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Nephrotoxicity Risk of Cyclophosphamide in Lupus Model

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Abstract

Cyclophosphamide is one of the standard therapies for lupus, especially lupus nephritis based on its immunosuppressive effect. However, cyclophosphamide is also known as a nephrotoxic agent. Therefore, this research was aimed to measure the effect of cyclophosphamide at the dose that comparable to the human dose of 1 mg/kg BW on the kidney of lupus mice induced by means of 2,6,10,14-tetramethylpentadecane (TMPD). In this research, the IL-6 as a pro-inflammatory cytokine was tested by using flow cytometry method. In addition, the structural damage of the kidney tissues was assessed by means of Moroni's kidney organ scoring method for lupus. The result showed that cyclophosphamide reduced the IL-6 significantly with the value of 36.72±22.79% for the TMPD-treated group; 32.59±9.97% for the cyclophosphamide group; and 30.25±4.48% for the naïve group. Moreover, the damages of the kidney tissues on the cyclophosphamide group were more severe than the TMPD-treated group. In conclusion, despite its anti-inflammatory effect which is useful for lupus, cyclophosphamide has a severe nephrotoxic effect which harms the patient. The effects may be a cause of the long interval use of cyclophosphamide. It can be a consideration for the further research and the next revision of the guideline for lupus nephritis treatment.

Keywords: cyclophosphamide, nephrotoxic, lupus treatment, IL-6, lupus mice

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1. Introduction

Cyclophosphamide is a non-selective immunosuppressive agent for lupus. It is widely used for lupus nephritis patients since cyclophosphamide is one standard for moderate to severe lupus treatment beside corticosteroids [1–4]. Cyclophosphamide has a non-specific immunosuppressive activity for lupus nephritis patients in a dose of 1-3 mg/kg BW [2, 5]. However, like other drugs for lupus, cyclophosphamide is used in a long term treatment, so there are big chances for the side effects to appear.

One of cyclophosphamide side effects is nephrotoxicity [6,7]. It is opposite to the aim of cyclophosphamide as the lupus nephritis standard treatment. Therefore, this research observed the effect of the cyclophosphamide on lupus nephritis. The mice model induced by means of 2,6,10,14-tetramethylpentadecane (TMPD) to became the severe lupus nephritis mice with the severity marker of proteinuria at the level of ++ (100mg/dL) [8–11]. The mice were treated by using cyclophosphamide for three weeks, and then the spleen cells were isolated to measure the IL-6 relative percentages. Finally, the mice kidneys were prepared for the structural tissue damage observation.

2. Experimental section

2.1 Materials

The materials used in this research were TMPD with the brand of Pristane was obtained from a Sigma-Aldrich distributor in Singapore. Then, cyclophosphamide with the purity of 98% was obtained from Kimia Farma, Indonesia; female BALB/c mice aged four weeks and pathogen-free species have been achieved from LPPT Unit 4, Gadjah Mada University, Indonesia.

Other materials such as antibody anti-IL-6 and anti-CD68 from Biogenesis were obtained from Molecular Biology Laboratory, Brawijaya University, Indonesia.

2.2 Methods

The female Balb/c mice used were seven weeks old when treated by using 0.5 mL TMPD intraperitoneal injection. The induction time lasted for six months. At the end of the induction time, the semi-quantitative data of proteinuria level showed a severe lupus manifestation. Then, the mice were divided into three experimental groups (n=5 per group), i.e., the TMPD-treated group, the cyclophosphamide group, and the naïve control group. The compounds tested were orally administered on the daily treatment until 21 days. Finally, the mice were sacrificed. The $CD68^{+}IL\text{-}6^{+}$ T cells were measured by using flow cytometer BD FACS Calibur. Then, the data were analyzed by using BD CellQuest program. The data was analyzed by facilitating of Oneway ANOVA by means of SPSS Statistics 22 version. Furthermore, the kidney tissues were observed by facilitating of hematoxylin-eosin (HE) staining and the use of microscope Olympus CKX41. The assessment was performed by means of Moroni scoring system. This research procedure was approved by local ICUC of Faculty of Veterinary Medicine, Universitas Airlangga, Indonesia with the number of 512-KE.

3. Results and Discussion

The principle of current lupus nephritis treatment is reducing the manifestations. Cyclophosphamide has a likely non-selective immunosuppressive effect which is beneficially used in lupus nephritis [1,2,12]. Therefore it becomes the standard treatment for severe lupus nephritis. The outcome of the immunosuppressive effect is the inhibition of pro-inflammatory biomarkers, such as IL-6.

In the present study, the IL-6 was measured by using flow cytometry method. The relative percentages of IL-6 expressed by IL-6-producing cells were analyzed well so that the difference between the TMPD-treated group, the cyclophosphamide group, and the naïve group was calculated as shown in Figure 1.

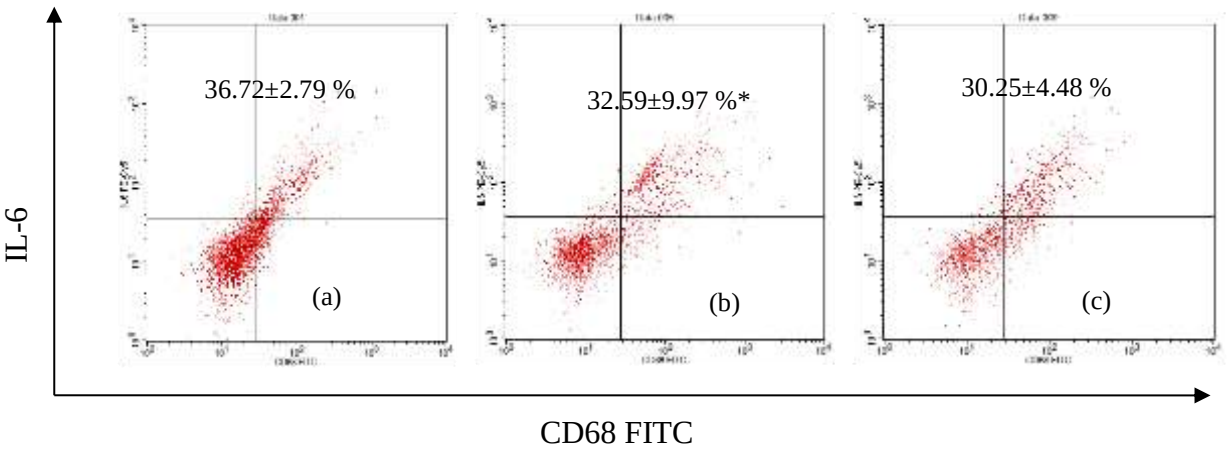
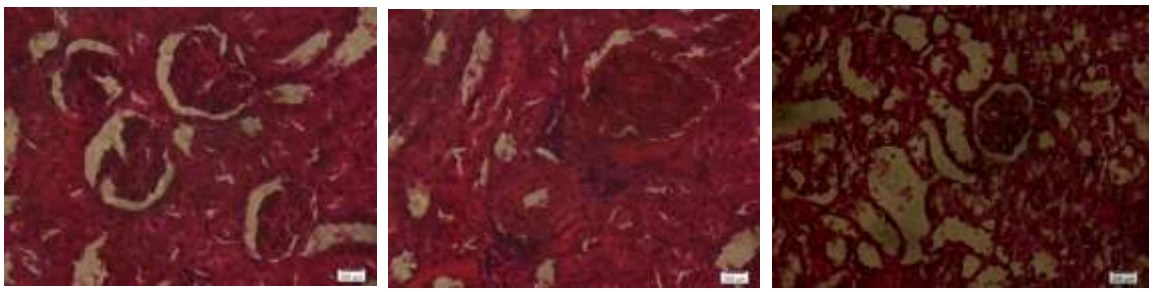


Figure 1 The relative percentage of IL-6+ of all producing cells in (a) TMPD-treated group, (b) cyclophosphamide group, and (c) naïve group



(a) (b) (c)

Figure 2 The structural damages of the kidney tissues of the (a) TMPD-treated group, (b) cyclophosphamide group, and (c) naïve group at the magnitude of 400x by means of Microscope Olympus CKX41. The ruler in the figures shows the length of 200μm.

The results show that the cyclophosphamide reduces the pro-inflammatory cytokine IL-6 significantly ($p<0.05$). It is a good sign of its efficacy to maintain the lupus patient's condition [13–15]. Although there was also a macrophage autophagy process which can inhibit the IL-6 secretion by itself, the IL-6 as the result of adaptive immune response seemed decreased since the total IL-6 assessment in this research calculates all IL-6 from all sources.

Figure 2a and 2b show the thickening of the glomerular basement membrane, the proliferation of inflammatory cells in the glomeruli, and also the unregular structure pattern of outside areas of glomeruli compared to naïve mice (Figure 2c). The glomeruli of the TMPD-treated mice are partially attached to the Bowman's capsule area and then make the Bowman's space narrower than normal. It leads the low filtration function of the glomeruli because of the damage of its podocytes [17–19]. Unfortunately, the cyclophosphamide cannot heal the inflamed glomeruli, but the worse sign of glomeruli attachment is seen in Figure 2b. The Bowman's spaces are too narrow, and some of the glomeruli are fully attached to the outside areas lead to the total damage of filtration function of the glomeruli [20–22]. The figures represent all mice in all groups tested.

Furthermore, the kidney tissue damage was assessed for its semi-quantitative data by

using Moroni's scoring method [23] for lupus nephritis. The results are shown in Table 1.

Table 1 Assessment of the structural damages of the kidneys in the three groups tested

Groups	Mean of severity grade according to Moroni's scoring method ± SD
TMPD-treated group	2.9±0.18
Cyclophosphamide group	3.3±0.83*
Naïve group	0.23±0.15

The result of cyclophosphamide group shows the grade of kidney-structural damage which is more severe significantly ($p<0.05$) than the TMPD-treated group. This data supports the descriptive data. It means the nephrotoxicity of cyclophosphamide might contribute in damaging the kidney structure and function. In spite of its effectiveness in maintaining immune system in lupus [24-26] the long term-use of cyclophosphamide results in a serious problem in the kidney so its use must be restricted.

Another issue in lupus nephritis is the limited choice of drugs used [27, 28]. It is overwhelming since the patients need the treatment all time. The suggestion is the use of other immunosuppressive drugs which are not nephrotoxic, like corticosteroids since it is also included in the guideline of lupus nephritis treatment.

However, the further research to support this research are necessary. The development of lupus nephritis drugs is also needed since the prevalence of lupus increase in this last decade.

4. Conclusion

Beside its immunosuppressive activity, cyclophosphamide in a long term use has side effects on inflammation and damage the structure of kidney tissue.

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Conflict of Interest

The authors declare there is no conflict of interest.

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