Evaluation of nanoparticle accumulation and treatment efficacy for a combined heavy-ion-beam irradiation and drug-delivery tumor therapy

Daisuke Kokuryo[1], Eiji Yuba[2], Kenji Kono[2], Tsuneo Saga[1], and Ichio 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*

Target audience Preclinical cancer research and molecular imaging with nanoparticle-based contrast agents.

Introduction and Purpose The combination of radiotherapy and chemotherapy is a common strategy for noninvasive cancer treatment. Each radiotherapy and chemotherapy technique has developed following advances in mechanical and chemical technology. For radiotherapy, high linear-energy-transfer beam therapy, using protons and heavy-ions such as carbon, has been developed based on the absorption properties of tissue [1]. For chemotherapy, drug-delivery treatment using nanoparticles (e.g. micelles & liposomes) containing anticancer drugs has improved treatment efficacy with minimum side-effects in preclinical trials [2, 3]. The dynamics of the nanoparticles in tumor and normal tissues can be measured by adding a contrast agent [4]. Previously, we reported that a multi-modal thermo-sensitive polymer-modified liposome (MTPL) containing a MR contrast agent, a fluorescence dye and an anticancer drug can be utilized to visualize accumulation in tumor and drug release via MR signal enhancement after heat-triggering [5] (Figure 1). As a next step, we have been applied the MTPLs to a combination treatment with carbon-ion irradiation. In the present study, MTPL accumulation in the tumor region before and after carbon-beam irradiation, and the subsequent treatment effects of the combination therapy were evaluated using *in vivo* high-field MR imaging.

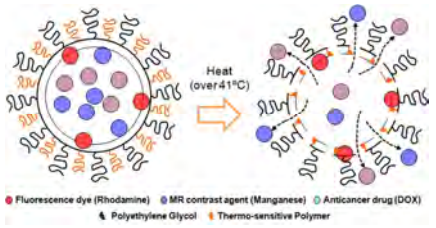

Fig. 1 MTPL structure before and after heating.

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 (Figure 1). Female BALB/c nude mice were used, and colon 26 murine cancer cells (1.0×10^6 cells/50 μl) were transplanted into the right and left back muscles of the mice at 8 to 10 days before the experiments. The mice were maintained in accordance with the guidelines of our institute, and all experiments were reviewed and approved by the institute's Committee for Care and Use of Laboratory Animals.

Strategy Figure 2 shows the treatment strategies in this study. A carbon-beam of 5 Gy was used to irradiate the left tumor (with collimators to protect normal tissue), with power tuned so that power deposition was maximum in the tumor centre [6]. A 0.2 ml MTPL dose was administered to the tail vein of the tumor model mice. To break the MTPLs, the irradiated tumor region was heated to 42.5 ºC for 10 minutes using a home-made warm water circulation system. The order of MTPL administration and irradiation were swapped for the "MTPL+Carbon-beam" and "Carbon-beam+MTPL" groups (Figure 2).

MTPL accumulation MTPL accumulation was evaluated from T_1-weighted images and quantitative T_1 maps acquired at 12 hours after irradiation. The MR images were acquired using a 7.0 Tesla preclinical MR scanner (Magnet: Kobelco+Jastec, Kobe, Japan, Console: Bruker-biospin, Ettlingen, Germany) with a 35-mm diameter volume coil for transmission and reception (Rapid Biomedical, Lymper, Germany). T_1-weighted images were acquired using a conventional spin-echo sequence with the following parameters: TR = 400 ms; TE = 9.6 ms; FOV = 38.4 × 19.2 mm^2; number of slices = 9; slice thickness = 1.0 mm; slice gap = 0.5 mm; Matrix = 256 × 128 and NEX = 4. T_1 mapping was performed using a rapid acquisition with relaxation enhancement (RARE) sequence with variable TR and TE. The parameters were as follows: TR = 5000, 3000, 1500, 800, 400 and 200 ms; TE = 11, 33, 55, 77 and 99 ms; RARE factor = 2; number of slice = 1; NEX = 1. Other parameters were the same as for the T_1-weighted images. T_1 maps were calculated using an imaging processing tool in the Paravision 5.1 software (Bruker-biospin).

Treatment effect To evaluate treatment effects, the tumor size was monitored every two days (up to 8) for each treatment strategy. The initial tumor sizes were chosen to be between 100 and 200 mm^3.The tumor sizes for each treatment group were compared using two-way ANOVA with Bonferroni correction (Prism, Ver. 5, GraphPad Software, CA, USA). A significance level of 0.05 was used.

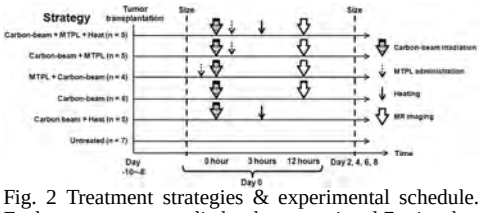

Fig. 2 Treatment strategies & experimental schedule. Each strategy was applied to between 4 and 7 mice, but MR imaging was only performed on three mice.

Results and Discussion Figure 3 presents typical T_1-weighted images for "Carbon-beam" and "Carbon-beam+MTPL+Heat" groups at 12 hours after irradiation. For the former group, there were no signal differences between the irradiated and unirradiated tumors (blue and white arrows). For the latter group, the MR signal after irradiation and heating with MTPL administration (yellow arrow) increased significantly in comparison to the signal from the contralateral tumor site (black arrow). Figure 4 presents R_1 (=1/T_1) measured in the tumor region at 12 hours after irradiation. There were no significant tumor signal differences between the "Carbon-beam+MTPL" and "MTPL+Carbon-beam" groups. Also, the thermosensitivity of the MTPLs worked effectively because R_1 of the irradiated and heated tumors in the "Carbon-beam+MTPL+Heat" group was significantly higher than that of unirradiated tumor that was not heated. Thus, carbon-beam irradiation at the applied dose did not have a significant influence on the dynamics of MTPL accumulation and thermosensitivity. Figure 5 shows the tumor growth curve up until 8 days after irradiation. The relative tumor volume of the "Carbon-beam+MTPL+heat" group was significantly smaller than that for the other groups. This indicates that the proposed combination treatment using carbon-ion beam irradiation and MTPLs with heat-triggering has the potential to enhance treatment efficacy. Also, this strategy has the potential to decrease the dose necessary to obtain the same treatment effect when using carbon-beam irradiation alone, meaning that the treatment can be optimized so that the irradiated dose is minimized. In future, it is expected that the combination of heavy-ion beam irradiation and nanoparticles (e.g. polymeric micelles containing cisplatin [7] or epirubicin [8], liposomes containing doxorubicin) will increase treatment efficacy.

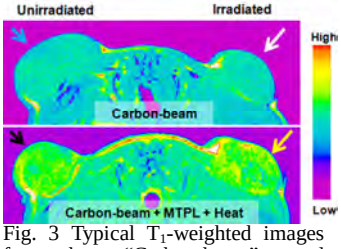

Fig. 3 Typical T_1-weighted images for the "Carbon-beam" and "Carbon-beam+MTPL+Heat" groups at 12 hours after irradiation. Blue arrow: no treatment. White arrow: carbon-beam irradiation. Black arrow: MTPL administration. Yellow arrow: carbon-beam irradiation and heating after MTPL administration.

Conclusion: MTPLs were reliably delivered to the tumor regardless of whether heavy-ion irradiation had been previously applied or not. Our combination strategy using heavy-ion beam irradiation and MTPLs provided effective treatment.

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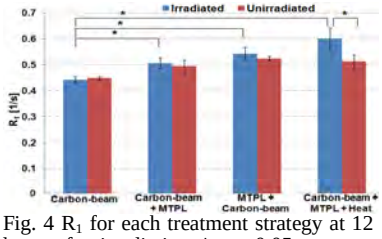

Fig. 4 R_1 for each treatment strategy at 12 hours after irradiation. *: $p < 0.05$, *t*-test.

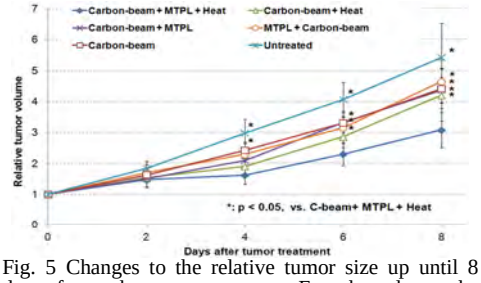

Fig. 5 Changes to the relative tumor size up until 8 days after each treatment strategy. Error bars denote the standard deviation across animals in the same group.