Multimodal Thermo-sensitive Polymer-modified Liposome for Visualization and Treatment of Disseminated Cancer

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Introduction The purpose of this study is to visualize the dynamics of accumulation of and the drug release from a nano-carrier using a multimodal temperature-sensitive liposome (TSL) that contains an anticancer drug, a fluorescent dye and a MR contrast agent for disseminated cancer treatment. A drug delivery system (DDS) using the TSL with doxorubicin has previously been developed to increase the treatment efficacy in the heated area and to decrease the side-effects of the anticancer drug in the intact organ [1, 2]. Additionally, the dynamics of drug release in fibrosarcoma has been visualized using MR contrast agent-containing TSLs [3, 4]. In previous work, we reported that a multimodal thermo-sensitive 'polymer-modified' liposome (MTPL) allows more accurate drug-release and that it can be loaded with various functional agents such as doxorubicin, rhodarmine, and $MnSO_4$ [5]. The MTPL accumulated in the subcutaneous tumor for over 8 hours after administration, and the temperature-triggered drug-release was visualized as a signal enhancement in T_1-weighted MRI [5]. As the next step, the MTPL was applied to an advanced-stage disseminated/metastatic cancer. Clinically, the benefits of treatment are not yet satisfactory although with advances in the methodology improvement is anticipated [6]. To improve the applicability of MTPL to the treatment of disseminated/metastatic cancer, the dynamics of MTPL accumulation and the detection of drug release were examined using both MRI and optical imaging.

Materials and Methods Our MTPL was composed of EYPC/DOPE/Cholesterol/PEG2000-PE/E087/Rhodarmine-PE (23.4/54.6/15/4/2/1 mol/%) and contained $MnSO_4$ (300mM, pH 5.3) and doxorubicin inside. *In vitro* MTPL signal intensity was characterized for temperatures between 38 and 45 ºC in a 7.0 Tesla animal MRI (Magnet: Kobelco, Japan; Console: Bruker Biospin, Germany) with a rat volume coil. All the MTPL samples (× 10 dilution with HEPES) were heated for 10 minutes. For *ex vivo* and *in vivo* experiments, female Bulb/c nude mice were used. Colon26 cancer cells (1.0×10^6 cells) were transplanted intraperitoneally under the 2.0 % isoflurane anesthesia and were allowed to grow for 7 to 10 days before the MRI experiments. The MTPL was administered via the tail vein more than 8 hours before MRI scanning.

In order to confirm the accumulation of MTPL after administration, *ex vivo* optical images were acquired using an IVIS-Lumina System (Caliper Life Sciences, CA). The mice were sacrificed 4, 8, and 16 hours after 0.2 ml (stock solution) MTPL administration, after which the liver, kidney, pancreas, spleen and mesentery were extracted for optical imaging.

MTPL drug release was visualized by acquiring horizontal multi-slice T_1-weighted MR images (TR/TE, 400/9.5 msec; slice thickness, 1.0mm; matrix, 256 × 256; FOV, 38.4 × 38.4 mm; Number of Acquisitions, 4) with a mouse volume coil or rat brain coil (RAPID Biomedical GmbH, Germany) before and after heating. The 0.3 ml (stock solution) MTPL was intravenously administered to the mice at 8 to 16 hours before heating. Just before MRI measurement, muscle relaxant (pancuronium bromide, 0.04 mg / kg) and parasympatholytic (butylscopolamine bromide, 1.0 mg / kg) agents were administered subcutaneously to suppress spontaneous breathing and peristalsis. The body was heated to a temperature of around 42.0 ºC, which was maintained for 3 minutes. Body temperature was measured rectally using an optical thermometer (FOT-H, FISO Technology Inc., Quebec, Canada). After heating the mice were allowed to cool to the temperature they had been before the MRI measurement.

Results Figure 1 contains MR T_1-relaxation maps of the *in vitro* MTPL sample at different temperatures. The observed T_1-longtudinal relaxation rate of the MTPL sample was 1.2 times higher at 42 ºC than at 38 ºC. In Figure 2, the *ex vivo* fluorescence images demonstrate MTPL accumulation in the internal organs. The fluorescence intensity drastically decreased in the liver and kidney area 16 hours after the administration. On the other hand, the fluorescence intensity in the mesenteric tumor increased over the first 8 hours and was still present 16 hours after the administration. Figure 3 presents the MR T_1-weighted *in vivo* images acquired before and after heating. A change in the signal intensity in the bowel (enteric canal) and the kidney after heating was observed after heating.

Discussions At temperatures above 42 ºC, the *in vitro* MTPL sample ruptured and released the MR contrast agent (Figure 1). Even though the T1 relaxation values above 43 ºC were slightly smaller than at 42 ºC, heating was limited to 42 ºC for the *in vivo* experiment in order to avoid protein affection. The dynamics of MTPL were different for tumor and intact organs (Figure 2) with the MTPL accumulating more slowly and remaining in the mesenteric area longer than in the intact organs. This indicates that heating to trigger drug release should be applied 8-16 hours after the MTPL administration. In Figure 3, the heating-trigger was applied 8 hours after the MTPL administration. Although the anticancer benefit *in vivo* has not yet been thoroughly evaluated, an effect is expected in the enhanced area. In future work, high resolution MR images are essential to identify small tumors in the abdomen and to evaluate the anticancer benefits of the MTPL.

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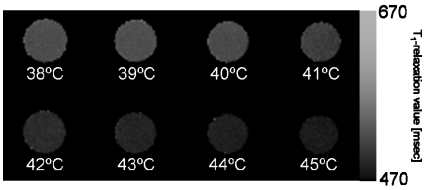

Figure 1 T_1 maps of the *in vitro* MTPL samples at different temperatures. The T_1 clearly shortened when the temperature was over 42 ºC.

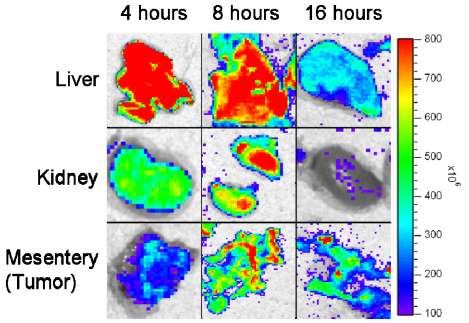

Figure 2 *Ex vivo* longitudinal fluorescence images reflecting the MTPL accumulation.

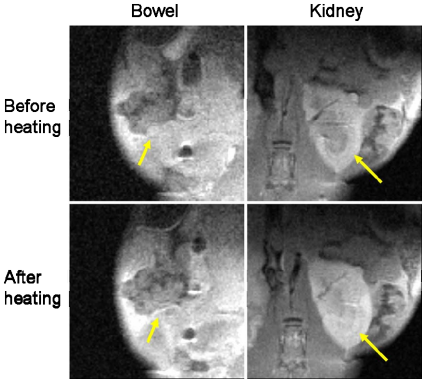

Figure 3 T_1-weighted images before and after heating. The signal intensity in the bowel and kidney changed after heating (Yellow arrows).