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Effect of Lycopene on Level of Malondialdehid (MDA) in Preeclampsia-Induced Placental Trophoblast Cells

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Abstract : Preeclampsia is a mjor cause in both maternal and perinatal mortality and morbidity. Underlying mechanism of preeclampsia remains unclear. It is assumed that preeclampsia is caused by imbalance in free radicals and antioxidant in blood and placenta. Lycopene, known to possess antioxidant properties, is therefore a promising agent to decrease preeclampsia risk. This study aimed to observe lycopene on MDA level in placental trophoblast which is induced by preeclampsia *in vitro*. Level of MDA was measured with TBARS (*thiobarbituric acid-reactive substances*). In preeclampsia-induced trophoblast, MDA level significantly reduced ($p<0,001$) from 18,8923 μM to 8,5773 μM after treated with lycopene of 31,25 μg/ml incubated for 24 hours, and from 18,899 μM to 8,6671 μM after incubation for 48 hours. Lycopene possess high antioxidant and antiangiogenesis that plays role as precursor in scavenging reactive oxygen and reduce free radicals that recover trophoblast cells induced by preeclampsia as indicated by decrease in MDA level. Further studies regarding the optimal concentration of lycopene on embryo cell for clinical trial, are encouraged.
Keywords : lycopene, MDA, preeclampsia.

Introduction

Preeclampsia is a major cause in both maternal and perinatal mortality and morbidity[1]. It has been reported that preeclampsia cases were 5-8% in developing countries[2,3], whilst according to World Health Organization (WHO) were 0,51%-38,4% in 2005. Preeclampsia ranked second in Indonesia as the main cause in maternal mortality after hemorrhage[4]. Referring to Department of Obstetrics and Gynecology in Hasan Sadikin Hospital (RSHS), Bandung, number of preeclampsia occurence was approximately 4-10% in 2005. Preeclampsia contributed about 10,4% to maternal mortality in RSHS. Maternal mortality was 228/100.000 living birth compared to desired target by government in 2010 which was 125/100.000 living birth[5].

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Underlying mechanism of preeclampsia remains unclear. Placenta and endothelial dysfunction have been proposed as the main pathophysiology of preeclampsia. Vascular disease and excessive trophoblast can promote trophoblast invasion on spiral arteries at early first and second trimester. This causes dilatation of spiral arteries that leads to reduced placental blood circulation. Incomplete spiral artery remodelling in preeclampsia causes inadequate response to increased blood supply along with development of pregnancy, which casuses reduced perfusion of utero-placenta and imbalance between pro- and antiangiogenic. These events will lead to ischemia on placental vascular[6,7].

Preeclampsia is also caused by imbalance in free radicals and antioxidant in blood and placenta[8]. Oxidative stress occurs due to disturbance in pro-oxidant and antioxidant. Previous researches show that antioxidant decrease preeclampsia risk after exposure of vitamin E, vitamin C and lycopene in pregnant women[9,10]. Lycopene is a strong antioxidant commonly found in tomatoes, watermelon, guava, papaya, red winde etc. Lycopene is a electron-rich compound and unstable which makes it easily reacted to oxygen and peroxide as well as free radicals. Lycopene has been proved to possess activities in prevention of various cancers such as prostate and cataract, cardiovascular dysfunction, endometriosis, osteoporosis, etc[11]. This study aimed to observe lycopene on MDA level in placental trophoblast which is induced by preeclampsia *in vitro*.

Materials and Methods

Lycopene was isolated from tomatoes [SIGMA Aldrich] and placental trophoblast was primary culture from Laboratory of Cell Culture, Faculty of Medicine, Universitas Padjadjaran. Serum was obtained from normal pregnancy and preeclamptic women which fulfillef inclusi and exclusi criteria

Cell Culture

Trophoblast cell was cultured in media containing Amniomax and *growth factor* supplemented with 10% serum (normal pregnancy and preeclampsia at 34-42 gestational age), and antibiotic-antimikotic (1%Penicillin G-Streptomycin *Solution Stabilised* and 1% Fungizone Amphotericin B). Cells were incubated for 24 hours at 37°C 5% CO_2 (v/v) untill confluent. Viability was measured with *trypan blue* in *haemocytometer* under light microscope with 400x magnification[12-14].

Measurement of MDA level

Cell of $6x10^5$ cell/ml containing 10% serum both normal and preeclampsia was replaced into 96-well microplate, and then incubated at 37°C 5% CO_2 (v/v) untill *confluent*. Wells were washed 3-4 times with PBS 37°C. Lycopene in various concentration were distirbuted in each well, and then incubated at 24 and 48 hours 37°C 5% CO_2 (v/v). Each well was washed with PBS pH 7,4 once for 5 minutes. Level of MDA was measured with TBARS (*thiobarbituric acid-reactive substances*) from NWLSS™ *Malondialdehyde Assay* Northwest (NWK-MDA01). Cells were treated with liquid containing 15% w/v *trichloroacetic acid*, 0,375 w/v *thiobarbituric acid*, 0,25 *hydrichloric acid* and 0,2% triton X. Furthermore, cells were carried and suspended with heating at 100°C for 15 minutes, and centrifuged at 4500 rpm for 10 min. Supernatant was measured with spectrophometer at 532 nm wavelength[13].

Data Analysis

Data was analyzed with T-test if normally distributed, and Mann Whitney test if not normally distributed. Data was quantitavely analyzed with ANOVA DMRT (Duncans's Multiple Range Test) to determine the significance among variables in each treatment with SPSS 22.

Result

Effect of lycopene on MDA level

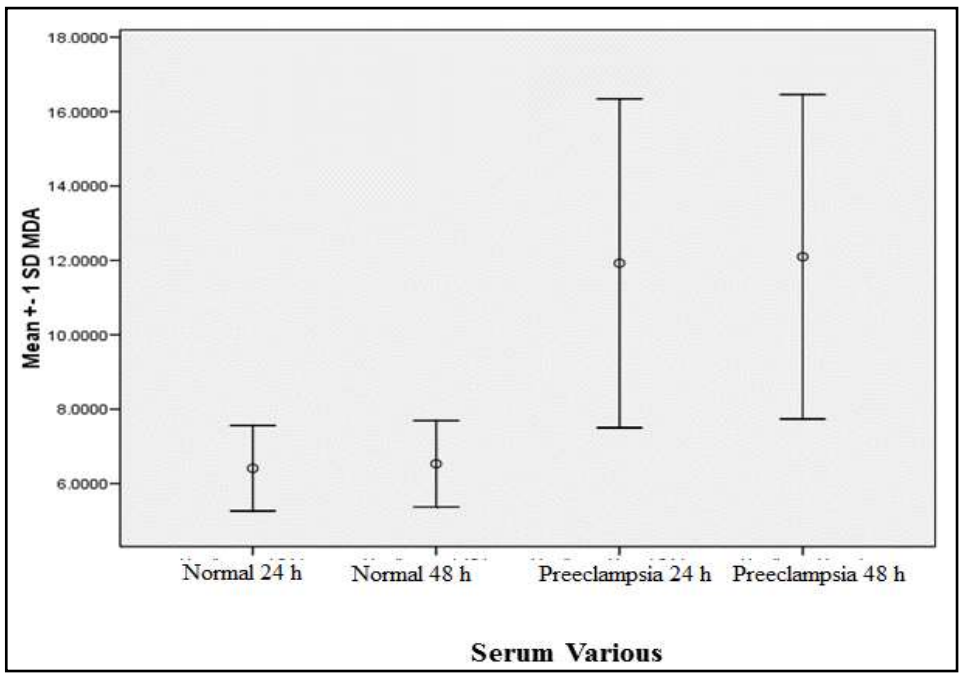

Figure 1. Level of MDA in trophoblast cell based on serum various

Figure 1 shows there was difference in MDA level affected by serum ($p<0,001$). In preeclampsia-induced trophoblast exhibited higher MDA level compared to normal. Lycopene lowered the MDA level in concentration-dependent manner. Thus, MDA level in preeclampsia-induced trophoblast treated with lycopene was comparable with that in normal.

Effect of incubation time on MDA level can be seen in Figure 2. Figure 2 showed there was decrease in MDA level on trophoblast after incubated 24-48 hours.

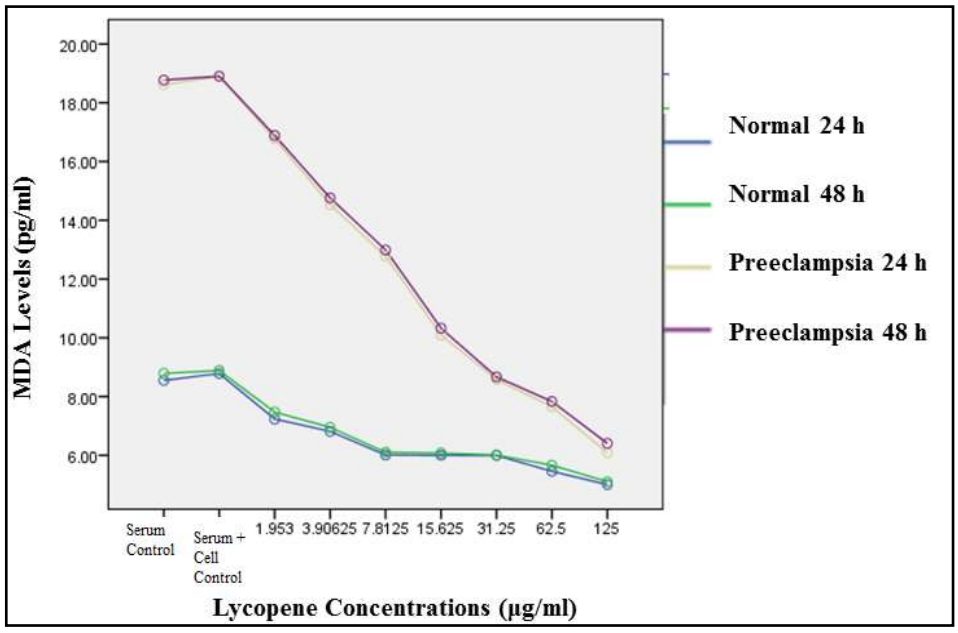

Figure 2. Comparison of MDA level in preeclampsia-induced trophoblast treated with lycopene in various incubation time.

As shown in Figure 2, MDA level reduced along with longer incubation time and higher lycopene concentration. Highest decrease after incubated for 24 hours. This might be due that trophoblast requires time to contact with compounds given that will reduce MDA level.

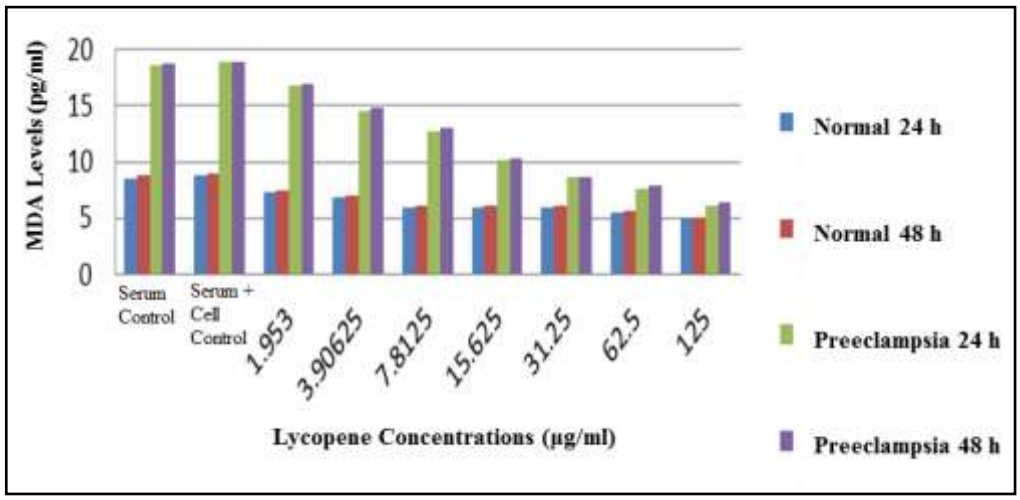

Figure 3. Level of MDA in serum various and incubation time.

In preeclampsia-induced trophoblast, MDA level significantly reduced ($p<0,001$) from 18,8923 µM to 8,5773 µM after treated with lycopene of 31,25 µg/ml incubated for 24 hours, and from 18,899 µM to 8,6671 µM after incubation for 48 hours (Figure 3).

Discussion

For our best knowledge, there is no studies regarding potency of lycopene as antioxidant and antiangiogenesis in preeclampsia. Angiogenesis occurrs in preeclampsia that leads to blood vessels derived from extravillous trophoblast invade to uterus that reduce placental vascular viability and affects placental oxygen supply due to dimished blood vessels. These events cause ischemic that promote damage in villous trophoblast (cell damage)[15,16]. Ischemic causes imbalance in pro-oxidant and antioxidant, release of free radicals that increase gradually in time leading to oxidative stress[17]. Free radicals cannot be neutralized by preeclamptic patients that causes cell damage, disturbance in cell integrity, endothelial lysis, reactivity and increase in vascular permeability[13].

Oxidative stress causes increase in MDA level that causes endothelial dysfunction, vasocontriction, disturbance in blood coagulation, lipid peroxidation, biomolecule oxidative damage, and DNA damage. Increase MDA also causes nitrite oxide that worsen oxidative stress[11,13,14]. In preeclampsia, there is decrease in NADPH which theoritically can be prevented by antioxidant that can provide protective effect and synergistic to internal antioxidant. Antioxidant is obtained through food or drug derived from plants which has been known to possess antioxidant properties[11,13].

The result of present study showed lycopene had antioxidant and antiangiogenesis activities in trophoblast induced by both preeclampsia and normal serum. Referring to study done by Wilcox (2003), Basu and Imrhan (2007), and Srinivasan (2007), tomatoes contain lycopene and is believed to prevent many diseases due to its high antioxidant content[18-20]. Lycopene is the most dominant antioxidant in tomatoes that possess activities such as antimicrobial, antithrombogenik, antivirus, reduce blood cholesterol and inhibit cell proliferation. Lycopene inhibits lung cancer growth in mouse[21]. Lycopen abundantly in cell membrane inhibits lipid peroxidase due to free radicals[22].

Lycopene is a electron-rich compound and unstable which makes it more reactive to oxygen and peroxidase and free radicals[11]. The main characteristic of lycopene is catalytic and effectively scavenge superoxid and peroxil radical[22]. Agarwal and Sekhon (2010) reported that lycopene protect lipid membrane and DNA damage due to oxidative stress *in vitro*[23]. Activity of lycopene is cosidered two-folds higher than β-caroten and ten-folds higher than α-karoten[24]. *In vitro* study reported that lycopene gives protetection on lipoprotein of cell membrane, DNA and vasculer against oxidant[18-20].

In summary, lycopene possess high antioxidant and antiangiogenesis that plays role as precursor in scavenging reactive oxygen and reduce free radicals that recover trophoblast cells induced by preeclampsia as indicated by decrease in MDA level. Further studies regarding the optimal concentration of lycopene on embryo cell for clinical trial, are encouraged.

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