Conference Paper

Oncothermia Basic Research at In Vivo Level: The First Results in Japan

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This paper summarizes the first results of oncothermia basic research conducted in Tottori University, Japan, and had two parts. In the first part C26 murine colorectal cancer model was investigated and oncothermia treatment induced histomorphological and some molecular changes which were examined. In the second study 9L rat glioma model was used to investigate the oncothermia treatment effects on tumor tissue oxygenization. Results of these investigations are very important in oncothermia research because this was the first time when independent research laboratory has repeated oncothermia experiments and proved the significant antitumoral and beneficial effects of oncothermia treatment.

1. 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.

2. Materials and Methods

2.1. Study I. In the first study we examine the effect of oncothermia treatment in a mouse tumor model.

2.1.1. Animal Model. Colon 26 (murine colorectal cancer) cell line derived allograft mouse tumor model was used for this study with double tumors (see Figure 1). The use of the mice and the procedures used in this study were approved by the Animal Research Committee of Tottori University.

2.1.2. Experimental Setup and Treatment. A single shot 30 min 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) (see Figure 2).

2.1.3. Study Design. 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.

FIGURE 1: Experimental mouse tumor model. Every animal had two tumors on the femoral regions, the right side was treated and the left side was individual control.

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

There were 3 treated animals and 1 untreated control animal in all sampling group in every time point (see Figure 3).

2.1.4. 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 were performed (HE staining and IHCH detection were performed 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.

2.2. Study II. In the second study we examined the effects of OTM to tumor oxygenization using a rat tumor model.

2.2.1. Animal Model. 9L (rat glioma) cell line derived allograft rat tumor model was used. All animals had 2 tumors on 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.

2.2.2. Oxygen Level Measurement. Tumor tissue oxygenization level was measured using an O_2 sensitive electrode system (Eikon Kagaku Ltd. 150D model).

2.2.3. Study Design. In 11 rats, tumor tissue oxygenization 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 teflon catheter, and 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

FIGURE 3: Oncothermia treated experimental animals in this study.

FIGURE 4: The study design. The 9L glioma cell line derived rat allograft tumor model (a), the oncothermia treatment procedure (b), and the tissue oxygenization measurement system (c).

thetreatment the tumor oxygenization level was measured again (see Figure 4).

3. Results

(i) Study I

- (a) Histomorphological Changes in a Qualitative and a Quantitative Way (see Figure 5).
- (b) Histomorphological Changes in Details (see Figure 6).
- (c) TUNEL Reaction (see Figure 7).
- (d) Ki-67 Expression Changes (see Figures 8 and 9).

FIGURE 5: All the tumor samples involved in this study and the result graph of the quantitative analysis of the living/dead area ratio measurement. Drastic and selective tumor destruction was detected after 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.

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

(ii) Study II

(a) Results of the Tumor pO_2 Level Measurement in a Rat Tumor Model (see Figure 10).

4. Conclusions

(1) In the mouse study, oncothermia treatment can significantly destroy the tumor tissue in a large volume

of the tumor even with single shot way. Oncothermia treatment induces apoptotic cell death in the destroyed tumor tissue and effectively inhibits cell proliferation in the living part of the tumor.

(2) In the rat study, oncothermia treatment can significantly increase the tumor tissue oxygenization which creates the basis of the strong synergism with radiotherapy and some chemotherapy.

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.

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 was 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 chosen area of the living part of the tumor samples. In a very interesting way the number of Ki-67 positive cells was significantly decreased in the living part of the treated tumor compared to the control tumors.

FIGURE 10: Result of the tumor tissue oxygenization level measurement in each animal (a) and in average (b). Tumor tissue pO₂ level was significantly higher right after the oncothermia treatment compared the pO₂ level measured right before the treatment in case of 10 out of the total 11 animals. The pO₂ level was almost double after the treatment in average.

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