Assessment of Pravastatin effects on healing of bone defect in rats

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Abstract: Statins are a commonly prescribed cholesterol-lowering drug; however, it has recently been shown that they also have the beneficial side effect of enhancing bone matrix formation. As a result, this study is to assay probability effect of Pravastatin on osteogenesis which is made in experimental flaw and it is as a laboratory pattern in rat femur. This experimental study was conducted on 30 male SD rats. Animals were divided randomly into 3 groups (control and experimental). After induction of general anesthesia, a hole in size of 2 mm in diameter was made using a dental bit in femur width to medullary channel. After surgery, the control group received orally sterile water daily and experimental groups 1 and 2 respectively received daily 10 and 20 mg/kg/PO of Pravastatin. Histopathological and histomorphometrical studies for evaluation of bone healing were carried out in experimental rats, which were euthanized after 45 days of the experiment. In control group, defect seemed to be filled with woven bone and bone marrow spaces and in spite of a poor osteogenic activity. In experiment groups, many osteoblasts groupings, and young bone trabeculas increased in number and bone trabeculas more organized. Histomorphometric results, observed that Pravastatin has significant effect on bone healing in experimental groups 2 and 3 than control group (p=0/000), analyzing obtained results show that Pravastatin has significant effect in group 3 that received high dosage of Pravastatin than group 2 (p=0/000). The results of this study show that Pravastatin could stimulate osteogenesis in rats.

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1. Introduction

Statinic drugs are competitive inhibitors of enzyme Hydroxymethyl Glutaryl CoA Reductase (HMG-CoA reductase) and act as lowering of blood lipids and cholesterol. Nowadays, these drugs have common uses in curing the heart disease (1). Studies on statinic drugs show the effectiveness of these drugs on bones (2,3). Also, to anti-inflammatory effects of it due to reducing interleukin-6 and interleukin-8 have been mentioned (4). There are many studies in proving the supportive effect of statins on bones in topical administration in animal models. As well as, the effect of simvastatin in condensation of bone tissue, increase bone formation and its strength have been proved (5). Mundy et al., (1999) for the first time have reported that statins have positive effect in bone condensation. In that study they shown that the mass of trabecular bone increased in ovariectomized rats that were receiving simvastatin at the dose 5-10 mg for 35 days (2). In the case of statin drugs and bone metabolism having such overviews are important. These compounds stimulate bone formation by increase in osteoblasts activities which apply by BMP-2. Another effect of statins is inhibition of enzyme HMG-CoA reductase so preventing the synthesis of mevalonate which yields to disturbance in osteoclasts activity and apoptosis, finally, prevents bone reabsorption (6). The third effect of statins is their anti-inflammatory effects, that inflammation is seen during the bone formation in the involved bone and surrounded tissues which help to bone healing (7). The aim of present study was evaluation of efficacy of pravastatin in healing of bone defect in femoral bone in rats.

2. Materials and methods

30 healthy male Sprague-Dawley rats (about 250-300 g body weight) were used for this study. All animals were obtained from the animal laboratory center of Islamic Azad University Tabriz Branch. Management and husbandry conditions were identical in all groups with 12/12 h light/dark cycle at 21±2°C. Food and water were provided *ad libitum*. After transmission to the department of surgery, for avoiding of stress and adaptation with environment, they did not receive such an experiment for a week. The rats were randomly divided into 3 control and experiments groups of 10 animals. Investigations using experimental animals were conducted in accordance with the internationally accepted principles for laboratory animal use and care and our ethical committee on animal care approved the protocol. Animals were anesthetized with Ketamine hydrochloride (Ketamine 10%, Alfasan, Woerden-Holland, 50mg/kg) and Xylazine (Xylazin 2%, Alfasan, Worden-Holland, 5mg/kg) intraperitoneally.

The right hind limb was shaved and prepared aseptically with povidone iodine. A 2-cm skin incision was made on the lateral site of femur. The muscles were dissected bluntly to expose the bone. A confined cortical defect was drilled using a low-speed dental bit, saline-cooled in a stepwise fashion. A hole in size of 2 mm was made as monocortical and left untreated. Tissue was closed in layers. Animals were monitored postoperatively, and then returned to their cages. Animals received intramuscular injections of Penicillin G, 60000 Iu/kg immediately after surgery and 24 h later. In first group as control group, after induction of defect received sterile water. In two other groups, Pravastatin was used orally at 10 and 20 mg/kg, each rat was given pravastatin dissolved in sterile water, administered by direct injection into the stomach using a blunt-ended needle inserted via the esophagus. Animals were euthanized after 45 days postoperatively under general anesthesia, with an injection of over dosage of Thiopental sodium (60 mg/kg). The femurs include osseous defect were harvested, stripped of soft tissues, and prepared for analyses.

The samples were fixed by immersing it in 10% neutral-buffered formalin. The sample was then embedded in paraffin, sliced into 5 µm sections, and stained with hematoxylin-eosin for blinded histological assessment. Slides were assayed by light microscope model NIKON ECLIPSE E200. For histomorphic evaluations we used of linear measurement method by crossed reticulate lines through the one ocular reticulate lens containing 100 cubical nests and by determination the percentage of bone defect by 1) bone marrow, 2) immature bone, and 3) occupied lamellar bone was performed. Considered tissue contents were measured by 40X magnification and mouse. Bone marrow and connective tissue were detected by presence of abundant fat cells and fibroblasts and collagen strains respectively. For comparative assessments, a part of normal femoral bone from contralateral side also was obtained.

Statistical evaluation of data was performed using the software package SPSS version 18.0 (SPSS Inc., Chicago, IL). Data are reported as mean±SEM. The significant level was set at $p<0.05$. Statistical comparisons were used analysis of variance (ANOVA). Tukey HSD multiple comparison testing was used to determine experimental defects with normal bone.

3. Results

Microscopic findings showed that induced defect in rats of control group has been filled by immature bone spicules in middle part of diaphysis of femur and newly immature bone formed at its junction with the old bone and is being replaced by primary bone trabeculae (fig 1&2). The second group of rats (Pravastatin 10 mg/kg), A thin layer of immature bone has closed bone defect. Primitive cortical trabeculae gradually formed. Bone marrows between the immature and primitive condensed trabeculae have been reduced and these spaces were denser. Compared with the control group, density of new bone tissue was relatively higher (fig 3&4). The third group of rats (Pravastatin 20 mg/kg) bone defect was almost completely filled with new bone formation. Formed bone was denser and more organized. Bone marrows between the immature and primitive condensed trabeculae almost lost. Immature bone reduced and major part of bone defect was filled by lamellar bone. Haversian bone systems gradually occur. There was continuity between newly formed bone and old bone (fig 5&6).

Evaluation of Histomorphometry results obtained indicates that amounts of lamellar bone in the third group significantly higher than other groups, this value is lower than normal bone ($p=0.000$). The bone marrow and immature bone in control group was significantly more than experiments groups ($p=0.000$). In comparison between studied groups, in terms of lamellar bone formation, bone marrows and immature bones rates, there were a significant differences between low dose of Pravastatin group and control group ($p=0.000$). Also among the high dose of Pravastatin group and control group is significant ($p=0.000$). Significant differences between high and low dose of Pravastatin in terms of lamellar bone formation, bone marrows and immature bones rate was observed ($p=0.000$). Histomorphometry evaluation results are shown in Table 1.

Table 1: Comparison of mean and standard deviation of the bone tissue healing area components between the groups studied

	Normal bone	Group 1 (control)	Group 2 (Pravastatin 10 mg/kg)	Group 3 (Pravastatin 20 mg/kg)
Lamellar bone	95.10 ± 1.59^{a}	15.10 ± 1.78^{b}	44.1 ± 1.13^{c}	60 ± 1.2^{d}
Immature bone	4.20 ± 1.31^{a}	34.15 ± 2.2^{b}	32.1 ± 1.26^{c}	19.21 ± 1.16^{d}
Bone marrow	0.70 ± 0.63^{a}	50.75 ± 2.86^{b}	23.8 ± 2.1^{c}	20.79 ± 1.13^{d}

a,b,c,d,e: Dissimilar letters indicate significant differences in each row.

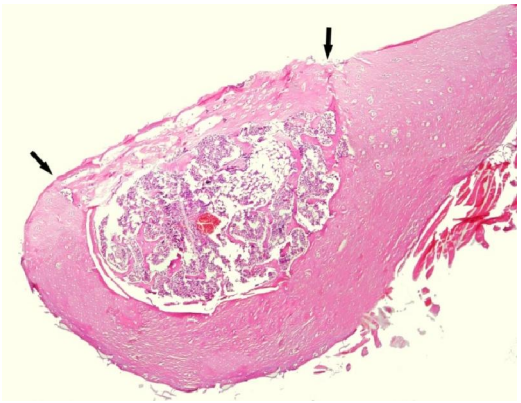

Fig 1: Microscopic view from experimental induced defect in middle part of the diaphysis of femoral bone in rats of control group. Newly formed immature bone trabeculae (woven bone) and wide spaces of bone marrow which filled the major areas of defect (H&E 250x).

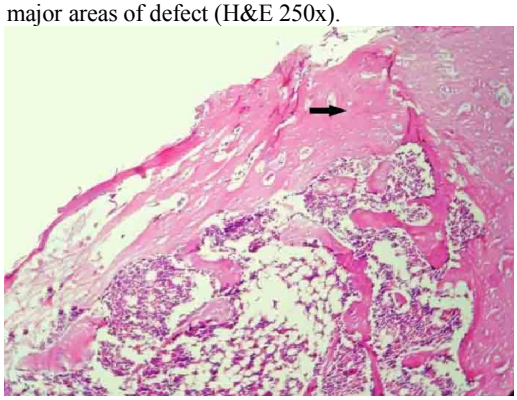

Fig 2: Microscopic view from experimental induced defect in middle part of the diaphysis of femoral bone in rats of control group. Immature bones has filled the major areas of healing tissue in location of defect. Newly immature formed bones confluence with the old bone adjacent to the defect is being replaced with primary trabeculae bone (H&E 600x).

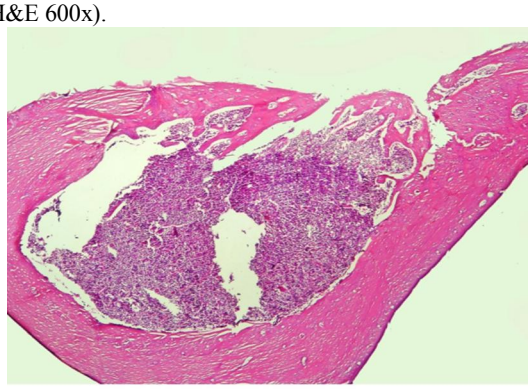

Fig 3: Microscopic view from experimental induced in middle part of the diaphysis of femoral bone in rats of group 2 (Pravastatin 10 mg/kg). Defect filled with woven bone and primitive compact bone and wide spaces of bone marrow is observed. Trabeculaes were greater dispersion. There is not enough coordination between the trabeculaes (H&E 250x).

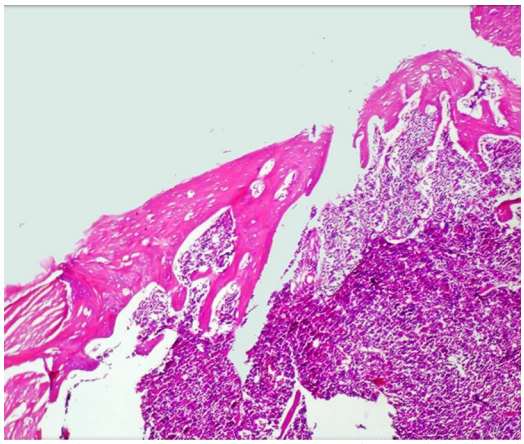

Fig 4: Microscopic view from experimental induced in middle part of the diaphysis of femoral bone in rats of group 2 (Pravastatin 10 mg/kg). The bulk of the bone defect is filled with wide spaces of bone marrow between woven bone and primitive compact bone (H&E 600x).

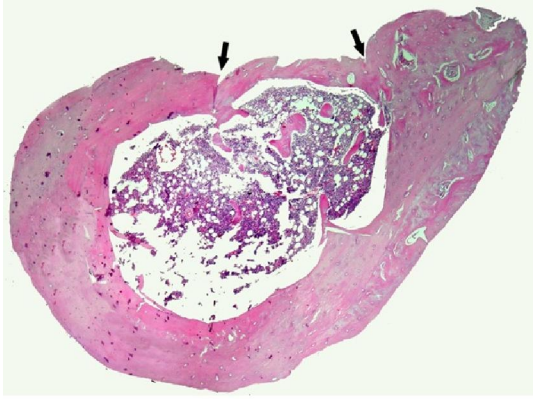

Fig 5: Microscopic view from experimental induced in middle part of the diaphysis of femoral bone in rats of group 3 (Pravastatin 20 mg/kg). Bone defect blocked with a thin layer of new formed bone. New formed bone and the "old" bone tissue have been joined together (H&E 250x).

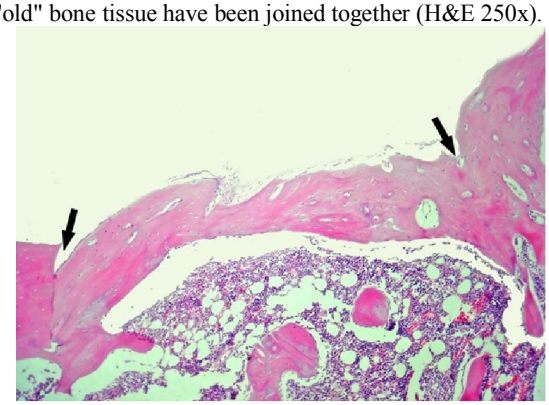

Fig 6: Microscopic view from experimental induced in middle part of the diaphysis of femoral bone in rats of group 3 (Pravastatin 20 mg/kg). Primary newly bone is remodeling. So immature and primitive lamellar bone conversion to compact bone. Haversian bone system gradually occur (H&E 600x).

4. Discussion

In the present study, microscopic evaluations indicate bone formation in the induced defect in femoral bone. In control group, changing the connective tissue to bone and starting the healing processes was not more severe and powerful and after 45 days, only a thin layer of immature bone with a wide spaces of bone marrow in it was saw. As well as histopathologic findings, in groups treated with pravastatin, after 45 days, organized and regular bone trabeculaes was seeing. In group treated with 10mg/kg pravastatin, only a thin layer of immature and primitive bone have filled bone defect. However, by increasing the dose to 20 mg/kg whole of the defect was filled by newly formed bone, which was more condensed and organized and haversian system was forming. After 45 days, amount of the a little mature and regular bone trabeculaes was changing to lamellar bone. Comparison results between groups show positive effects of high dose pravastatin than low dose. In a study by Wong et al., 2003, it has been shown that combination of collagen and astatine is an osteo-inductive agent which results in rapid healing of bone when used in skull bone fractures (8). In another study by Wong et al., in 2005, it has been shown that soluble astatine also can increase BMP; it yields to increase in osteoblasts and bone formation (9). Majima during a study in 2006 showed the positive effects of atorvastatin in increasing the bone density in the patients with hypercholesterolemia. In that study, the short term effect of atorvastatin has been shown (10). In another study by Kawane et al., (2004), the bone density of ovariohysterectomized rats was increased significantly subsequent use of atorvastatin (11). Nyan et al., (2007) reported that combined use of simvastatin and calcium sulfate accelerates bone healing (12). Montagnani et al., (2003) demonstrated that simvastatin increases the bone density, bone formation and spongy tissue pressure (13). Also, in another study by Ayukawa et al., (2004) it has been reported that use of simvastatin around the titanium implants increases the bone density near by the implant (14). After digestive absorption, 95% of astatines metabolize in the liver and only a small part passes from liver and reach in the bone tissue. So, astatinic drugs which are used widely for treatment the hypercholesterolemia not bone tissue, this research suggests to produce new generation of astatines by focus on the bone tissue target, and for reaching to the appropriate dose we need to other studies many more.

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Conclusion:

The results of this study indicate that Pravastatin can increase the bone formation and to accelerate healing process of defect.

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