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. Hyperthermia 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 hyperthermia and hypothermia showed a time-dependent toxicity; hence viability was reduced in a significant manner at all time points, whereas mild hyperthermia 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 45°C and recovery at 37°C 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 hyperthermia 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 chlori.

Trabeculectomy is the gold standard procedure performed in glaucoma when topical medication and laser intervention fail to maintain the ideal intraocular pressure (IOP) of patient’s eye. However, excessive accumulation of extracellular matrix components (ECM) mediated by Tenon’s fibroblast (HTF) leads to significant cases of surgical failure. Anti-vascular endothelial growth factor (VEGF) has become the focus in current scar modulation strategy. Improved bleb morphology following trabeculectomy augmented with ranibizumab has been reported. However, mechanism of actions of ranibizumab on HTF is not well understood. Therefore, this in vitro study was conducted to elucidate mechanism of actions of ranibizumab on HTF. HTF used in this study were propagated from Tenon’s capsule obtained from patients undergoing trabeculectomy. Firstly, isolated and characterized HTF were treated with different concentrations of ranibizumab in serum and serum-free media for 24 and 48 hours and then HTF viability was measured using MTT assay. Then, HTF were extracted to measure the expression of collagen Type 1 (COL1A1), fibronectin (FN), transforming growth factor-β1 and -β2 (TGF-β1 & TGF-β2) using qRT-PCR and ELISA. The experiment was followed with metabolomics profiling which was performed to identify the most significant metabolite regulated by ranibizumab. Finally, the expression of regulatory genes and proteins involved in the cell cycle regulation and angiogenesis including p21, p53, CDK2, CDK4, PTEN, AKT1 and THBS1 were measured by RT2 Profiler PCR Array and Western Blot. Findings from the MTT assay showed that ranibizumab at the concentration of 0.5 mg/ml induce significant reduction in HTFs viability. The optimum degree of reduction was observed in serum-free media incubated for 48 hours. Furthermore, results suggested that ranibizumab mediates the down-regulation of COL1A1 and TGF-β1 gene level, but not at the protein level. No relevant changes were observed in FN and TGF-β2 mRNA level, but the proteins level was up-regulated. In metabolomics study, ranibizumab was shown to induce significant reduction in spermide and spermidine level. Therefore in subsequent experiment, ranibizumab effects were compared to DFMO, a potent irreversible inhibitor in spermidine synthesis. Findings show that ranibizumab exerts similar mechanism to DFMO in regulating spermide expression by HTF, where it reduces PTEN, AKT1 and THBS1 expression. Moreover, ranibizumab administration increase p53 and p21 expression and reduces CDK2 and CDK4 expression. These observations suggest that ranibizumab might exert its anti-scarring property by enhancing the activities of p53 and p21, thus lead to reduction in CDK2 and CDK4. This shows that cell cycle of ranibizumab-treated HTF could be arrested, particularly at G1 phase.