Original article:

Effect of Secretome Mesenchymal Stem Cells On Expression Interleukin 10 And Interleukin 17 in Mice Lupus Model

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Abstract

Background: Systemic Lupus Erythematosus (SLE) is a chronic autoimmune inflammatory disease with extensive clinical features. The role of Interleukin 10 (IL-10) is to promote autoantibody production and autoreactive B cell proliferation. Interleukin 17 (IL-17) associated with pathogenesis of SLE and positively correlated with disease activity. Intraperitoneal pristane can induce lupus in mice. Mesenchymal stem cells secretome work in paracrine effects asanti-inflammatory and immunomodulation agent by suppressing autoreactive T and B cell, which play an important role in pathogenesis of SLE. The aim of this study is to evaluate effect of mesenchymal stem cells secretome on the expression of IL-10 and IL-17 in murine lupus models induced by pristane. ***Methods*:** A randomized experimental study, post-test only control group design, samples using 21 female Mus Musculus mice strain Balb/C, divided into 3 groups: control group with intraperitoneal injection of 0.5 mL of 0.9% NaCl, treatment group with intraperitoneal injection of 0.5 mL pristane, therapy group with intraperitoneal injection of 0.5 mL pristane and 0.45 mL of secretome. Statistical analysis using ANOVA test. A p-value < 0.05 was considered statistically significant. ***Results:*** The results showed significant relationship between control and pristane groups both at the levels of IL-17 (control 6.9±1.95,pristane 9.9±2.27, pristane+secretome 6.1±1.95 p=0.016), and there are significant differences in the expression of IL-10 in the control group vs pristane group (-4,42±1,43 per 100 lymphocyte; p=0,006), pristane group vs pristane+secretome group (4,00±1,43 per 100 lymphocyte; p=0,012).
Conclusion: Mesenchymal stem cells secretome decreased the expression of IL-10 and IL-17 levels in murine lupus models induced by pristane.

Keywords: Interleukin 10; Interleukin 17; Pristane; Secretome mesenchymal stem cell; Systemic Lupus Erythematosus.

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Background

Systemic Lupus Erythematosus (SLE) is a complex autoimmune disease characterized by autoantibody in nucleus and this condition includes organ system in the body. Etiopathology of SLE was to involve complex and multifactorial interaction between genetic variation and environmental factor[1]. The annual incident of SLE in America was 5.1 per 100.000 people, meanwhile SLE prevalence in America was reported about 52 cases per 100.000 people with the ratio between female and male population about 9-14:1. There had not been SLE epidemiology data in Indonesia yet. Data at Cipto Mangunkusumo Hospital Jakartain 2002 informed

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about 1,4% SLE cases based on total of patients visit in rheumatology polyclinic. However, there were 291 SLE patients or about 10.5% occurred at Hasan Sadikin Hospital Bandung based on total patients that had treatment in rheumatology polyclinic during 2010[2]. Recent SLE therapy only inhibits disease progress and prevent a serious disease. There has not been found SLE definitive therapy which can cure SLE. Therefore, there are many new researches about SLE treatment.

Factor interaction in SLE will cause immune response that increases T-cell and B-cell activation, thus autoantibody (DNA-anti DNA) will increase. Some of autoantibody will form immune complex, then this will make precipitation that can damage tissues[1].

Mesenchymal Stem Cells are one of new promising therapy in SLE. Secretome of Messenchymal stem cells consists of cytokine, micro RNA (miRNA), exosomes and microvesicle which can cause therapy effect[5] and this is not because of cell differentiation[6]. Mechanism taken from secretome includes anti-apoptosis[7], anti-inflammation[8], antifibrosis[9], angiogenic[10] and the effect of tissue regeneration. The role of IL-10 is to promote autoantibodyproduction and autoreactive proliferation B cell. IL-17 associated with pathogenesis of SLE and positively correlated with disease activity. Giving pristane via intraperitoneal can induce lupus in mice. Mesenchymal stem cells secretome work in paracrine effects as anti-inflammation andimmunomodulation agent by suppressing autoreactive T and B cell, which play animportant role in pathogenesis of SLE. This study evaluates effect of mesenchymal stem cells secretome on the expression of IL-10 and IL-17 in murine lupus models induced by pristane.

Materials and method

This research was an experimental research with post-test only controls group design. Subject using 21 female mice with sub-species musculus Balb/C aged 3-4 months old, weighted was 20 -30 gram and they were divided into 3 groups. Control group injected with5 mL NaCl 0,9% via intraperitoneal in the first treatment, third week later re-inject with 45mL NaCl 0.9%. Lupus control group injected with 0.5 mL pristane via intraperitoneal and third week later injected with 0.9% NaCl. Treatment group injected with of 0.5 mL pristane via intraperitoneal and 0.45 mL secretome of mesenchymal stem cells via intraperitoneal. In day 24, we examined IL-10 and IL-17 from their serum.

Data taken was described with mean standard deviation, test of normality was assessed with Shapiro Wilk test and homogeneous variance test was described using Levene's test. F anova test was continued to Least Significant Difference (LSD) post-hoc test, finally Kruskal-Wallis test was continued by mann whitney test. The significant level was $p \leq 0,05$. Ethical approval was taken prior the study.

Results

The results showed significant between control and pristane groups both at the levels of IL-17 (control 6.9±1.95, pristane 9.9±2.27, pristane+ secretome 6.1±1.95; p=0.016), and there are significant differences in the expression of IL-10 in the control group vs pristane group (-4,42±1,43 per 100

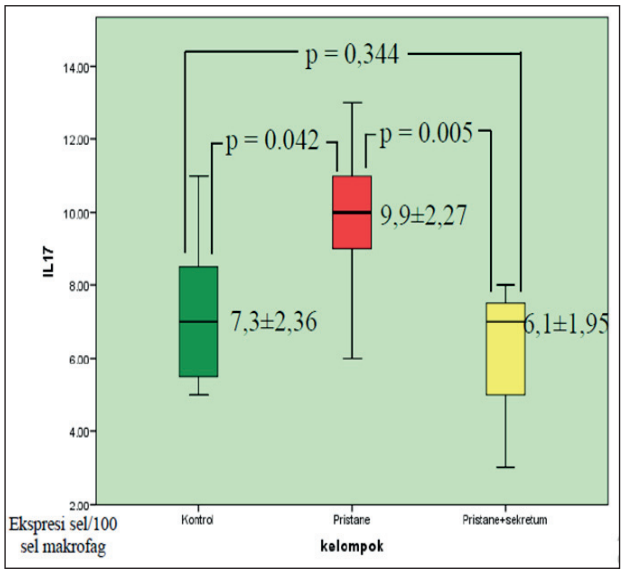

Figure 1. Expression IL 17 between control, pristine group, and secretome

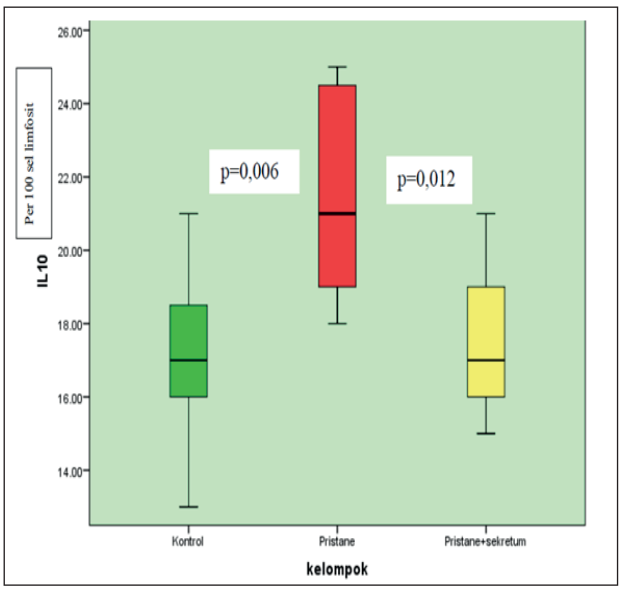

Figure 2. The level of prolactin in the first day postpartum mothers in hospitals in Semarang in 2016 (n = 32)

lymphocyte; p=0,006), pristane group vs pristane+ secretome group (4,00±1,43 per 100 lymphocyte; p=0,012).
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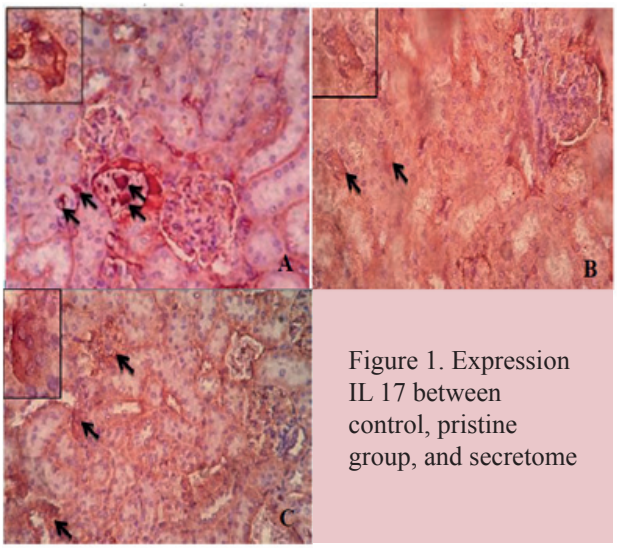

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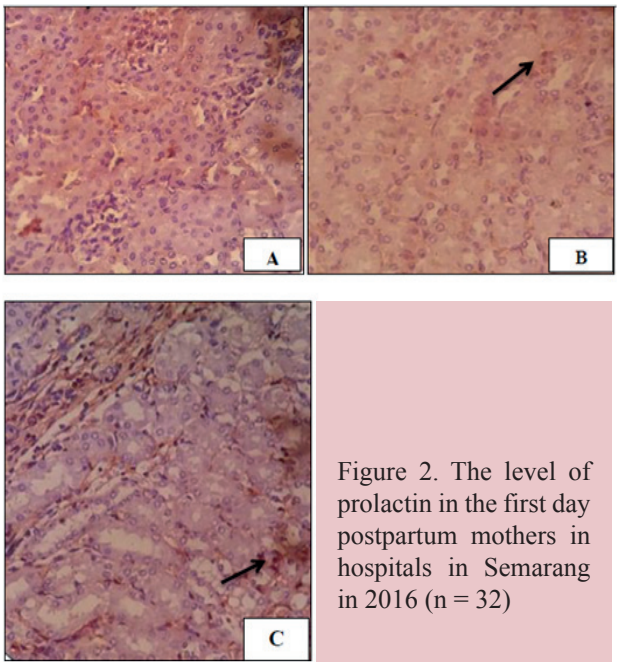

Figure 2. The level of prolactin in the first day postpartum mothers in hospitals in Semarang in 2016 (n = 32)

Discussion

The use of pristane to induce mice model with lupus had been used widely in the research. Lupus process begun by giving pristane that would cause apoptosis. Apoptosis body occurred was source of autoantigen and this could cause lupus by using IFN-1[15,16]. IFN-1 could damage tissues. Tissue damage could be detected by molecular level (increase of dsDNA antibody and decrease of Complement C3) through immunological examination using ELISA method and description of histological damage (Pulmonary vasculitis description and lupus nephritis in kidney). Secretome of mesenchymal stem cells had immunomodulatory agents that could improve clinical description of SLE.

Intraperitoneal injection of pristane in normal mice would cause SLE. Dysregulation of apoptosis and disorder of apoptosis bodies clearance caused autoimmune process. Pristane known as a compound which induced lupus through immune response depended on T cell[15]. Pristane was an active compound that interacted with phospholipid compound of cell membrane. Biophysical disorder in cell membrane would cause cell apoptosis. Calvani et al in 2007 had found apoptosis pathway taken from pristane in peritoneal cell culture and lymphoid cell culture which was through both intrinsic apoptosis pathway and extrinsic apoptosis pathway. Intrinsic apoptosis pathway was detected by increasing of cytochrome c and caspase- 9 in cell culture. Meanwhile, extrinsic apoptosis pathway could be seen from the increase of caspase-8, Fas and Fasl in cell culture. Finally, the increase of caspase-3 existed, and apoptosis occurred in this process[15].

Apoptosis bodies resulted by pristane induction would be source of autoantigen which would be presented to APC. Immature monocyte cells Ly6^{Chi} /plasmacytoid cell DC had a role as APC. Immature monocyte cells migrated from bone marrows to peritoneum caused by MCP1/CXCL1 stimulation. The increase of MCP1/CXCL1 was caused by pristane induction to lymphoid and peritoneal cells. Apoptosis bodies would stimulate adaptor molecule MyD88[16]. Apoptotic body would be recognized by TLR 7 as autoantigen. Stimulation of endosomal TLR-7 would stimulate immature monocyte expressing Ly6Chi on its surface and this caused gene transcription of IFN-1, then IFN-1 production occurred[16]. Autoantigen was formed from lymphoid nucleus which had apoptosis in cavum peritoneal and cytokine milieu disorder caused autoimmune[15].

This research was similar with previous research in mice where stem cell therapy of allogeneic bone marrow in mice with lupus either in early stage (9 weeks old) or in advanced stage (16 weeks old) showed the decrease of IGG and IGM anti dsDNA antibody compared to mice getting cyclophosphamide[12]. Other researches mentioned that clinical test of stem cell in mesenchymal stem cells therapy in human was mesenchymal stem cells therapy in refractory

severe SLE[12,13]. Those researches showed that there was a decreased anti ds DNA antibody level in the end of therapy. This research result supported other researches with higher samples i.e 15 patients of refractory SLE including 4 cases reported previously. In this research one third of previous patients failed in therapy using mycophenolate mofetil(1-2 gr/day x 3 months)[13]. Patients got intravenous allogeneic mesenchymal stem cell taken from bone marrow of 3-5 healthy family member without HLA suitability. In that research, there was the decrease of antibody anti ds DNA level after therapy.

In general, benefit of this research was by giving secretome of mesenchymal stem cells in SLE which could decrease disease activity of SLE disease. This research result supported more protocol in the use of mesenchymal stem cell secretome as a new therapy in SLE management.

Conclusion

Mesenchymal stem cells secretome decreased the expression of IL-10 and IL-17 levels in murine lupus models induced by pristane.

This research was still conducted in animals . However, SLE in human consisted of etiology, pathogenesis, and more complex management. Therefore, further research in human was still needed to investigate the benefit of mesenchymal stem cells secretome in SLE.

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