Journal of Orthopaedics 16 (2019) 230–233

Contents lists available at ScienceDirect

Journal of Orthopaedics

journal homepage: www.elsevier.com/locate/jor

Recombinant platelet derived growth factor-BB and hyaluronic acid effect in rat osteoarthritis models

I Gusti Ngurah Yudhi Setiawan*, I Ketut Suyasa, Putu Astawa, I Wayan Suryanto Dusak, I Ketut Siki Kawiyana, I Gusti Ngurah Wien Aryana

Department of Orthopaedic and Traumatology, Udayana Faculty of Medicine, Indonesia

ARTICLE INFO

Keywords:
Platelet derived growth factor
Knee joint osteoarthritis
Chondrocytes
Procollagen amino terminal N-Propeptide type IIA
C-telopeptide fragment of type II collagen

ABSTRACT

Osteoarthritis (OA) arises from imbalance of cartilage metabolism between the synthesis and degradation of type II collagen by the chondrocyte. Collagen type II degradation is characterized by increase in the biomarker of C-telopeptide fragment of type II collagen (CTX-II), while the anabolic process of cartilage is characterized by an increase in the biosynthesis of procollagen amino terminal N-propeptide type IIA (PIIANP). Platelet derived growth factor (PDGF) with Hyaluronic Acid (HA) as a potent growth factor can be used to stimulate the higher formation of chondrocyte and PIIANP levels and lower CTX-II levels in mouse knee osteoarthritis model.

1. Introduction

Osteoarthritis is a joint disease of cartilage degeneration with the loss of joint cartilage tissue. It is caused by imbalance of cartilage metabolism between the synthesis and degradation of type II collagen as the most specific cartilage matrix ingredient. Osteoarthritis is still difficult to handle because of the irreversibility and progressivity of the disease.[1]

Collagen type II degradation is specifically characterized by an increase in the biomarker of C-telopeptide fragment of type II collagen (CTX-II),[1] while the anabolic process of cartilage is characterized by an increase in the biosynthesis of procollagen amino terminal N-propeptide type IIA (PIIANP).[2] In osteoarthritis, chondrocytes fail to synthesize matrix with good quality. The biomechanical and biochemical changes in the joint due to osteoarthritis further increase the destruction process of cartilage.[1]

Platelet derived growth factor (PDGF) as a potent growth factor can be used as an alternative for cartilage regenerative therapy. *Platelet Derived Growth Factor-BB* has proangiogenic characteristic which upregulates VEGF to stimulate the growth of new capillaries.[3] Meanwhile, Hyaluronic Acid (HA) is viscosupplement for knee joint which can also act as growth scaffold and carrier of PDGF.[4] This study aims to prove that Recombinant PDGF-BB (rrPDGF-BB) with HA as scaffold can stimulate higher formation of chondrocyte and PIIANP levels and lower CTX-II levels in mouse knee osteoarthritis model.[1]

2. Methods

The authors wanted to know the effect of rrPDGF-BB and hyaluronic acid as scaffold on mouse knee cartilage metabolism, thereby reinforcing the theory of OA pathogenesis as well as recommendation of rrPDGF-BB growth factor augmentation in intraarticular HA to slow pathogenesis and assist cartilage regeneration in OA genu.

This research is an experimental research using randomized post-test only control group design. The research was conducted at pharmacology laboratory and at the laboratory of veterinary pathology from February 2018 until March 2018.

The study used 32 white Wistar rat aged 12 weeks and weighed 200–250 g. Prior to intervention, induction of OA in rat knee joint was by intraarticular injection of monosodium iodoacetate (MIA) at a dosage of 0.3 mg per 150 mg per body weight. Monosodium iodoacetate (MIA) is dissolved in isotonic saline solution to a final concentration of 40 mg/mL. The total volume of the solution to be injected into the synovial space of the joint is 50 μL. After 2 weeks, rats were given intervention. The rats with MIA inducted-OA were then divided into 2 groups, the control group without intraarticular injection of rrPDGF-BB and HA and the intervention group with intraarticular injection ofrrPDGF -BB and HA.

The rrPDGF-BB injection was administered at 10 ng/mL dose in 10 mg/ml of 110 kDa HA hydrogel intraarticular 2 times in the first week and second week after MIA injection. At week 4, rats are euthanized using ketamine injection at dose 132–300 mg/kgBW

* Corresponding author. Department of Orthopaedic and Traumatology, Udayana Faculty of Medicine, Udayana University - Sanglah General Hospital, Jl. Kesehatan 1, Denpasar-Bali, 80114, Indonesia.
E-mail address: igyudhisetiawan@gmail.com (I.G.N.Y. Setiawan).

https://doi.org/10.1016/j.jor.2019.02.028
Received 23 September 2018; Accepted 17 February 2019
Available online 02 March 2019

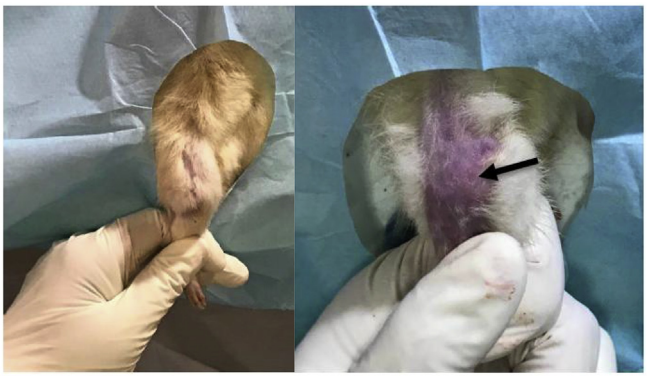


Fig. 1. Swelling and palpable osteophytes over the rat knee joint.

intramuscular.

About 2 mL of serum samples were taken with 3 mL syringe to examine serum levels of PIIANP and CTX-II by ELISA method. The rats knee joint was exposed and macroscopic documentation was performed and histopathology examination was performed microscopically to count the number of chondrocyte cells in haematoxylin-eosin (HE) staining of rat cartilage joints in 5 fields of view at 400 times magnification of light microscope.

The data obtained were analyzed using SPSS for Windows version 22.0 and independent *t*-test with 95% significance level.

3. Result

The data from this experimental study include clinical data, histopathology and laboratory data support the feature of OA following injection of MIA. The clinical signs of the rat knee joint showed marked swelling of the knee joint and palpable osteophytes as shown in Fig. 1.

The gross appearances of rat knee joint with OA in the control and intervention group with rrPDGF-BB and HA after the harvest shown in Fig. 2.

The knee joint of the control group appeared with thickened synovium tissue, and osteophytes in the distal femur and proximal tibia. The joint surface over the cartilage in the medial and lateral femoral condyle showed chondrolysis lesions. The knee joint of the intervention group that has been given the injection of rrPDGF-BB and intraarticular HA showed hypertrophy of synovium and the osteophytes were less prominent than the control group, and the cartilage surface was still smoother than the control group.

The histopathological features of rat knee joint cartilage in the control group without injection of rrPDGF-BB and HA showed cartilage surface fibrillation, less superficial chondrocyte cells. The type II collagen fiber configuration was loosely arranged with invasion of blood vessels in calcification zone as shown in Fig. 3.

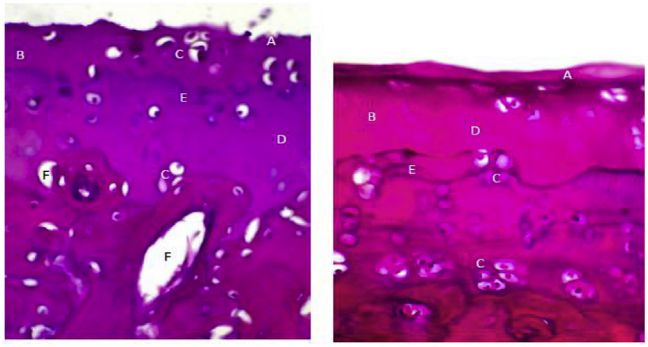


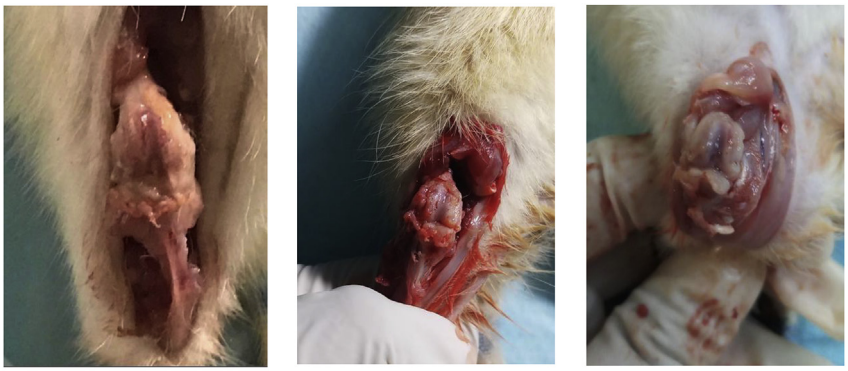
Fig. 3. The histological features of rat knee joint: Surface fibrillation (A), reduced superficial chondrocyte cells (B), clustered chondrocyte cells (C), loose type II collagen fiber (D), tidemark split and reduplication (E), and invasion of blood vessels in calcification zone (F).

The histopathological features of rat knee joint cartilage in the intervention group with rrPDGF-BB and HA showed more superficial chondrocyte cells with less clustered chondrocyte cells and the type II collagen fiber configuration was medium arranged (Fig. 4).

The results of this study showed an average number of chondrocyte cells count in the intervention group amounted to 58.25 ± 6.816, while the control group amounted to 49.81 ± 6.997. The lowest counted chondrocyte cells were found in the control group amounted to 32 cells/fields of view while the most counted cells found in the intervention group amounted to 70 cells/fields of view. The mean of PIIANP levels in the intervention group was 5,119 ± 3,513 ng/mL while in the control group was 2,723 ± 1,582 ng/mL. The highest level of PIIANP was in the intervention group with 12,04 ng/mL while the lowest level was in intervention group that was 0,478 ng/mL. The mean of CTX-II in the intervention group was 10,130 ± 4,133 ng/mL while in the control group that was 14,670 ± 6,291 ng/mL. The CTX-II level was highest in the control group amounted to 25,260 ng/mL while the lowest level was in the intervention group amounted to 2,275 ng/mL.

The significance of the data in unpaired two groups with the normal distribution was tested with independent *t*-test that compares the average of post-test data from each group as shown in Table 1.

The above table shows that the number of chondrocyte cells counts in the intervention group was higher than the control group, and the mean difference between the intervention and control groups was statistically significant with $p = 0.002$ ($p < 0.05$). The PIIANP levels in the intervention group were higher than the control group, and the mean difference between the intervention and control groups was statistically significant with $p = 0.021$ ($p < 0.05$). While the level of CTX-



(a) (b) (c)

Fig. 2. The gross appearance of the knee rat joint in the control group (a) and intervention group (b) and (c).

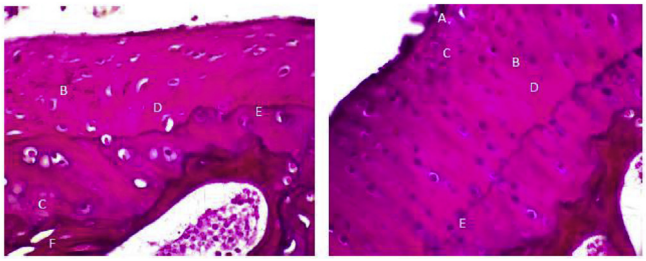


Fig. 4. The histopathological features of rat knee joint cartilage in the intervention group with rrPDGF-BB and HA showed surface fibrillation (A), more superficial chondrocyte cells (B), less clustered chondrocyte cells (C), medium arranged of type II collagen fiber (D), tidemark split and reduplication (E), and invasion of blood vessels in calcification zone (F).

II in the intervention group was lower than the control group, and the mean difference between intervention and control groups was statistically significant with p = 0,023 (p < 0.05).

4. Discussion

4.1. Effects of rrPDGF-BB and HA on total chondrocyte cells count

In this study, there were significant differences in the number of chondrocytes in knee joint cartilage in the OA intervention group given rrPDGF-BB with HA compared with the control. The formation of chondrocytes resemble the regenerative activity can be explained because the role of rrPDGF-BB in mediation of tissue repair processes including chemotaxis (monocytes, neutrophils, fibroblasts), proliferation (fibroblasts, smooth muscle cells, capillary endothelial cells, chondrocytes), and induction of matrix molecules (fibronectin, hyaluronic acid, collagen).[5]

This is consistent with a prospective study by Schmidt et al. (2006) who found indirect evidence for the role of active rrPDGF-BB in the healing process can be seen from the healing response to cartilage damage by microfracture.[6] The formation of a clot in the defect attached to the bone by the rough bony surface produced by microperforations. Growth factors such as PDGF-BB are released later to the site of the defect, giving chemotactic and mitogenic effects on cells around cartilage and mesenchymal stem cell infiltration. It provides a good environment for the formation of new tissues that can be augmented by administering scaffolds in autologous cells.[5]

Studies by Li F et al. also show that rrPDGF-BB can help promote fibroblast proliferation, matrix synthesis, neovascularization and mechanical components of the ligamentation process in anterior cruciate ligament ruptures that have been reconstructed and given PDGF-BB growth factor. This shows the role of PDGF-BB in cell proliferation and matrix synthesis required for healing.[7]

Another study by Montaseri et al. also showed results in line with this study. In the in vitro study, PDGF-BB administration in primary chondrocytes undergoing differential induction by IL-1β showed apoptotic resistance and increased anabolic activity leading to proliferation of chondrocytes.[8]

Giving of rrPDGF-BB can inhibit the expression of MMP-9, MMP-13, caspase-3 breakdown process and inhibit the activation of NF-kB by IL-1β, so that the chondrocytes cell apoptosis process can be prevented and the degradation of cartilage extracellular matrix can be inhibited. In addition, the administration of rrPDGF-BB can also help to improve the adaptation of Shc, phosphorylated Erk1/2 adapter protein and cartilage master transcription factor SOX-9, resulting in increased production of cartilaginous specific matrix which plays an important role in the differentiation and proliferation of chondrocytes, while preventing acceleration cell maturation along the endochondral pathway.[8] The ability of the chondrocytes to be capable of proliferation and differentiation is essential in the regeneration process to fill the defects with cartilage resulting from the process of chondrocytes cell apoptosis and the degradation of the cartilage extracellular matrix. This is particularly important in cartilage and subchondral bone to prevent delamination of the regenerated tissue.[6]

In addition, rrPDGF-BB also induces chemotaxis and proliferation of MSC and progenitor cells,[9] where MSCs are abundant in joint tissue including synovium, fat pad, synovial fluid, bone marrow, and cartilage, and release trophic factors that can cause cartilage repair conditioned by the chondrocytes.[10] Growth factors such as PDGF, FGF-2 or EGF will induce MSC cell differentiation from progenitor pool to osteogenic or chondrogenic. The PGDF-BB assists the chondrogenesis process through the activation of mediators such as Wnt, Sox-9 or TGF-β and BMPs to then form the chondrocytes.[11]

4.2. The influence of rrPDGF-BB and HA against PIIANP levels

The research indicated that there is a process of synthesizing collagen type II with a higher rrPDGF and HA showed by a significant difference from PIIANP levels. Some previous research showed that there is increase of synthesizing matrix collagen type II levels from chondrocytes after treated with rrPDGF-BB and/or IGF-1 marked by increased PIINP levels.[8,12] This research also supported by research from Rousseau et al., shows that there is a decrease of PIIANP levels of patient with OA knee compared to the normal indicates decrease of synthesizing collagen from joint disease.[1,13] The research from Berry et al. with 117 OA knee subject also found that low serum PIIANP levels or less from its mean value associated with decreasing volume of cartilage medial.[14] A research by Zhu et al., shows that PIIANP produced by chondroprogenitor cells and deposited to extracellular matrix to form the cartilage and have a bounding with BMP-2 and TGF-β1 which is the factor of tissue that can induce chondrogenesis in vivo.[15] Platelets Derived Growth Factor BB then modulate BMP-2 and TGF-β1 factors so initiate the differentiation process of chondrocytes cell.[11]

The provision of rrPDGF-BB can induce an anabolism mediator for the metabolism process of cartilage through activation B1-Integrin and shc protein adapter, then can stimulate *MAP kinase intracellularsi gnaling pathway*, so there is a rise in expression regulations of SOX-9 on chondrocytes cells.[8] This process can trigger the formation of *cartilage specific proteoglycan* and collagen synthesis type II that marked by an increase in the type IIA Procollagen Amino-N Propeptides (PIIANP).[11]

4.3. The influence of rrPDGF-BB and HA against CTX-II levels

In this study obtained significant differences in CTX-II levels in cartilage of the knee joint on the OA group that given rrPDGF-BB with HA compared to the p value of 0,023 (p < 0,05). This shows that there

Table 1
The result of the post-test data comparability test with independent *t*-test within the control and intervention groups.

Variables	Group		Mean difference	95% CI	p value
	Intervention with PDGF & HA (n = 16)	Control without PDGF & HA (n = 16)			
Number of Chondrocytes Cells Count	58,25 ± 6,816	49,81 ± 6,997	8,437	3,449–13,425	0,002
PIIANP levels	5,119 ± 3,513	2,723 ± 1,582	2,396	0,391–4,400	0,021
CTX-II levels	10,130 ± 4,133	14,670 ± 6,291	−4,539	−8,408 – (−0,670)	0,023

is higher degradation process of collagen type on the control group compared with intervention group.[11]

Montaseri et al. suggested that the provision of rrPDGF-BB and/or IGF-1 inhibits expression of protein mmp-9 and mmp-13 then lead to inhibits the degradation process of the cartilage.[8] Olsen et al., in his research on chondrocyte cells bovine ex vivo said that low levels of CTX-II in cartilage after induced by the growth factors like IGF-1 that shows the role of degradation products type II collagen as CTX-II able to look back on a katabolic process in cartilage.[12] This is supported by a study from Bruyere et al., which states that elevated levels of CTX-II significantly associated with the process of degradation cartilage besides MMP and could predict lose thickness of cartilage from medial tibia ($p = 0.03$) and lateral tibia ($p = 0{,}001$). In this study, cartilage turnover measured by specific marker that forming matrix collagen type II of PIIANP and CTX-II. Compared with other studies, this study shows the results of consistent higher levels of PIIANP accompanied with lower the CTX-II after given the PDGF-BB with HA indicates of increasing the cartilage metabolism process/turnover. Improving metabolism indicates the process of cartilage regeneration shown by the number of higher chondrocytes.[8]

5. Conclusion

From the data above it can be concluded that the recombinant PDGF-BB significantly improving the anabolism process of collagen type II by chondrocyte cells and inhibit the catabolism that marked with the higher cell volume of chondrocyte and levels of serum PIIANP and low levels of serum CTX-II. The result of this research can be used as the basis for the development of the intervention of osteoarthritis based on growth factor for regenerative therapy. But, the research still needed a different sample or more total sample for getting clinical effect, especially in humans.

Conflicts of interest

None.

Ethical approval

Medical Research Ethics Committee of Faculty of Medicine Udayana University.

Contributorship

All authors have contributed intellectually to the work, participated in the work to the extent that he or she can defend the contents. All authors reviewed and edited the manuscript and approved the final version of the manuscript.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jor.2019.02.028.

References

1. Rousseau JC, Garnero P. Biological markers in osteoarthritis. *Bone.* 2012;51(2):265–277https://doi.org/10.1016/j.bone.2012.04.001.
2. Wollheim FA. Serum markers of articular cartilage damage and repair. *Rheum Dis Clin N Am.* 1999;25(2):417–432 viii https://doi.org/10.1016/S0889-857X(05)70076-4.
3. Rodrigues M, Griffith LG, Wells A. Growth factor regulation of proliferation and survival of multipotential stromal cells. *Stem Cell Res Ther.* 2010;1(4):32https://doi.org/10.1186/scrt32.
4. Chen J, Yang L, Guo L, Duan X. Sodium hyaluronate as a drug-release system for VEGF 165 improves graft revascularization in anterior cruciate ligament reconstruction in a rabbit model. *Experimental and Therapeutic Medicine.* 2012;4(3):430–434https://doi.org/10.3892/etm.2012.629.
5. Yoshimoto T, Yamamoto M, Kadomatsu H, Sakoda K, Yonamine Y, Izumi Y. Recombinant human growth/differentiation factor-5 (rhGDF-5) induced bone formation in murine calvariae. *J Periodontal Res.* 2016;41(2):140–147https://doi.org/10.1111/j.1600-0765.2005.00847.x.
6. Schmidt MB, Chen EH, Lynch SE. A review of the effects of insulin-like growth factor and platelet derived growth factor on in vivo cartilage healing and repair. *Osteoarthritis Cartilage.* 2006;14(5):403–412https://doi.org/10.1016/j.joca.2005.10.011.
7. Li F, Jia H, Yu C. ACL reconstruction in a rabbit model using irradiated Achilles allograft seeded with mesenchymal stem cells or PDGF-B gene-transfected mesenchymal stem cells. *Knee Surg Sports Traumatol Arthrosc.* 2007;15(10):1219–1227https://doi.org/10.1007/s00167-007-0385-x.
8. Montaseri A, Busch F, Mobasheri A, et al. IGF-1 and PDGF-bb suppress IL-1??-induced cartilage degradation through down-regulation of NF-??B signaling: involvement of Src/PI-3k/AKT pathway. *PLoS One.* 2011;6(12)https://doi.org/10.1371/journal.pone.0028663.
9. Hollinger JO, Hart CE, Hirsch SN, Lynch S, Friedlaender GE. Recombinant human platelet-derived growth factor: biology and clinical applications. *The Journal of Bone and Joint Surgery-American.* 2008;90(Suppl 1):48–54https://doi.org/10.2106/JBJS.G.01231.
10. Mastbergen SC, Saris DBF, Lafeber FPJG. Functional articular cartilage repair: here, near, or is the best approach not yet clear? *Nat Rev Rheumatol.* 2013;9(5):277–290https://doi.org/10.1038/nrrheum.2013.29.
11. Augello A, De Bari C. The regulation of differentiation in mesenchymal stem cells. *Hum Gene Ther.* 2010;21(10):1226–1238https://doi.org/10.1089/hum.2010.173.
12. Olsen AK, Sondergaard BC, Byrjalsen I, et al. Anabolic and catabolic function of chondrocyte ex vivo is reflected by the metabolic processing of type II collagen. *Osteoarthritis Cartilage.* 2007;15(3):335–342https://doi.org/10.1016/j.joca.2006.08.015.
13. Quintana DJ, Garnero P, Huebner JL, Charni-Ben Tabassi N, Kraus VB. PIIANP and HELIXII diurnal variation. *Osteoarthritis Cartilage.* 2008;16(10):1192–1195https://doi.org/10.1016/j.joca.2008.03.006.
14. Berry PA, Maciewicz RA, Wluka AE, et al. Relationship of serum markers of cartilage metabolism to imaging and clinical outcome measures of knee joint structure. *Ann Rheum Dis.* 2010;69(10):1816–1822https://doi.org/10.1136/ard.2009.124420.
15. Zhu Y, Oganesian A, Keene DR, Sandell LJ. Type IIA procollagen containing the cysteine-rich amino propeptide is deposited in the extracellular matrix of prechondrogenic tissue and binds to TGF-??1 and BMP-2. *JCB (J Cell Biol).* 1999;144(5):1069–1080https://doi.org/10.1083/jcb.144.5.1069.