

Induksi Interleukin-6 Memicu Apoptosis Melalui Jalur IL-17 dan Stat3 yang Ditekan dengan Pengobatan Phycocyanin

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Abstrak

Penelitian ini bertujuan untuk menganalisis dosis optimal induksi IL-6 pada tikus hamil yang dapat memicu peningkatan tekanan arteri dan protein sebagai dua gejala preeklampsia. Sebanyak 25 tikus hamil dibagi menjadi 5 kelompok, yaitu tikus hamil kelompok kontrol (tanpa induksi IL-6), 1 kelompok tikus hamil diberi induksi IL-6 dalam dosis 1,25 ng / hari, 1 kelompok tikus hamil diberi induksi IL-6 dosis 2,5 ng / hari, 1 kelompok tikus hamil diberi induksi IL-6 dosis 5 ng / hari, dan kelompok tikus hamil diberi induksi IL-6 dosis 10 ng / hari. Induksi IL-6 dilakukan pada hari kesepuluh kehamilan selama 5 hari. Ekspresi caspase-3, IL-17, dan STAT3 dianalisis dengan mikroskop confocal. Ekspresi caspase-3, IL-17 dan STAT3 secara signifikan lebih tinggi pada kelompok preeklampsia dibandingkan kelompok kontrol ($p < 0,05$). Ekspresi caspase-3 menurun secara signifikan pada semua kelompok yang diobati dengan phycocyanin dibandingkan kelompok preeklampsia ($p < 0,05$), namun belum dapat mencapai ekspresi yang sebanding dengan kelompok kontrol ($p < 0,05$). Ekspresi IL-17 menurun secara signifikan pada kelompok tersebut dengan pemberian dua dosis tertinggi phycocyanin dibandingkan dengan kelompok preeklampsia ($p < 0,05$). Ekspresi STAT3 menurun secara signifikan pada semua kelompok yang diberi phycocyanin dibandingkan kelompok preeklampsia ($p < 0,05$), mencapai ekspresi yang sebanding dengan kelompok kontrol pada kelompok yang menerima phycocyanin pada dosis 10 dan 20 ng ($p > 0,05$). Kesimpulannya, IL-6 pada tikus hamil mampu meningkatkan apoptosis melalui jalur IL-17 dan STAT. Penghambatan apoptosis akibat pengobatan phycocyanin tidak hanya melibatkan pembentukan IL-17, data ini ditemukan dalam dosis phycocyanin 10 dan 20 ng.

Kata Kunci: model preeklampsia, apoptosis, IL-17, STAT3, tikus

Induction of Interleukin-6 Trigger an Apoptosis Through IL-17 and Stat3 Pathway that Alleviated by Phycocyanin Treatment

Abstract

This study was to analyze the optimum dose of IL-6 induction in pregnant rats that could trigger an increase in mean arterial pressure and protein as two symptoms of preeclampsia. A total of 25 pregnant rats was divided into 5 groups, including pregnant rats the control group (without induction of IL-6), a group of pregnant rats were given an induction in IL-6 doses of 1.25 ng/day, a group of pregnant rats given doses of IL-6 induction 2.5 ng/day, a group of pregnant rats were given an induction of IL-6 doses of 5 ng/day, and groups of pregnant rats were given an induction in IL-6 doses of 10 ng/day. Induction of IL-6 was performed on the tenth day of gestation for 5 days. The expression of caspase-3, IL-17, and STAT3 were analyzed by confocal laser scanning microscopy. The expression of caspase-3, IL-17 and STAT3 was significantly higher in preeclampsia group than the control group ($p < 0.05$). The expression of caspase-3 decreased significantly in all groups were treated by phycocyanin compared to the preeclamptic group ($p < 0.05$), but has not been able to reach expression comparable to the control group ($p < 0.05$). Expression of IL-17 decreased significantly in the group given the two highest doses of phycocyanin compared to the preeclampsia group ($p < 0.05$). STAT3 expression decreased significantly in

all groups were treated by phycocyanin compared to the preeclamptic group ($p < 0.05$), reached expression comparable to the control group in the group received phycocyanin at doses of 10 and 20 ng ($p > 0.05$). In conclusion, IL-6 on pregnant rats were able to increase apoptosis through IL-17 and STAT pathway. Inhibition of apoptosis due to phycocyanin treatment not only involve the formation of IL-17, this data is found in phycocyanin doses of 10 and 20 ng.

Keywords: preeclampsia model, apoptosis, IL-17, STAT3, rats

INTRODUCTION

Preeclampsia is one of the causes of maternal death. In Indonesia, the prevalence of maternal deaths due to preeclampsia by 28-30%, is the second leading cause of death of pregnant women after bleeding (1). Preeclampsia is caused by inadequate invasion of trophoblast cells to the maternal uterine blood vessels, resulting in failure of the remodeling of the spiral arteries that supply blood to the fetal maternal (2,3). The clinical characteristics of preeclampsia are increased blood pressure and the presence of protein in the urine of pregnant women (4).

Interleukin-6 (IL-6) is a cytokine pleiotropic which has various functions, are pro-inflammatory, anti-inflammatory, and activation of other immune system. Various types of cells, including dendritic cells, macrophages, lymphocytes, fibroblasts, epithelial cells and placental trophoblast cells produce IL-6 (5,6). In pathological conditions of pregnancy, an increase in IL-6 is a pro-inflammatory due to the dominance of immunological T-helper 1 (Th1) against T-helper 2 (Th2) (7). Environment that is pro-inflammatory result in decreased resistance trophoblast cells become susceptible to apoptosis (8). IL-6 together with TGF- β 1 will stimulate increased secretion of IL-17 through Stat3 pathway. Increased levels of IL-17 will further induce apoptosis of endothelial cells (5). On the other hand, excessive apoptosis in trophoblast remodeling resulting in failure of the spiral arteries thus creating an environment of inflammation and hypoxia in placenta. Furthermore, this condition will trigger the production of vasoactive substances, angiotensin II type 1 receptor (AT-AA), which plays a role in increased blood pressure and protein urine (8-11).

Phycocyanin is one of the pigments in the micro-algae *Spirulina*, form oligomers consisting of alpha and beta subunits as well as the chromophore. Phycocyanin was utilized in

various countries as nutritional supplements and pharmacological function as an antioxidant, anti-inflammatory, neuroprotective and hepatoprotective (12-15). Until now, the use of phycocyanin in the treatment of pre-eclampsia has not been done. Therefore, this study aimed to analyze the effect of phycocyanin to apoptosis induced by IL-6 in mice pregnant involving the production of IL-17 and STAT3 pathway.

MATERIAL AND METHODS

Animals

Rats (*Rattus norvegicus*) Wistar, nulliparous aged 12-14 weeks, with a weight of 120-200 grams were obtained from the Faculty of Pharmacy, University of Gadjah Mada, Yogyakarta, Indonesia. Rats were maintained in laboratory test animals Faculty of Medicine, University of Wijaya Kusuma, Surabaya, East Java, Indonesia on conditions of light and dark cycle of 12 hours, the temperature of 25°C and a pressure of 1 atmosphere. Feed brand Pars Confeed (BR1) and mineral water was provided ad libitum.

Mating

Before the rats mated with males, to get the same gestational age and to increase the success of pregnancy, all the rats synchronized in the estrus cycle by providing both leet effect, pheromones and Whitten in female rat. Seventy-two hours after Whitten effect, female rats were mated for one night in pairs (1:1). The next day after mated considered the first day of pregnancy. To ensure the success of pregnancy, vaginal examination is done by looking at the blockage of the vagina (vaginal plug) and a vaginal swab.

Induction of IL-6

A total of 25 pregnant rats were divided into 5 groups of pregnant rats the control group (without induction of IL-6), a group of pregnant rats were given induction of IL-6 doses of 1.25

ng/day, a group of pregnant rats given doses of IL-6 induction 2.5 ng/day, a group of pregnant rats were given induction of IL-6 doses of 5 ng/day, and groups of pregnant rats were given induction of IL-6 doses of 10 ng/day. Induction of IL-6 was performed on the tenth day of gestation for 5 days. Injection of IL-6 was done via the tail vein. IL-6 was obtained from BIOS Inc., USA, No: bs-0003R in the form of lyophilized powder, used together with solvent for injection (Aquadest brands Otsuka, Pasuruan, East Java, Indonesia).

Phycocyanin

Phycocyanin powder were purchased from Sigma Aldrich product code P20711 Singapore. The content of CPC in one ampoule is 5 mg. The phycocyanin powder were treated at a dose of 10, 20, 40, 80 ng/100 grams body weight by oral gavage for 5 days in conjunction with the induction of IL-6. Phycocyanin was dissolved with aquadest and emulsifier until the volume of 100cc, in order to obtain phycocyanin dosage levels of 5 ng/0.1ml.

Labeling immunofluorescence staining

Paraffin-embedded placenta sections (10 μ m thick) were immunostained according to the manufacturer's instructions (Santa Cruz Biotechnology, Dallas, TX, USA). Briefly, placenta sections were deparaffinized in xylene and dehydrated through graded ethanol series. Nonspecific protein bindings were blocked with 2% skim milk powder in PBS at RT for 20 min, followed by washing with PBS. Next, placenta sections were incubated with Anti IL-17 mouse, Bioss, USA no. cat 14563 (Santa Cruz Biotechnology, US), anti-STAT3 rat no. cat 558099 antibodies (Santa Cruz Biotechnology, US), and mouse anti-caspase-3 no. cat 611048 (Bioss, US) at specified dilutions for 1 h, followed by washing with PBS. The primary antibody bindings were then detected with goat anti-rabbit rhodamine (Santa Cruz Biotechnology) and goat anti-mouse FITC (Santa Cruz Biotechnology) antibodies at specified dilutions for 1 h in the dark, followed by washing with PBS. All PBS washed steps consisted of three washes of 5 min each. The expressions of caspase-3, IL-17, and STAT3 were analyzed by counting fluorescent intensity of cells (in arbitrary units; AU) in five random high-power (x400) microscope fields. The

fluorescent images were recorded under a confocal laser scanning microscope (Olympus).

Statistical analysis

To determine the level of significance of the caspase-3, IL-17, and STAT3 between groups, the data were analyzed using statistical parametric two-way ANOVA test (two-way ANOVA) with significance level of 0.05.

RESULTS

The expression of caspase-3 was significantly higher in the preeclampsia group than the control group ($p < 0.05$). The expression of caspase-3 decreased significantly in all groups were treated by phycocyanin compared to the preeclamptic group ($p < 0.05$), but has not been able to reach expression comparable to the control group ($p < 0.05$).

Expression of IL-17 was significantly higher in the preeclampsia group than the control group ($p < 0.05$). Expression of IL-17 decreased significantly in the group given the highest doses compared phycocyanin preeclampsia group ($p < 0.05$), although it has not yet reached an expression that is comparable to the control group ($p > 0.05$).

STAT3 expression was significantly higher in the preeclampsia group than the control group ($p < 0.05$). STAT3 expression decreased significantly in all groups were treated by phycocyanin compared to the preeclamptic group ($p < 0.05$), reached expression comparable to the control group in the group received phycocyanin at doses of 10 and 20 ng ($p > 0.05$).

DISCUSSION

In this study, the induction of IL-6 on pregnant rats proved to increase the expression of caspase-3 compared to the control group ($p < 0.05$). This increase was followed by an increase in STAT-3 and IL-17. This suggests that the increase in caspase-3 induced by IL-6 at least through increased IL-17 and STAT3. This is consistent with earlier findings that IL-6 together with TGF- β stimulates an increase in expression of IL-17 through STAT3. Furthermore, the increase in IL-17 triggers apoptosis of endothelial cells (5).

In this study, administration of a dose of 10-80 ng phycocyanin shown to decrease the expression of caspase-3 in rats preeclampsia induced by IL-6, but has not been able to reach expression comparable to the control group ($p < 0.05$). These findings show that phycocyanin can inhibit apoptosis induced by IL-17 in pregnant rats. This study was consistent and extended previous findings on the effects of phycocyanin as an antiapoptosis. Phycocyanin apoptosis modulation was occurs through mitochondrial pathway [16-19]. To decrease the expression of IL-17 is found only in the dosage of phycocyanin doses of 40 and 80 ng. This finding indicates that the inhibition of apoptosis due to phycocyanin treatment not only involving the formation of IL-17, this data is found phycocyanin treatment at doses of 10 and 20 ng. On the other hand, phycocyanin was able to reduce STAT3 in a dose of 10-80 ng, which reached an expression that is comparable to the control group (doses of 10 and 20 ng) ($p > 0.05$). This confirms that the inhibition of apoptosis as the inhibition of the expression of STAT3, which triggers the production of IL-17 as well as the other factors.

In conclusion, IL-6 on pregnant rats were able to increase apoptosis through IL-17 and STAT pathway. Inhibition of apoptosis due to phycocyanin treatment not only involve the formation of IL-17, this data is found in phycocyanin doses of 10 and 20 ng.

Declaration of interest

The author(s) declare(s) that there is no conflict of interests regarding the publication of this article.

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