Thermosensitive Polymer-modified Liposome as a Multimodal and Multifunctional Carrier for MRI and Optical Imaging: Tumor Detection, Visualization of Triggered Drug Release, and Chemotherapy

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Introduction: A liposomal drug delivery system will be help to avoid the side effects of chemotherapy by releasing anticancer drug at the tumor site. Recently, using doxorubicin-containing lysolipid-based temperature-sensitive liposomes (Dox-LTSLs) it has been reported that tumor doxorubicin concentrations were up to 30 times higher than those of mice treated with free drug administration (1,2). The temporal and spatial pattern of drug delivery in a fibrosarcoma model was visualized during treatment using LTSLs containing doxorubicin and a $MnSO_4$ MRI contrast agent (Dox/Mn-LTSLs) (3). Conventional thermosensitive liposomes, such as a gel-to-liquid-crystalline transition-based material, have the potential shortcoming that drug molecules may leak through the incomplete membrane of the liposome. On the other hand, our thermo-sensitive polymer-modified liposomes (TPL) can provide precise drug release using hydrophobicity change of the copolymer as a thermo-triggering (4). Dehydrated copolymer chains strongly destabilize the liposomal membrane within a limited temperature range (40-42 ˚C), and therefore the TPL provides both highly accurate temperature sensitivity and long-term stability *in-vivo* (8 hours). To improve the liposomal drug delivery system, we developed a multimodal-TPL (MTPL) and added three new factors, long-term stability of the liposome *in-vivo*, passive accumulation in the tumor, and multimodal *in-vivo* observation with both MRI and (fluorescent) optical imaging. The purpose of this study was to investigate whether 1) MRI and optical imaging can visualize accumulation of MTPL in the tumor after administration, 2) MTPL allows visualization of the drug release after being triggered by mild-heating (42.5 ˚C) eight hours after administration, and 3) MTPL can provide sufficient anti-tumor effects after treatment.

Materials and Methods: EYPC, DOPE, Chol, PEG(2000)-PE, EOEOVE-ODVE and Rho-PE (23.4/54.6/15/4/2/1, mol%) (53.3 mg) were dissolved in a mixture of chloroform and methanol, and the solvent was removed by evaporation. The obtained thin lipid/copolymer-mixed membrane was further dried under vacuum for 4 h and dispersed in an aqueous $MnSO_4$ solution (300 mM, pH5.3). ADR-loaded liposomes were purified using a Sepharose 4B column with a mixture of 20 mM Hepes and 150 mM NaCl (pH 7.4). Female Bulb/c nude mice (n = 13, 17.5 ± 2.5 g) were divided into three groups: heated group (n = 5), non-heated group (n = 5), and short-term observation group (n = 3). Colon 26 cancer cells were transplanted subcutaneously in the femurs of the mice under 2.0 % isoflurane anesthesia. Tumors were allowed to grow for 7 days before treatment. For both the heated and non-heated group, the MTPL (0.3 ml) was administrated via the tail vein under 2.0 % isoflurane anesthesia. In vivo optical imaging (IVIS-100 system, Caliper Life Sciences, CA) was performed 0, 4 and 8 hours after the MTPL administration under 2.0 % isoflurane anesthesia. For the heated group, transversal and horizontal multi-slice T_1-weighted MR images (TR/TE = 250/9.57 ms, slice thickness = 1.5 mm, matrix = 256*256, field of view = 32.0 mm, average = 4) were acquired prior to heating using a 7.0 T-MRI (Magnet: Kobelco, Japan. Console: Bruker, Germany) in combination with a volume coil for transmission (Bruker) and 2ch phased array coil for receiving (Rapid Biomedical, Germany) 8 hours after the MTPL administration. Thereafter, one side of the tumor site was heated at 42.5 ˚C for 10 minutes by hot-water circulation with temperature monitoring in the rectum and at the tumor site using a fiber optic thermometer. MRI acquisition was performed again in same manner after heating. For the short-term group, continuous MRI acquisitions were performed for 3 hours in the same manner and after MTPL administration inside the magnet under 1.5 % isoflurane anesthesia. Mice were allowed to recover from anesthesia after the MRI measurement and kept in normal cages for 14 days.

Results and Discussion: MTPLs that contain doxorubicin for chemotherapy, $MnSO_4$ as an MRI contrast agent, and the fluorescent dye Rhodamine for *in-vivo* optical imaging were synthesized (Fig. 1). Moderate signal enhancements on the T_1-weighted MRI (Fig. 2) and definitive signals on the optical imaging (Fig. 3) were observed in the tumor after MTPL administration without heating. MRI signal intensity was further enhanced over an expanded area after mild-heating (42.5 ˚C) eight hours after the administration (Fig. 4, yellow arrows). The anti-tumor effect of the MTPL was clear for 14 days after the treatment (Fig. 5).

Conclusions: We developed MTPLs and demonstrated long-term stability with tumor accumulatability. This method provides a system consisting of diagnostic, treatment, and evaluation methods. In addition, it will be useful for "active" targeting using antibodies or peptides with highly specific accumulation.

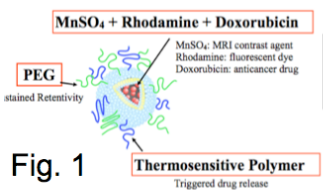

Fig. 1

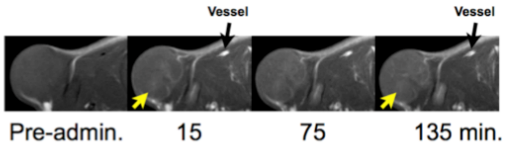

Fig. 2

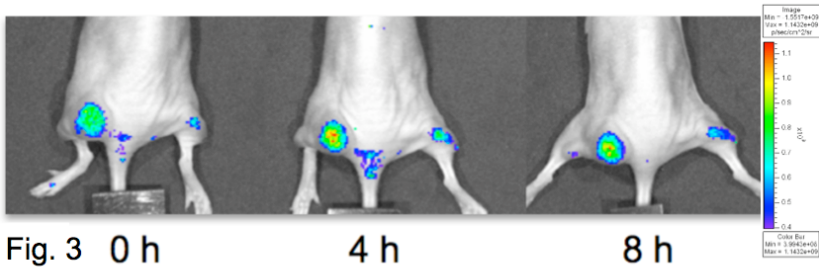

Fig. 3

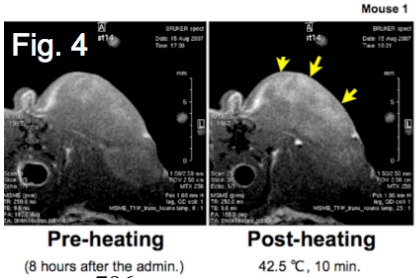

Fig. 4

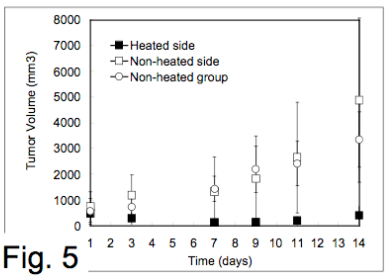

Fig. 5

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