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Khrishna Murti, PhD
Head of Language Institute-HM Publisher
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Submitted to the journal "Sriwijaya Journal of Ophthalmology" (February 2nd, 2023)

Activated Platelet Rich Plasma As A New Therapeutic Modality of Cataract Disorders : In Vivo Study

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

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

[2]Cattleya Mental Health Center, Palembang, Indonesia

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Abstract

Introduction: Cataracts are the leading cause of visual impairment and vision loss in the world, where 33% of the world's population experiences decreased vision due to cataracts. This study aims to explore the role of platelet rich plasma (PRP) in inhibiting the pro-inflammatory cytokine, interleukin-1 ß, thus triggering tissue repair in the case of cataracts in vivo studies.

Methods: This study is an experimental study with a posttest only with control group design approach. A total of 30 white rats (*Rattus norvegicus*) of the Wistar strain were included in this study (male, 150-200 g, 8-10 weeks). Rats were divided into 3 groups, P1 (the group that was notinduced by cataracts and was not treated with platelet rich plasma), P2 (the cataract-induced group and given intraocular injection saline 10 uL), and P3 (cataract-induced group and given intraocular injection platelet rich plasma 10 uL).

Results: The results showed that the P3 group that received platelet rich plasma treatment showed a significant decrease in IL-1B levels when compared to the cataract-induced P2 group but without PRP administration ($p<0.05$).

Conclusion: Activated platelet rich plasma has the potential to be a new therapeutic modality in cataract conditions through chronic inflammatory response inhibition in invivo studies.

Keywords: Cataract, Interleukin, Platelet rich plasma, Sodium selenite, Experimental study.

1. Introduction

Cataracts are one of the most serious health disorders experienced quite a lot in elderly patients. As a person gets older, the performance of each organ will decrease, including one of them is the lens of the eye. [1,2] Cataracts are the leading cause of visual impairment and vision

loss in the world, where 33% of the world's population experiences decreased vision due to cataracts. The World Health Organization (WHO) estimates that 18 million people are blind in both eyes due to cataracts and this condition constitutes 48% of blindness cases worldwide. [3]

Cataracts are caused by various factors, all of which play a role in the initiation of chronic inflammatory processes. Chronic inflammation will cause activity from various pro-inflammatory cytokines that will prevent the activation of anti-inflammatory cytokines and inhibit the resolution of the tissues of the lens of the eye. [4-6] This will cause damage and turbidity to the lens of the eye. The current therapeutic modality is surgery and replacement of the lens of the eye, but this modality is not without risks. The invasive process carried out has the potential to cause various complications, disorders and may even cause more widespread damage. [7.8] The option of exploring new, minimally invasive therapeutic modalities is the best solution.

Platelet rich plasma is one of the blood products that is rich in growth factors. [9-11]Platelets are derived from one of the components of the blood, namely platelets. Platelets are components that play an important role in the regeneration or repair of body tissues that are damaged or disturbed. [12.13] This is due to the richness of platelets with growth factors. Growth factors are part of anti-inflammatory cytokines that play a role in inflammatory inhibition and activation of tissue regeneration processes . [14-16] This potential is sufficient for the improvement of chronic inflammatory processes in cataract cases. This study is one of the earliest exploratory studies that has the potential to determine the role of platelet rich plasma in inhibiting the pro-inflammatory cytokine, IL-1B, thereby triggering tissue repair in cataract cases in vivo study.

2.Methods

This study is an experimental study with a post test only with control group design approach. A total of 30 white 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. First, the white rats carried out an acclimatization process for 7 days, then divided into 3 groups (P1, P2, and P3) randomly, where each group consisted of 10 heads white mouse. The P1 group was a group of mice that were notinduced by cataracts and were not treated with platelet rich plasma; Kgroup P2 was a group of rats induced by cataracts by intraperitoneal injection of sodium selenite 4 mg / kgBB and given intraocular injection of saline 10 uL ; groupP3 was a group of rats induced by cataracts by intraperitoneal injection of sodium selenite 4 mg /

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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. Sodium selenite was injected intraperitoneally in experimental rats. Rats are monitored daily for signs of distress and signs of infection. Evacuation of the eye is carried out by performing transpalpebral enucleation (the enucleated lens is the most cloudy lens) followed by making a palpebra incision and freeing the eyeball from the surrounding tissue, trace the back of the eyeball with tweezers until the optical nervus can be reached. Next, gunting nervus opticus and remove the eyeball. Then an eculate of the anterior segments with scissors is carried out. Identify the lens, remove it and rinse it with physiological fluid so that it does not mix other tissues. The mouse eyepiecewas 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 interleukin (IL)-1 levelsβ was carried out by the enzyme linked immunosorbant assay (ELISA) method according to the instructions of the manufacturer (CloudClone®).

After the data is collected, data cleaning, coding and tabulation are carried out. All results were assessed with an average ± standard deviation accompanied by a normality test (Saphiro Wilk) and a data homogeneity test (Levene Statistic). The test used in this study is the one way Anova. The result is said to be meaningful if $p \leq 0.05$. Data analysis was performed using SPSS version 25 for Windows.

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Table 1 shows the degree of inflammatory markers (kadar IL-1β). Higher levels of IL-1β indicate inflammation of the lens tissue as occurs in cataract conditions. The P3 group that

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*oneway ANOVA, $p<0.05$

Discussion

Cataracts are caused by chronic inflammation of the lens tissue due to various precipitating factors. [1,3] Various precipitating factors such as trauma or the aging process trigger a series of inflammations that result in the activation of various pro-inflammatory cytokines, namely IL-1β, IL-6 and TNF alpha. Chronic activation of β the cytokine IL-1 leads to the inability to activate the antiiflammatory cytokine, TGF-β. [16-18] It causes no repair of lens tissue, even chronic inflammatory processes cause activation of various processes of apoptosis and necrosis. Platelet rich plasma rich in growth factor shows potential in suppressing the activation of inflammatory cytokine, IL-1β. The ability of platelet rich plasma in suppressing this IL-1β indicates the potential of this biological agent in reducing chronic inflammation and preventing lens damage . Of course, these results show the promising potential of platelet rich plasma as a biological agent modality in overcoming cataracts.

4. Conclusion

Activated platelet rich plasma has the potential to be a new therapeutic modality in cataract conditions through chronic inflammatory response inhibition in invivo studies.

5. References

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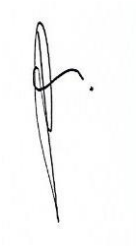
Submission acknowledgement

Dear author(s),

Rachmat Hidayat*, Patricia Wulandari has submitted the manuscript “Activated Platelet Rich Plasma as a New Treatment Modality for Cataract Disorders: In Vivo Study“ to Sriwijaya Journal Of Ophthalmology. The paper will be screened by editor and reviewed by peer review.

Cordially,



Prof. Paula Magnano, PhD

Editor HM Publisher

(*) Corresponding author

Sriwijaya Journal of Ophthalmology

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Peer Review Results

Dear author(s),

Rachmat Hidayat*, Patricia Wulandari has submitted the manuscript "Activated Platelet Rich Plasma as a New Treatment Modality for Cataract Disorders: In Vivo Study" to Sriwijaya Journal of Ophthalmology. The decision : Revision Required.

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Editor HM Publisher

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Reviewer 1: Revision required

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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

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

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

[2]Cattleya Mental Health Center, Palembang, Indonesia

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loss in the world, where 33% of the world's population experiences decreased vision due to cataracts. The World Health Organization (WHO) estimates that 18 million people are blind in both eyes due to cataracts and this condition constitutes 48% of blindness cases worldwide. [3]

Cataracts are caused by various factors, all of which play a role in the initiation of chronic inflammatory processes. Chronic inflammation will cause activity from various pro-inflammatory cytokines that will prevent the activation of anti-inflammatory cytokines and inhibit the resolution of the tissues of the lens of the eye. [4-6] This will cause damage and turbidity to the lens of the eye. The current therapeutic modality is surgery and replacement of the lens of the eye, but this modality is not without risks. The invasive process carried out has the potential to cause various complications, disorders and may even cause more widespread damage. [7.8] The option of exploring new, minimally invasive therapeutic modalities is the best solution.

Platelet rich plasma is one of the blood products that is rich in growth factors. [9-11]Platelets are derived from one of the components of the blood, namely platelets. Platelets are components that play an important role in the regeneration or repair of body tissues that are damaged or disturbed. [12.13] This is due to the richness of platelets with growth factors. Growth factors are part of anti-inflammatory cytokines that play a role in inflammatory inhibition and activation of tissue regeneration processes . [14-16] This potential is sufficient for the improvement of chronic inflammatory processes in cataract cases. This study is one of the earliest exploratory studies that has the potential to determine the role of platelet rich plasma in inhibiting the pro-inflammatory cytokine, IL-1B, thereby triggering tissue repair in cataract cases in vivo study.

2.Methods →3

This study is an experimental study with a post test only with control group design approach. A total of 30 white 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. First, the white rats carried out an acclimatization process for 7 days, then divided into 3 groups (P1, P2, and P3) randomly, where each group consisted of 10 heads white mouse. The P1 group was a group of mice that were notinduced by cataracts and were not treated with platelet rich plasma; Kgroup P2 was a group of rats induced by cataracts by intraperitoneal injection of sodium selenite 4 mg / kgBB and given intraocular injection of saline 10 uL ; groupP3 was a group of rats induced by cataracts by intraperitoneal injection of sodium selenite 4 mg /

kgBB and given intraocular injection platelet rich plasma 10 uL, treatment was carried out every one once a week for 4 weeks. This study has received approval from the CMHC-Science and Research Center Research Ethics Commission, with number No.35/CMHC/KEPK/2021.

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. Sodium selenite was injected intraperitoneally in experimental rats. Rats are monitored daily for signs of distress and signs of infection. Evacuation of the eye is carried out by performing transpalpebral enucleation (the enucleated lens is the most cloudy lens) followed by making a palpebra incision and freeing the eyeball from the surrounding tissue, trace the back of the eyeball with tweezers until the optical nervus can be reached. Next, gunting nervus opticus and remove the eyeball. Then an eculate of the anterior segments with scissors is carried out. Identify the lens, remove it and rinse it with physiological fluid so that it does not mix other tissues. The mouse eyepiecewas 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 interleukin (IL)-1 levelsβ was carried out by the enzyme linked immunosorbant assay (ELISA) method according to the instructions of the manufacturer (CloudClone®).

After the data is collected, data cleaning, coding and tabulation are carried out. All results were assessed with an average ± standard deviation accompanied by a normality test (Saphiro Wilk) and a data homogeneity test (Levene Statistic). The test used in this study is the one way Anova. The result is said to be meaningful if $p \leq 0.05$. Data analysis was performed using SPSS version 25 for Windows.

3. Results →4

Table 1 shows the degree of inflammatory markers (kadar IL-1β). Higher levels of IL-1β indicate inflammation of the lens tissue as occurs in cataract conditions. The P3 group that

received platelet rich plasma treatment showed a significant decrease in level β 1 when compared to the cataract-induced P2 group but only given saline treatment ($p<0.05$).

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

Group	**IL-1β (g/mL)** levelsρ **Mean ± Elementary School**	**p value***
P1	23.56 ± 1.87	0,002
P2	245.87 ± 12.32	
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*oneway ANOVA, $p<0.05$

Discussion →5

Cataracts are caused by chronic inflammation of the lens tissue due to various precipitating factors. [1,3] Various precipitating factors such as trauma or the aging process trigger a series of inflammations that result in the activation of various pro-inflammatory cytokines, namely IL-1β, IL-6 and TNF alpha. Chronic activation of β the cytokine IL-1 leads to the inability to activate the antiiflammatory cytokine, TGF-β. [16-18] It causes no repair of lens tissue, even chronic inflammatory processes cause activation of various processes of apoptosis and necrosis. Platelet rich plasma rich in growth factor shows potential in suppressing the activation of inflammatory cytokine, IL-1β. The ability of platelet rich plasma in suppressing this IL-1β indicates the potential of this biological agent in reducing chronic inflammation and preventing lens damage . Of course, these results show the promising potential of platelet rich plasma as a biological agent modality in overcoming cataracts.

4. Conclusion →6

Activated platelet rich plasma has the potential to be a new therapeutic modality in cataract conditions through chronic inflammatory response inhibition in invivo studies.

5. References →7

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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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Activated Platelet Rich Plasma as a New Treatment Modality for Cataract Disorders: In Vivo Study

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A B S T R A C T

Introduction: Cataract is the main cause of visual impairment and vision loss in the world, where 33% of the world's population has decreased vision due to cataracts. This study aims to explore the role of platelet-rich plasma (PRP) in inhibiting the pro-inflammatory cytokine interleukin-1ß, thereby triggering tissue repair in cataract cases in vivo study. **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 (male, 150-200 g, 8-10 weeks). The rats were divided into 3 groups, P1 (the group that was not induced by cataract and not treated with platelet-rich plasma), P2 (the group that was induced by cataract and given 10 uL of intraocular saline injection), and P3 (the group that was induced by cataract and given an intraocular injection of platelet-rich plasma). Plasma 10 uL). **Results:** The results showed that the P3 group that received platelet-rich plasma treatment showed a significant decrease in IL-1B levels when compared to the P2 group with cataract induced but without PRP administration ($p<0.05$). **Conclusion:** Activated platelet-rich plasma has potential as a new therapeutic modality in cataract conditions through inhibition of chronic inflammatory response in vivo studies.

1. Introduction

A cataract is one of the serious health problems experienced by quite a lot of elderly patients. As a person gets older, the performance of each organ will decrease, including the eye lens organ.[1,2] Cataracts are the leading cause of visual impairment and vision loss in the world, where 33% of the world's population has decreased vision due to cataracts. The World Health Organization (WHO) estimates that 18 million people are blind in both eyes due to cataracts, and this condition constitutes 48% of blindness cases worldwide.[3]

Cataracts are caused by various factors, all of which play a role in the initiation of the chronic inflammatory process. Chronic inflammation will cause the activity of various pro-inflammatory cytokines, which will prevent the activation of anti-inflammatory cytokines and inhibit the resolution of the lens tissue of the eye.[4-6] This will cause damage and cloudiness to the eye lens. Current treatment modalities are surgery and lens replacement, but these modalities are not without risks. Invasive processes that are carried out have the potential to cause various complications. Disturbances may even cause

widespread damage.[7,8] Optional exploration of new minimally invasive therapeutic modalities is the best solution.

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Table 1 shows the levels of inflammatory markers (IL-1β levels). Higher levels of IL-1β inflammation of the lens tissue occur in cataracts. The P3 group that received the platelet-rich plasma treatment showed a significant decrease in the level of 1β when compared to the P2 group that was induced by cataracts but was only given saline treatment ($p<0.05$)

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4. Discussion

Cataracts are caused by chronic inflammation of the lens tissue due to various precipitating factors.[1,3] Various precipitating factors such as trauma or the aging process trigger a series of inflammation that results in the activation of various pro-inflammatory cytokines, namely IL-1β, IL-6, and TNF alpha. Activation of the IL-1cytokine inflammatory cytokine, TGF-β.[16-18] This causes no repair of lens tissue, and even chronic inflammatory processes lead to activation of various processes of apoptosis and necrosis. The platelet-rich plasma that is rich in growth factors shows the potential to suppress the activation of the inflammatory cytokine IL-1β. The ability of platelet-rich plasma to suppress IL-1β indicates the potential of this biological agent in reducing chronic inflammation and preventing lens damage. Of course, these results show the promising potential of platelet-rich plasma as a biologic agent modality in treating cataracts.

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Activated platelet-rich plasma has potential as a new therapeutic modality in cataract conditions through inhibition of chronic inflammatory response in vivo studies.

6. References

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Letter of Acceptance

Manuscript “Activated Platelet Rich Plasma as a New Treatment Modality for Cataract Disorders: In Vivo Study“ by Rachmat Hidayat*, Patricia Wulandari, has been accepted to publish in Sriwijaya Journal Of Ophthalmology Vol 6 issue 2 in February 2023.

Cordially,

Prof. Paula Magnano, PhD

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(*) Corresponding author

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3. Results

Table 1 shows the levels of inflammatory markers (IL-1β levels). Higher levels of IL-1β inflammation of the lens tissue occur in cataracts. The P3 group that received the platelet-rich plasma treatment showed a significant decrease in the level of 1β when compared to the P2 group that was induced by cataracts but was only given saline treatment ($p<0.05$)

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, $p<0.05$

4. Discussion

Cataracts are caused by chronic inflammation of the lens tissue due to various precipitating factors. Various precipitating factors such as trauma or the aging process trigger a series of inflammation that results in the activation of various pro-inflammatory cytokines, namely IL-1β, IL-6, and TNF alpha. Activation of the IL-1cytokine inflammatory cytokine, TGF-β.[16-18] This causes no repair of lens tissue, and even chronic inflammatory processes lead to activation of various processes of apoptosis and necrosis. The platelet-rich plasma that is rich in growth factors shows the potential to suppress the activation of the inflammatory cytokine IL-1β. The ability of platelet-rich plasma to suppress IL-1β indicates the potential of this biological agent in reducing chronic inflammation and preventing lens damage. Of course, these results show the promising potential of platelet-rich plasma as a biologic agent modality in treating cataracts.

5. Conclusion

Activated platelet-rich plasma has potential as a new therapeutic modality in cataract conditions through inhibition of chronic inflammatory response in vivo studies.

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