P-07: Andocs G., Okamoto Y., Osaki T., Tsuka T., Imagawa T., Minami S., Balogh L., Meggyeshazi N., Szasz O. (2012) Oncothermia basic research at in vivo level. The first results in Japan

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

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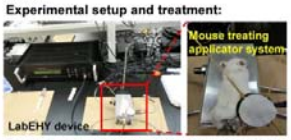
Background: Oncothermia method (OTM) is a long time (since 1989) applied method in oncology.[1] with great clinical success.[2] Oncothermia research group conducts investigations to reveal the basic mechanism of action of this tumor treatment method in basic research level performing a huge number of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

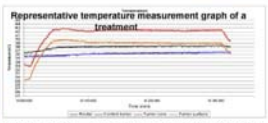
Methods

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 model with double tumors. Every animal had two tumors on the femoral region, the right side (○) was treated, the left side (○) was individual control.

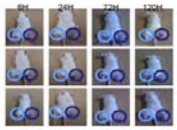
Experimental setup and treatment:





A single shot 30 min oncothermia treatment was done, reaching maximum 41-42°C intratumoral temperature, using the LabEHY system (Oncotherm Ltd.), under precise tumor temperature control using fluoroptic temperature measurement system (Lumasense m3300).

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. All time-group there were 3 treated animals and 1 untreated control animal.



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 detection were performed. Samples were evaluated using complex histomorphological methods.

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 heterotopic allograft rat tumor model with double tumors in both femoral region. Tumor tissue oxigenization was measured in the tumor on the right side.

Oxygen level measurement: pO_2 sensitive elektrode system (Eikon Kagaku Ltd.150D model)

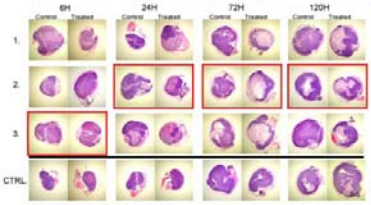
Study design:

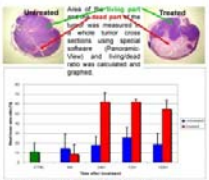
In 11 rats, tumor tissue oxigenization level was measured using a pO2 sensitive electrode system right before the treatment. Then a single shot, 30min oncothermia treatment was performed reaching maximum 42oC intratumoral temperature. Right after the treatment the tumor oxigenization level was measured again.

Results

Study I. :

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





Result graph of the living/dead area ratio measurement of the whole tumor cross sections

Drastic and selective tumor-destruction was detected after a single shot OTM. The tumor destruction was not immediate, it had a time-delay. Samples marked with a red rectangle are evaluated in details.

References:

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[2]Szasz A. Szasz N. Szasz O. (2010) Oncothermia. Principles and Practices, Springer Verlag.
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[3]Andocs G, Szasz O, Szasz A. (2009): Oncothermia treatment of cancer: from the laboratory to clinic. Electromagn Biol Med. 28(2):148-65.
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[5] Meggyeshazi N. Programmed cell death induced by modulated electro-hyperthermia. ICHS 2012
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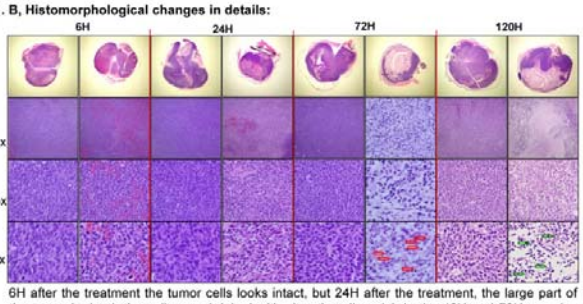
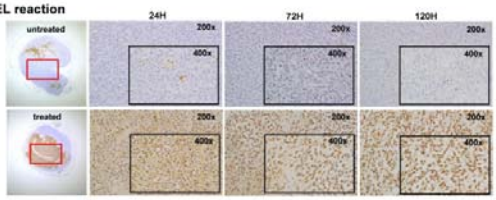
1. B, Histomorphological changes in details:



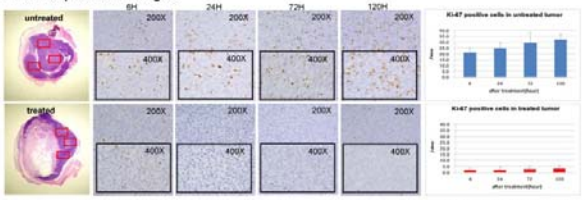
6H after the treatment the tumor cells looks intact, but 24H after the treatment, the large part of the tumor is dead, the cells are shrinked with picnotic cell nuclei. In the 48H and 72H samples definite late morphological signs of apoptotic cell death was observed: extremely high number of apoptotic bodies(▭). 120H after the treatment morphological signs of leukocyte (mostly neutrophiles▭) invasion can be visible.

2.TUNEL reaction



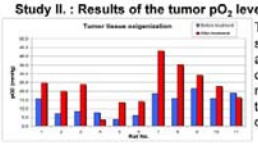
TUNEL assay enzymatically labels the DNA fragments resulted by apoptotic cell death process. In the dead tumor area a huge number of TUNEL-positive cells were observed after a single shot OTM treatment.

3. Ki-67 expression changes

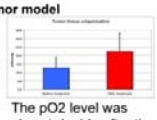


The Ki-67 proliferation marker protein is expressing in the nuclear membrane only in the dividing cells. That is why sampling for Ki-67 positive cell counting was done from the living part of the tumors (▭). In a very interesting way the number of Ki-67 positive cells were significantly decreased in the living part of the treated tumor compared to the control tumors.

Study II. : Results of the tumor pO_2 level measurement in a rat tumor model



Tumor tissue pO_2 level was significantly higher right after the OTM treatment compared the pO_2 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.

Conclusions

1. In the mouse study, OTM treatment can significantly destroy the tumor tissue in a large volume of the tumor even with a single shot way. OTM 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, OTM treatment can significantly increase the tumor tissue oxigenisation which creates the basis of the strong synergism with radiotherapy and some chemotherapy.

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TATEYAMA KAGAKU GROUP