Dexamethasone Activities toward Population of B cells, Gr-1, and TNF-α cytokine in Mice

*(Mus musculus)* Balb/c Biliary Atresia Model

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ABSTRACT

Biliary atresia is condition caused by Rotavirus (RRV) infection. The aims of this study were to know the immune responses of mice model of biliary atresia treated with corticosteroid. Mice were split into 3 treatment groups: control (K), RRV injection (R), and RRV injection in the presence of dexamethasone (R+D). In R treatment, the baby mice born in <24 hours were injected with 20 µl of phosphate buffered saline containing 1.5 x 10⁶ fluorescence-forming units Rhesus Rotavirus (RRV). First termination was performed in the day 7 to 14, while second termination was done in the day 14 to 21. The dosage of dexamethasone which is applied in this experiment is 0.5mg/kg body weight. Immunocompetent cells were isolated from spleen, and cell surface molecules were then analyzed by flowcytometry. The data was tested by SPSS 16.0 for Windows program. The results showed that dexamethasone given as corticosteroid for biliary atresia therapy could suppress TNF-α production as well as Gr-1 proliferation. In the other hand dexamethasone can promote B²²⁺ cell proliferation in rotavirus infected mice.

Keywords : Baby mice, biliary atresia, dexamethasone, flowcytometry, rotavirus

INTRODUCTION

Biliary atresia is the most of general cause in pathologic jaundice and acholic stools disease in children around the world. BA (Biliary Atresia) entails a progressive, inflammatory injury of one or any bile ducts, leading to fibrosis and obliteration of both the extrahepatic and intrahepatic bile ducts. The function of bile systems are disposing metabolic waste from liver and transport of bile salts which required to digest fat in the small intestine. In biliary atresia, a blockage of bile flow take place from the liver to the gallbladder. It may lead to liver damage and cirrhosis of the liver, which if left untreated could be fatal [17].

Causes of biliary atresia is derived from various things such as: viruses, especially rotavirus infection, genetic disorders, toxic materials which interfere the growth of the biliary tract as well as the bile duct damage during delivery of perinatal. There are three types of biliary atresia: Type I, atresia of the common bile duct; type II, atresia of the hepatic ducts whereas type III, obstruction or blockage of the bile duct to upstream the porta hepatis and above the porta hepatis. Most patients included in biliary atresia type III, which reached 90% [14].

This disease occurs in 1/14.000 up to 1/10.000 live births. The ratio of biliary atresia in girls and boys is 4:1. From 904 cases of biliary atresia were enrolled in over 100 institutions, biliary atresia widely experienced by Caucasians (62%), blacks (20%), Hispanic (11%), Asia (4.2%) and American Indians (1.5%) [15].

At the time of diagnosis, a Kasai portoenterostomy is performed in an attempt to re-establish bile flow. Despite this surgical intervention, the intrahepatic bile duct injury continues, leading to cirrhosis and the need for liver transplantation during childhood in the majority of patients. This surgery must carried out before the baby is 2 months old [9]. Originated from an attempt to treat infants with biliary atresia (BA) after hepatic portoenterostom (HPE) [6], Corticosteroids (steroids) became therapies which commonly used in the post-portalenterostomy and believed to improve clinical outcomes in BA. Basic theory of the use of steroids are anti-inflammatory effect that has aimed to reduce viral infection of the biliary tract by increasing the bile flow, as well as reducing inflammation and edema periduktal [5].

Therefore, this study was conducted to determine the activity of corticosteroids which in this study is used dexamethasone as an alternative
therapy in the treatment of biliary atresia in infants that could be known the effectiveness their use compared with other treatments.

**METHODS**

**Research Design**

Treatments in this study were divided into three treatments, with K treatment as Control, R treatment as RRV infection, and D14 n D21 treatment as RRV infection+ Dexamethasone injection.

### Grouping of Mice

<table>
<thead>
<tr>
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<th>Termination 14th day (K14)</th>
<th>Termination 21st day (R21)</th>
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<tbody>
<tr>
<td>K</td>
<td>6 mice babies</td>
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<td>R</td>
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<td>D21</td>
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<td>21 mice babies</td>
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#### Table 1. Grouping of mice in each treatment

**RRV(Rhesus rotavirus) Injection**

Baby mice BALB / c born in <24 hours is taken to be experimental animals. Control mice (C) were not given any treatment, while the treatment group mice R and D (D14 and D21) were injected with 20µl 106 PFU RRV strain MMU 18006 subcutaneously.

**Dexamethasone Injection**

Dexamethasone dose which injected in baby mice is 0.5 mg / kg body weight of mice babies. D14 group given steroids from day 7 and terminated after observed until day 14. D21 group given steroids from day 14 and terminated after observed until day 21.

**Lymphocytes Cell Isolation**

Spleen were crushed with wire, by adding 1ml PBS to obtain homogenates. The homogenates then moved into propylene tubes, added PBS till 10 ml. Then centrifugated in 2500 rpm, 4ºC for 5 minutes. Supernatant removed and obtained pellet was homogenized with 1 ml PBS, taken 40 µl, moved into microtube and add with 500µl PBS, then centrifugated again in 2500 rpm, 4ºC for 5 minutes. Pellets added with cytofix / cytosperm 100µl then incubated for 20 min, 4 º C. Then add 1 ml washperm then centrifuged 2500 rpm, 4 º C, 5 minutes. Pellets ready to immunostaining.

**Flowcytometry Analysis**

Isolated lymphocytes cells of spleen then added with antibody staining, FITC anti-mouse CD4 clone GK 1.5, PE rat anti-mouse CD8a clone 53-6.7, PE anti-mouse TNF-α clone MP6-XT22 and FITC Rat anti-mouse Ly-6G and Ly-6C clone RB6-8C5, PE anti-mouse CD45R/B220 clone RA3-6B2, PE/Cy7 anti-mouse IgM clone RMM-1. Conjugated results incubated for 15 minutes in ice box. The sample then added with 350 µl PBS placed inside flowcytometer cuvette. Then choose acquire and the flowcytometer calculated the total cell number and the number of cells detected by labeling antibody. Obtained results then processed by BD cellquest Pro TM program to be analyzed with Complete Randomized Design by SPSS I6 for Windows program. Observed variables are absolute cell B lymphocytes, Gr-1, and TNF-α cytokine.
RESULT AND DISCUSSION

Population of CD4+TNF-α+ cells on Spleen Organ

The results of CD4+TNF-α+ in the RRV induction treatment and control not only on the first termination but also on the second termination (Figure 1) showed difference in the absolute number of cells. In first termination, there were amounted to 61,560 cells in the control, while the RRV induction treatment was 65,100 cells. Then in D14 treatment were lower than RRV induction. In second termination, there were amounted to 65,190 cells in the control, while the RRV induction treatment was 215x 10^3 cells.

![Figure 1. Absolute cell number of CD4+TNF-α+ at first and second termination](image)

RRV (R) treatment able to increase TNF-α expression by CD4+ cells. Immunity responses for virus infection commonly mediated by T helper cells type 1 (Th1) as inflammation process where IFNγ and TNF-α play a main role in this case [2]. About 1-2 weeks after RRV infection, CD4+ cells produce IFN-γ which found in baby mice hepatic with rising of macrophage quantity that produce TNF-α [7]. The increasing of quantity and size of Kupffer cells is caused by fibrosis forming in hepaticrelated to the increasing of IL-18 serum on Biliary atresia. IL-18 is macrophage derived cytokine which work together with IL-12 cytokine to increase proliferation and differentiation of Th1 cells which will cause T cells release pro-inflammatory cytokine[18].

Population of Gr-1 cells on Spleen Organ

The result of D14 treatment and D21 treatment showing the decreased of CD4+TNF-α+ population when compared with control. This could be caused by corticosteroid activity in biliary atresia therapy with induce bile to enhance bile-salt flow with induce Na+K+ATPase enzyme to increase electrolyte transport in canalliculi as well as inhibit inflammation response [16]. Besides that, Corticosteroid able to work as immunosuppressan which have ability to inhibit lymphocyte and macrophage migration to bile duct, enhance gen coding transcription for anti-inflammation protein, and regulate IL-4, IL-10, and IL-13 production by Th2 cells. By this mechanism, Th1 expression will be pressing so T helper will work on Th2 cells, where could suppress TNF-α cytokine secretion eventually[3].

![Figure 2. Absolute cell number of Gr-1 at first and second termination](image)

The results of Gr-1 cells in the RRV induction treatment and control not only on the first termination but also on the second termination (Figure 2) showed difference in the absolute number of cells. In first termination, there were amounted to 718x10^3 cells in the control, while the RRV induction treatment was 980x10^3 cells. Then the RRV + Dexamethasone induction were lower than RRV induction. In second termination, there were amounted to 662x10^3 cells in the control, while the RRV induction treatment was 2,1x 10^6 cells.

Total absolute cell number of Gr-1 in control are higher than R treatment at first treatment as well as second treatment. This can caused by neutrophils and macrophage activation in R treatment which is innate immunity strong responses against Rotavirus infection. Macrophages have surface receptor which bind and phagocyte every kinds of pathogen. This will cause inflammation responses which make plasma protein accumulation, including complement components that are part of the humoral innate immunity, and phagocytic activity by neutrophils.
in the area of infection which is one of non-specific immune responses[11, 12]. Migration of neutrophils during an infection and inflammation influenced the presence of chemotactic signals in the form of KC/CXCL1, MIP-2/CXCL2/3 and CXCR2 receptors. Secretion of substances that resemble toxins by rotavirus can stimulate CXC chemokine, so neutrophils moving towards the area of inflammatory [1].

The absolute cells number of D14 and D21 treatment decreased when compared with the treatment of R. This may caused by anti-inflammatory and immunosuppressive of Dexamethasone which potentially inhibits the migration of lymphocytes and macrophages into the bile duct to suppress the cytokine IL-3 which has the potential to trigger the proliferation of a variety of hematopoietic cells into myeloid progenitor cells, which trigger proliferation of various cells including myeloid cells that one of them is granulocytes. IL-3 plays a role in various cellular activities, such as cell growth, cell differentiation and apoptosis. Generally, IL-3 secreted by activated helper T cells as immune response to stimulate the T cells producing from bone marrow [19], so that it may reduce the absolute number of Gr-1.

Population of B220+ cells on Spleen organ

The results of B220+ cells in the RRV induction treatment and control on the first termination (Figure 3) showed difference in the absolute number of cells. In first termination, there were amounted to 8.3x10^6 cells in the control, while the RRV induction treatment was 7.4x10^6 cells. Then the RRV + DexamethasoneInduction were higher than RRV induction. In second termination, there were amounted to 1.6x10^6 cells in the control, while the RRV induction treatment was 1.7x10^6 cells. In R+D treatment, the absolute cells were higher than RRV induction treatment.

Figure 3. Absolute cell number of B220+ at first and second termination (C= Control, R= Infection, R+D= Infection+ Dexamethasone)

Total absolute cells of B220+ in control was higher than R induction treatment at first termination. This may happen because Rotavirus injection which given to 1-2 weeks old mice baby only cause weak antibody responses. This condition was estimated by the characteristic of hypo-responsive cells in immunity system which not mature yet, or lymphoid tissue disemination so the form of antibody production in the baby is low [4].

In D14 treatment, B220+ cells produced higher number of population compared with the control or treatment R. Dexamethasone can suppress Th1 cell activity where the cells act as a pro-inflammatory agent by increasing the activation of Th2 cells which produce the cytokines IL-4, IL-5, and IL-10[8]. IL-4 other than its activity in affects the B cells to make IgD and IgE antibodies, IL-4 also plays a role in regulating the activation of Th1 cells and Th2 cells, so the immune system homeostasis remains within the normal threshold conditions[10].

At second termination, absolute number of B220+ cells is higher R treatment compared with controls. This can be caused by antibodies produced by B cells to eliminate foreign antigens particularly hazardous substances [13]. Antibodies are the first molecules known to be involved in specific antigen recognition. Antibody molecule has two separate roles: first bind pathogenic molecules to enhance the immune response, second to recruit immunocompetent cells and other effector molecules such as antibodies which have bind to the target. For example, binding of antibodies to the virus will give neutralization reaction besides provide the marker on the virus for easy recognized by phagocytic cells and complement[12].

In D21 treatment, the absolute number of B220+ cells was higher than R treatment. B lymphocytes more active when obtaining the right cytokine which is secreted by T lymphocytes [12]. Here, dexamethasone may improve the proliferation and differentiation of B cells to induce macrophages to inhibit the production of
IL-12, thus reducing the ability of IFN-γ secretion as well as increase Th2 cells to synthesize IL-4 which serves as a factor of proliferation and differentiation of B cells and T cells [20].

CONCLUSION

The dexamethasone which given as corticosteroid for biliary atresia therapy could act as immunosupressan as well asimmunostimulator which able to suppress the population of TNF-α and Gr-1 and able to increase the population of B220+ in mice which infected by rotavirus.

ACKNOWLEDGMENTS

Gratitude we give to Allah SWT so we can completed this journal well. Not forgetting our prayers and best regards be addressed to the Prophet Muhammad. Writers say many thanks to all those who have helped in finishing this journal. And finally this journal may be helpful for anyone who needs.

REFERENCES

