Treatment protocol for studying the effect of modulated electro-hyperthermia on melanoma lung metastasis in a mouse model

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Introduction

Modulated electro-hyperthermia (mEHT) is a non-invasive method for locally targeting tumor cells by applying radiofrequency (RF) of 13.56 MHz. Tumors have elevated glycolysis due to the Warburg effect. As a result, there is increased lactate production and reduced electric impedance in tumor cells, leading to increased permittivity and conductivity, which support mEHT to selectively induce apoptosis in malignant tumor cells. Here we look at the protocol, optimized for treating melanoma lung metastasis using mEHT in a mouse model.

Methods

Lung vs laryngopharyngeal temperature correlation setup

Measuring lung temperature is crucial to ensure that the target temperature of 41-42 °C is reached and maintained during treatment. Direct measurement of lung temperature during treatment, however, is highly invasive and could result in extensive damage to the treated lungs. Here, we sort out to establish a method of measuring lung temperature indirectly by demonstrating that a strong correlation exist between the main bronchi and laryngopharyngeal temperature.

Treatment setup and protocol – pilot studies

Lung metastasis was induced by tail vein injection of B16-F10 melanoma cells into C57Bl/6 mice. The following day mice were treated with mEHT (n=6). mEHT treatment of the lungs was performed every third day for a total of 6 times with LabEhy200 (Oncotherm TM) with a treatment protocol set up to maintain 41-42 °C inside the lungs. Treatment was done with a regular round electrode that covers the thorax. Mice were sacrificed on day 18 and metastatic nodules were counted.

Electrode re-design

Pilot studies with regular round electrode covering the thorax revealed extensive burning on the skin underlying the upper treatment electrode. This may have been caused by the relative high impedance of structures (sternum, ribs, air in lungs) in the thoracic region causing a higher power concentration on the overlying skin. Although the target temperature range of 41-42 °C in the lungs was achievable, an unavoidable side effect was the observed burning. We therefore aimed to redesign a customized electrode for lung treatment, capable of preventing this burning.

Results and discussion

Text Our results demonstrate that a temperature correlation exist between the main bronchi and the laryngopharynx in mice with an average laryngopharyngeal-bronchial temperature difference of 1.44 ± 0.46 °C (n = 4). Pilot studies also demonstrated that, when mice induced with B16-F10 melanoma in the lungs were treated with mEHT, a relative reduction in the number of metastatic nodules was observed compared to the control group. In addition, our redesigned electrode, customized for the lung treatment showed markedly reduced skin damage.

Conclusion

Taken together, our results demonstrate that a temperature correlation exist between the main bronchi and the laryngopharynx in mice which proved useful in estimating the lung temperature during treatment by only measuring the laryngopharyngeal temperature non-invasively. Our pilot studies indicated that mEHT treatment may have a beneficial effect in reducing the number of melanoma metastasis in the lung.

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Introduction

Modulated electro-hyperthermia (mEHT) is a non-invasive method of locally targeting tumor cells for destruction applying a radiofrequency (RF) of 13.56-MHz. Tumors have elevated glycolysis due to Warburg effect. As a result, there is increase lactate production and reduced electric impedance in tumor cells, leading to increase permittivity and conductivity, which support mEHT to selectively induce apoptosis in malignant tumor cells. Here we look at the effect of mEHT on B16F10 melanoma metastasis in a mouse lung model.

MATERIALS AND METHODS

Treatment setup and protocol

Lung metastasis was induced by tail vein injection of B16F10 melanoma cells into C57BL/6 mice. The following day mice were treated with mEHT alone, with mEHT and aspirin (ASA, 11.1 mmol/L) administered in drinking water during the entire experiment or left untreated. 30 min mEHT treatment of the lungs was performed every third day for a total of 6 times with LabEhy200 (Oncotherm TM) with a treatment protocol set up to maintain 41-42 °C inside the mice lung.

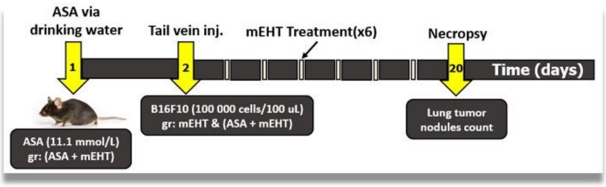


Figure 1 Experimental timeline for lung treatment with mEHT

Lung temperature correlation setup

Measuring lung temperature is crucial to ensuring that the target temperature of 41-42 °C is reached and maintained during treatment. Direct measurement of lung temperature during treatment however is highly invasive. Here, we sort out to establish a method of measuring lung temperature indirectly by demonstrating that a correlation exist between the lung and laryngopharyngeal temperature.

	Mice 1	Mice 2	Mice 3	Mice 4
Average temp. diff. (°C)	1.16	1.76	0.95	1.89
SD	0.1114	0.1515	0.1075	0.2365

Table showing correlation between lung and laryngopharyngeal temperature in mice (n = 4). Average temperature difference was calculated from time 10 min to 30 min of mEHT treatment across all animals. Average difference across all animals was 1.44 ± 0.4559 °C. Based on this, a laryngopharyngeal temperature of approx.. 40 °C was decided optimal to ensure a temp. range of 41 – 42 °C in the lungs for all subsequent treatments.

Electrode design for lung treatment

Pilot studies with regular round electrode covering the thorax revealed extensive burning on the skin underlying the upper treatment electrode. This may have been caused by the relative high impedance of structures (sternum, ribs, air in lungs) in the thoracic region causing a higher power concentration on the overlying skin. Although the target temperature range of 41-42 °C in the lungs was achieved, an unavoidable side effect was the observed burning. We therefore aimed to redesign a customized electrode for lung treatment, capable of preventing or reducing the extent of burning.

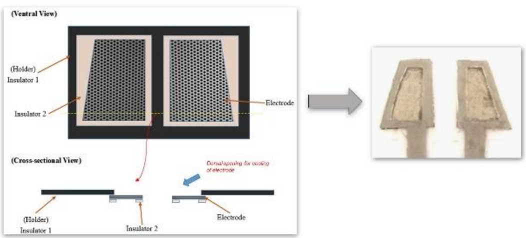
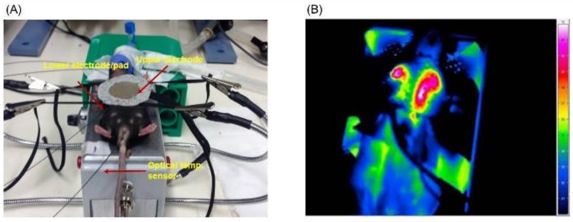


Figure 2 Customized electrode for lung treatment

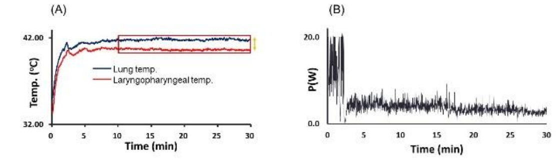
(A) Schematics of customized electrode designed for lung treatment (B) Lung treatment electrode designed based on schematics. Silicon material was used as insulator. Significant reduction in burning relative to regular round electrode was observed when used in treatment of mice with B16F10 melanoma primary nodules in the lungs.

MATERIALS AND METHODS

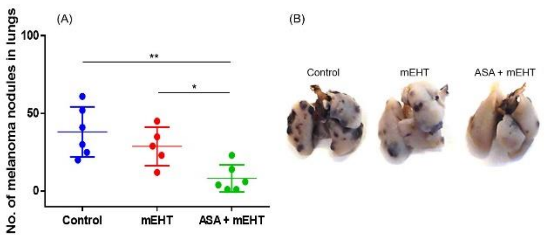


(A) Experimental setup showing positioning of mouse, treatment electrodes and optical temperature sensors. Optical temperature sensors were positioned in laryngopharynx, skin beneath electrode, rectum and pad. (B) Thermal camera imaging showing localized increased in temperature in lung region.

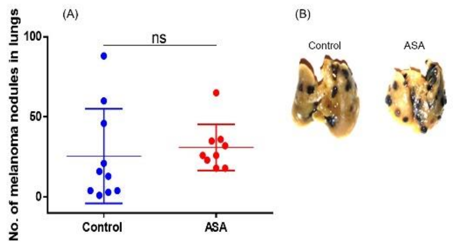
RESULTS



(A) Representative plot showing correlation between lung and laryngopharyngeal temperature in mouse under treatment with mEHT. Lung temp. was maintained at 41 – 42 °C during course of treatment for all animals. (B) Representative plot showing power variation during course of mEHT treatment.



(A) Plot of number of B16F10 melanoma metastatic lung nodules in control, mEHT and (ASA + mEHT) experimental groups. mEHT treatment alone showed some reduction in number of metastatic nodules, although this was not significant. mEHT when combined with aspirin (ASA) however showed a significant reduction in the number of metastatic nodules (* $p < 0.05$, ** $p < 0.01$, one-way ANOVA). (B) Representative lungs in each experimental groups showing B16F10 melanoma metastatic lung nodules. Significant reduction in nodules was observed when ASA was combined with mEHT treatment.



(A) Plot of number of B16F10 melanoma metastatic lung nodules in control and ASA groups. (B) Representative lungs with metastatic nodules for control and ASA given experimental groups. No significant difference in number of metastatic nodules was observed between both groups.

CONCLUSIONS

Our results demonstrate that a temperature correlation exist between the main bronchi and the laryngopharynx in mice which proved useful in estimating lung temperature during treatment by only measuring the laryngopharyngeal temperature non-invasively. Pilot studies indicated that aspirin (ASA) when combined with mEHT treatment resulted in a significant reduction in the number of B16F10 melanoma metastatic nodules in the lungs. Aspirin alone, under the concentration used in this experiment did not seem to inhibit the growth of B16F10 melanoma nodules in the lungs. This suggests that this inhibitory effect may only exist when ASA and mEHT are combined together and not separately.

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