UNIVERSITI TEKNOLOGI MARA

EFFECTS OF HYPO- AND HYPERTHERMIA, BERBERINE AND THE COMBINATION OF BERBERINE WITH HYPERTHERMIA ON OSTEOSARCOMA CELLS

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AUTHOR'S DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Teknologi MARA. It is original and is the results of my own work, unless otherwise indicated or acknowledged as referenced work. This thesis has not been submitted to any other academic institution or non-academic institution for any degree or qualification.

I, hereby, acknowledge that I have been supplied with the Academic Rules and Regulations for Post Graduate, Universiti Teknologi MARA, regulating the conduct of my study and research.

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ABSTRACT

Osteosarcoma is the most common primary malignant bone tumour with a high resistance to chemo- and radiotherapy. Hyperthermia is a well-established type of cancer treatment. However, the molecular changes and responses of osteosarcoma cells to hyperthermia are not well understood. Hypothermia has been proven to be protective in certain medical situations such as brain surgery, but there is no published study about its effect in bone cancer. According to literature, about 60% of all cancer patients take supplements without informing their oncologists during therapy. Little is known about the effect of antioxidants like berberine chloride on osteosarcoma cells, especially in combination with hyperthermia. Berberine is a natural alkaloid available in several traditional herbs, and it can help treat many pathological conditions. The overall objective of this study is to investigate the short- and long-term effects of various stages of hyper- and hypothermia on osteoblast-like osteosarcoma cells and its underlying mechanism of action. It also seeks to study the long-term effect of a single short-term treatment with severe hyperthermia (45°C, 1 h) on osteosarcoma cells and its underlying causes. An additional objective is to investigate the effect of hyperthermia alone and in combination with berberine chloride on osteosarcoma cells and its underlying mechanisms. Osteoblast-like osteosarcoma cells (MG-63 cells) were treated with hyper- and hypothermia for short, medium and long-term periods. Some cells were also treated with berberine chloride and a combination of berberine chloride with mild, moderate, and severe hyperthermia. Severe hypothermia and hyperthermia showed a time-dependent toxicity; hence viability was reduced in a significant manner at all time points, whereas mild hypothermia showed a protective effect. Severe hyperthermia induced significant DNA damage at all time points. Severe and mild hyperthermia (1 h) in the present study resulted in the downregulation of CIRBP, which may explain the significant cell death. Caspase-3/7, 8, and 9 showed very low activity at 12, 24 and 72 h post-treatment with severe hyperthermia due to RNA degradation and massive cell death. On the other hand, the effect of severe hyperthermia on the cytoskeleton was lethal at 12 h and onward. The long-term effect of severe hyperthermia (1 h at 45ºC and recovery at 37ºC for 72 h) activates caspase-3/7, 4, 8, 9, and 12 in association with a significant reduction of Hsp90-alpha expression and induced apoptosis. Additionally, hyperthermia suppressed RANKL mRNA expression and elevated Osterix, whereas RUNX2 showed levels similar to untreated control. The changes in RANKL and Osterix expression in this study indicate that hyperthermia may be inducing differentiation of osteosarcoma. Berberine chloride (80 μg/ml) induced apoptosis in a significant manner. Mild hyperthermia (39°C) resulted in the attenuation of berberine chloride cytotoxicity against osteosarcoma cells in a significant way. All treatments of berberine, hyperthermia, and hyperthermia combination with berberine chloride induced apoptosis and suppressed enzymatic activity and mRNA expression of caspase-3/7, 8, and 9. In conclusion, severe hypothermia showed an anti-proliferative apoptotic effect; severe hyperthermia was more effective in bone cancer killing at 12 h and above, and mild hyperthermia attenuated the cytotoxicity of berberine chloride.

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