α-Mangostin Exposure on Viability and Migration of Osteoblast Cell Post Inflammatory Induction

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Abstract

Bone regeneration in dentistry happens in the jawbone after simple tooth extraction or bone fracture. The bone regeneration process consists of a continuous and directed phase and takes place in three phases, which are inflammation, proliferation, and remodelling. α-Mangostin has been known to have potential anti-inflammatory effects and is most likely a potential therapeutic agent to inhibit bone resorption caused by the inflammatory process.

Objectives to investigate the effect of α-mangostin exposure on osteoblast cell migration and viability during inflammatory induction.

In vitro laboratory experimental study on 7F2 cell line culture. Cell cultures were divided into 5 groups, those were the cell culture group with osteogenic media, with lipopolysaccharide, with α-mangostin, a combination of lipopolysaccharide and α-mangostin, and a combination of lipopolysaccharide and α-mangostin after 24 hours. The sample will undergo 5 replicates of each of the MTT and scratch wound assays. The One-Way Anova test is used to assess the data results, and a significance level of 0.05 is required.

There was a significant difference in viability and migration between the experimental groups (p=0.000). There was a correlation between viability and migration of osteoblast cell post-inflammation (p=0.000).Exposure to α-mangostin had an impact on the migration and viability of osteoblast cells after inflammation.

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Introduction

The process of bone regeneration is closely related to dentistry, especially in oral and maxillofacial surgery. One example of the process of bone regeneration can be seen in bone healing after tooth extraction. The bone healing process consists of a continuous and directed phase and takes place in three phases, namely inflammation, proliferation, and remodelling. The initial phase of inflammation, which is crucial for bone healing and is influenced by both local and systemic reactions to noxious stimuli, can impair bone healing if it continues.[1–5]

To prevent the occurrence of impaired bone healing, herbal ingredients can be used as an alternative for socket preservation after tooth extraction by reducing inflammatory factors, one of which is mangosteen (*Garcinia mangostana*). Mangosteen consists of active chemical components such as xanthones, phenols, flavonoids, gartanins, saponins, garcinons, tannins, anthocyanins, terpenes, vitamins B1, B2, and other bioactive compounds. Xanthones in the pericarp of mangosteen contain a lot of α-Mangostin and γ-Mangostin.[6–8]

One of the benefits of α-Mangostin is that it can prevent resorption by decreasing the inflammatory response, which will increase osteoclastogenesis, as well as increase cell resistance to oxidative stress produced during the inflammatory process, so that cell death can be suppressed. Previous studies have shown that α-Mangostin inhibits intracellular ROS

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activity. Inhibition of intracellular ROS activity will inhibit the activity of enzymes that activate nuclear factor kappa B (NF-κB) for translocation into the nucleus and regulate proinflammatory mediators such as IL-1, IL-6, TNF-α.[6,9–12]

This research purpose is to investigated the effectiveness of α-mangostin on the bone healing process with parameters of viability and migration of 7F2 osteoblasts after Lipopolysaccharides (LPS) exposure, through the MTT assay and wound healing assay.

Materials and methods

Research Samples

The study was performed use of an experimental laboratory study using 7F2 ATCC CRL-12557 osteoblast cell culture (Food Industry Research and Development Institute, Taiwan) with a pre and post-test control group design.

Research Methods

The cell cultures were divided into 5 groups, which are 7F2 cell cultures with osteogenic medium (K); 7F2 cell culture with osteogenic medium and LPS (L2630, Sigma Aldrich, Inc, St. Louis, Missouri) (P1); 7F2 cell culture with osteogenic medium+ *α*-Mangostin (M3824, Sigma Aldrich, Inc, St. Louis, Missouri) (P2); 7F2 cell culture with osteogenic medium and LPS + *α*-Mangostin (P3); 7F2 cell culture with osteogenic medium and LPS + *α*-Mangostin after 24 hours of LPS exposure (P4).

Each group was tested with an in vitro scratch assay and an MTT assay with 5 replications each determined using the Federer formula. Based on research by Tantra et al., (2021) using the MTT (Invitrogen, Life Technologies Corporation, Eugene, Oregon) assay method, LPS concentration of 10 ng/ml and *α*-Mangostin concentration of 5 g/ml were used.

Through incubating the dishes overnight at 4°C for two hours at 37°C without rotation or shaking, 60-mm dishes containing an ECM substrate compatible with 7F2 osteoblasts were ready for the in vitro scratch assay procedure.
The unbound ECM substrate was taken out and blocked on coated dishes for an hour at 37°C with 3 ml of bovine serum albumin 2 mg/ml. Before plating the cells, dishes were cleaned once with saline phosphate-buffered (PBS) and then refilled with 3-5 ml of media (Dulbecco's Modified Eagle with supplement).

Sub confluent growing cells were washed twice with PBS, trypsin-containing verine (EDTA), and serum-containing medium before being reconstituted in a tissue culture dish. From aliquots of cell suspensions, cell counts were measured using a hemocytometer. To create a confluent monolayer, cells were placed on a 60 mm plate that had been prepared. At 37°C, the dishes were correctly incubated for 6 hours. The p200 pipette tip was then used to scratch the dish in a straight line. By washing the cells once with 1 ml of growth medium and replacing it with 5 ml of specific medium, debris was eliminated and scratch edges were smoothed.

Markers were made for dish observations so that the field of view was the same during shooting. The dish was positioned beneath a phase-contrast microscope after the reference point had been established, and the first image of the scratch was captured. Once more, the dish spent 24 hours at 37°C in a tissue culture incubator. The second dish image was then captured after that. The images obtained for each sample can be analysed quantitatively using the software. The scratch closure rate was calculated by subtracting the remaining width of the scratch line from the initial width of the initial scratch at 24 hours. This value was then divided by two to account for the two "healing" surfaces and converted to the closure rate expressed in micrometers per hour, the wound width calculation was performed using *ImageJ* software.

Statistical Analysis

The data from the study were processed using SPSS version 24. (IBM Corporation, Illinois, Chicago, US). The data were examined using the Shapiro-Wilk and Levene tests, to checking the normality and homogeneity, respectively. If the data is normally distributed, that will continue to the One-Way ANOVA comparative test and the Tukey HSD follow-up the Pearson correlation test used as a comparative measure. The study's acceptable margin of error is 5% (α= 0.05).

Ethical clearance had been obtained from the Ethics Commission of the Faculty of Dental Medicine, Universitas Airlangga, Surabaya (No.210/HRECC.FODM/V/2022), 2022.

Results

Figure 1 showed osteoblast cell viability was calculated by MTT assay. The highest osteoblast cell viability was found in the group

with LPS and treated with α-Mangostin (P4) after 24 hours (96.07%), close to the viability of the control group (K) (100%). The (P2) group that was given α-Mangostin without LPS showed high viability (82.73%), then the (P3) group that was given LPS and α-Mangostin (77.43%), while the lowest cell viability was found in the (P1) group, the group that was added LPS without administration of α-Mangostin (73.12%).

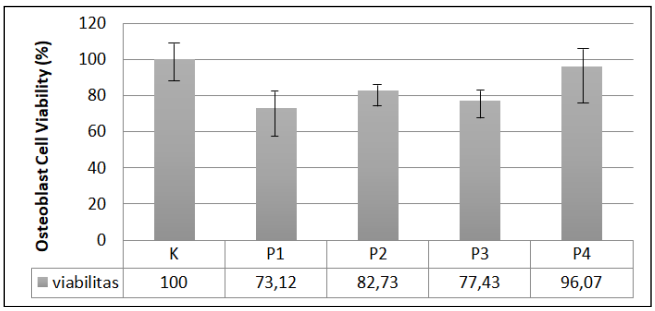

Figure 1. Osteoblast Cell Viability using different groups with osteogenic medium (K); osteogenic medium and LPS (P1); osteogenic medium and α-Mangostin (P2); osteogenic medium, LPS, and α-Mangostin (P3); osteogenic medium, LPS, α-Mangostin after 24 hours of LPS exposure (P4).

Shapiro-Wilk and Levene's test results both have significance values above 0.05 (p>0.05), indicating that the research data are normal and homogeneous and can be analysed using parametric tests. Given that the One-Way ANOVA test revealed a significant difference in osteoblast cell viability in the study group with a significance value of 0.000 (p=0.000), it can be concluded that administering α-Mangostin had an impact on boosting osteoblast cell viability following inflammation induction.

Osteoblast cell migration was observed using a light microscope with 400x magnification after a scratch assay was performed, as shown in figure 2 to figure 5.

The results shown by figure 6 showed the greatest migration of osteoblasts in the treatment group P3 (607.25 nm), then the P4 group (592.81 nm), and P2 (450.90 nm), where the migration of osteoblasts in the P2 group seemed close to the cell migration of the control group (K) (480.30 nm). The smallest osteoblast cell migration was found in the P1 group given LPS without α-mangostin (190.82 nm).

The data was confirmed normally distributed and homogeneous (p>0.05) and the One-Way ANOVA test revealed significant differences in osteoblast cell migration in the study group, with a significance value of 0.000 (p=0.000). This demonstrates that administering α-mangostin causes an increase in osteoblast cell migration following inflammation induction.

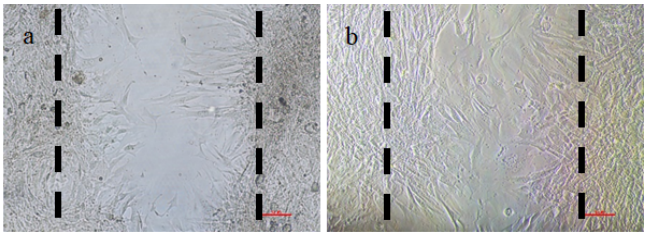

Figure 2. Scratch Assay Results of P1 Group: a) after 24 hours, b) after 48 hours.

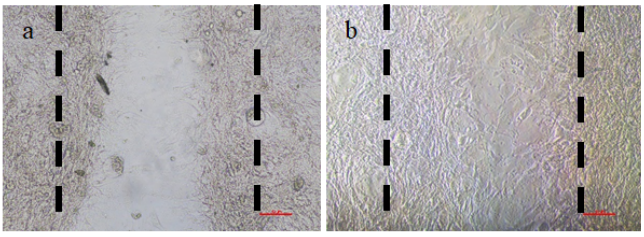

Figure 3. Scratch Assay Results of P2 Group: a) after 24 hours, b) after 48 hours.

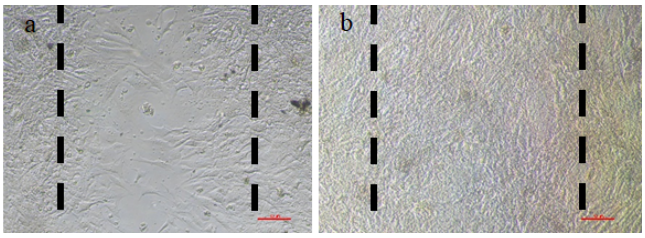

Figure 4. Scratch Assay Results of P3 Group: a) after 24 hours, b) after 48 hours.

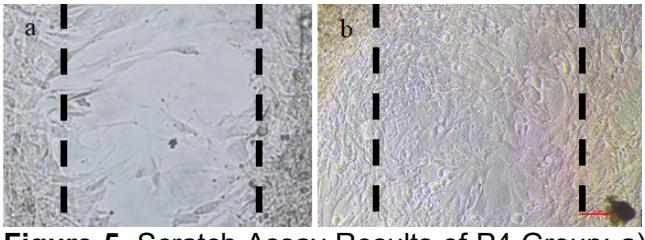

Figure 5. Scratch Assay Results of P4 Group: a) after 24 hours, b) after 48 hours.

The relationship between the viability and migration of osteoblasts following the induction of inflammation was examined using the Pearson test. Since the Pearson test resulted in a significance value of 0.000 (p = 0.000), showed that viability and osteoblast cell migration following inflammation induction are related. A strong positive correlation is indicated by a

Pearson correlation value of 0.700. The rate of osteoblast cell migration increases with osteoblast cell viability.

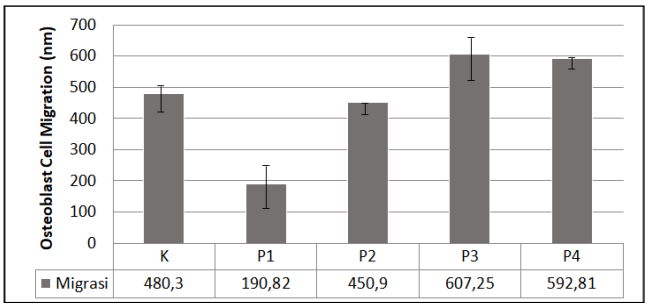

Figure 6. Osteoblast Cell Migration using different groups with osteogenic medium (K); osteogenic medium and LPS (P1); osteogenic medium and α-Mangostin (P2); osteogenic medium, LPS, and α-Mangostin (P3); osteogenic medium, LPS, α-Mangostin after 24 hours of LPS exposure (P4).

Discussion

Osteoblast cell viability increased in the treatment group treated with *α*-Mangostin, both with LPS induction, without LPS, and those given *α*-Mangostin after 24 hours. Research shows that there is an effect of giving *α*-Mangostin to increase the viability of osteoblast cells after inflammation induction. In this study, osteoblasts were induced using LPS to trigger inflammation in the cells. When osteoblasts recognize an inflammatory process, they release pro-inflammatory cytokines like TNF-α, IL-1, and IL-6. These cytokines then activate metabolic pathways involving diacylglycerol (DAG), protein kinase C (PKC), and NADPH oxidase, resulting in the formation of reactive oxygen species (ROS), which heighten oxidative stress and cell death.[13–15]

α-Mangostin can inhibit the production of the cyclooxygenase enzyme (COX), NO, PGE2, and TNF-α which is the cause of inflammation. *α*-Mangostin can also inhibit intracellular ROS activity which will suppress enzyme activity, then activate nuclear factor kappa B (NF-κB) to regulate the release of pro-inflammatory cytokines such as IL-1, IL-6, and TNF-α, so that inflammation is reduced. This is evidenced by the effect of *α*-Mangostin in reducing TNF-α and IL-1 levels, as well as reducing the expression of IL-6, ICAM-1, and NF-κB in Wistar rats.[5,7] This mechanism explains the increase in osteoblast cell viability in the study group after administration of *α*-Mangostin. The above mechanism explains the decrease in osteoblast cell viability in the LPS-induced group. Research conducted by Syam et al. (2014) showed that *α*-Mangostin can inhibit LPS activity by attenuating the signalling pathway of NF B and MAPK.[10,12,15–17]

The scratch method was carried out to determine cell migration activity in vitro because it is considered to be able to resemble cell migration examinations in vivo and facilitates measuring various cell migration parameters such as speed, polarity, and intracellular signalling activity. The results showed the greatest migration of osteoblasts in the group given *α*-Mangostin therapy, both with LPS induction, without LPS, and those given *α*-Mangostin after 24 hours. These results indicate the effectiveness of *α*-Mangostin in accelerating wound healing. The bone healing process requires the expression of important genes including TGF-β, FGF, PDGF, IGF, BMP, osteonectin, osteocalcin, osteopontin, fibronectin, BMPR, Smads, IL-1, IL-6, GMCS, and various collagen isotopes. The expression of well-regulated genes allows cellular interactions to improve bone morphology. Initial proinflammatory responses include the secretion of tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), IL-6, IL-11, and IL-8. TNF-α concentrations peak within 24 hours and return to normal within 72 hours after trauma. During this period, osteoblasts detecting the induction of inflammation will secrete TNF-α, which stimulates secondary inflammatory signals and acts as a chemotactic agent to recruit the necessary cells.[13,18]

The results of the Pearson test showed that there was a relationship between viability and post-inflammatory osteoblast migration. The greater the value of osteoblast cell viability, the greater the migration of osteoblasts. It appears that the viability and migration of osteoblasts were the smallest in the group given LPS without *α*-Mangostin administration. These results show the effect of giving *α*-Mangostin in increasing the viability and migration of osteoblast cells. The migration of osteoblasts is influenced by various inflammatory mediators produced by living osteoblasts. So that the more the number of living osteoblasts, the more inflammatory mediators that can be produced for osteoblast

migration. Osteoblast cells can respond to both injury and inflammation induction by producing pro-inflammatory cytokines, such as tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), IL-6, IL-11, and IL-8. TNF-α functions to stimulate secondary inflammatory signals and acts as a chemotactic to recruit the necessary cells.[13,19]

Osteoblast cell viability in the LPS-induced group and directly given *α*-Mangostin was significantly different from that in the LPS-induced group and given *α*-Mangostin 24 hours later, but osteoblast cell migration was not significantly different between the two groups. These results indicate that *α*-Mangostin is effective in increasing the viability of osteoblast cells 24 hours after inflammation. However, there has been an increase in optimal osteoblast cell migration after direct administration of *α*-Mangostin at the onset of inflammation, so it can be concluded that the administration of *α*-Mangostin immediately after an injury can accelerate osteoblast cell migration.

The limitation of this study is that observations were made only at one time so the migration of osteoblasts that occurred has not been observed specifically. In addition, this study was conducted in vitro on cell culture. The effect of exposure to α-Mangostin on living tissue needs to be investigated further.

Conclusions

Giving *α*-Mangostin 5% concentration can increase the viability and migration of osteoblast cells after inflammation induction. The greater the value of osteoblast cell viability, the greater the migration of osteoblasts after inflammation induction.

Declaration of Interest

The authors report no conflict of interest.

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