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Influence of Lycopene on the mRNA Expression of Vascular Endothelial Growth Factor (VEGF) in Osteoblast Cells

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Abstract

Introduction*: This research investigates the impact of lycopene, a natural carotenoid found in various fruits and vegetables, on the mRNA expression of Vascular Endothelial Growth Factor (VEGF) in MG-63 human osteoblast cells. VEGF plays a crucial role in angiogenesis, a process vital for bone formation and repair. By exploring the influence of lycopene on VEGF expression, this study aims to shed light on its potential role in enhancing bone health.*

Materials and Methods: *The MG-63 cells were cultured and treated with lycopene at concentrations of 1µM/ml and 10µM/ml for 24h and 48h. Total RNA was extracted, converted to cDNA, and subjected to quantitative real-time PCR to analyze VEGF gene expression. Statistical analysis employed one-way ANOVA and Newman-Keuls tests for significance.*

Results: *Results revealed a significant upregulation of VEGF mRNA expression in MG-63 cells after lycopene treatment at both 24 and 48 hours. This suggests that lycopene administration may positively influence VEGF expression in osteoblast cells, potentially impacting angiogenesis and bone regeneration processes. Mean ± SD for Lycopene 1.0µM/ml is 2.15±0.49 and Lycopene 10µM/ml is 3.35±1.20 with a significance of 0.00.*

Conclusion: *This study demonstrates that lycopene treatment modulates VEGF mRNA expression in MG-63 human osteoblast cells, indicating its potential to promote angiogenesis and contribute to bone development and regeneration. These findings underscore the beneficial effects of lycopene on bone health, warranting further exploration of its therapeutic potential in bone-related disorders.*

Keywords: Lycopene, Vascular Endothelial Growth Factor (VEGF), Osteoblast Cells, Mrna Expression, Angiogenesis, Bone Health

Introduction

Osteoporosis, a debilitating skeletal disorder characterized by reduced bone mass and deterioration of bone microarchitecture, poses a significant global health burden, particularly in aging populations. Among many factors contributing to bone health, angiogenesis, the formation of new blood vessels, plays a crucial role in maintaining bone homeostasis [1]. Vascular endothelial growth factor (VEGF) is a pivotal regulator of angiogenesis and has been implicated in the regulation of bone remodeling and repair. Dysregulation of VEGF expression in osteoblasts, the bone-forming cells, has been associated with pathological conditions such as osteoporosis and delayed fracture healing [2].

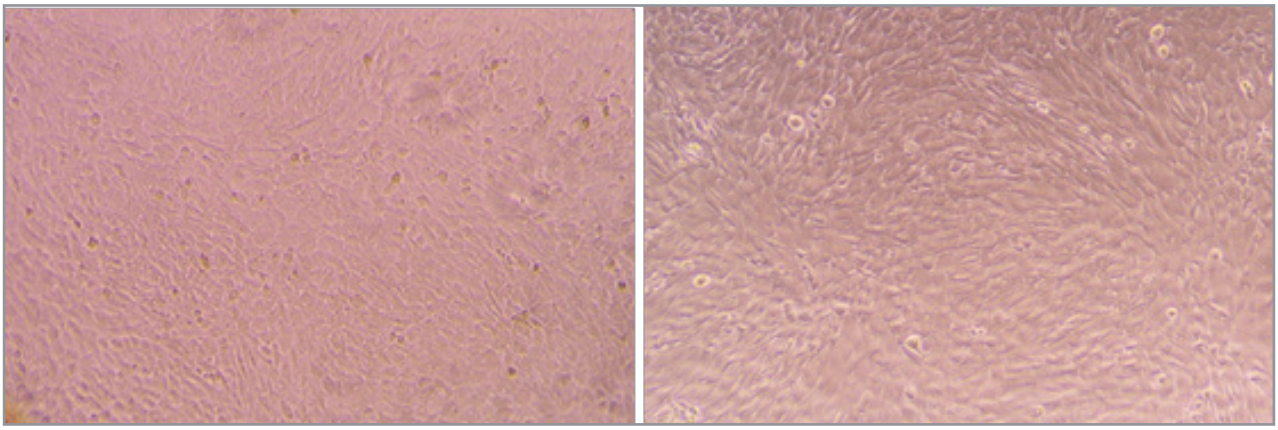
Lycopene, a naturally occurring carotenoid found abundantly in tomatoes and other fruits, has garnered considerable attention for its potential health benefits. Extensive research has linked lycopene consumption to reduced risks of chronic diseases, including cardiovascular disease, cancer, and osteoporosis. Moreover, recent studies have suggested that lycopene may modulate VEGF expression in various cell types, indicating its potential role in angiogenesis regulation [3]. Despite growing evidence supporting the association between lycopene and VEGF expression in different cellular contexts, there is a paucity of data on its specific effects on VEGF mRNA expression in osteoblast cells [4]. Understanding the impact of lycopene on VEGF expression

in osteoblasts holds significant implications for bone health and may offer new insights into therapeutic approaches for managing osteoporosis and related skeletal disorders [5].

Previously, a study investigated the potential benefits of lycopene, a natural antioxidant, on bone metabolism and the functional activity of osteoblastic cells in ovariectomized female rats, a model for postmenopausal osteoporosis. The combined in vitro and in vivo findings suggest that lycopene may offer protective benefits to bone health in the context of postmenopausal osteoporosis. The observed increase in osteoblastic cell proliferation and ALP activity, along with the upregulation of key osteogenic genes, supports the idea that lycopene positively influences the functional activity of osteoblasts [6]. In this study, we aim to investigate the effect of lycopene on VEGF mRNA expression in osteoblast cells. We will utilize a well-established osteoblast cell culture model and employ quantitative real-time polymerase chain reaction (qRT-PCR) techniques to measure changes in VEGF mRNA levels upon lycopene treatment. Furthermore, we will explore potential underlying molecular mechanisms to elucidate how lycopene modulates VEGF expression in osteoblasts. By shedding light on the relationship between lycopene and VEGF expression in osteoblast cells, this research may pave the way for novel therapeutic strategies to improve bone health and potentially provide a basis for the development of lycopene-based supplements or interventions for individuals at risk of osteoporosis and related bone disorders.

Materials and Methods Reagents and Chemicals

Trypsin-EDTA, fetal bovine serum (FBS), antibiotics-antimycotics, Dulbecco's modified Eagle's medium (DMEM), Lycopene, and phosphate-buffered saline (PBS) were purchased from Gibco, Canada. Chloroform, isopropanol, Tris, glycine, EDTA, sodium bicarbonate, BSA and TRI reagent were purchased from Sigma-Aldrich (St. Louis, USA). Oligonucleotide primers for VEGF, and β-actin (Sigma-Aldrich Company St. Louis, MO, USA), iScriptc DNA synthesis kit (Bio-Rad, USA) and quantitative real-time RT-PCR reaction KAPA SYBR® FAST PCR master mix kit (Kapa Biosystems, USA) were also used in the present study.



a) 24hr **b)** 48hrs

Figure 1: The human osteoblast cell line MG-63.

Human Osteoblast cell line MG-63 was procured from National Center for Cell Sciences (NCCS), Pune, India. The cells were cultured in DMEM containing 10% heat- inactivated fetal bovine serum and antibiotics at 37°C in 5% CO2 and 95% air. Lycopene (1µM/ml and 10µM/ml) was treated in Human Osteoblast cell line MG-63 for 24h and 48h.

Quantitative Real-time PCR

The Control and Lycopene (1µM/ml and 10µM/ml) treated in MG-63 cells were washed with PBS and added 100 ul of Trizol reagent. Total RNA was extracted using the protocol mentioned in the kit and quantified using Nanodrop (Thermo Scientific). The RNA was converted to cDNA using the cDNA conversion kit (Promega). cDNA, the target primer for VEGF gene, was processed with master mix (SYBR Green master mix, Life Technologies, 4385612) using a PCR system. Results were analyzed with a 2-ΔΔCT method, □- actin used as an internal control, and normalized for this study.

Statistical Analysis

Data were subjected to one-way analysis of variance (ANOVA) and Newman-Keuls test. $P < 0.05$ was considered statistically significant.

Results

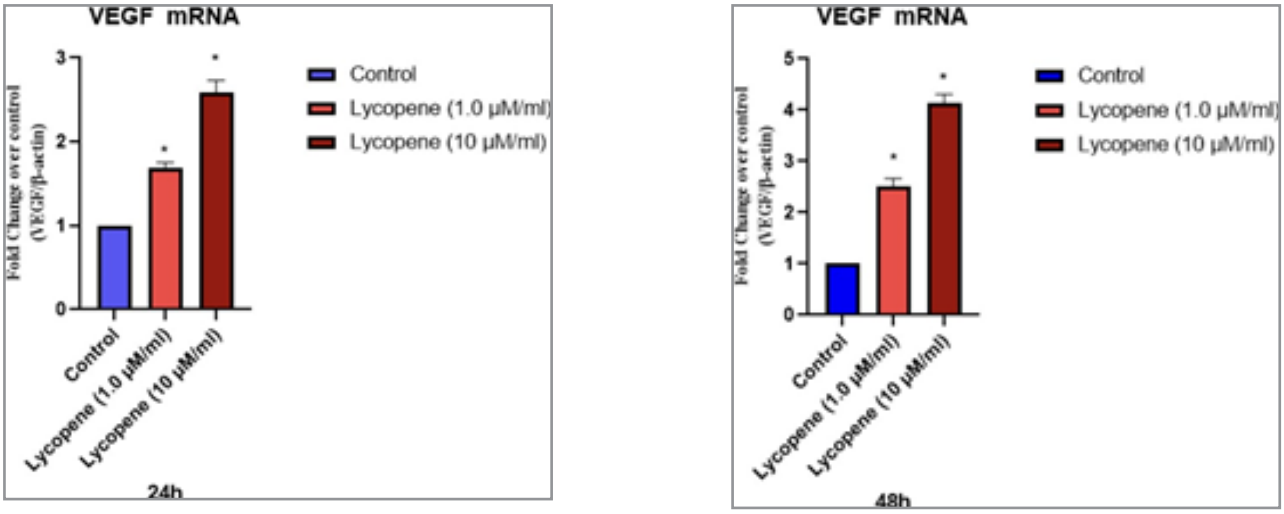


Figure 2: Effect of Lycopene on VEGF mRNA expression in Osteoblast cells. Representative graph showing real-time RT-PCR amplification of VEGF mRNA expression treated with Lycopene (24h & 48h) in (MG-63) Osteoblast cells. The 2-ΔΔ Ct method of relative quantification was used to determine the fold change in expression with β-actin. Values are mean ± SD of triplicate of 3 independent experiments. '*' denotes statistical significance at the level of $p < 0.00$ when compared with control.

The present study investigated the effect of lycopene on VEGF mRNA expression in MG-63 osteoblast cells using real-time RT-PCR amplification. The 2-ΔΔCt method was employed to determine the fold change in VEGF mRNA expression, with β-actin serving as the reference gene for normalization. The results obtained from this analysis revealed significant changes in VEGF mRNA expression upon lycopene treatment at both 24 and 48 hours. Mean ± SD for Lycopene 1.0µM/ml is 2.15±0.49 and Lycopene 10µM/ml is 3.35±1.20 with a significance of 0.00.

The representative graph (Figure 2) illustrates the relative expression levels of VEGF mRNA in osteoblast cells treated with lycopene compared to the control group. Statistical analysis revealed that the observed changes in VEGF mRNA expression were highly significant ($p < 0.00$) compared to the control group, further emphasizing the robustness of the results. The mean ±

SEM values, derived from triplicate experiments conducted independently, provided a reliable estimate of the effect of lycopene on VEGF mRNA expression.

Discussion

Lycopene, a potent carotenoid and antioxidant known for its singlet oxygen quenching ability, has garnered attention for its role in reducing oxidative stress and promoting bone health. This study delves into the intricate relationship between lycopene and vascular endothelial growth factor (VEGF) mRNA expression in osteoblast cells, shedding light on the potential implications for bone formation and repair. The appreciation of vasculature's role in osteogenesis dates back to 1763 when Albrecht von Haller proposed that blood vessels play a crucial role in bone formation [7]. This historical perspective laid the groundwork for understanding the intricate interplay between vascular factors and bone health. Vascular endothelial growth factor (VEGF) has emerged as a key player in bone repair, as evidenced by studies such as that conducted by [8]. The multifaceted role of VEGF extends beyond angiogenesis, encompassing its involvement in osteogenesis. During bone repair processes, VEGF is expressed in a pattern reminiscent of its role in developmental stages [9].

This sets the stage for exploring the potential modulatory effects of lycopene on VEGF expression in osteoblast cells. The present study contributes to the growing body of evidence supporting the positive impact of lycopene on osteoblast activity, a pivotal factor in bone formation. Reports from various sources underscore the significance of VEGF in both chondrogenesis and osteogenesis, highlighting its multifunctional role in skeletal development and repair [10]. The key finding of the study reveals that lycopene administration leads to a significant increase in VEGF mRNA expression in MG-63 osteoblast cells. This result aligns with the broader understanding of lycopene's ability to modulate cellular processes and underscores its potential as a regulator of VEGF-mediated pathways in bone biology. To comprehend the observed increase in VEGF mRNA expression, it is essential to delve into the potential mechanistic pathways involved.

Lycopene, with its potent antioxidant properties, may act as a modulator of oxidative stress within osteoblast cells. Oxidative stress has been implicated in the regulation of VEGF expression, and lycopene's ability to quench singlet oxygen radicals may contribute to a cellular environment conducive to elevated VEGF mRNA levels [11]. Understanding the interplay between lycopene and VEGF in the context of osteoblast activity holds promise for clinical applications. The promotion of osteoblast function and the concurrent increase in VEGF expression suggest that lycopene supplementation could be explored as a po-

tential therapeutic intervention for conditions involving compromised bone health or impaired bone repair processes [12].

While the current study provides valuable insights, it is essential to acknowledge its limitations. Future research could explore the underlying molecular mechanisms by which lycopene modulates VEGF mRNA expression in osteoblast cells, investigate the impact of lycopene on other angiogenic factors and signaling pathways, and evaluate the in vivo effects of lycopene supplementation on bone regeneration and fracture healing.

Conclusion

In conclusion, our investigation demonstrates that the administration of lycopene induces a modulation in Vascular Endothelial Growth Factor (VEGF) mRNA expression within human osteoblast cells (MG-63). The observed upregulation of VEGF expression suggests that lycopene holds promise in fostering angiogenesis, thereby playing a role in the facilitation of bone development and regeneration. These results offer valuable insights into the positive impact of lycopene on bone health, shedding light on its potential therapeutic applications in conditions related to bone disorders. The findings emphasize the need for further exploration and investigation into the therapeutic potential of lycopene, particularly in the context of bone-related disorders. Ultimately, this research contributes to the growing body of evidence supporting the beneficial effects of lycopene, encouraging continued research endeavors to unlock its full therapeutic potential in promoting skeletal health and addressing associated pathological conditions.

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Conflict of Interest

The authors would like to declare no conflict of interest in the present study.

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