Sys Rev Pharm 2021;12(1):1551-1554
A multifaceted review journal in the field of pharmacy

Low-dose Aspirin Reduces Caspase-3 Expression in Maternal Preeclampsia Serum-Induced Vascular Smooth Muscle Cells (VSMCs)

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ABSTRACT

Preeclampsia is a multisystem disorder of pregnancy characterized by hypertension and proteinuria after 20 weeks of gestation. Trophoblast apoptosis is known to take place in preeclampsia which is regulated by cysteine aspartate specific proteases (caspase), an executor protein to assign apoptosis. To date, effective treatment for preeclampsia has not been established yet. Aspirin is currently the most widely prescribed treatment in the prevention of cardiovascular complications. In this study, we evaluated level of caspase-3 on preeclampsia induced VSMC cells performed with enzyme-linked immunosorbent assay (ELISA). Low-dose aspirin recuded apoptosis of VSMC during trophoblast invasion as indicated by lower expression of caspase-3 compared to control. Thus, aspirin is believed to reduce pro-apoptotic compounds and to compensate cells function including preeclampsia induced VSMC by controlling trophoblast apoptosis. Further studies to measure other apoptosis-related markers, as well as in vivo and clinical studies, are encouraged.

Keywords: Aspirin, caspase 3, preeclampsia induced VSMC

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INTRODUCTION

Preeclampsia is a multisystem disorder of pregnancy characterized by hypertension and proteinuria after 20 weeks of gestation[1]. Preeclampsia can lead to liver and kidney failure, seizures (eclampsia), and abnormalities of the clotting system[2]. Its occurence is 1–8% of pregnant women. Preeclampsia is the second cause of maternal mortality worldwide[3] and is one of the five leading causes of maternal mortality.[4] To date, effective treatment for preeclampsia has not been established yet.

It has been hypothesized that the underlying mechanism of preeclampsia involve genetic factor, immunologic factor, vascular disease, and failure in trophoblast invasion into spiral artery at the first trimester. These cause spiral artery to dilate inadequately and disturbance in endothelial and muscular wall of blood vessel, leaves the blood vessel be smaller in diameter, leading to diminished perfusion and placental ischemia.[5-7] During trophoblast invasion into spiral artery, signal transduction is disrupted that further generates apoptosis. Increased apoptosis leads to TRAIL binding to apoptosis receptor in vascular smooth muscle cells (VSMC) which activates TNF-α via FasL pathway.[8,9] Apoptosis is an essential process in normal VSMC development in which cells are damaged to maintain vascular physiological balance. Apoptosis is involved in attachment, trophoblast invasion, spiral artery transformation, trophoblast transformation, and immune tolerance of paternal antigen expressed by trophoblast.[10] It is regulated by cysteine aspartate specific proteases (caspase), an executor protein to assign apoptosis.[11,12.]

Aspirin is currently the most widely prescribed treatment in the prevention of cardiovascular complications.[13] At low doses, aspirin is also usually used to prevent pregnancy-related vascular disorders, such as preeclampsia and intrauterine growth restriction, and maternal disorders like antiphospholipid syndrome. The indications for the use of aspirin during pregnancy are, however, the subject of much controversy. Proof of its efficacy is not established in a good number of these indications, yet it is being prescribed in an ever-increasing proportion of pregnant women.[14-16] In this study, we evaluated pathogenesis of preeclampsia in VSMC and treated with low dose aspirin.

MATERIAL AND METHODS

Study design

This in vitro study adopted a post-test only control group design. Vascular Smooth Muscle Cells (VSMCs) ATCC CRL 1999 (purchased from American Type Collection Culture) was used and induced with normal and preeclamptic sera that were collected from the normal pregnant women and preeclamptic pregnancy, respectively. Low-dose aspirin purchased from BAYER, with a variety of concentrations, was administered to each serum group to investigate its effect on the level of caspase 3.

VSMC cell culture preparation

VSMC cells were cultured in ham's F-12K medium supplemented with 10% (v/v) FBS (30 minutes, 56°C), 10% endothelial cell growth supplement, 1% ascorbic acid, 1% insulin, 1% transferin, 1% sodium selenite, 1% HEPES, and antibiotic-antimicotic (1%Penicillin G-Streptomycin Solution Stabilised and 1% Fungizone Amphotericin B) and incubated at 37°C 5% (v/v) CO2. Confluent cells were washed with PBS three times and followed by tripzinisation with 0,05% Tripsin-EDTA, incubated at 37°C for 30 sec. Tripsin-EDTA was discarded, then added with complete medium at equal volume.[17-20.]

Exposure of VSMC cells with healthy and preeclamptic sera

VSMC cells was grown in RPMI 1640 supplemented with 10% serum (normal and preeclampsia) with supernatant of trophoblast cells, and dengan supernatan antibiotic-antimicotic (1%Penicillin G-Streptomycin Solution

Stabilised and 1% Fungizone Amphotericin B). Cells were incubated at 37°C 5% CO2 (v/v) for 24h. Furthermore, aspirin in various dose (0,25-2 mM) was added, and cells were incubated at 37°C 5% CO2 (v/v).[15-17.]

Measurement of Caspase 3

Cells (10^6 cells/ml) containing 10% serum (normal and preeclampsia) with or without supernatant, were placed into 96-wells microplate, and incubated at 37°C 5% CO2 (v/v). Confluent cells were washed 3-4 times with PBS 37°C to remove medium and unattached cells. Subsequently, cells of 100 µl were aliquoted into wells of aspirin in various concentrations and incubated for 24 and 48 hours at 37°C 5% CO2 (v/v). Each well was washed with PBS pH 7,4 every 5 minutes, followed by trypsinization with 0,05% Tripsin-EDTA, incubated for 30 seconds at 37°C. Afterward, complete medium of equal volume was added into wells.[11,12] Cells were lysed with vortex for 10 minutes, and centrifuged at 3.000 rpm for 20 min. Measurement was performed with enzyme - linked immunosorbent assay (ELISA).[15.]

Data analysis

Data were statistically analyzed with a two-way analysis of variance (ANOVA) and continued with Duncan test using SPSS 21 (IBM Corp, Armonk, NY, USA). The analysis aimed to test the significance of the effect of low-dose aspirin in a varied concentration on caspase 3 levels in preeclamptic serum-induced VSMC cell line.

Ethical clearance

The study was conducted after the approval from Ethical Review Boards of Health Research, Faculty of Medicine and Dr. Hasan Sadikin Hospital, Bandung. All research subjects were voluntarily required to sign informed consent prior to the study.

RESULTS

Standarization of normal and preeclampsia serum

Serum which comprised of normal and preeclampsia, was measured for its characteristics. Measurement was conducted using electrophoresis which included albumin, alfa 1 globulin, alfa 2 globulin, beta 1 globulin, beta 2 globulin, gamma globulin and A/G ratio each group to obtain standarized concentration of serum used in this study (data are not shown). Results showed that normal serum was classified in normal category for each fraction (albumin, α-1 globulin, α-2 globulin, β-globulin, and gamma globulin). Meanwhile, preeclamptic serum showed some differences which was excluded from normal category (i.e higher α-globulin and β-globulin). Morphologically, blood samples carried from preeclamptic women had higher viscocity which is line with previous studies.[17-19.]

Expression of Caspase-3 of normal and preeclampsia-induced VSMC

Based on Table 1, expression of caspase-3 were higher in VSMC cells (prior to induction) with supernatant compared to that in cells without supernatant. This indicates role of trophoblast supernatant as communication media between trophoblast and VSMC. In normal pregnancy, expression caspase-3 was high which might be due to VSMC apoptosis during high trophoblast invasion in normal pregnancy. Expression of caspase-3 was higher in VSMC cells with preeclampsia serum compared to that in cells without preeclampsia. This indicates effects of preeclampsia serum was higher than supernatan toward VSMC apoptosis. Preeclampsia-induced VSMC showed higher expression of caspase 3 compared to normal gorup, This result indicate apoptosis occurence marked by increased level of caspase-3 in preeclampsia-induced VSMC cells.

Table 1. Expression of Caspase-3 in normal and preeclampsia-induced VSMC

Group	Expression (pg/ml)		
	Normal	Preeclampsia	p-value
Caspase 3	2,839 ± 0,001	4,707 ± 0,020	p<0,0001

Data are presented in Mean±Standard Deviation. Significant difference is indicated p-value (p<0,05) analyzed with Duncan test.

Expression of Caspase-3 in preeclampsia-induced VSMC treated with aspirin.

Based on Figure 1, caspase-3 level was highly expressed in preeclampsia-induced VSMC without aspirin, compared to normal group. There was difference in caspase 3 level between normal and preeclampsia-induced VSMC. Aspirin of 1,5 mM showed significant (p<0,05) reduced caspase-3 in preeclamptic cells which was almost ccomparable to normal level.

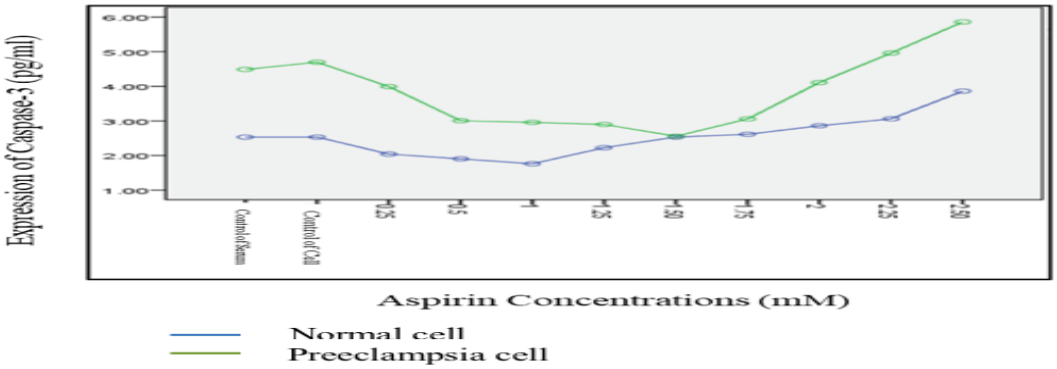

Figure 1. Expression of caspase-3 in normal and preeclampsia-induced VSMC at various incubation time after treatment of aspirin

DISCUSSION

VSMC effects on flexibility of spiral artery in placental physiology.[20] VSMC dysfunction during normal placental development depends on trophoblast invasion and differentiation, and apoptosis as main component of placenta.[21,22] Apoptosis in VSMC elevates following apoptosis in trophoblast due to miscommunication between trophoblast cells and VSMC during pregnancy which remains unclear. Thus, we aimed to observe the interaction between trophoblast and VSMC during pregnancy in vitro by adding supernatant of trophoblast into VSMC growth media or vice versa. In the present study, VSMV cells grow accordingly after supernatant addition that indicates similarity, mainly in nutritional characteristics of trophoblast and VSMC.

It has been reported that trophoblast cells initiate VSMC apoptosis in vitro.[8,23-27] Failure of trophoblast invasion in preeclamptic patients is allegedly due to imbalance of soluble protein interaction such as sFlt-1 and PlGF, as well as other protein TNF-α and caspase 3, which leads to trophoblast apoptosis. Subsequently, trophoblast releases TRAIL that binds to VSMC trimeric death receptors 4 and 5 (DR4/5), that promote FADD recruitment to activate procaspase-8 into caspase-8. This promote caspase cascade and VSMC apoptosis.[8,9] Apoptosis is a programmed-cell death which aims to regenerate tissues.[11] Apoptosis is an essential process in normal VSMC development to maintain vascular physiological balance. Apoptosis is involved in attachment, trophoblast invasion, spiral artery transformation, trophoblast transformation, and immune tolerance of paternal antigen expressed by trophoblast.[15] It is regulated by caspase, an executor protein to assign apoptosis.[11,12] In preeclampsia, failure in cells signaling and communication occurs in which trophoblast cells is unable to invade into spiral artery to dilate due to increased apoptosis.[28,30] Incomplete trophoblast invasion disrupts decidualization, resulting in inadequate maternal placental blood supply that leads to ischemia and endothelial damage. In this study, caspase-3 levels of preeclampsia-induced VSMC-supernatant were higher in preeclampsia-induced VSMC-supernatant than those in normal group. Similar findings are also found in previous study done by Pramatirta et al (2016) (17). Referring to Lydia et al.[8] and Pijnenborg et al.[9] apoptosis of trophoblast is followed by VSMC apoptosis mediated by TNF-α. In this study, supernatants can be one of alternatives as media for cells communication in pathogenesis study.

Furthermore, aspirin was used to observe pathogenesis of preeclampsia allegedly affected by VSMC and trophoblast. Low dose aspirin is known to possess anti-apoptotic and anti-inflammatory properties. In the present study, aspirin affects apoptosis of VSMC during trophoblast invasion as indicated by expression of caspase 3. This is line with study done by Pramatirta et al.[17] that low dose of aspirin reduces apoptosis in trophoblast cells indicated by decreased apoptotic index, caspase 3 and TNF-α. However, at high dose, aspirin induce apoptosis by stimulating cytocrome-c release and activating Apaf-1 which further activate caspase.

Antiapoptotic of aspirin is assumably precursor in trapping proinflammatory compounds such as TNF-α which is produced due to disrupted signal transduction affected by genetic (i.e., hypertension) and environmental factors (i.e pollutant during trophoblast apoptosis). Thus, aspirin is believed to reduce pro-apoptotic compounds and to compensate cells function including tropholast apoptosis and TRAIL production along with its binding to Fas in VSMC. Cells balance is indicated by reduced apoptosis which directly effect on reduced caspase 3. Further studies to measure other apoptosis-related markers, as well as in vivo and clinical studies, are encouraged.

CONCLUSION

Low-dose aspirin significantly reduced caspase 3 levels. These findings suggest the potential of low-dose aspirin to be used in treating preeclampsia, by restoring the balance between apoptotic factor (PlGF) and antiapoptotic factor. Further studies including in vivo studies and clinical trials of low-dose aspirin are encouraged.

ACKNOWLEDGEMENTS

We would like to thank Universitas Padjadjaran, which has funded this research through Internal Grant, Universitas Padjadjaran, Bandung, Indonesia

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