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Oncothermia basic research at in vivo level. The first results In Japan

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Background

Oncothermia method (OTM) is a long time (since 1989) applied method in oncology [1] with great clinical success.[2] Oncothermia research group conducts investigations to reveal the basic mechanism of action of this tumor treatment method in basic research level performing a huge number of in vivo studies. The tumor destruction efficacy and the role of temperature independent effects of the OTM were proven earlier and presented elsewhere [3], [4], as well as the recent in vivo results [5], [6]. In this paper we summarize the first results we have achieved in Tottori University, Japan.

Materials and methods

Study I.

In the first study we examine the effect of oncothermia treatment in a mouse tumor model. **Animal model:** Colon26 (murine colorectal cancer) cell line derived allograft mouse tumor modelwas used for this study with double tumors. The use of the mice and the procedures used in this study were approved by the Animal Research Committee of Tottori University.

Figure 1. Experimental mouse tumor model. Every animal had two tumors in both the femoral regions, the right side was teated, the left side was indivicual control

Experimental setup and treatment: A single shot 30min oncothermia treatment was done reaching maximum 42°C intratumoral temperature, using the LabEHY system (Oncotherm Ltd.), under precise tumor temperature control using fluoroptic temperature measurement device (Lumasense m3300)

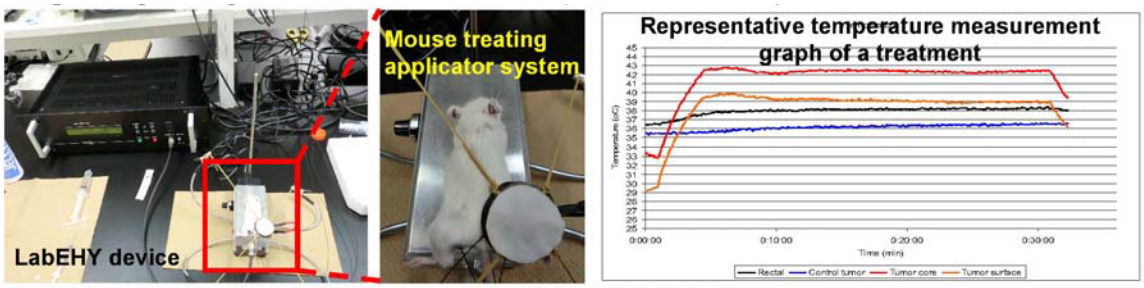

Figure 2. The experimental setup with the LabEHY system and a representative temperature measurement graph of the temperature curve of the tumors

Study design: A time course study was performed. After a single shot oncothermia treatment animals were sacrificed at 6H, 24H, 72H, and 120H later and tumors were removed. In all time-group there were 3 treated animals and 1 untreated control animal.

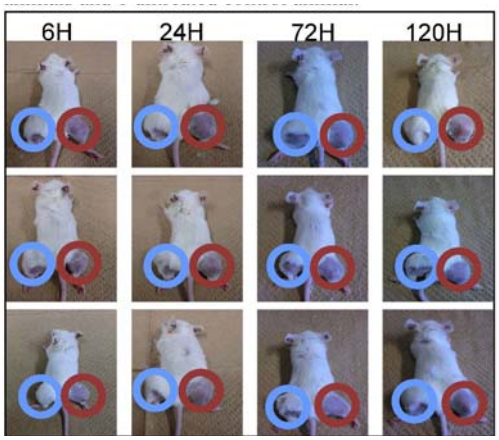

Figure 3. Oncothermia treated experimental animals in this study

Tumor sample processing: All the removed tumors were cut accurately at their centerline. After a standard histological process the samples were stained with HE and TUNEL reaction and Ki-67 immunohistochemical (IHCH) detection was performed (HE staining and IHCH detection were perfomed by Sapporo Byori Kensa Center, Japan). Samples were evaluated using complex histomorphological methods. Besides the qualitative analysis, a quantitative microscopical evaluation was also performed in the tumor samples stained with Ki-67. In ten randomly chosen high magnification (400x) microscopic view area of the living part of the tumor tissue samples the Ki-67 positive cell nuclei were counted, recorded and evaluated.

Study II.

In the second study we examined the effects of OTM to tumor oxigenization using a rat tumor model.

Animal model: 9L (rat glioma) cell line derived allograft rat tumor model was used. All animals had 2 tumors in both femoral regions. The use of the rats and the procedures used in this study were approved by the Animal Research Committee of Tottori University.

Oxygen level measurement: Tumor tissue oxigenisation level was measured using an O_2 sensitive electrode system (Eikon Kagaku Ltd. 150Dmodel).

Study design: In 11 rats, tumor tissue oxigenization level was measured using a pO_2 sensitive electrode system right before the treatment. The sensor probe of the system was inserted into the tumor tissue with the help and guidance of a teflone catheter, then the measured pO_2 value was recorded. Then the probe and the catheter were removed and a single shot, 30min oncothermia treatment was performed using a LabEHY system (Oncotherm Ltd.), reaching maximum 42°C intratumoral temperature. Right after the treatment the tumor oxigenization level was measured again.

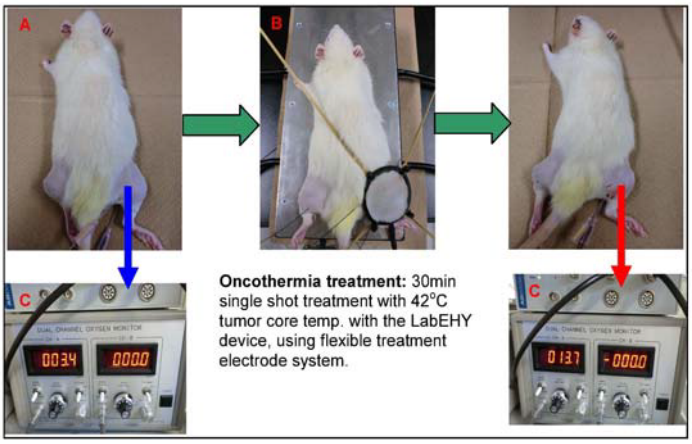

Figure 4. The study design. The 9L glioma cell line derived rat allograft tumor model (A), the oncothermia treatment procedure (B) and the tissue oxigenization measurement system (C)

Results

Study I.

1. A. Histomorphological changes in a qualitative and a quantitative way

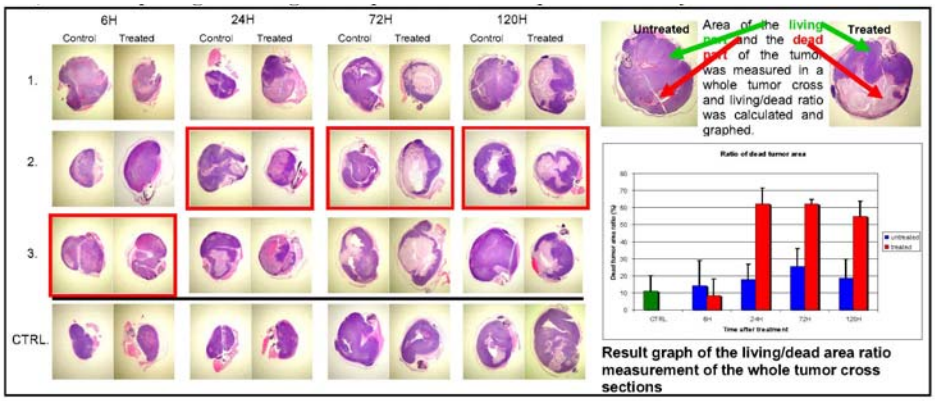

Figure 5. All the tumor samples involved in this study and the result graph of the quantitative analysis of the living/dead area ratio measurements. Drastic and selective tumor-destruction was detected adter a single shot oncothermia treatment. The tumor destruction was not immediate it had a time-delay. Samples marked with a red rectangle are evaluated in details

1. B. Histomorphological changes in details

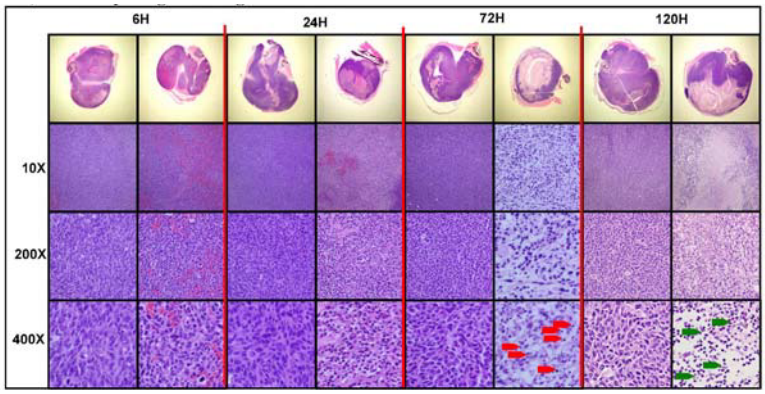

Figure 6. Detailed morphological analysis of the tumor samples marked with red rectangle in Fig. 5. 6H after the treatment the tumor cells look intact, but 24H after the treatment, the large part of the tumor is dead, the cells shrank with picnotic cell nuclei. In the 48H and 72H samples definite late morphological signs of apoptotic cell death was observed: extremely high number of apoptotic bodies (marked with red arrow). 120H after the treatment morphological signs of leukocyte (mostly neutrophiles, marked with green arrow) invasion is visible

2. TUNEL reaction

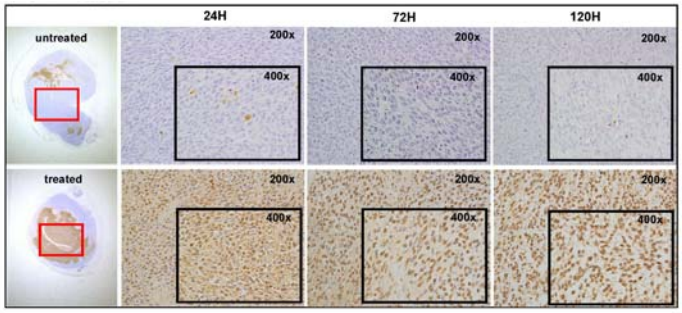

Figure 7. Result of the qualitative evaluation of TUNEL staining. TUNEL assay enzymatically labels the DNA fragments resulted by apoptotic cell death process. In the dead tumor area a huge number of TUNEL-positive cells were observed after a single shot OTM treatment

3. Ki-67 expression changes

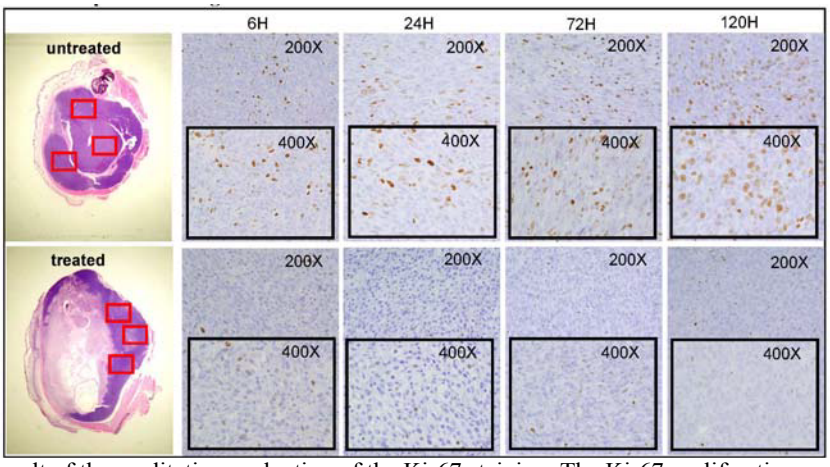

Figure 8. Result of the qualitative evaluation of the Ki-67 staining. The Ki-67 proliferation marker protein is expressed in the nuclear membrane only in the dividing cells. That is why sampling for Ki-67 positive cell analysis and counting were done from the living part of the tumors. The high magnification images from the living part of the tumor samples (marked with red rectangle in the whole cross sections)

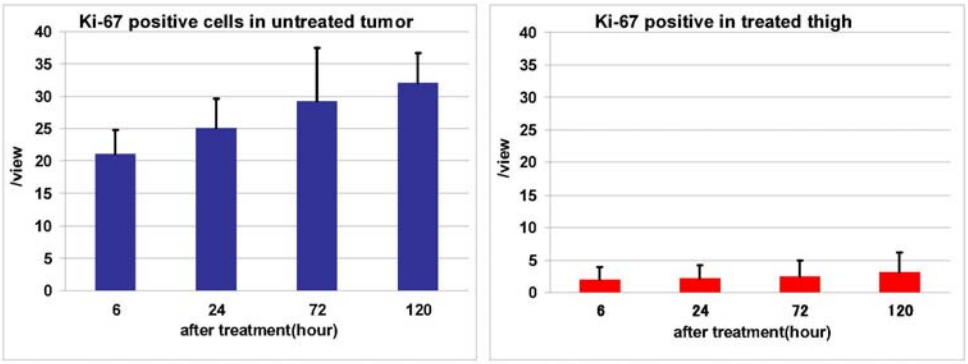

Figure 9. Result of the quantitative evaluation of the Ki-67 staining. Ki-67 positive cell nuclei were counted in 10 randomly choosen area of the living part of the tumor samples. In a very interesting way the number of Ki-67 positive cells were significantly decreased in the living part of the treated tumor compared to the control tumors

Study II.
Results of the tumor pO2 level measurement in a rat tumor model

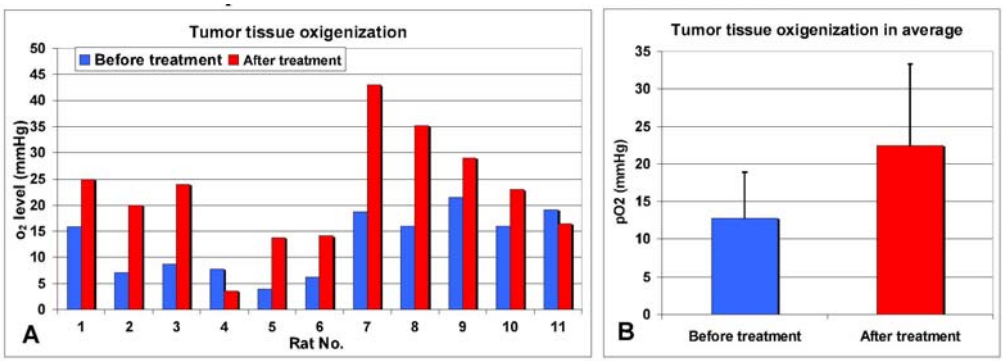

Figure 10. Result of the tumor tissue oxigenization level measurement in each animal (A) and in average (B). Tumor tissue pO_2 level was significantly higher right after the oncothermia treatment compared to the pO_2 level measured right before the treatment in case of 10 out of the total 11 animals. The pO_2 level was almost double after the treatment in average

Conclusions

1. In the mouse study, oncothermia treatment could significantly destroy the tumor tissue in a large volume of the tumor with only a single shot. Oncothermia treatment induce apoptotic cell death in the destroyed tumor tissue and effectively inhibit cell proliferation in the living part of the tumor.
2. In the rat study, oncothermia treatment could significantly increase the tumor tissue oxigenisation which created the basis of the strong synergism with radiotherapy and some chemotherapy.

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