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Research

Intrafracture Recombinant Platelet-Derived Growth Factor Improves Fracture Healing Process on Wistar Rat

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ABSTRACT

Background: Femoral fracture is associated with high morbidity and mortality, especially in young adults with high-energy injuries. Abnormal fracture healing presents as a significant matter in management of femoral fracture. Platelet Derived Growth Factor (PDGF-BB) is proposed as potential agent in fracture healing. Up to this point, PDGF has been scientifically studied improve wound healing by enhancing fibroblast proliferation, extracellular matrix synthesis, and re-vascularization. This study aims to evaluate role of PDGF on fractured bone healing.
Subjects and Method: This was a randomized controlled trial conducted at Animal and Biomedical Laboratory of Udayana University, from March to September 2022. 36 male and healthy Wistar Rats at 3-4 months old and weighs 140-160 grams were selected for this study. The dependent variables were Allen score, type I and type III collagen levels. The independent variable was administration of 10 mcg PDGF-BB following femoral osteotomy and intramedullary wire fixation. Type I and III collagen levels are assessed using ELISA methods. Statistical analysis is performed using independent T-test in SPSS ver 25.0 software.
Results: Allen score in the PDGF-BB group (Mean= 3.89; SD= 1.40) was higher than control group (Mean= 2.28; SD= 1.48), with p=0.002. Type I collagen in the PDGF-BB group (Mean= 4.97; SD= 0.54) was higher than control group (Mean= 4.41; SD= 0.74), with p=0.014. Type III collagen in the PDGF-BB group (Mean= 12.1; SD= 2.49) was higher than control group (Mean= 10.01; SD= 1.61), with p=0.005.
Conclusion: In comparison to the non-PDGF-BB group, Wistar rats that had their femurs osteotomized and joined with intramedullary wire in the PDGF-BB group has higher mean of Allen score, type I and type III collagen levels.

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

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BACKGROUND

Femoral fracture is considered as one of the most deliberating health problems. It carries significant complications and mortality potential within each cases occurring. As one of the strongest long bones in human body, femoral fracture is usually affected by high energy trauma. Male gender is highly

prevalent in high energy trauma. Similarly, young adults are also susceptible to femoral fracture caused by high-energy injuries, hence, associated with higher morbidity and mortality. The complication causing most morbidity is fracture that fail to unite (Khan et al., 2017).

There are many surgical method of femoral shaft fixation available. Intramedullary nailing has several advantages over other implants such as, superior rotational stability, ability to coat the implant with medication, even pressure distribution, minimal invasive, high fatigue strength, and high union rates with low impant failure rate (Kang et al., 2021). Nevertheless, certain problems may occur regardless the superiority of all treatment management.

Abnormal fracture healing presents as a significant matter in management of femoral fracture. Delayed union, malunion and non-union in femoral fracture is not unfamiliar. A study by Tay et al. (2014) reported among a total of 285 cases of femoral shaft fractures undergoing treatment, almost 50% of the cases (138 cases) become delayed union and non-union (Tay et al., 2014). Another study by Ma et al. (2016) found an incidence of 2.8% non-union cases in patients who underwent intramedullary nailing with locking procedures. It includes a hypertrophic non-union in 11 cases (Ma et al., 2016). Hence, revision surgeries are required.

Platelet Derived Growth Factor (PDGF-BB) is proposed as potential agent in fracture healing. Up to this point, PDGF has been scientifically studied improve wound healing by enhancing fibroblast proliferation, extracellular matrix synthesis, and re-vascularization. Platelets produce Platelet Derived Growth Factor, which is class of proteins composed of PDGF-AA, PDGF-AB, PDGF-BB, PDGF-CC, and PDGF-DD, when a fracture occurs (Solchaga et al., 2012). Recent research suggests that PDGF-BB is involved in chemotaxis, especially process of tissue healing mechanism (includes fibroblast, fibroblasts, monocytes), proliferation (includes cells of smooth muscle, capillary endothelial and fibroblasts), matrix molecules initiation (fibronectin, and hyaluronic acid), along with collagenase fibroblasts fabrication and release mechanism (Yoshimoto et al., 2006). When stimulated, cells of vascular endothel proliferation results in the new vascular formation on affected fracture area, an increase in VEGF will accelerate bone healing (Graham et al., 2009; I Made Iman Antariksa, 2012).

This study examined recombinantion of PDGF-BB on recovery of fracture (measured by the Allen score), collagen levels of type I and type III in the shaft Femur of Wistar-type rats that had undergone osteotomy followed by wire on intramedullar implantation, compared to rats that not administered by PDGF-BB. This study aims to evaluate role of PDGF on fractured bone healing.

SUBJECTS AND METHOD

1. Study Design

An experimental randomized post-control-only study is performed on Animal and Biomedical Laboratory Udayana University from March to September 2022.

2. Population and Sample

Simple random sampling is done on Wistar rat population. A total of 36 male and healthy Wistar Rats sample at 3-4 months old and weighs 140-160 grams are included in this study. Diseased rats, femoral pathology and inactive rats are excluded in this study. Samples are divided into 2 groups of 18 case and 18 control groups. The intervention given is administration of PDGF-BB intrafracture during fracture fixation. Group 1 is test animal with its shaft of femur had

undergone osteotomy and Wires on Intramedullar Open Reduction Internal Fixation that had administered of PDGF-BB. Group 2 is test animal with its shaft of femur undergone osteotomy and Wires on Intramedullar Open Reduction Internal Fixation without administion of PDGF-BB.

3. Study Variables

The independent variable in this study is administration of 10 mcg PDGF-BB following femoral osteotomy and intramedullary wire fixation. While the dependent variables are Allen score, type I and type III collagen levels. Control variables include: strain of the rats, male rats, age, body weight, food and environment for the experimental rats.

4. Operational definition of variables

Femoral shaft osteotomy is the procedure of cutting the bone in the diaphysis area of the femur which is right in the middle of the femoral bone and is broken using gigli and gives a bone defect of 8mm.

Intramedullary wire internal fixation is the procedure of inserting a metal rod into the bone medulla cavity and passing through the fracture fragments by the retrograde method to improve bone stabilization.

PDGF-BB is a polypeptide heparin binding chain with 4 types A, B, C, and D. PDGF used is PDGF-B which is derived from KLH conjugated synthetic peptide from human PDGF-B.

Type I collagen is collagen formed from the pro- α1(I) chain produced by the COL1A1 gene**.**

Type III collagen is fibrilar-forming collagen consisting of three α1(III) chains and is expressed in the initial embryo and throughout embryogenesis. The number of collagen levels of type I with type III obtained through ELISA examination and with µg/µl unit results.

5. Study Instruments

At the end of 4th week, femoral extraction is performed on sedated rats. Allen score measures fracture healing from microscopic anatomical pathology results. On the contrary, type I and III collagen levels are assessed using ELISA methods.

6. Data analysis

Data collection of Allen score, type I and type III collagen levels are listed. Descriptive analysis is performed and demonstrated in form of tables. Statistical analysis is performed using independent T-test in SPSS ver 25.0 software.

RESULTS

1. Sample Characteristics

Among the two groups, a descriptive analysis of certain variables, including: age and weight of the rats, was conducted before statistical analysis were performed to compare the characteristics between the two groups (see Table 1). The profile of Wistar Rats include: age Mean= 13.44; SD= 1.50 weeks (PDGF) and, Mean= 13.33; SD= 1.60 (control); weight Mean= 150.67; SD= 5.47 weeks (PDGF) and Mean= 149.28; SD= 6.83 (control). In both groups, the data is distributed normally and homogenous.

2. Bivariate Analysis

For all measured variables, mean value is compared between the 2 groups, and statistical analysis is performed using independent t-test as shown in table 2. In this study, we found PDGF-BB significantly improves fracture healing according to Allen score, type I and type III collagen levels. The mean value of Allen scores Mean= 3.89; SD= 1.40 (PDGF) and, Mean= 2.28; SD= 1.48 (control). The mean value of type I collagen Mean= 4.97; SD= 0,54 (PDGF) and, Mean= 4.41; SD=0.74 (control). The mean value of type III collagen Mean= 12.1; SD= 2.49 (PDGF) and, Mean= 10.01; SD= 1.61 (control). Among all the parameters measured, there are significant difference with better results in the PDGF group indicating improved fracture healing.

Table 1. Sample Characteristics

Variable	With PDGF BB (N=18)				Without PDGF BB (N=18)			
	Mean	SD	Min.	Max.	Mean	SD	Min.	Max.
Age (week)	13.44	1.50	10	16	13.33	1.60	10	16
Weight (gr)	150.67	5.47	140	160	149.28	6.83	140	160
Allen Score	3.89	1.40	2	6	2.28	1.48	0	5
Levels of Type 1 Collagen	4.97	0.54	3.5	5.7	4.41	0.74	2.6	5.7
Levels of Type III Collagen	12.10	2.49	8.2	17.6	10.01	1.61	6.9	13.3

Table 2. Independent T-test Results for Allen Score, Type I Collagen and Type III Collagen Levels

Variable	With PDGF BB (N=18)			Without PDGF BB (N=18)		
	Mean	SD	p	Mean	SD	p
Allen Score	3.89	1.40	0.020	2.28	1.48	0.020
Type 1 Collagen Levels	4,97	0.54	0.014	4.41	0.74	0.014
Type III Collagen Levels	12.10	2.49	0.005	10.01	1.61	0.005

DISCUSSION

PDGF-BB plays a crucial part in the fracture healing process, and it is anticipated that injection of PDGF-BB will stimulate angiogenesis, hence enhancing production of callus in fracture of bone (Rodrigues et al., 2010). Femoral bone and surrounding soft tissue were used to collect a sample for histology. Allen score was utilized to histologically evaluate the healing score (Harmantya Mahadhipta and Achmad Fauzi Kamal, 2013). According to studies on process of bone recovery evaluated callus level production using the Allen score on fractures treated with PDGF-BB, the fracture healing process was more advanced (p 0.05) based on the mean Allen score of callus developed on PDGF-BB-injection fracture area. Sources claiming that PDGF-BB has no remarkable effect on trigger formed of callus and recovery of fracture process had not widely studied as of yet. On this scientific finding has similar results with previous research indicating PDGF-BB-injection triggers formation of callus (Caplan and Correa, 2011).

There have been few studies on the level of type I collagen in patients who received treatment with PDGF-BB and those who did not. The objective of this study was to determine the level of collagen in the fractures that were treated with and without PDGF-BB. Compared to the patients who were not treated with PDGF-BB, the individuals who received treatment with this drug had significantly higher levels of type I collagen (p 0.05). On this finding, it showed PDGF-BB significantly raises levels of type I collagen are consistent with those of 13 others. According to studies done by Hanaoka et al., PDGF-BB causes an increase in type I collagen synthesis, with the greatest increase occurring 24 hours after administration (Hanaoka et al., 2006; Ojima et al., 2003). In addition, it had proposed PDGF-BB doesn't influence collagen synthesis directly, but more as potent chemoattractant for wound macrophages and fibroblasts, which triggers elevated endogenous in TGF and triggers synthezied of new collagen also wound recovery improvement (Evrova and Buschmann, 2017). However, studies claims

that while PDGF-BB doesn't give effect to collagen levels directly, it powerfully stimulates collagenase expression, which may be important for the remodeling phase (Tan et al., 1995).

Type III collagen levels in fractures treated with PDGF-BB compared to groups which isn't treated with PDGF-BB were found to be significantly greater in the treatment group, according to the study's findings (P0.05). We may conclude that the findings of this study could be utilized as recommendation for future studies in order to establish either PDGF-BB can elevate type III collagen levels because few other studies already demonstrated elevated type III collagen trigger by PDGF-BB. Furthermore, the amount of type III collagen were roughly three times higher than type I collagen levels in this study. When compared to collagen type I, the level of collagen type III is three times higher under normal bone conditions. Type III collagen is crucial in bone because of its rigidity, which can be improved with the help of PDGF-BB. Currently, the intervention in this study has only been tested on rats, but it is hoped that this will change so that it could utilized to higher animals, like rabbit, chimpanzees, or even humans in clinical trials in the future. There is optimism that PDGF-BB will one day be used in human trials, which would advance research and optimize treatment for fractures.

In conclusion, the femoral shaft of the Wistar-type rats which has undergone osteotomy and wire on intramedullar implantation and additional intrafractured PDGF-BB healed more quickly compared to group that not intrafractured PDGF-BB. Comparing individuals with and without intrafractured PDGF-BB, also discovered that ype I and type III collagen levels were higher. We support the use of PDGF in improving fracture healing.

AUTHOR CONTRIBUTIONS

IGNWA: designed and coordinated the study, performed the experiments, acquired and analyzed data, interpreted the data, and wrote the manuscript.
KGBG: performed the experiments, acquired and analyzed data, interpreted the data, and wrote the manuscript.
IKS, and AAWL: supervised and finalized the manuscript. All authors approved the final version of the article.

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CONFLICT OF INTEREST

The authors declare that the study was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest

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