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Effects of Lisinopril on Bone Healing

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Abstract

This study set out to investigate whether treatment with lisinopril could aid in fracture healing, in addition, this study further aimed to closely understand the effects of lisinopril in terms of bone tissue healing time. Rabbits were obtained from the market and utilized as target samples for future experimental procedures after authorization. Lisinopril was used as the test drug in the trial. An ACE inhibitor drug called lisinopril is used to treat hypertension, heart failure and after a heart attack. Over several days, lisinopril was injected locally into the fracture site. The inflammation and re-epithelialization were measured and recorded for each study group on days 7, 14, and 21 after the fracture. Bone tissue samples were collected from the injured animals and fixed in formalin overnight. Tissue sections were then prepared, stained with eosin-hematoxylin and examined for pathological changes under the microscope. The effect of the drug was accompanied by low levels of granulation tissue and osteomalacia, as well as high levels of angiogenesis. Overall, bone regeneration was encouraged with the drug and there was no sign of bone loss in the experimental group, indicating that the drug is safe.

Key words: Lisinopril, bone healing, inflammation, bone injury.

Microstructural bone, reduced bone mass and tissue degeneration leading to bone fragility and increased susceptibility to fracture are characteristics of osteoporosis (Schütz *et al*., 2018). Although most anti-osteoporosis medications reduce the incidence of osteoporotic fractures, even though fractures can occur in people who are taking those medications (Rothe *et al*., 2019). To assess whether most anti-osteoporosis treatments should be discontinued or continued after a fracture occurs, the safety and efficacy of these drugs on fracture healing have been studied. In addition, it is known that medications used to treat osteoporosis also help with fracture repair. An endocrine system called the renin-angiotensin system regulates the body's electrolyte balance, the concentration and composition of body fluids, and blood pressure (Zhao *et al*., 2019). Angiotensin II is one of the other major effector peptides of this system and is usually produced by angiotensin I through a chemical reaction using angiotensin-converting enzymes (Zhao *et al*., 2019). An important molecule in the systemic mechanism of angiotensin II production is the angiotensin-converting enzyme. Antihypertensive drugs, particularly ACE inhibitors and angiotensin receptor blockers, have identified the RAS as a key target (Velliou *et al*., 2022).

Angiotensin-converting enzyme inhibitors such as lisinopril are used to treat heart failure, high blood pressure, and heart attacks. It is usually the first-line drug for treating high blood pressure (Gaber *et al*., 2020). It is also used in diabetic patients to avoid kidney problems. Based on experimental evidence-based studies, the effects of angiotensin II may potentially result in the promotion of osteoblast proliferation, moreover, it results in the stimulation of osteoclast activity through its effects on osteoblasts. In many studies which have been based on similar studies, it has been found that bone

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mineral density (BMD) in the populationtaking the following drug was higher in elderly men and women taking ACE inhibitors (Hussein and Taqa, 2023). A case-control study also showed that ACE inhibitors were associated with a somewhat lower risk of fracture. In a prospective, uncontrolled study in hypertensive patients, quinapril and enalapril reduced calciuria and serum 1,25-hydroxyvitamin D, but did not significantly affect urinary deoxycytidine which is a marker of resorption of bone (Su *et al*., 2022; Li and Li, 2022).

This study set out to investigate the possibility that treatment with lisinopril could aid in fracture healing, and in addition, this study further aimed to closely understand the effects of lisinopril in terms of bone tissue healing time. In this work, for the drug study, the mandible of rabbits was intentionally injured and then the fractured bone was treated with lisinopril. Lisinopril was given orally over several days. On the seventh, fourteenth, and twenty-first days after the fracture, the mandible mass was measured and recorded for each study group.

Materials and Methods

Animal: Rabbits were obtained from the market and utilized as target samples for future experimental procedures after receiving ethical permission. The ideal room temperature for these animals was between 23 and 28 degrees Celsius, with a relative humidity of about 51%. They were also kept in a diurnal balance with 12-hour cycles of light and 12-hour cycles of darkness. The rabbits were given sterile solid food that was suited to their needs throughout the experiment and was not prevented from drinking water.

A total of 24 male rabbits were categorized into four groups (6 rabbits for each group). One group is a control group and three are experimental groups. All groups are subjected to the surgical operation of the mandibular bone and make two holes at the site of the mandible with a critical size bone deficit of 5mm depth and diameter of 3mm with 2cm space between them. The three experimental groups received lisinopril in a daily dose of 3mg/kg in drinking water. The control group received only drinking water.

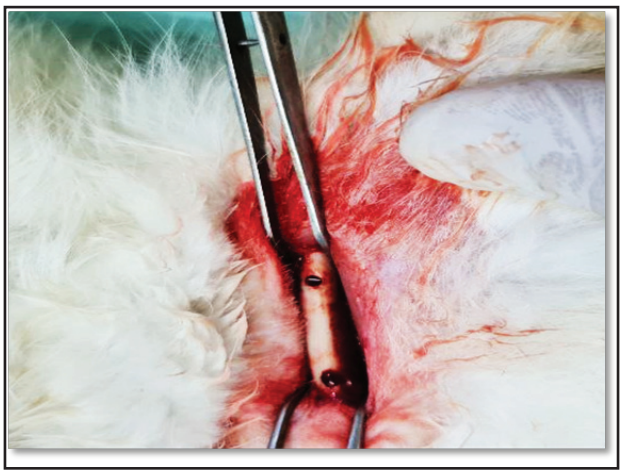

Fig 1. Site of injury in the mandible of rabbit used in the study.

The experimental groups were euthanized in 1, 2 and 3 weeks intervals. The control group was divided into three subgroups, two rabbits for each interval and euthanized at the same time as the experimental groups.

Test drug: In the trial, Lisinopril was used as a test drug. An ACE inhibitor drug called lisinopril is used to treat high blood pressure, heart failure, and after a heart attack. It is said to have the chemical formula C21H31N3O5 and a molecular weight of about 405.488 g/mol. Its half-life is about 12.6 hours and its bioavailability is 90%. It is metabolized in the liver and eventually excreted through the urine.

Histological study: The bone tissue samples were collected from the injured animal and fixed in formalin overnight. The tissue slices were then prepared, stained with eosin-hematoxylin and examined under a microscope for pathological changes.

Statistical analysis: The results of each experiment were expressed as the mean and S.D. One-Way ANOVA followed by a Posthoc test was used to assess the significance of differences between groups in multiple group comparisons. For comparisons between the two groups, the Student's t-test was used. All values were statistically significant with p-values less than 0.05.

Results :

The results of the following experimental study were collected during three different durations

of the study, which were recorded separately for both the study groups (Fig 1; Table I). The first group was the control group which revived the normal saline as the placebo. The experimental group received the drug in a biologically safe composition.

In the first week of the experiment, the results were obtained after seven days. In the control group, the obtained results indicated that there was no bone formation with infiltration of a large number of inflammatory cells with good granulation tissue formation. However, in the drug group, it was seen that there was moderate inflammatory cell infiltration with good granulation tissue formation and with the obvious bone trabecular formation in many areas of the tissue section and many new capillary formations.

The second session of obtaining the results of the following experiment was recorded after the fourteenth day. The results of the controlled group represented that there was infiltration of bone trabeculae formation with few capillaries (angiogenesis) and a decrease in the number of inflammatory cells. In addition to it, the results of the experimental group showed that there was very good angiogenesis with good bone formation and decrease in the granulation tissue amount and nil inflammatory infiltration.

The last results were obtained after the twenty-first day of the experiment. The results showed that in the controlled group there were many areas of bone formation with good angiogenesis and a decrease in granulation tissue but the inflammatory reaction still notes. However, in the experimental group, the results showed that there was very will bone formation and minimum granulation tissue and with angiogenesis, there is a large number of osteoblast count to osteocyte in the thick bone trabeculae.

This represents that overall the healing of bone was facilitated by the administration of the drug and there was no evidence of bone decay in the experimental group therefore, the drug is safe.

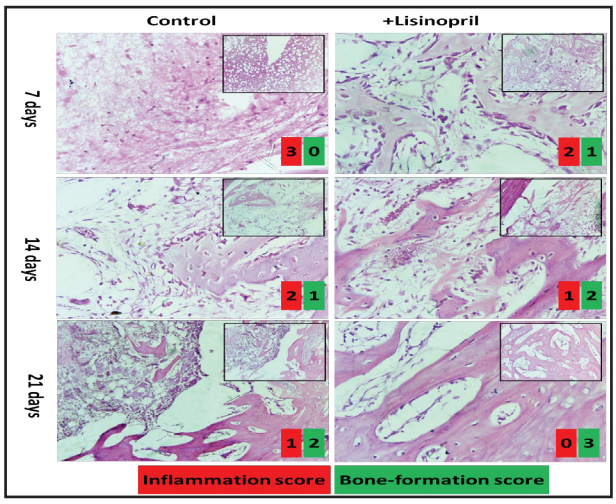

Fig 2. Lisinopril-induced bone osteogenesis and suppressed inflammation.

Table I. Results diagnosis by histopathology

Time points	Control	+Lisinopril
Day 7	There is no bone formation with infiltration of a large number of inflammatory cells with good granulation tissue formation.	Moderate inflammatory cell infiltration with good granulation tissue formation and will obliviously bone trabeculae formation in the many areas of tissue suction and many new capillary formations.
Day 14	There is infiltration of bone trabeculae formation with few capillaries (angiogenesis)and a decrease in the number of inflammatory cells.	Very good angiogenesis with good bone formation and decrease in the granulation tissue amount and nil inflammatory infiltration.
Day 21	Many areas of bone formation with good angiogenesis and a decrease in granulation tissue but the inflammatory reaction still note.	Very well bone formation and minimum granulation tissue and will angiogenesis there is a large number of osteoblast count to osteocyte in the thick bone trabeculae.

Discussion

Based on histological findings, lisinopril enhanced fracture healing in rabbits in the current study when injected subcutaneously into tissue near the fracture site. Lisinopril drug treatment increased bone formation and reduced inflammation in all three groups at days 7, 14, and 21 after fracture compared to the untreated rabbit group. It also improved the microstructure of the bone, increased healing strength, and accelerated fracture healing (Glowacki *et al*., 2021; Mo *et al*., 2020). To the best of our knowledge, this is an adequate and meaningful experimental study showing that lisinopril treatment accelerates fracture healing in rabbits, thus validating the main hypothesis of the study and demonstrating the safe efficacy of the drug.

According to evidence-based studies and previously completed experimental studies, the primary and most important goals of fracture healing in clinical practice are mechanical rehabilitation of broken bone and complete functional morphology of bone. According to earlier studies, the textural geometry and mechanical properties of broken or injured bone are restored in a closely controlled osteoporotic fracture healing system (Savasky *et al*., 2018). For this reason, geometric and histological repair are equally important to complete natural fracture healing; mechanical repair is not the only option. In this study, we investigated the effect of lisinopril on the microstructural and biomechanical properties of bone callus (Giusti and Scotlandi, 2021). Based on a three-point bending test, the results of the study showed that the mechanical parameters of the bone callus were higher in the lisinopril group than in the control group. In addition, qualitative analyses based onhistological staining showed that the microstructure of bone callus in the perindopril group was superior to that of the control group (Pitzurra *et al*., 2020). These findings imply that lisinopril promotes the healing of osteoporotic fractures by improving the mechanical properties of the bone and encouraging microstructural recovery of the healing zone (Lavery *et al*., 2020).

In a previous study, the effect of Lisinopril on femur fracture healing was investigated. A mouse female fracture model of femur fracture was established, and repaired with a bone marrow nail, and perindopril was administered systemically at a high dose (3 mg/kg/day) (Giusti and Scotlandi, 2021). Compared to controls, perindopril-treated animals exhibited considerable periosteal callus development at 2 and 5 weeks post-fracture (Oktaviono *et al*., 2021). This study also showed that torsional stiffness and failure torque were higher in perindopril-treated rats after duration. In this trial, Lisinopril was injected subcutaneously into the fracture site at a relatively low dose; at 2 weeks after fracture, it increased re-epithelialization and granulation and improved fracture strength (Borchert *et al*., 2020; Wagner *et al*., 2020). At 4 weeks post-fracture, when the callus size difference disappeared, we found that those treated with perindopril had higher fracture strength at the fracture site (Chappuis *et al*., 2018).

There are current views regarding the potential involvement of the renin-angiotensin system in the movement and deposition of mesenchymal stem cells favourably at the site of bone damage initiating healing and stem cell deposition (Ahmadian *et al*., 2017; Durik *et al*., 2012). This involvement of stem cells is important because stem cells themselves carry immunomodulatory properties potentiating healing and they do release a plethora of trophic factors initiating growth and cellular regeneration mediating bone healing (Forsyth *et al*., 2018; Chen *et al*., 2019; Merkhan *et al*., 2021; Shephard *et al*., 2022). Thereby renin-angiotensin inhibition might strongly participate as a positive parameter in bone healing and osteogenesis.

Conclusion

The purpose of this study was to investigate whether treatment with lisinopril could help fracture healing, and in addition, this study further aimed to closely understand the effect of lisinopril in terms of time to healing of bone tissue. The inflammation and re-epithelialization were measured and recorded for each study group on days 7, 14 and 21 after the fracture. The first group was the control group and was resuscitated with normal saline as a placebo. The experimental group was then given a drug at a biosafety concentration. The effect of the drug was accompanied by low levels of granulation tissue and bone proliferation as well as high levels of angiogenesis. Overall, bone regen-

eration was encouraged with the drug and there was no sign of bone loss in the experimental group, indicating that the drug was safe.

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