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The effectiveness of Triamcinolone Acetonide (TA) and Interleukin-6 (IL-6) levels in preventing intraperitoneal adhesion

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ABSTRACT

Background: Intraperitoneal adhesions are formed due to the inflammatory response in releasing proinflammatory cytokines such as IL-6. Triamcinolone Acetonide (TA) acts as an anti-inflammatory by inhibiting the production and release of IL-6 cytokines. This study aims to determine the effectiveness of triamcinolone acetonide in preventing intraperitoneal adhesions and its effect on assessing IL-6 levels in post-exploratory laparotomy in rats.

Methods: An experimental study was conducted among 40 male Wistar rats from January and March 2021 in the Animal Laboratory of Universitas Padjadjaran, Bandung, Indonesia. Blood samples were taken before treatment and on the 14th day after treatment. In rats, laparotomy and cecum abrasion were performed with a needle. Rats in the treatment group were given triamcinolone acetonide at a dose of 0.1 mg, 0.3 mg, or 0.5 mg. On the 14th day, a microscopic examination of the degree of adhesion was performed. Data were analyzed using SPSS version 20 for Windows.

Results: The results of the Kruskal-Wallis test analysis on the formation of intraperitoneal adhesions microscopically showed a significant difference between the control group and the treatment group ($p < 0.001$) with the lowest mean rank in the TA group of 0.3 mg (11.4), which stated that this dose was more effective in preventing intraperitoneal adhesions. The One-Way ANOVA test on IL-6 examination had a significant difference in postoperative IL-6 reduction between the control and treatment groups ($p < 0.001$).

Conclusion: Triamcinolone acetonide was effective in preventing intraperitoneal adhesions and reducing IL-6 levels in post-exploratory laparotomy rats.

Keywords: IL-6, Intraperitoneal adhesion, Triamcinolone Acetonide
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INTRODUCTION

Abdominal surgery or laparotomy causes the formation of intraperitoneal adhesions in almost 60%-80% of patients.[1] Generally, it causes complications in the short term and the medium and long term.[1] Postoperative adhesion is formed after trauma to the peritoneal cavity and results from biochemical response and cellular that occurs in an attempt to repair the peritoneum. The rate of adhesion formation varies from patient to patient and is highly dependent on the type and magnitude of the surgery performed and whether any postoperative complications develop.[2]

The formation of postoperative adhesions is also involved in the process of wound healing or tissue repair.[3] Immediately following injury, bleeding occurs, and vascular permeability increases with fluid leaking from the wound surface. Simultaneously, there is a posttraumatic inflammatory response, with inflammatory cell infiltration, the release of proinflammatory cytokines and activation of complement and coagulation cascades. Cytokines are protein compounds that act as intermediaries in various cellular responses.[2] The use of drugs as adjuvants has also been widely used to prevent postoperative adhesions. These drugs include: steroid and non-steroidal anti-inflammatory drugs, antihistamines, progesterone, GnRH agonists, fibrinolytic and anticoagulants.[4]

Triamcinolone is a synthetic corticosteroid that acts as an anti-inflammatory. The most commonly used forms of Triamcinolone are Triamcinolone Acetonide (TA) and Triamcinolone Hexacetonide (TH).[5] In animal trials, intravitreal injection of TA is effective in reducing the incidence of neovascularization. Oxidative stress can induce Vascular Endothelial Growth Factor (VEGF) and Connective Tissue Growth Factor (CTGF). VEGF has a vital role in the post-injury or trauma neovascularization process. Neovascularization is an essential factor in the formation of adhesions. CTGF plays a critical role in the pathogenesis of the fibrotic disease. TA has been shown to suppress VEGF and CTGF.[5]

TA has been shown to inhibit proinflammatory cytokines and stimulate the release of anti-inflammatory cytokines.[5] TA can suppress the target of the Interleukin-6 (IL-6) receptor, which is

one of the proinflammatory cytokines that play a role in the formation of adhesion. An important anti-inflammatory mechanism of TA is mediated by inhibition of Nuclear Factor Kappa-B (NF-kappa-B), which causes a decrease in the expression of the protein Interleukin-6 (IL-6), Interleukin-8 (IL-8), Monocyte Chemoattractant Protein-1 (MCP-1), and COX-2.[3,5]

Based on those mentioned above, this study aims to evaluate the effectiveness of TA in preventing the formation of postoperative intraperitoneal adhesions as believed to play a role in the process of neovascularization, fibrosis and inflammatory cytokines.

METHODS

The subjects of this study were male rats (*Wistar sp*) obtained from the Pharmacology laboratory of the Faculty of Medicine, Universitas Padjadjaran, Bandung, Indonesia. The calculation of the size of the rat sample used was calculated based on the Federer formula and 40 rats have obtained the sample requirement in four treatments or as many as ten rats in each group. The first treatment group underwent exploratory laparotomy without TA administration. The second treatment group underwent exploratory laparotomy and was administered TA at a dose of 0.1 mg. The third treatment group underwent exploratory laparotomy and was administered TA at a dose of 0.3 mg. Finally, the fourth treatment group underwent exploratory laparotomy and was administered TA at a dose of 0.5 mg.

Measurement of IL-6 levels was carried out by taking blood samples from mice before treatment. Then a second blood sample was also taken after 14 days of treatment. The rat blood taken was put into a sample tube and sent to the Clinical Pathology Laboratory of Dr. Hasan Sadikin General Hospital Bandung

TA solution was prepared by dissolving Triamcinolone Acetonide with sterile water. The dose of 0.1 mg was made by dissolving TA 1 mg to 1 ml and given to mice as much as 0.1 ml. Meanwhile, for a dose of 0.3 mg, the solution is given as much as 0.3 ml, and for a dose of 0.5 mg, it is presented as much as 0.5 ml.

Each rat was anaesthetized using ketamine. All rats used in this study were treated the same way as making an incision on the paramedian abdominal wall with a Bisturi surgical blade of size 21. Then the peritoneal cavity was opened, and an incision was performed on the caecum wall of the rat with a 23 G needle, then administered TA according to the dose. The abdominal wall was again closed with a size 3.0 silk thread. All rats were treated for two weeks, and on day 14, an exploratory laparotomy was performed to take the caecum part of the rats. The cecum of the rats taken was put into a tube containing buffered formalin and sent to the Anatomical Pathology Laboratory of Dr. Hasan Sadikin General Hospital, Bandung, to assess the degree of adhesion using the Yilmaz criteria based on the previous study.[6]

The IL-6 data obtained were presented in numerical form and multiple comparisons One-Way ANOVA test was performed to see differences between groups after treatment. The adhesion data were presented in categorical form. The differences in the degree of intraperitoneal adhesion microscopically between the treatment groups were analyzed using the Kruskal-Wallis test. The difference in mean adhesion in each group was carried out using multiple comparisons test with a One-Way ANOVA test. Data were analyzed using SPSS version 20 for Windows.

RESULTS

From the results of manipulation in the control group and treatment with different doses of TA (TA), it was found that microscopic intraperitoneal adhesion formation after laparotomy was found in rats. On microscopic examination by an Anatomical Pathologist, 1, 2 and 3-degree adhesions were found with the distribution as shown in Table 1.

In the Kruskal-Wallis test, there was a significant difference in the degree of microscopic intraperitoneal adhesion between the control group and each TA treatment group (TA 0.1 mg, TA 0.3 mg and TA 0.5 mg) ($p<0.05$) (Table 1). Therefore, it is necessary to test multiple comparisons with the One-Way ANOVA test to determine which treatment groups are different. There was a significant difference in the mean degree of intraperitoneal adhesion between the control group with the 0.1 mg TA ($p=0.001$), 0.3 mg TA ($p=0.0001$), and 0.5 mg TA ($p=0.0001$) treatment groups microscopically (Table 2).

Table 1. Distribution of intraperitoneal adhesion microscopically

Group	Degree of adhesion microscopically				Total	p
	0	1	2	3		
Control group	0	0	4	6	10	
TA 0.1 mg	0	3	7	0	10	
TA 0.3 mg	0	8	2	0	10	<0.05
TA 0.5 mg	0	4	6	0	10	
Total	0	15	19	6	40	

TA: Triamcinolone Acetonide; *Kruskal-Wallis test: statistically significant if p-value less than 0.05

Table 2. The results of differences in the degree of microscopic adhesion between the control and each treatment group

Group	Mean Difference	Standard Error (SE)	p
Control group			
TA 0.1 mg	0.9	0.217	0.001*
TA 0.3 mg	1.4	0.217	0.0001*
TA 0.5 mg	1.0	0.217	0.0001*

TA: Triamcinolone Acetonide; *Multiple Comparison One Way-Anova test: statistically significant if p-value less than 0.05

Table 3. The differences in IL-6 level post-procedure among groups

Groups	N	Mean	SE	Minimum	Maximum	p
Control group	10	3.13	0.26	2.77	3.53	0.001*
TA 0.1 mg	10	2.69	0.38	2.22	3.39	
TA 0.3 mg	10	2.32	0.33	2.03	2.91	
TA 0.5 mg	10	1.90	0.46	1.29	2.91	
Total	40	2.51	0.58	1.29	3.53	

TA: Triamcinolone Acetonide; *One Way-ANOVA test: statistically significant if p-value less than 0.05

Table 4. The mean differences in IL-6 level post-procedure

Groups	Mean Difference (MD)	Standard Error (SE)	p
Control group			
TA 0.1 mg	0.444	0.165	0.051
TA 0.3 mg	0.806	0.165	0.0001*
TA 0.5 mg	1.232	0.165	0.0001*

TA: Triamcinolone Acetonide; *Multiple Comparison One Way-ANOVA test: statistically significant if p-value less than 0.05

Based on the data above, it can be seen that the 0.5 mg TA group indicated the average difference in the pre-treatment IL-6 examination and the lowest average post-treatment IL6 value (mean = 1.90). The control group indicated the highest (mean = 3.13) with a significance value between groups was 0.001 using the One-Way ANOVA test (Table 3). Based on the data above, it can be concluded that there was a significant post-treatment decrease in IL-6 among the treatment groups, with the most significant effect seen in the 0.5 mg TA group, followed by the 0.3 mg TA group, then the 0.1 mg TA group ($p<0.05$) (Table 3).

In the One-Way ANOVA test, there was a significant difference in postoperative IL-6 reduction between the control and TA treatment groups (TA 0.1 mg, TA 0.3 mg and TA 0.5 mg). Therefore, it is necessary to test multiple comparisons with the One-Way ANOVA test to determine which treatment groups are different. There was a non-significant decrease in the average of post-treatment IL-6 between the control group with 0.1 mg TA treatment group ($p=0.051$). However, there was a significant decrease in the average of post-treatment IL-6 between the control group with 0.3 mg TA ($p=0.001$) and 0.5 mg TA ($p=0.001$) treatment groups (Table 4).

DISCUSSION

Adhesion is a physiological process in the healing of the peritoneum, which generally can be formed due to inflammatory conditions of the abdomen.[7] Postoperative adhesions are formed after trauma to the peritoneal cavity and result from biochemical and cellular responses that occur to repair the peritoneum.[2] In this study, rat caecum abrasion was performed to induce peritoneal injury, leading to tissue repair. This abrasion model is similar to the peritoneal injury in a real laparotomy. This technique can then be used to observe adhesions caused by an imbalance between the processes of fibrogenesis and fibrinolysis according to previous studies.[8,9]

The formation of postoperative intraperitoneal adhesions is unavoidable, even with the use of microsurgery or laparoscopically. So that additional therapy is needed in the form of materials that can reduce or overcome adhesion formation. Peritoneal injury, which then causes an acute inflammatory state shows an increase in the concentration of proinflammatory cytokines.[10-12] TA was chosen because it is a corticosteroid class, where corticosteroids in previous studies have been shown to inhibit the production and release of cytokines, such as IL-1, IL-6, and Tumor Necrosis Factor (TNF)-Alpha.[9]

The research data involving 40 rats with one control group and three treatment groups stated that different degrees of adhesion were found microscopically. Still, it can be seen that the highest degree of adhesion was found in the control group. In the treatment group with TA, a lower degree of adhesion was found, so this supports the hypothesis of TA as an anti-adhesion agent.[3,12]

The rats in 1 group, both the control and treatment groups, also had different degrees of adhesion. This is as reviewed from previous research, which states that the formation of adhesions is influenced by several factors such as the patient's disease status, age, nutritional status, the difficulty level of laparotomy, number of operations that have been performed.[8]

The Kruskal-Wallis test was performed in testing the comparison of the incidence and degree of microscopic intraperitoneal adhesions between the control and treatment groups. The test results obtained $p<0.05$, which means a significant difference between the control and treatment groups in the formation of microscopic intraperitoneal adhesions. These results are similar to a previous study that states TA can reduce VEGF and CTGF, which play an essential role in preventing neovascularization in the pathogenesis of fibrosis, to maintain a balanced state between fibrogenesis and fibrinolysis to ensure wound healing.[12-13]

The researcher also compared each treatment group with the control group to determine the most effective dose of triamcinolone in its anti-adhesion role. Based on the Kruskal-Wallis test followed by the multiple comparisons test, there were significant differences between the control group with 0.1 mg TA treatment ($p= 0.001$), 0.3 mg TA treatment ($p= 0.001$), and 0.5 mg TA treatment group ($p= 0.001$). However, from the assessment of the mean rank with the Kruskal-Wallis test, it was found that the lowest mean rank value was in the 0.3 mg TA group, while the highest value was in the control group. This indicated that the 0.3 mg TA group was more effective in preventing the formation of intraperitoneal adhesions. In other words, it provided the best

benefit in preventing the formation of intraperitoneal adhesions.

The results of this study are consistent with the therapeutic dose of TA in humans, which is 0.11-1.6 mg/kg BW. This dose was then converted to a dose of rats weighing 300 grams to 0.6-3mg/kg BW, where the administration of TA 0.3 mg and 0.5 mg in this study was based on doses of 0.1 mg/kgBW and 1.6 mg/kgBW. This dose is within the therapeutic dose range of TA in rats to show significant results in this study. TA doses administered within the therapeutic dose range are generally clinically and statistically significant.[13,14]

In the control group, there was an increase in IL-6 after the procedure. This is in accordance with the theory that the rise in IL-6 may occur due to local inflammation that accompanies mesothelial damage in the process of tissue repair in peritoneal injury.[15] Proinflammatory cytokines (IL-1, IL-6, IL-8, and TNF-α) play an important role in the early phase of wound healing and are produced by activated macrophages in the peritoneal fluid.[16] IL-1 and TNF-α will also induce the release of IL-6. The tissue repair process then involves plasminogen activator and plasminogen inhibitor. Plasmin can then cause the mobilization and release of TNF- α, IL-1, and IL-6. IL-6 is consistently reported to be adhesiogenic.[17] Thus, if proinflammatory anti-cytokine agents are not given, it is suspected that IL-6 levels will continue to increase, which triggers the formation of intraperitoneal adhesions. Another study also stated that administration of IL-6 into the peritoneal cavity of mice reported an increase in adhesion formation.[11,12,14] Based on previous research by Saharui A et al., which states that TA has been shown to inhibit proinflammatory cytokines so that it can suppress the target of the Interleukin-6 (IL-6) receptor, which also plays a role in the formation of adhesions, this study examined the levels of IL-6 before and after treatment in the control and treatment groups.[8]

On administration of TA, there were statistically significant differences in the formation of intraperitoneal adhesions microscopically in experimental rats compared to controls. TA 3 mg provided the best benefit in preventing the formation of microscopic intraperitoneal adhesions, which was statistically significant when compared to controls. Administration of TA can reduce the average value of IL-6 after the procedure, which is statistically significant when compared to controls.

CONCLUSION

This study found that Triamcinolone Acetonide (TA) effectively prevented intraperitoneal adhesions and reduced IL-6 levels in post-exploratory laparotomy rats.

CONFLICT OF INTEREST

There is no competing interest regarding the manuscript.

ETHICS CONSIDERATION

The research was conducted at the Animal Pharmacology Laboratory of Padjadjaran University in February-April 2021. Ethical approval was obtained from the Health Research Ethics Commission, Faculty of Medicine, Universitas Padjajaran with Number 872/UN6.KEP/EC/2021.

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AUTHOR CONTRIBUTIONS

All authors contribute equally to the study from the conceptual framework, data acquisition, and data analysis until reporting the study results through publication.

REFERENCES

1. van Steensel S, van den Hil LCL, Schreinemacher MHF, Ten Broek RPG, van Goor H, Bouvy ND. Adhesion awareness in 2016: An update of the national survey of surgeons. PLoS One. 2018;13(8):e0202418.
2. Tabibian N, Swehli E, Boyd A, Umbreen A, Tabibian JH. Abdominal adhesions: A practical review of an often overlooked entity. Ann Med Surg (Lond). 2017;15:9-13.
3. Sungkar A, Widyatmoko D, Yarso KY, Wasita B. The effect of duration of wound skin tissue on MDA, TNF-α, IL-6, Caspase 3, VEGF levels, and granulation tissue thickness in the white rat (Rattus novergicus). Bali Medical Journal. 2020;9(3):918-923.
4. van Steensel S, Liu H, Mommers EHH, Lenaerts K, Bouvy ND. Comparing Five New Polymer Barriers for the Prevention of Intra-abdominal Adhesions in a Rat Model. J Surg Res. 2019;243:453-459.
5. Brochhausen C, Schmitt VH, Planck CN, Rajab TK, Hollemann D, Tapprich C, et al. Current strategies and future perspectives for intraperitoneal adhesion prevention. J Gastrointest Surg. 2012;16(6):1256-74.
6. Atta HM. Prevention of peritoneal adhesions: a promising role for gene therapy. World J Gastroenterol. 2011;17(46):5049-5058.
7. Roberts LM, Sanfilippo JS, Raab S. Effects of laparoscopic lavage on adhesion formation and peritoneum in an animal model of pelvic inflammatory disease. J Am Assoc Gynecol Laparosc. 2002;9(4):503-507.
8. Saharui A, Lahunduitan I, Kalitouw F. Peranan triamnicolone acetate terhadap adhesi intraperitoneal pasca laparotomi. Jurnal Biomedik: JBM. 2017;9(1):13-6.
9. Arung W, Meurisse M, Detry O. Pathophysiology and prevention of postoperative peritoneal adhesions. World J Gastroenterol. 2011;17(41):4545-4553.
10. Kim SG, Song KY, Lee HH, Kim EY, Lee JH, Jeon HM, et al. Efficacy of an antiadhesive agent for the prevention of intra-abdominal adhesions after radical gastrectomy: A prospective randomized, multicenter trial. Medicine (Baltimore). 2019;98(19):e15141.
11. Takagi K, Araki M, Fukuoka H, Takeshita H, Hidaka S, Nanashima A, et al. Novel powdered anti-adhesion material: preventing postoperative intra-abdominal adhesions in a rat model. Int J Med Sci. 2013;10(4):467-74.
12. Siebelt M, Korthagen N, Wei W, Groen H, Bastiaansen-Jenniskens Y, Müller C, et al. Triamcinolone acetonide activates an anti-inflammatory and folate receptor-positive macrophage that prevents osteophytosis in vivo. Arthritis Res Ther. 2015;17:352.
13. Iwasaki K, Ahmadi AR, Qi L, Chen M, Wang W, Katsumata K, Tsuchida A, Burdick J, Cameron AM, Sun Z. Pharmacological Mobilization and Recruitment of Stem Cells in Rats Stops Abdominal Adhesions After Laparotomy. Sci Rep. 2019;9(1):7149
14. Beyene RT, Kavalukas SL, Barbul A. Intra-abdominal adhesions: Anatomy, physiology, pathophysiology, and treatment. Curr Probl Surg. 2015;52(7):271-319.
15. Fielding CA, Jones GW, McLoughlin RM, McLeod L, Hammond VJ, Uceda J, et al. Interleukin-6 signaling drives fibrosis in unresolved inflammation. Immunity. 2014;40(1):40-50.
16. Grellner W, Georg T, Wilske J. Quantitative analysis of proinflammatory cytokines (IL-1beta, IL-6, TNF-alpha) in human skin wounds. Forensic Sci Int. 2000;113(1-3):251-264.
17. Saba AA, Kaidi AA, Godziachvili V, Dombi GW, Dawe EJ, Libcke JH, et al. Effects of interleukin-6 and its neutralizing antibodies on peritoneal adhesion formation and wound healing. Am Surg. 1996;62(7):569-572.