down to the temperature determined before the start of HT. In group B (N=2) the same experiment was performed but without HT.

Imaging was performed using a 3T clinical MRI scanner (Magnetom Verio or Skyra, Siemens Healthcare). Before and after hyperthermia T1-mapping using variable flip angles (α =5°; 7°, 10°; 12°, 15°,18°, 23°) was performed. Dynamic $T1(t)_{1\alpha}$ (α = 23°) was quantified as recently recommended[7] throughout the experiment. T1 and T1-related parameters (absolute and relative difference of $T1_{1\alpha}$ before and after HT ($\Delta T1_{1\alpha}$) and area under the curve (AUC) of dynamic $T1(t)_{1\alpha}$ during hyperthermia) were correlated with DOX concentrations in tumor tissue. DOX in tumor tissue was determined by HPLC after sacrificing the animal at the end of the experiment.

Results: In group A mean tumor temperature was 41.1±1.2°C during one 1 h after injection while mean body temperature was 33.4±1.3°C. A highly significant linear relationship of DOX and all investigated T1 parameters was found and used to calculate DOX in tumor tissue (see Tab. 1). The best match (Fig.1) of DOX determined by HPLC and MRI was found using AUC $T1(t)_{1\alpha}$ (relative).

DOX by MRI-Parameter:	Slope (SE)	Intercept (SE)	R^2	Correlation coefficient
Absolute $\Delta T1_{1\alpha}$	1.21 (± 0.27)	-2.56 (± 1.92)	0.535	0.731
relative $\Delta T1_{1\alpha}$	2.07 (± 0.31)	-7.70 (± 2.03)	0.719	0.848
AUC $T1(t)_{1\alpha}$ (abs.)	1.37 (± 0.38)	-2.24 (± 2.91)	0.646	0.803
AUC $T1(t)_{1\alpha}$ (rel.)	1.03 (± 0.18)	-0.05 (± 1.14)	0.657	0.811

Tab. 1: Linear regression and correlation coefficient (Pearson) of DOX concentration determined by MRI and HPLC for 15 samples from 5 animals. SE denotes standard error.

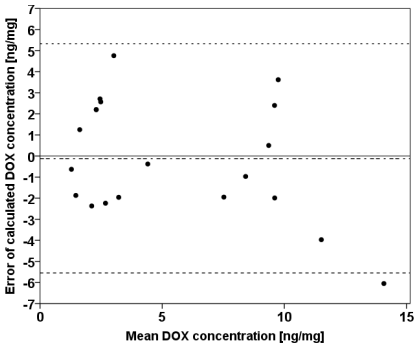

Fig. 1 Bland-Altman plot using DOX concentration found by HPLC and using AUC $T1(t)_{1\alpha}$ (relative)

Discussion: This was the first time that a mixture of TSL loaded with a clinically approved CA and TSL loaded with DOX has been investigated for *in vivo* chemodosimetry[8]. Although there is a potential need to consider different kinetics of DOX or CA-release from mixed TSL results allowed assessment of DOX deposited in heterogeneous tumors using T1-based MRI-parameters. The use of a newly developed non-invasive laser based hyperthermia device allowed a tumor specific, non-invasive heating of the tumor[6]. Local differences in T1 maps in the heterogeneous tumors demonstrate the need for visualization of drug release from TSL for improved targeting of HT to a specific tissue volume.

Conclusion: The preliminary results show that visualization of the CA-release and possibly quantification of DOX in the treated tumor tissue is also feasible when using a mixture of CA- and DOX-TSL.

References:

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