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Potential of Activated Platelet-Rich Plasma Against Osteoarthritis: In Vivo Study

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ABSTRACT

Introduction: Osteoarthritis (OA) is a disorder that causes a decrease in the quality of life in elderly patients. The current treatment is only symptomatic in reducing inflammation. This study is one of the exploratory studies to examine the potential of platelet-rich plasma (PRP) in optimizing the improvement of OA patients through the inhibition of inflammatory signals in joint tissue in vivo. **Methods:** This study is an experimental study with a post-test-only approach with a control group design. A total of 30 rats (*Rattus norvegicus*) Wistar strain was included in this study and met the inclusion criteria in the form of the male gender, weight between 150-200 grams, and age 8-10 weeks. The rats were divided into 3 groups, namely the group that was not induced by OA and not given PRP (P1), the OA group and given 50 uL saline injection (P2), the OA group and given PRP 50 uL (P3), the treatment was carried out once a week for 4 weeks. **Results:** The results showed that the P3 group that was treated with platelet-rich plasma showed a significant decrease in interleukin-1ß levels when compared to the P2 group that was induced by OA but was only treated with saline ($p<0.05$). **Conclusion:** Platelet-rich plasma has the potential as a biological agent against osteoarthritis in an in vivo study.

Keywords: osteoarthritis, in vivo study, platelet-rich plasma, interleukin, experimental study.

Introduction

Osteoarthritis (OA) is a joint movement disorder, especially in the knee joint, caused by chronic inflammation in the joints.[1-3] The chronic inflammation that occurs in OA is caused by loss of joint cushion (joint cartilage), which causes trauma when the joint is moved. Trauma that occurs chronically triggers chronic inflammation that triggers complaints of pain and difficulty moving joints.[4] OA disorders are common in elderly patients, along with joint cartilage degeneration. According to epidemiological data, there has been a threefold increase in OA patients in the last 10 years. This is due to the increasing life expectancy, where more and more patients are more than 60 years old. This increase in life expectancy should also be accompanied by an increase in the quality of life of elderly patients.[5]

OA is one of the disorders that cause a decrease in the quality of life of sufferers, where the majority of patients are elderly. The current treatment is only symptomatic in reducing inflammation. The use of non-steroidal anti-inflammatory drugs that are very massive and used in the long term, on the one hand, triggers a series of unwanted side effects in the form of gastrointestinal disorders and triggers various other metabolic and endocrine disorders. This triggers the exploration of new therapeutic modalities for the development of definitive therapeutic modalities. Management of OA should not only refer to the chemical aspect in the form of drugs, but it is necessary to develop stem cell-based biological agents. One of the promising modalities to be developed is platelet-rich plasma.[6]

Platelet-rich plasma is one of the modalities of biological agents obtained from the patient's own blood. Platelet-rich plasma is the result of processing the patient's blood, where platelets are isolated from plasma through a structured separation process. Platelets are blood components that are responsible for repairing tissue damage. This makes platelets rich in growth factors that are important in repairing damaged tissue. Proper isolation and activation of platelets are important factors in optimizing the growth factors present in platelet-rich plasma.[7,8] This study is one of the exploratory studies to examine the potential of platelet-rich plasma in optimizing the improvement of OA patients through the inhibition of inflammatory signals in joint tissue in vivo.

Methods

This study is an experimental study with a post-test-only approach with a control group design. A total of 30 rats (*Rattus norvegicus*) Wistar strain was included in this study and met the inclusion criteria in the form of the male gender, weighing between 150-200 grams, and of age 8-10 weeks first, rats were acclimatized for 7 days, then divided into 3 groups (P1, P2, and P3) randomly, where each group consisted of 10 rats. The P1 group was a group of mice that were not induced by OA and were not treated with platelet-rich plasma; The P2 group was a group of rats that were induced by OA by intra-articular injection of monosodium iodoacetate (MIA, 2 mg/50 uL) and administered an intra-articular saline injection of 50 uL; A P3 group is a group of rats that were induced by OA by intra-articular injection of monosodium iodoacetate (MIA, 2 mg/50 uL) and given an intra-articular injection of 50 uL of platelet-rich plasma, the treatment was administered once a week for 4 weeks. This study has been approved by the CMHC-Science and Research Center research ethics commission, number No.33/CMHC/KEPK/2021.

Platelet-rich plasma was obtained by first taking 3 mL of rat blood, then the process of isolation of platelet-rich plasma was carried out by mixing with 0.5% citrate buffer and centrifuged at 1200 rpm for 15 minutes. Next, the platelets were isolated and activated by adding 1% thrombin. The process of making platelet-rich plasma is carried out at the Eureka Research Laboratory, Palembang, Indonesia. Induction of OA was carried out by first anesthesia in rats using ketamine (dose of 0.015 mg/gBW) intramuscularly and chlorate (dose of 0.0025 mg/gBW) subcutaneously. Monosodium iodoacetate (MIA) was injected intra-articularly genu dextra region of experimental rats. Mice were monitored daily for signs of distress and signs of infection. Evacuation of genu dextra was carried out by anesthesia first in mice, then perfusion was carried out, and genu dextra was taken. The genu dextra rat tissue was put into a closed microtube container containing 0.9% NaCl liquid, one container for one sample. The samples were temporarily stored in a cooler bag (temperature $\leq$ 20ºC) and immediately stored in the freezer (temperature -20ºC). Analysis of IL-1B levels was carried out using the enzyme-linked immunosorbent assay (ELISA) method according to the manufacturer's instructions (CloudClone®).

After the data is collected, data cleaning, coding, and tabulation are carried out. All results were assessed by means ± standard deviation accompanied by a normality test (Shapiro-Wilk) and data homogeneity test (Levene Statistic). The test used in this study is one-way

ANOVA. The results are said to be meaningful if $p \leq 0.05$. Data analysis was performed using SPSS version 25 for Windows.

Results and Discussion

Table 1 shows the assessment of inflammatory markers (IL-1βlevels). The higher levels of IL-1β indicate inflammation in the synovial tissue, as occurs in OA conditions. The P3 group that received the platelet-rich plasma treatment showed a significant decrease in IL-1β levels when compared to the P2 group that was induced by OA but was only treated with saline ($p<0.05$).

Table 1. Comparison of IL-1β levels between groups

Group	IL-1β levels (ρg/mL) Mean ± SD	value*
P1	23.56 ± 1.87	0.002
P2	245.87 ± 12.32	
P3	55.64 ± 2.43	

*one-way ANOVA, $p<0.05$

OA was caused by chronic inflammation due to trauma initiated by degeneration of joint cartilage.[9-11] Chronic trauma causes inflammation that results in the activation of various pro-inflammatory cytokines, namely interleukin-1ß (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor (TNF)-α.[12] Chronic activation of the IL-1ß cytokine results in the inability to activate the anti-inflammatory cytokine TGF-β.[13] This causes no repair of cartilage tissue. Even the chronic inflammatory process causes osteoclast activation and further degrades bones and joints. The platelet-rich plasma that is rich in growth factors shows the potential in suppressing the activation of the inflammatory cytokine IL-1β.[14] The ability of platelet-rich plasma to suppress IL-1β suggests the potential of this biological agent in reducing chronic inflammation and preventing increasingly severe cartilage and bone damage.14 Of course, these results show the promising potential of platelet-rich plasma as a biological agent modality in overcoming OA.

Conclusion

Platelet-rich plasma has the potential as a biological agent against osteoarthritis in an in vivo study.

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Submission acknowledgement

Dear author(s),

Rachmat Hidayat*, Patricia Wulandari has submitted the manuscript “Potential of Activated Platelet-Rich Plasma Against Osteoarthritis: In Vivo Study“ to Journal of Anesthesiology & Clinical Research. The paper will be screened by editor and reviewed by peer review.

Cordially,

Prof. Paula Magnano, PhD

Editor HM Publisher

(*) Corresponding author

Journal of Anesthesiology and Clinical Research

HM Publisher

Peer Review Results

Dear author(s),

Rachmat Hidayat*, Patricia Wulandari has submitted the manuscript “Potential of Activated Platelet-Rich Plasma Against Osteoarthritis: In Vivo Study“ to Journal of Anesthesiology and Clinical Research. The decision : Revision Required.

Cordially,

Prof. Paula Magnano, PhD

Editor HM Publisher

(*) Corresponding author

Reviewer 1: Revision required

Potential of Activated Platelet Rich Plasma Against Osteoarthritis: In Vivo Study →1

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[1]Department of Biology, Faculty of Medicine, Universitas Sriwijaya, Palembang, Indonesia

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Abstract →3

Osteoarthritis (OA) is one of the disorders that causes a decrease in the quality of life in elderly patients. The current management is only symptomatic in reducing inflammation and inflammation. This study is one of the exploratory studies to examine the potential of platelet rich plasma (PRP) in optimizing the improvement of OA patients through inhibition of inflammatory signals in joint tissues in depth Vivo. This study is an experimental study with a post test only with control group design approach. A total of 30 rats (*Rattus norvegicus*) of the Wistar strain were included in this study and met the inclusion criteria in the form of male sex, weighing between 150-200 grams, and after 8-10 weeks. The mice were divided into 3 groups, namely the group that was not OA induced and not given PRP (P1), the OA group and given the injection of a 50 uL saline solution (P2), the group OA and given PRP 50 uL (P3), the treatment is carried out once a week for 4 weeks. The results showed that the P3 group that received platelet rich plasma treatment showed a significant decrease in interleukin-1ß levels when compared to the OA-induced P2 group however, it is only given saline treatment ($p<0.05$). In conclusion, platelet rich plasma has the potential to be a biological agent against osteoarthritis disorders in invivo studies.

Keywords: Osteoarthritis, In vivo study, Platelet rich plasma, Interleukin, Experimental study. →2

1. Introduction →4

Osteoarthritis (OA) is a disorder of joint movement, especially the knee joint caused by chronic inflammation of the joints. [1-3] Chronic inflammation that occurs in OA is caused by loss of joint pads (joint cartilage), which causes trauma when the joint undergoes movement. The chronic trauma triggers chronic inflammation, triggering complaints of pain and difficulty

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OA is one of the disorders that causes a decrease in the quality of life of sufferers, where the majority of patients are elderly. The current management is only symptomatic in reducing inflammation and inflammation. The use of nonsteroidal anti-inflammatory drugs that are very massive and used in the long term, on the one hand triggers a series of undesirable side effects in the form of gastroinstestinal disorders and triggers various other metabolic endocrine disorders. This triggers the exploration of new therapeutic modalities for the development of definitive therapeutic modalities. OA management should not only refer to chemical aspects in the form of drugs, but it is necessary to develop stem cell-based biological agents. One of the modalities that is quite promising to be developed is platelet rich plasma. [6th]

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Platelet rich plasma was obtained and first took the blood of mice as much as 3 mL, then the process of isolating platelet rich plasma by mixing with a 0.5% citrate buffer and centrifugation was carried out at a speed of 1200 rpm for 15 minutes. Next, platelet isolation and activation are carried out with the addition of 1% thrombin. The process of making platelet rich plasma was carried out at the Eureka Research Laboratory, Palembang, Indonesia. OA induction was performed by first performing anesthesia in White Rats using ketamine (dose 0.015 mg / gBW) intramuscularly and chlorate (dose 0.0025 mg / gBW) subcutaneously. Monosodium iodoacetate (MIA) was injected intra-articularly in the genu dextra region of experimental rats. Rats are monitored daily for signs of distress and signs of infection. Evacuation of genu dextra was carried out by performing anesthesia first on mice, then perfusion was carried out and genu dextra was taken. The mouse's dextra genu tissue was inserted into a lidded microtube container containing 0.9% NaCl liquid, one container for one sample. The samples are temporarily stored in a cooler bag (temperature $\leq 2^0$ C) and immediately stored in a freezer (temperature -20^0 C). Analysis of IL-1B levels was carried out by the enzyme linked immunosorbant assay (ELISA) method according to the instructions of the manufacturer (CloudClone®).

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3.Results and discussion →6

Table 1 shows the assessment of inflammatory markers (kadar IL-1B). Higher levels of IL-1 B indicate inflammation of synovial tissue as occurs in OA conditions. The P3 group that

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4. Conclusion →7

Platelet rich plasma has the potential to be a biological agent against osteoarthritis disorders in invivo studies.

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Reviewer Comment:

1→ Title of Manuscripts should be explained independent variable and dependent variable also subject of study.

2→ Keywords should be showed the main words of the study, the authors can use MeSH to develop keywords.

3→ Abstract should be showed the main of background, methods, results and conclusion of study.

- Background abstract should be showed the urgency of study and why the study important, in simple way.
- Conclusion should be wrote in simple way, specific to the main results. Conclusion in abstract should not showed statistic results.

4→Introduction should be showed the urgency of study (epidemiology data), biological plausibility concept, and lack of knowledge in the study.

- Paragraph 1→ need improvement in urgency of study and explain more about epidemiology data. Authors do not only show the data, but try to elaborate and make comparison about the data from year to year.
- Paragraph 2 and 3 need improvement to focus in biological plausibility concept.

5→ Methods should be showed more about how the study develop. Methods should be showed the design of study; population, sample and sample size of study; inclusion criteria; place of study; ethical clearence steatment; independent and dependent variable; data analysis.

- Methods need to showed the design of study; population, sample and sample size of study; inclusion criteria; place of study; ethical clearence steatment; independent and dependent variable; data analysis, more specific but not to long.

6→ Results should be showed baseline characteristics subject of study, main results of study. Authors must be focused and try to make results no more table and figure.

7→ Discussion should be explored more biological plausibility, not only showed about statistical results.

8→ Conclusion should more specific and not more showed statistical results

9→ Authors must check the references for make update references. References should no more than 10 years.

Reviewer 2: Revision required

Potential of Activated Platelet Rich Plasma Against Osteoarthritis: In Vivo Study

Rachmat Hidayat[1*], Patricia Wulandari[2]

[1]Department of Biology, Faculty of Medicine, Universitas Sriwijaya, Palembang, Indonesia

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Platelet rich plasma was obtained and first took the blood of mice as much as 3 mL, then the process of isolating platelet rich plasma by mixing with a 0.5% citrate buffer and centrifugation was carried out at a speed of 1200 rpm for 15 minutes. Next, platelet isolation and activation are carried out with the addition of 1% thrombin. The process of making platelet rich plasma was carried out at the Eureka Research Laboratory, Palembang, Indonesia. OA induction was performed by first performing anesthesia in White Rats using ketamine (dose 0.015 mg / gBW) intramuscularly and chlorate (dose 0.0025 mg / gBW) subcutaneously. Monosodium iodoacetate (MIA) was injected intra-articularly in the genu dextra region of experimental rats. Rats are monitored daily for signs of distress and signs of infection. Evacuation of genu dextra was carried out by performing anesthesia first on mice, then perfusion was carried out and genu dextra was taken. The mouse's dextra genu tissue was inserted into a lidded microtube container containing 0.9% NaCl liquid, one container for one sample. The samples are temporarily stored in a cooler bag (temperature $\leq 2^0$ C) and immediately stored in a freezer (temperature -20^0 C). Analysis of IL-1B levels was carried out by the enzyme linked immunosorbant assay (ELISA) method according to the instructions of the manufacturer (CloudClone®).

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4. Conclusion →5

Platelet rich plasma has the potential to be a biological agent against osteoarthritis disorders in invivo studies.

5.References →6

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Reviewer Comment:

1→ Abstract should be showed the main of background, methods, results and conclusion of study.

- Background abstract should be showed the urgency of study and why the study important, in simple way.
- Conclusion should be wrote in simple way, specific to the main results. Conclusion in abstract should not showed statistic results.
- Keywords should be showed the main words of the study, the authors can use MeSH to develop keywords.

2→Introduction should be showed the urgency of research which supported by epidemiology data, biological interaction concept, lack of knowledge in the research and also objective of research.

3→ Authors should be wrote methods about how the study develop. Methods should be showed the design of study; population, sample and sample size of study; inclusion criteria; place of study; ethical clearence steatment; independent and dependent variable; data analysis.

4→ Authors should be wrote results with baseline characteristics subject of study, main results of study. Authors must be focused and try to make results with no more table and figure.

5→ Discussion should be explored more biological plausibility, not only showed about statistical results.

6→ Conclusion should more specific and not more showed statistical results

7→ Authors must check the references for make update references. References should no more than 10 years.

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Potential of Activated Platelet-Rich Plasma Against Osteoarthritis: In Vivo Study

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ABSTRACT

Introduction: Osteoarthritis (OA) is a disorder that causes a decrease in the quality of life in elderly patients. The current treatment is only symptomatic in reducing inflammation. This study is one of the exploratory studies to examine the potential of platelet-rich plasma (PRP) in optimizing the improvement of OA patients through the inhibition of inflammatory signals in joint tissue in vivo. **Methods:** This study is an experimental study with a post-test-only approach with a control group design. A total of 30 rats (*Rattus norvegicus*) Wistar strain was included in this study and met the inclusion criteria in the form of the male gender, weight between 150-200 grams, and age 8-10 weeks. The rats were divided into 3 groups, namely the group that was not induced by OA and not given PRP (P1), the OA group and given 50 uL saline injection (P2), the OA group and given PRP 50 uL (P3), the treatment was carried out once a week for 4 weeks. **Results:** The results showed that the P3 group that was treated with platelet-rich plasma showed a significant decrease in interleukin-1ß levels when compared to the P2 group that was induced by OA but was only treated with saline ($p<0.05$). **Conclusion:** Platelet-rich plasma has the potential as a biological agent against osteoarthritis in an in vivo study.

Keywords: osteoarthritis, in vivo study, platelet-rich plasma, interleukin, experimental study.

Introduction

Osteoarthritis (OA) is a joint movement disorder, especially in the knee joint, caused by chronic inflammation in the joints.[1-3] The chronic inflammation that occurs in OA is caused by loss of joint cushion (joint cartilage), which causes trauma when the joint is moved. Trauma that occurs chronically triggers chronic inflammation that triggers complaints of pain and difficulty moving joints.[4] OA disorders are common in elderly patients, along with joint cartilage degeneration. According to epidemiological data, there has been a threefold increase in OA patients in the last 10 years. This is due to the increasing life expectancy, where more and more patients are more than 60 years old. This increase in life expectancy should also be accompanied by an increase in the quality of life of elderly patients.[5]

OA is one of the disorders that cause a decrease in the quality of life of sufferers, where the majority of patients are elderly. The current treatment is only symptomatic in reducing inflammation. The use of non-steroidal anti-inflammatory drugs that are very massive and used in the long term, on the one hand, triggers a series of unwanted side effects in the form of gastrointestinal disorders and triggers various other metabolic and endocrine disorders. This triggers the exploration of new therapeutic modalities for the development of definitive therapeutic modalities. Management of OA should not only refer to the chemical aspect in the form of drugs, but it is necessary to develop stem cell-based biological agents. One of the promising modalities to be developed is platelet-rich plasma.[6]

Platelet-rich plasma is one of the modalities of biological agents obtained from the patient's own blood. Platelet-rich plasma is the result of processing the patient's blood, where platelets are isolated from plasma through a structured separation process. Platelets are blood components that are responsible for repairing tissue damage. This makes platelets rich in growth factors that are important in repairing damaged tissue. Proper isolation and activation of platelets are important factors in optimizing the growth factors present in platelet-rich plasma.[7,8] This study is one of the exploratory studies to examine the potential of platelet-rich plasma in optimizing the improvement of OA patients through the inhibition of inflammatory signals in joint tissue in vivo.

Methods

This study is an experimental study with a post-test-only approach with a control group design. A total of 30 rats (*Rattus norvegicus*) Wistar strain was included in this study and met the inclusion criteria in the form of the male gender, weighing between 150-200 grams, and of

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References

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Letter of Acceptance

Manuscript "Potential of Activated Platelet-Rich Plasma Against Osteoarthritis: In Vivo Study" by Rachmat Hidayat*, Patricia Wulandari, has been accepted to publish in Journal of Anesthesiology & Clinical Research Vol 3 issue 1 in June 2022.

Cordially,

Prof. Paula Magnano, PhD

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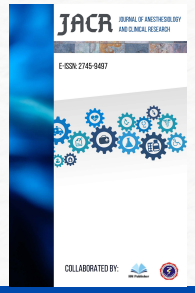

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