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Rosuvastatin-Induced CD34 Bone Healing Marker

Hiba H. Saeed, Maha T. Al-Saffar, Abdulsattar S. Mahmood
College of Dentistry, University of Mosul, Mosul, Iraq
*hiba.20dep10@student.uomosul.edu.iq

Abstract

Rosuvastatin is primarily used for controlling abnormal lipids and cholesterol in the blood; however, evidence proves that when rosuvastatin was administered to patients with fractured bones, the administration demonstrated dramatic improvement in the wound healing process and regeneration characteristics of the bones. Physiologically, in the cases of the fractured bone, the bone marrow releases $CD34^+$ cells into the peripheral blood, which circulates to the site of the fracture and recruits them there. The following study it is aimed to examine the effect of rosuvastatin on the promotion of expression of CD34. The study further aims to evaluate the impact of rosuvastatin on the repair and healing of fractures through a randomized control trial. 20 Rabbits were divided into two equal groups, which had ten rabbits each. Group one was the controlled group which received the placebo, which was normal saline, while the second group was the experimental group which was labelled as the rosuvastatin group. The results of the following study suggested that there was significant evidence of early osteogenesis in the group which received rosuvastatin as compared to the controlled group. In addition to it, the study confirmed that rosuvastatin is responsible for the early healing of the fracture and effective bone regeneration and repair.

Keywords: Rosuvastatin, bone, CD34, surface markers.

Introduction

One of the leading issues which result after any conventional allogeneic and autologous bone grafting is the impairment in the healing of the surgical site or the failure in the healing of the fracture (Li *et al.*, 2020). Although most of the fractures heal appropriately, a marked number of them fail due to multiple reasons, which include local and systemic factors (Sybil *et al.*, 2021). These factors include external forces, trauma, interruption in blood supply, and many other reasons (Rezazadeh *et al.*, 2019). Out of all these reasons, inadequate blood supply is one of the most prevalent causes of delayed healing and regrowth, malunion and nonunion of fractures (Wang *et al.*, 2019). Conventionally, it is believed that the supply to the soft tissue and complex vascular structures are responsible for transferring the blood supply to fracture sites and promoting healing in various injuries. When considering regenerative medicine and tissue engineering, neovascularization has been identified as an attractive method for bone repair and regeneration (Lin *et al.*, 2018). It appears to take great advantage of the reciprocal osteogenic relationship that exists between endothelial cells and osteoblasts. To demonstrate the efficacy of circulating CD34-positive cells with osteogenic and vasculogenic or angiogenic properties for fracture repair, a number of studies have been performed (Li *et al.*, 2020). Physiologically, in the cases of the fractured bone, the bone marrow releases $CD34^+$ cells into the peripheral blood, which circulates to the site of the fracture and recruits them there. By secreting vascular endothelial growth factor (VEGF), which differentiates osteoblasts and endothelial cells, the attracted $CD34^+$ cells near the injured site create an

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environment conducive to fracture healing(Bowman *et al.*, 2021). Finally, these interconnected pathways promote osteogenesis and vasculogenesis, which ultimately accelerates the development of the sternal callus and results in functional recovery after fracture (Wang *et al.*, 2019).

Although that bone materials mainly involve a combination of vitamins and minerals; which are the requirement of healing process. Rosuvastatin, a drug which belongs to the pharmacological class statin, is primarily used for controlling abnormal lipids and cholesterol in the blood, however, evidence proves that when rosuvastatin was administered to patients with fractured bones, the administration demonstrated dramatic improvement in the wound healing process and regeneration characteristics of the bones (Leutner *et al.*, 2019; Li *et al.*, 2020). These findings are supported by the phenomenon that states that the drugs which belong to the statin class, which includes rosuvastatin, induce the expression increase of CD34 protein, which further promotes the regeneration of bones.

The following study it is aimed to examine the effect of rosuvastatin on the promotion of expression of CD34. The study further aims to evaluate the impact of rosuvastatin on the repair and healing of fractures through a randomized control trial.

Methodology

Material: In the following research, the chemical substance which has been used is rosuvastatin, which has the chemical formula C22H28FN3O6S. It belongs to the class statin, which is also known as HMG-CoA reductase inhibitors, with a molecular mass of 481.539g/mol. It is primarily used for controlling the level of cholesterol. However, the effects on bone repairing and healing are markedly highlighted, which are studied in the following trial.

Study design: The following study is based on an experimental design which follows the protocols of the randomized control trials. It is the most suitable method to evaluate the impact of the drug on the subject. Furthermore, a randomized control trial enables the evaluation of the difference in the impact of the drug on a condition in comparison to the controlled group, which does not receive the intervention.

Study subject: In the following study, the subject animals which are used for the experiment are the six-month-old rabbits. All these animals were taken under the protocols and guidelines of animal welfare acts which ensured that all the animals' rights were preserved and these animals were kept under standard care.

Experimental Design: A total of 20 rabbits were randomly selected to include in the experiment. All these animals were kept at room temperature, which is approximately 19±1ºC, and the humidity of the room where the rabbits were stored was approximately 55%. All the rabbits were healthy, and they weighed around 3.0kgs to 3.5kgs each. Fluorescent lighting in the cages was 20lux in the back, and 130 lux in front, which maintained the 12 hours day and 12 hours night cycle. These rabbits were provided with tap water and food as per the requirement.

A total of 20 Rabbits were divided into two equal groups, which had ten rabbits each. Group one was the controlled group which received the placebo, which was normal saline, while the

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second group was the experimental group which was labelled as the rosuvastatin group or (RVS) group. After the intervention, each group was studied in the span of seven, fourteen, and twenty-one days. The results were carefully recorded to learn the impact of the drug and build a conclusion from it. The immunohistochemistry of the bone was carried out by evaluating the bone marker CD34.

Data Analysis

The mean and SEM of all data were analyzed separately. In addition to it, a Non-parametric analysis (Mann-Whitney U-test) using SPSS for Windows, version 24, was used to assess differences between groups. The results were considered statistically significant at a value of P=0.05 and a confidence interval of 95%.

Results

The results of the following experiment have been made on the bases of two separate groups, the controlled group and the intervention group, which received rosuvastatin. In addition to it, the results have been recorded three different times. Firstly, the results were analyzed after seven days of the administration of the respective intervention. A second analysis was done after fourteen days of analysis, and lastly, the third intervention was done after the experiment. All these analyses were done based on the histopathology reports of the bone. The CD34 marker represents the status of osteogenesis in the bones.

On the seventh day after the intervention, the results of the controlled group represented that there were no signs of changes in the histophysiology. Correspondingly, the results of the intervention group represented that there were positive findings which indicated mild angiogenesis and mild osteoprogenitor cells, which are the stem cells that play a significant role in the growth of the bone and repair. The presence of these osteoprogenitors indicates the regeneration of bone at the site of the injury.

The next evaluation was done on the fourteenth day of the experiment. In the controlled group, there were signs of angiogenesis and cell differentiation of osteoblast. This indicated that there was new vascular growth and the formation of osteocytes. On the other hand, the results of the controlled group indicated mild to moderate bone formation. Progress in angiogenesis and marked generation of bones at the site of injury. In addition to it, there were marketing patches of CD34 at the bone trabecular, indicating activation of stem cells of the bone marrow.

Lastly, the results which were obtained on the 21st day of the experiment indicated that in the controlled group, there was a mild formation of bone and mild angiogenesis. In addition to it, there was mild CD34 expression indicating a normal bone healing process or secondary bone healing process. However, the findings of the intervention group indicated that there was obvious secondary bone healing and evident regeneration of bone at the site of the bone injury. In addition to it, there were well-developed blood vessels which indicated healthy angiogenesis, which was accompanied by the formation of thick bone trabeculae with narrowing of bone marrow spacing that indicates an accelerated bone healing process.

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Table 1. Results diagnosis by histopathologist

Time points	Control	+Rosuvastatin
Day 7	No any marketing immunohistochemical changes by CD34	Mild angiogenesis and mild osteoprogenitor cell differentiation
Day 14	Starting angiogenesis in the site of bone surgical procedure with differentiation of osteoblast	Mild to moderate bone formation Angiogenesis Bone regeneration at site of artificial defect Marketing patches of CD34 at bone trabecule indicating activation of stem cells of the bone marrow
Day 21	Mild bone formation and mild angiogenesis with mild CD34 expression indicating normal bone healing process or secondary bone healing process	Bone regeneration at site of artificial defect Secondary bone healing is obvious with well-developed blood vessels and thick bone trabecule formation with narrowing of bone marrow spacing that indicates accelerated bone healing process

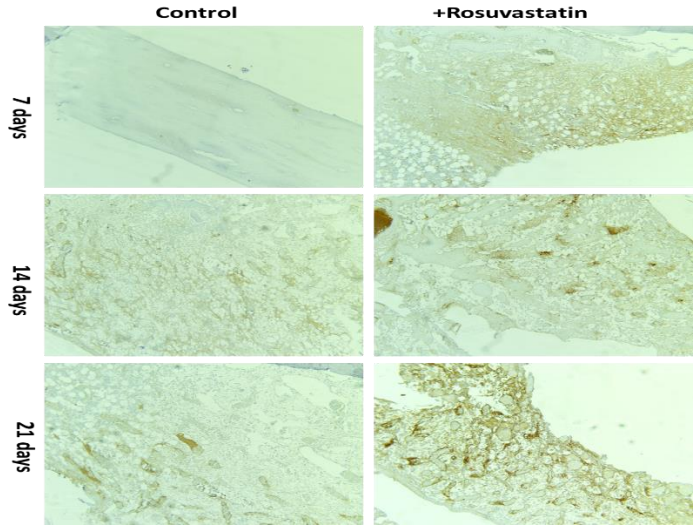

Figure 1. Rosuvastatin-induced bone osteogenesis marker CD34

Discussions

It has been reported by multiple studies that rosuvastatin is commonly utilized to manage abnormal lipids and cholesterol in the blood (Almukhtar et al., 2020; Althanoon *et al.*, 2020; Almukhtar et al., 2021; Almukhtar et al., 2022) However, the emerging evidence suggested that when rosuvastatin was used in patients with fractures, the wound healing process and bone regeneration function were significantly improved(Li *et al.*, 2020). In the following experimental study, the effects of rosuvastatin were examined on the rabbits induced with bone injuries.

The results of the following study suggested that there was a marked increase in the bone regeneration process after the administration of rosuvastatin. In addition to it, the comparison suggested that the healing process in the controlled group was delayed and slow as compared to the intervention group, which received rosuvastatin (Rezazadeh *et al.*, 2019). The following findings are supported by the study, which stated that BMPs are the most effective biological

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growth factors that promote bone growth and thus contribute to bone healing in the repair of bone defects(Wang *et al.*, 2019). These proteins promote the differentiation of osteoprogenitor cells into mature osteoblasts and other specific cell types into osteoblast lineage cells, respectively. In addition, it has been found that an investigation tested the use of ACS as an RSV carrier and produced critical-sized cortical bone lesions next to titanium implants in rabbits(Chatterjee *et al.*, 2019). They found that when administered topically, RSV enhanced bone growth. In light of these investigations, another study decided to repair the abnormalities in the cervical bone of rats by topical application of RSV using ACS. The results of this study showed that topical RSV treatment had a positive effect on auto graft-induced bone growth, which is consistent with the results of other investigations(Akbari et al., 2020; Safakheil and Safakheil, 2020). The findings of this study are similar to the above-mentioned studies as there has been an evident sign of rapid bone regeneration.

In addition to bone repair and bone regeneration, the results of the following study represented that rosuvastatin is markedly associated with facilitating angiogenesis in the cells. An examination performed to assess the effect of rosuvastatin administration on EPC-mediated neovascularization, and EPC mobilization in any ischemia-related injury was the basis for the results of this study. The results of the study showed that a low dose of rosuvastatin (0.1 mg/kg) greatly increased capillary density and accelerated blood flow recovery compared to saline-treated controls (Gokdemir, 2021). The following findings are also seconded by another study which stated that rosuvastatin when administered at lower doses, promotes the mobilization of endothelial progenitor cells, which in turn enhances the formation of ischemic neovascularization (Hu *et al.*, 2019). Therefore, optimizing the dose of rosuvastatin may increase its efficacy in the prevention and treatment of ischemic disease.

Although the following study has presented a clear comparison between the regeneration of bone and the repairing process of bone, however, the study did not discuss the optimum dosing of the drug and safety percussion. Moreover, the following study did not discuss the adverse effects associated with the drug too. Therefore more investigation and studies are required in this area.

The present trial showed a satisfied outcome to certain extent. However, additional trials need to be carried out in the future via combining rosuvastatin with vitamins (C, E, and D)(Merkhan and Abdullah, 2020; Sulaiman et al., 2022) or minerals (Zinc) (Younis *et al.*, 2022; Althanoon and Merkhan, 2021) which might boost the response to rosuvastatin. Similar studies could be carried out using fluvoxamine or fluoxetine (Abdulqader *et al.*, 2022; Faisal et al., 2022), allopurinol(Faisal et al., 2020; Abdulrazzaq et al., 2020; Alchalaby et al.2022), esomeprazole (Abdullah *et al.*, 2021; Merkhan et al., 2022), atorvastatin (Almukhtar et al., 2022; Almukhtar et al., 2020), antiepileptics(Althanoon et al., 2019), risperidone, and olanzapine(Faisal *et al.*, 2019).

Conclusion

The following study was conducted with the aim of evaluating the impact of rosuvastatin on fracture repair and healing and its effects on osteogenesis and angiogenesis at the site of bone injury. The findings of the study suggested that there was significant evidence of early osteogenesis in the group which received rosuvastatin as compared to the controlled group. In

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addition to it, the study confirmed that rosuvastatin is responsible for the early healing of the fracture and effective bone regeneration and repair. However, the study does not discuss the dosage and the adverse effects associated with rosuvastatin.

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