Increased efficacy in treatment of glioma by a new modulated electro-hyperthermia (mEHT) protocol

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Presented at 36th ICHS, Budapest, 2018

Cite this article as:
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Introduction
Modulated electro-hyperthermia (mEHT) is an effective and widespread supplemental therapy in cancer treatment using the radiofrequency (RF) of 13.56 MHz and a fractalphysiology-based modulation frequency based on selective heating of the tumors. From the Pennes equation... We used an animal model to demonstrate the hypothesis in vivo.

Methods
RG2 [D74] (ATCC®, CRL 2433™) glioma cell line was inoculated into the parietal lobe of syngeneic Fischer 344 rats. This model mimics the human malignant astrocytoma by having incompetent BBB. A gadolinium-based MRI contrast agent (MAGNEVIST®, 0.5 mmol/mL, 0.2 mL/kg bdw) was used to detect lesions associated with altered blood-brain barrier and the volume of the tumor was quantificated at the 8th and 15th days after inoculations (AMIDE® software). The animals was divided randomly in 4 groups: sham (3), treated with classical mEHT protocol (3), treated with new mEHT protocol (3), treated with classical mEHT protocol and with the temozolamide (30 mg/kg bdw for 5 days), an oral chemotherapy drug used as a second-line treatment for astrocytoma and a first-line treatment for glioblastoma multiforme (1). We applied the mEHT treatment at 6th, 9th, 11th and 13th days after inoculations.

Results
As a result of a tecnological improvement we used a new cooling system wich was able to prevent the overheating of the skin below the RF electrode and above the skull with high electrical impedance. Consequently based on a stepwise protocol we could apply extremly high energies (even 10 W) to reach as soon as possible the requested temperature into the brain. The brain temperature was evaluated indirectly by the measurement of the temperature in the middle ear and by using a correlation curve set up in an earlier experiment. The tumor growing rate between the 8th and 15th days after inoculations was in the case of sham animals: 23.73±12.15, treated with classical mEHT protocol: 19.08±0.49, treated with new mEHT protocol: 6.83±2.02, treated with classical mEHT protocol and with the temozolamide: 7.99.

Conclusion
Text The application of the new cooling system allowed us to set up in the case of glioma a new mEHT protocol which is based on that principle to reach a very high specific absorption rate in the treated tissue. This new protocol was more efficient as the classical one and
surprisingly looks like more efficient/similarly efficient than the classical one combined with chemotherapy.

This study was supported by the Hungarian National Research, Development and Innovation Office (NVKP_16-1-2016-0042)
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Selection of the malignant cells by biophysical differences

1 Selects the tumor
PET image

2 Selects the malignant cells
Cancer cell

3 Energy absorption on membrane rafts

4 Modulation (fractal physiology)

Challenge of the dose of oncological hyperthermia

mEHT heats the cell-membrane rafts

Quasi adiabatic energy transfer

Target: nano-clusters (rafts)

Energy flow

Signal transduction

References:
- Andocs G et al. Radiology and Oncology 185:120-26, 2009
- E. Papp et al. 2017
Power equation (Pennes equation)

\[
\rho c \frac{\partial T}{\partial t} = \nabla \left( k \nabla T \right) + w_b c_b (T_a - T) + q_m + \rho SAR
\]

where \( \rho, c, \) and \( k \) are the density (kg/m^3), the specific heat (J/(kg K)), and the tissue thermal conductivity (W/(m K)), respectively; \( w_b \) is the mass flow rate of blood per unit volume of tissue (kg/m^3); \( c_b \) is the blood specific heat; \( q_m \) is the metabolic heat generation per unit volume (W/m^3); \( T_a \) represents the temperature of arterial blood (K); \( T \) is the actual temperature risen above the ambient level; \( \Delta T/\Delta t \) is the rate of temperature rise. (SAR (Specific Absorption Rate) – (W/kg))

Simplified (no derivatives)

\[
\frac{\rho c A T}{\Delta t} = \frac{1}{\Delta x} \left( k \frac{A T}{A x} \right) + w_b c_b (\Delta T) + (\Delta T) + \rho SAR
\]

Our tasks:
1. Keep the time-dependent part (SAR) large
2. Keep the environmental (space-dependent) part small
3. Keep the compensating energy small

Compensates only the energy-loss by blood

Challenge of the dose of oncological hyperthermia

HepG2 cell-line, human hepatocyte carcinoma

Yuk-Wah Tsang (2017)

Apoptosis
Phase 1+2+3+4 = 31.6 %
Phase 1+2+3 = 31.2 %
Phase 1+2 = 25.9 %
Phase 1 = 13.3 %

42°C

Apoptosis

Power (energy pump)
Phase 1, 2 → 18 W
Phase 3, 4 → 7.5 W

Apoptosis measured by expression of Annexin

Annexin V positive cells (%)

Without AuNP
With AuNP

AuNP heats conventionally, produces the same apoptotic rate than without when heated to the same temperature

but

AuNP heated with oncothermia the effect of apoptosis decreases, because less energy is given to the malignant cells directly when AuNP is also heated
Challenge of the dose of oncological hyperthermia

\[
\frac{\Delta T}{\Delta t} = \left( \text{temperature growth by time in the tumor} \right) = c \cdot \text{SAR}
\]

Physiological washout time (~ 2 min, in rats)

Finish earlier

End of the session

Step-up protocol by physiology of the target

Higher dose was given
- in shorter time
- safer conditions

Time from start of heating

inoculation

RG2 (syngeneic)

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16

mEHT treatment

Time (days)
Development on an evaporation-based cooling system

Calibration curve:
The temperature in the brain area under the electrode is 1.78°C higher than in the middle ear.

The application of the new cooling system allowed us to achieve a very high quasi adiabatic specific absorption rate in the treated tissue.
Washout break,

\[ Y = Y_0 + M^{3/4} \]


Effective, evaporation-based cooling system

Mediso nanoScan 1T small animal MRI system
and a 3D image acquisition sequence

Sham

mEHT

imEHT

MAGNEVIST®,
0.5 mmol/mL,
0.2 mL/kg bdw
Tumor size after the treatment (15\textsuperscript{th} day)

Quantification of the MRI results

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>mEHT</th>
<th>imEHT</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>8\textsuperscript{th} day MRI (volume, mm\textsuperscript{3})</strong></td>
<td>13,76342773</td>
<td>321,4416504</td>
<td>12,35961914</td>
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<tr>
<td></td>
<td>3,479003906</td>
<td>125,5187988</td>
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<td>13,58032227</td>
<td>319,6105957</td>
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<tr>
<td><strong>Mean</strong></td>
<td>10,2742513</td>
<td>255,5237</td>
<td>11,98324</td>
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<tr>
<td><strong>SD</strong></td>
<td>5,885568985</td>
<td>112,5913</td>
<td>0,549599</td>
</tr>
</tbody>
</table>
HSP70  Ki67  HMGB1

Sham  

mEHT  

ImEHT  

Chemotherapy  Radiation  Adenoviral vectors

HMGB1

Angelopoulou et al, J Mol Med (2016)
microPET

12.8 MBq 18F-FDG i.v. through the tail vein

Continuous measurement for 50 minutes with microPET P4 (Concorde microsystems)

The image reconstructed after 10 minutes of measurement 40 minutes after FDG administration (spatial resolution 1.8x1.8x1.8 mm)

Manually MRI Corr registation with VivoQuant

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Thank you for your attention!