Optimization of Liposomal Theragnosis: Quantitative T_1 Measurement of Drug Distribution and Release in Deep-seated Tumor using Multimodal Thermo-sensitive Polymer-modified Liposome

D. Kokuryo[1], S. Nakashima[2], K-H. Chuang[3], I. Kanno[1], K. Kono[2], and I. Aoki[1]

[1]Molecular Imaging Center, National Institute of Radiological Sciences, Chiba, Chiba, Japan, [2]Graduate School of Engineering, Osaka Prefecture University, Sakai, Osaka, Japan, [3]Singapore Bioimaging Consortium, Singapore, Singapore

Introduction The purpose of this study is to achieve 'Theragnosis (therapy + diagnosis)' with thermo-sensitive polymer-modified nanotechnology. Previously, a temperature-sensitive liposome (TSL) based on the phase transition of lipid membrane was developed and applied as a drug delivery system (DDS) loaded with doxorubicin to increase treatment efficacy [1, 2]. The dynamics of drug release in fibrosarcoma was also visualized using MRI [3, 4]. In previous work, we reported that a multimodal thermo-sensitive polymer-modified liposome (MTPL) allows more accurate temperature-sensitivity and drug release than standard TSL. The MTPL can be loaded with various functional agents such as doxorubicin, rhodamine, and $MnSO_4$ [5]. The MTPL was shown to accumulate in subcutaneous and disseminated tumors for over 8 hours after administration, and the temperature-triggered membrane disruption was visualized as a signal enhancement in T_1-weighted MRI [5, 6]. As the next step, a heat-triggered DDS using the MTPL was applied to deep and intractable tumors such as pancreas or metastasis cancer. To reduce side-effects, the heat-trigger is timed so that the MTPL concentration is low in intact organs and optimized in tumor. In this paper, quantitative T_1-relaxation times were measured using a rapid imaging technique to estimate the dynamics of drug concentration in the tumor, healthy kidney and liver. The drug release was also visualized with T_1-weighted imaging and corresponding observation of the in vivo temperature distribution.

Materials and Methods The MTPL was composed of EYPC/DOPE/Cholesterol/PEG2000-PE/EOEOVE-ODVE/Rhodarmine-PE (23.4/54.6/15/4/2/1 mol/%) and contained $MnSO_4$ (300mM, pH 5.3) and doxorubicin. The structure of this thermo-sensitive polymer becomes hydrophobic at around 42.0 ºC, at which point the lipid membrane is sensitive to disruption by further incremental increases in temperature [6, 7]. Female BALB/c nude mice were used for *in vivo* experiments. Colon26 cancer cells (2.0×10^5 cells) were transplanted intramuscularly and were allowed to grow for about 8 days before the MRI experiments.

All MRI acquisitions were performed on a 7.0 Tesla animal MRI (Magnet: Kobelco, Japan; Console: Bruker Biospin, Germany). A modified Look-Locker sequence [8] was used to quantitatively measure T_1 in transverse slices before and after MTPL (0.25 ml) was administered via the tail vein. Measurements were made with a 35 mm inner-diameter volume coil (Rapid Biomedical, Germany). T_1-maps in the tumor, kidney and liver were acquired before, 0 (immediately after), 4, 8, 12, 24, 48 and 72 hours after administration. Look-Locker imaging parameters were: TR/TE, 10000/3.0 ms; interexcitation interval, τ, 400 ms; FA, 20º; FOV, 38.4 × 38.4 mm, 4 slices.

In order to visualize drug release, transverse multi-slice T_1-weighted MR images (TR/TE, 400/9.5 ms; FOV, 32.0 × 32.0 mm) were acquired before and after heating. Local RF heating and MR imaging were performed with a 17 mm inner-/25 mm outer-diameter surface coil (Takashima Seisakusho, Japan). MTPL (0.25 ml) was intravenously administered to the mice around 12 hours before heating to minimize side-effects. The temperature near the tumor and in the rectum was measured using optical thermometers (FISO Technology Inc., Canada). The tumor was heated to a temperature of around 42.5 ºC for 10 minutes. Before and during heating, several gradient-echo images (TR/TE, 40/12 ms; FOV, 32.0 × 32.0 mm) were obtained to estimate temperature distribution using the proton resonance frequency method [9].

Results Figure 1 shows the dynamics of the relaxation rate ($R_1 = 1/T_1$, normalized by the value before MTPL administration) up until 72 hours after MTPL administration. In the liver and kidney area, after MTPL administration R_1 rapidly increased before beginning to decrease at 4 hours for the liver and at 8 hours for the kidney. R_1 in the liver at 24 hours after MTPL administration had returned to the same level as before administration. At the same time, R_1 in the kidney was still twice as high as before administration. On the other hand, R_1 in the tumor was increased for the first 4 hours and then was maintained until 12 hours after the MTPL administration. Figure 2 presents the T_1-weighted images before and after applying a heat-trigger at 12 hours after the MTPL administration. Signal enhancement in the tumor was observed after the heating. Figure 3 (b) shows the temperature distribution overlaid on a T_1-weighted image. The optical fiber measured the absolute temperature near the tumor to be 36.0 ºC before heating and around 42.5 ºC during the 10 minute heating period. The temperature of the area on the upper edge of the tumor was over 42.5 ºC and signal change was evident in this area. The temperature below the tumor was under 42.5 ºC.

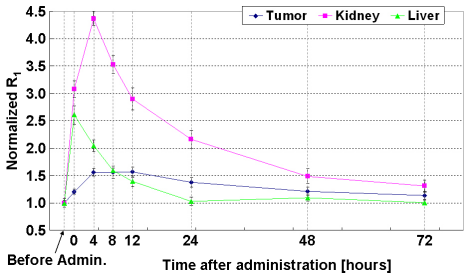

Figure 1 Dynamics of normalized R_1 from before MTPL administration until 72 hours after.

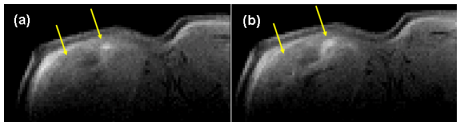

Figure 2 T_1-weighted images (a) before and (b) after heating. The signal intensity at the tumor edge increased after heating (yellow arrows).

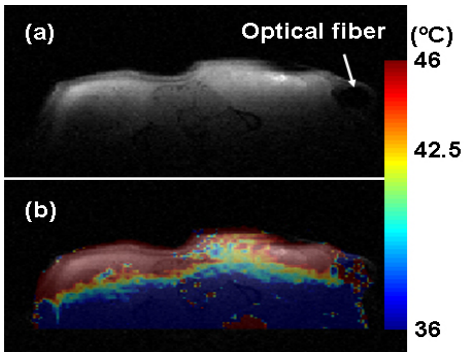

Figure 3 (a) T_1-weighted image and (b) temperature distributions superimposed on (a). The red area was over 42.5 ºC.

Discussion The time-course of R_1 after MTPL administration differed widely in tumor and intact organs such as kidney and liver (Fig. 1). Drug concentration in the tumor was reasonably well maintained for up to 24 hours due to the EPR (enhanced permeability and retention) effect [10] (Fig. 1). On the other hand, fluorescence imaging has previously shown that MTPL concentration in liver and kidney reduces significantly between 8 and 16 hours after administration [6]. Therefore, applying the heat-trigger at 12-24 hours after the MTPL administration is the best time to avoid side-effects.

After heating at 12 hours after the MTPL administration, signal enhancement was observed at the edge of the tumor area (Fig. 2) where the temperature was over 42.5 ºC (Fig. 3). Meanwhile, the signal did not change in the area where the temperature was under 42.5 ºC. Therefore, the area of drug release after heating can be visualized and compared to the temperature distribution maps. In future work, a heat-triggered DDS with temperature distribution mapping will be possible.

Acknowledgements The authors thank Miss Sayaka Shibata, Mr. Takeo Shimomura and Mr. Teppei Nakahara for technical assistance during *in vivo* experiments and Mr. Jeff Kershaw for proofreading.

References [1] Needham D, et al: Cancer Res, 2000; 60: 1197-1201, [2] Kong G, et al: Cancer Res, 2000; 60: 6950-6957, [3] Ponce AM, et al: J Natl Cancer Inst ,2007; 99: 53-63, [4] Peller M, et al: Invest Radiol, 2008; 43: 877-892, [5] Aoki I, et al: Proc. ISMRM, 2008; 796, [6] Kokuryo D, et al: Proc. ISMRM, 2009; 894, [7] Kono K, et al: Bioconjug. Chem, 2005; 16: 1367-1374, [8] Chuang KH, et al: Magn Reson Med., 2006; 55: 604-611, [9] Ishihara Y, et al: Magn Reson Med., 1995; 34: 814-823, [10] Matsumura Y, et al: Cancer Res, 1986; 46: 6387-6392.