Administration of Recombinant Platelet Derived Growth Factor-Bb (PDGF-Bb) Intrafracture on Shaft Femur Rat Wistar Performed Osteotomy and Intramedullary Wire Improves Healing Process of Fractures, Collagen Type I And III Levels

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ABSTRACT

Introduction: Of all the fractures that can occur in human body, one of the most lethal is femoral fracture. Treatment of femoral fracture can be divided into two, which is operative and non-operative. External fixation, intramedullary nailing, locked intramedullary nail, and plate and screw are the options for operative treatment. Platelet Derived Growth Factor (PDGF-BB) have essential role of wound healing, due to positive effect of upregulating proliferation of fibroblast, synthesize of extracellular matrix, and revascularization. In this study we aimed to see the effect of PDGF-BB fracture healing process, type I and III collagen levels in the Wistar rats femoral shaft treated with osteotomy and intramedullary nailing.

Methods: This study is an experimental randomized post-control only to animal with femoral shaft osteotomy and intramedullary wire installation in Wistar Rats that added PDGF-BB intrafracture, to the collected data then analyzed using Pearson test in SPSS 25.

Results: Based on the independent t test result, we found that callus formation, type I and III collagen expression on the femur shaft of wistar rats that has been administered PDGF-BB intrafracture had a statistically significant result compared to those who had not been administered PDGF-BB intrafracture with P<0.05.

Conclusion: Mean Allen score, type I collagen and type III collagen levels of shaft femur in Wistar rats group that been osteotomized and attached by intramedullary wire added with PDGF-BB is higher compared to non PDGF-BB.

Keywords: Allen score, femoral fracture, PDGF-BB, type I collagen, type III collagen.

I. INTRODUCTION

Bone fracture is one of the non-fatal musculoskeletal injuries that can cause morbidity and mortality so it interferes with daily activities [1]. One of the fractures that often occurs and has a high disability rate is a fracture of the femur. Fractures in the middle or shaft of the femur are a type of fracture that often occurs. Based on the study, it was more experienced by women compared to men with a ratio of 70% of 10,548 respondents, usually experienced in young adult patients with high-energy injury is associated with high morbidity and mortality and is prone to complications such as delayed union or nonunion [1]. In general, long bone fractures will be treated operatively, such as the use of implants as a deep fixation of the broken bone. There are many options for surgical fixation including external fixation, intramedullary nailing, also plate and screws. Currently, the use of intramedullary nailing on fractures of the femoral shaft gives satisfactory results. This technique provides early mobilization, reduces the duration of hospitalization, and reduces complications compared to other surgical procedures [2], [3]. Advantages of intramedullary nailing, include less scar formation, shorter operative time, less blood loss, faster mobilization, and less incidence of joint stiffness [4]. With the advancement of existing therapies to treat femoral shaft fractures, it is undeniable that complications are often found in this case before and after surgery. Complications often occur in the form of delayed union, malunion, and nonunion.
A study conducted in Scotland found an estimated incidence of non-union in the pelvis and femur of 13: 1000 cases [5]. Platelet-Derived Growth Factor (PDGF-BB) has an essential role in wound healing because it has a positive effect that increases fibroblast proliferation, synthesis of extracellular matrix, and the process of re-vascularization. Platelet-Derived Growth Factor is a group of proteins consisting of PDGF-AA, PDGF-AB, PDGF-BB, PDGF-CC, and PDGF-DD which are produced by platelets when the fracture occurs. Platelet-Derived Growth Factor BB is chemotactic and mitogenic so it can function as a factor that can improve wound healing [6]. Recent studies suggest that PDGF-BB mediates tissue repair processes including chemotaxis (monocytes, neutrophils, fibroblasts), proliferation (fibroblasts, smooth muscle cells, capillary endothelial cells), induction of matrix molecules (fibronectin, hyaluronic acid) and the production and secretion of collagenase fibroblasts [7]. Increased VEGF will accelerate the bone healing process by stimulating the proliferation of vascular endothelial cells resulting in the formation of new blood vessels or capillaries at the fracture site [4], [8]. In this study, the authors wanted to determine the effect of giving recombinant PDGF-BB by examining in terms of the effect of fracture healing (as seen by the Allen score), type I collagen levels, and type III collagen levels in the femoral shaft of Wistar rats which underwent osteotomy and intramedullary wire installation, with those who were not given PDGF-BB.

II. MATERIALS AND METHODS

This study used a randomized post-test only experimental design in test animals with the femoral shaft of Wistar rats undergoing osteotomy and insertion of intramedullary wires with intrafractured PDGF-BB added by assessing the fracture healing process which is assessed from the Allen score, the levels of collagen types I and III obtained calculated from the ELISA examination, then the sample is selected by simple random sampling. Rats were then divided into 2 groups, namely P0 = Rats with femoral shafts that underwent osteotomy and ORIF Intramedullary Wires that received PDGF-BB injection; P1 = Rats with a femoral shaft that underwent osteotomy and ORIF Intramedullary Wire that did not receive PDGF-BB injection. An osteotomy was performed on the diaphysis of the femur (right in the middle of the bone), then the fracture was fixed using the ORIF Intramedullary Wire technique. In the treatment group, 10 mcg of recombinant PDGF-BB was given at each end of the fracture fragment. On the last day of the fourth week, the rats were euthanized with barbiturate, then the right femur of the rats was taken for examination of callus formation and levels of collagen types I and III. The data obtained in the study were analyzed by descriptive analysis and the data obtained will be presented in tabular form. Followed by inferential analysis, where the normality test of the data uses the Shapiro Wilk test, the homogeneity test of the data uses the Levene test, the numerical comparison test uses the Independent T-Test if it is normally distributed and if it is not normally distributed then uses the Mann-Whitney Test. Assessment of test results using 95% CI and p value at the limit of significance of 0.05.

III. RESULTS

A descriptive explanation of several characteristics such as the age of the rats and the weight of the rats was carried out for the two groups and then statistical tests were carried out to compare the characteristics mentioned above from the two groups, which can be seen in Table I.

With a total of 18 data for each group (n<50), the normality test used was the Shapiro Wilk test, while the homogeneity of data variance was carried out using Levene's test. The data distribution is normal and homogeneous in both groups. From the normality test results for the Allen score variable, the expression of collagen types I and III was normally distributed with a P value>0.05. The homogeneity test results showed that the variable Allen score, collagen levels I and III were homogeneous in both groups with a P value>0.05. For numerical variables, a significance test was conducted for the data of two unpaired groups using an independent T-test for normally distributed data. The table below shows that the mean Allen score in the group with PDGF-BB (3.89 ± 1.41) was significantly higher than the group without PDGF-BB (2.28 ± 1.48) with the difference in the mean values in the two groups. 1.61 (p value = 0.02, 95% CI 0.63 - 2.59).

The inferential statistical test used in this study was the independent T-test because the data were normally distributed and homogeneous. Assessment of test results using 95% CI and P value at the limit of the significance of 0.05. Based on the results of the independent T-test, it was found that the administration of PDGF-BB showed a significantly higher level of collagen type I (4.97 ± 0.54) in Wistar rats that underwent intramedullary nailing compared to those who not given PDGF-BB (4.41 ± 0.74) with a mean difference between the two groups of 0.56 (P = 0.014, CI 0.12 - 1.00). The inferential statistical test used in this study was the independent T-test because the data were normally distributed and homogeneous. Assessment of test results using 95% CI and P value at the limit of the significance of 0.05.

Based on the results of the independent T-test, it was found that the administration of PDGF-BB showed a significantly higher level of collagen type III (12.1 ± 2.49) in Wistar rats that underwent intramedullary nailing compared to those who were not given PDGF-BB (10.01 ± 1.61) with a mean difference of 2.09 (P = 0.005, CI 0.66 – 3.51).

| Variable                  | Intervention          | p-value |
|---------------------------|-----------------------|---------|
| Age (week)                | Mean ± SB             | 0.952   |
| Weight (gr)               | Mean ± SB             | 0.964   |
| Allen Score               | Mean ± SB             | 0.02    |
| Type I Collagen Levels    | Mean ± SB             | 0.014   |
| Type III Collagen Levels  | Mean ± SB             | 0.005   |

**TABLE I: CHARACTERISTICS OF RESEARCH SUBJECTS**
IV. DISCUSSION

PDGF-BB has an important role in the healing process of fractures, and the administration of PDGF-BB is expected to stimulate the formation of angiogenesis which can increase the stimulation of callus formation in bone fractures [9]. Research on the fracture healing process was assessed from the degree of callus formation using the Allen score on fractures given PDGF-BB. In this study, it was found that the mean Allen score of callus formed at fracture sites treated with PDGF-BB showed a higher degree of the fracture healing process with p < 0.05. For the time being, sources that say that PDGF-BB does not yet have a significant effect on stimulating callus formation and fracture healing process have not been widely published. Therefore, this study is in line with other studies that support that PDGF-BB stimulates callus formation [10]. Studies on the levels of type I collagen in fractures treated with PDGF-BB have not been widely reported. One of the aims of this study was to assess the level of type I collagen in fractures given and without PDGF-BB. Thus, there was a significant result that the level of type I collagen was higher in fractures given PDGF-BB compared to those not given PDGF-BB with p<0.05. The results of this study are in line with research conducted by [11], that PDGF-BB significantly increases type I collagen levels. Based on research conducted by [12], it was said that PDGF-BB induces the level of type I collagen synthesis, and it was found that a significant increase was found 24 hours after administration of PDGF-BB [11], [12]. However, according to research by Ojima et al, it is said that PDGF-BB does not directly play a role in collagen levels, but PDGF-BB provides a potent stimulation of collagenase expression, which may play a role in the remodeling phase [12]. The results of the study on the levels of type III collagen in fractures that were given and not given PDGF-BB, it was found that the levels of type III collagen in fractures that were given PDGF-BB were higher than those who were not given PDGF-BB with P<0.05. Not many have been able to suggest that PDGF-BB can increase levels of type III collagen in other studies, so it can be concluded that this study has results that can be used as a reference for further research to assess whether PDGF-BB can increase levels of type III collagen [10]-[12]. In addition, in this study, the amount of type III collagen levels compared to type I collagen levels was about 3 times higher. Where in normal bone conditions, the ratio of collagen type III is 3:1 compared to the level of collagen type I. With the addition of PDGF-BB can increase the amount of collagen type III which is very necessary in bone tissue to provide rigidity properties to increase bone strength [12]. The limitation of this study is that the intervention carried out is still limited to rat samples, it is hoped that in the future this intervention can be expanded to higher samples such as rabbits, chimpanzees to clinical trials.

V. CONCLUSION

It is hoped that in the future the use of PDGF-BB can be applied to humans for scientific development and maximizing therapy in fracture cases.

CONFLICT OF INTEREST

Authors declare that they do not have any conflict of interest.

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TABLE II: INDEPENDENT T-TEST RESULTS FOR THE MEAN ALLEN SCORE AND COLLAGEN LEVELS DIFFERENCE BETWEEN THE TWO GROUPS

| Number | Variable | Intervention | Mean Difference | IK 95% | p-value |
|--------|----------|--------------|-----------------|--------|---------|
| 1      | Allen Score | Mean ± SB (N=18) | 3.89 ± 1.41 | 2.28 ± 1.48 | 1.61 | 0.63 - 2.59 | 0.020 |
| 2      | Type 1 Collagen Levels | Mean ± SB (N=18) | 4.97 ± 0.54 | 4.41 ± 0.74 | 0.56 | 0.12 - 1.00 | 0.014 |
| 3      | Type 3 Collagen Levels | Mean ± SB (N=18) | 12.1 ± 2.49 | 10.01 ± 1.61 | 2.09 | 0.66 - 3.51 | 0.005 |

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