The Effect of Dexamethasone Treatment to Humoral Immunity in BALB/C Mice Models

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ABSTRACT

Glucocorticoids (GCs) are a class of steroid hormones which regulate a variety of essential biological functions. The profound anti-inflammatory and immunosuppressive activity of synthetic GCs, combined with their power to induce lymphocyte apoptosis place them among the most commonly prescribed drugs worldwide. Endogenous GCs also exert a wide range of immunomodulatory activities, including the control of T cell homeostasis. Dexamethasone is kind of glucocorticoid which is used to control some deseases. The purpose of this experiment is to know the effect of dexamethasone on B220. There are three different doses of treatments applied: 0.0 mg/kg BW (control), 0.5 mg/kg BW, and 10 mg/kg BW. Each treatment uses 6 mice (2 weeks old) with intraperitoneal injection. The treated mice were observed for 7 days and spleen cells were isolated for flow cytometric immunophenotyping. The data were analyzed by BD CellQuest and tested using One-way ANOVA (p<0.05) then Tukey’s test with SPSS 16.0 for Windows program. The result of this experiment showed that the dose of 0.5 mg/kg BW did not decrease B220 cell number significantly, but the dose of 10 mg/kg BW did.

Keyword : B220, Dexamethasone, Glucocorticoids

INTRODUCTION

Dexamethasone is one of the medications used in the treatment of multiple myeloma. It is a synthetic adrenocortical steroid. Adrenocortical steroids, also known as glucocorticosteroids or corticosteroids, are produced naturally by the adrenal glands in the body \cite{1}. Adrenal glands produce hormones and steroids. The steroids influence many actions of the body’s systems. They are involved in regulation of carbohydrates, proteins, and fats. They also inhibit inflammatory and allergic \cite{2}.

Dexamethasone suppress certain actions of the immune system and also inhibit cytokines, which are chemicals in the body that control inflammation. Dexamethasone decreases inflammation or swelling by stopping white blood cells, which normally fight infection, from traveling to areas of the body where there is swelling. Its anti-inflammatory actions can actually stop the swelling around tumors (especially on the spine, brain, and bone) and the resulting pain and other symptoms caused by tumors pressing on nerve endings \cite{3}.

Dexamethasone can also alter normal immune system responses and is therefore useful in the treatment of conditions that affect the immune system, such as certain types of anemia (for example, aplastic anemia and hemolytic anemia), thrombocytopenia and purpura. It has been found that steroids can increase the ability of chemotherapeutic and immunomodulatory agents to destroy myeloma cells \cite{4}.

B220 is expressed on B lymphocytes throughout their development from early pro-B stages on and is down-regulated upon terminal differentiation to plasma cells. B220 is commonly used as a cell B marker. It also expressed on a small subset of dendritic cell (plasmacytoid Dendritic) \cite{5}.

Dexamethasone have been known to influence humoral immunity system (B cells) \cite{6}. Dexamethasone treatment with high dose can induce apoptosis on T cells and B cells \cite{7}. However immunological surveillance deeply affected by the existence of regulatory T cells \cite{8,9,10}. Based on this, we want to evaluate the effect of the Dexamethasone 0.5 mg/kg BW dose...
and 10mg/kgBW dose on B220 cells in Balb/C mice.

**RESEARCH METHODS**

**Dexamethasone Treatments.** Eighteen female albino mice (Balb/C) and 14 days old were obtained from animal house. Dexamethasone treatment with intraperitoneal injection was given to normal mice with 0.5 mg/kgBW dose and 10mg/kgBW dose. After 5 days, mice were terminated and spleen was isolated. In this research used three groups of treatment, control (without dexamethasone injection), D1 (0.5 mg/kgBW dose) and D2 (10 mg/kgBW dose).

**Isolation of Lymphocyte Cell.** Spleen was isolated then washed using PBS solution. Spleen was crushed on separate dish until became suspension. Suspension was taken in polypropylene tube until 10 ml on ice to keep the condition of cells well. Then centrifuged at 2500 rpm, 10 °C for 5 minutes at 4°C. The supernatant was discarded and the pellet resuspended with 1 ml PBS.

**Flow Cytometry Analysis.** Pellet suspension that obtained previously, was taken 200 µl and placed in 500 µl PBS microtube. The suspension was centrifuged at 2500 rpm, 10°C for 5 minutes at 4°C. Pellet stained with antibodies extracellular and then resuspended with 300 µl of PBS, then put in a cuvette and running in the BD FACS Calibur TM flow cytometer.

**Statistical Analysis.** This research was analysed through one-way analysis of variance (ANOVA). Difference among means have been analysed by applying Tukey’s HSD (Honesty Significant Difference) test at 99.95% (p<0.05) and used program SPSS 16.0 for Windows.

**RESULT AND DISCUSSION**

This result was focus on relativity data of B220. Relativity data can be assumed as the amount of cell which were observed toward amount of other cell. This result was also focus on the effect of dexamethasone to hummoral immunity. The parameter in this research is B220 as marker of B cell. Relative number of B220 were analysed in control, D1 (0.5 mg/kg BW dose) and D2 (10 mg/kg BW dose) treatments using statistical analysis.

**Total Number of B220 Cells Analysis**

Based on figure 1, dexamethasone treatment in 0.5 mg/kg BW (D1) dose did not show significant result (p>0.05) to decrease the amount of B220 compared with control treatment. The relative number of B220 was decrease significantly in 10 mg/kg BW (D2) dose treatment of dexamethasone. The amount of relativity number B220 in control was 35.31%; 0.5 mg/kg BW (D1) dose was 27.21% and 10 mg/kg BW (D2) dose was 18.97%.

![Figure 1](image1)

**Figure 1.** Relative number of B220 cell in spleen after dexamethasone treatment control (without dexamethasone injection), D1 (0.5 mg/kg BW dose) and D2 (10 mg/kgBW dose).

![Figure 2](image2)

**Figure 2.** Relative number of B220 cells in spleen after dexamethasone treatment, control (without dexamethasone injection), D1
(0.5 mg/kgBW dose) and D2 (10 mg/kgBW dose).

Information: Difference characters showed the significantly difference based on Tukey’s HSD test with P<0.05.

Antibodies, which were the first specific product of the adaptive immune response to be identified, are found in the fluid component of blood, or plasma, and in extracellular fluids. Because body fluids were once known as humors, immunity mediated by antibodies is known as humoral immunity [11]. Most antigens require an accompanying signal from helper T cells before they can stimulate B cells to proliferate and differentiate into cells secreting antibody. The ability of T cells to activate B cells was discovered long before it was recognized that a functionally distinct class of T cells activates macrophages, and the term helper T cell was originally coined to describe T cells that activate B cells. B220 as marker of B cell has important function to make identification the effect of dexamethasone on humoral immunity response [11,12].

There are some factors that make the relativity number of B220 decrease in this research. That result may happened because of mechanism of glucocorticoid in our body could make apoptosis in the cells. Both 0.5 mg/kgBW dose and 10 mg/kgBW dose were decrease, but on 10 mg/kgBW dose the amount was significant. Anti-inflammasi effect of glucocorticoid and dexsamethasone high dose could inhibit proliferation and survival of B cell. Dexsamethasone high dose also could make B cell apoptosis. Glucocorticoid as a trigger to increase the expression of pro-apoptotic gen so that B cell can do the apoptosis [6,7].

Molecular modes of GR action. GCs passively diffuse into the cell and bind to the GR. This results in the dissociation of the heat shock protein complex (Hsps) and translocation of the ligand-bound GR into the nucleus. There the GR modulates transcription either by binding to DNA or via interaction with other transcription factors. Non-genomic mechanisms of GR action include interference with cytosolic signaling molecules [13].

The major pathways of lymphocyte apoptosis, the ‘intrinsic’ pathway involves the activation of ‘BH3-only’ molecules (Bad, Bim etc.) which in turn activate the ‘multidomain’ proteins Bax and Bak. This leads to the formation of the ‘apoptosome’ and activation of caspase-3, a process which is counteracted by the anti-apoptotic proteins Bcl-2 and Bcl-xL. The ‘extrinsic’ pathway is initiated by oligomerization of death receptors followed by caspase-8 activation and also converges on caspase-3. An alternative pathway induced by lysosomal stress involves the release of cathepsins [7,14].

In the first half of the 20th century, scientists finally discovered the importance of cortical hormones which, in the 1940s, were shown not only to be involved in the stress response but also to exert potent anti-inflammatory activity. The function of dexamethasone on anti-inflammatory [15,16].

![Figure 3. Glucocorticoid high dose on inflammatory process.](image)

Glucocorticoid may increased the production of protein anti-inflammasi and also inhibit the expression of gen which were coding protein pro-inflammasi [15]. As an anti allergic agen, dexsamethasone can inhibit secretion of histamine from mast cell so that can inhibit the irritation was caused by allergen[16]. The chances of side effects caused by steroids, including dexamethasone, increase with length of treatment and dose of the medication. In other words, the longer you take the drug and the higher the dose you are taking, the greater are your chances of experiencing side effects [15].
CONCLUSION

The result of this research showed that in 0.5 mg/kgBW dose did not effect significantly to decrease B220 but 10 mg/kgBW dose can effect significantly to decrease B220.

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REFERENCE


