The Effect of Darapladib Therapy for the Expression of Lp-PLA₂ in Dyslipidemia and Type 2 Diabetes Mellitus Atherosclerosis Model

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ARTICLE INFO

Article history:
Received on: 04/10/2017
Accepted on: 15/03/2018
Available online: 29/04/2018

Key words:
darapladib, dyslipidemia, atherosclerosis, T2DM, Lp-PLA₂.

ABSTRACT

Atherosclerosis is the main cause of mortality and morbidity globally. Lipoprotein-associated phospholipase A₂ (Lp-PLA₂) activity is suspected to have a significant role in atherosclerosis. 50 Sprague-Dawley Rats were divided into five groups: normal, dyslipidemia, Type 2 diabetes mellitus (T2DM), dyslipidemia with darapladib administration and T2DM with darapladib administration. These groups were divided into two serial times: 8 and 16 weeks. mRNA Lp-PLA₂ was measured from blood and aortic tissue extraction. Aortic tissue Lp-PLA₂ was measured by immunofluorescence. Lp-PLA₂ expression in aortic tissue was consistently increased in dyslipidemia and T2DM. The expression of Lp-PLA₂ enzymatic was significantly suppressed (p < 0.05) with the administration of darapladib especially in 8 weeks groups in both dyslipidemia and T2DM. The administration of darapladib in dyslipidemia and T2DM didn’t significantly suppress the expression of mRNA Lp-PLA₂ in blood and aortic tissue. The failure of genetic expression suppression of Lp-PLA₂ was found in both 8 weeks and 16 weeks groups. The expression of Lp-PLA₂ protein also showed an inclined difference between dyslipidemia and T2DM. These results showed that administration of darapladib significantly decreased Lp-PLA₂ protein but prone to increase the expression of mRNA Lp-PLA₂ in blood and aortic tissue in dyslipidemia and T2DM model.

INTRODUCTION

Cardiovascular disease (CVD) has become a serious global health problem and the leading cause of death in both modern and developing countries (Zhang et al., 2008). Atherosclerosis is the main cause of mortality and morbidity globally. It is predicted that prevalence of CVDs will increase quickly in developing countries (Wihastuti et al., 2014, 2015). Atherosclerotic plaque formation is a long-term process and often without clinical symptoms. Atherosclerosis development is highly associated with lipid metabolic disorders and inflammation. Oxidized LDL (OxLDL) has been widely demonstrated to have an important role in vascular inflammation (Zhang et al., 2013) Ox-LDL is capable of inducing monocyte adhesion to endothelial cells (Heriansyah et al., 2015). OxLDL initiates chemoattractant release, which in this way attracts macrophage migration into the lesion, and triggers the formation of reactive oxygen species (ROS), leading to oxidative stress, and the death of vascular smooth muscle cells (Heriansyah et al., 2017). Lipoprotein-associated phospholipase A₂ (Lp-PLA₂) activity that highly correlated with oxLDL is suspected to have a significant part in atherosclerosis development and also contributes to plaque destabilization process in different pathways. Several epidemiology studies with 80.000 subjects have clearly shown that the higher level of Lp-PLA₂ in the circulation is associated with higher risk of cardiovascular event, either in primary prevention and in secondary prevention cohort (Vepa et al., 1999; Unno et al., 2000; Von der Thesen, 2003; Waehe et al., 2004; Tsimikas et al., 2009; van Dijk et al., 2013). The physiology and potential function of Lp-PLA₂ in atherosclerosis pathogenesis is still controversial and far from achieving an understanding. Several pieces of evidence from previous experiments showed that there are two parts of the Lp-PLA₂ enzyme, which are opposites of one another, as an anti-inflammatory agent and pro-inflammatory agent. As an anti-inflammatory agent, the Lp-PLA₂ enzyme has the ability to
hydrolyze mediators that have a potential role in atherogenesis (such as oxLDL and platelet activating factor (PAF)). In the same time, hydrolyzed products of this molecule which are mediated by Lp-PLA2 stimulate atherosclerosis lesion either in the human and animal trial. Analysis using reverse transcriptase-polymerase reaction (RT-PCR) showed an increased expression of mRNA Lp-PLA2 in human atherosclerotic lesions (Tsimihodimos et al., 2002; Wæhre et al., 2004).

In a recent study, Lp-PLA2 selective inhibitors [darapladib (DP)] have become a potential therapeutical candidate to decrease cardiovascular accident (Wæhre et al., 2004). Several epidemiology studies with CAD patients as a subject showed that DP has the ability to suppress Lp-PLA2 activity and inflammatory markers (Tsimihodimos et al., 2002; Tsimikas et al., 2009; Tousoulis et al., 2013; Tian, 2014; White, 2014; Taruya et al., 2015). A clinical trial using animal treated by DP also succeed to suppress Lp-PLA2 activity and advanced stage atheroma plaque size (Szasz et al., 2013). However, two large clinical trial in stable CAD patients (STABILITY) and acute coronary syndrome patients (SOLID-TIMI 52) reported that administration of DP did not lower major cardiovascular accident, and even in fact increase the risk of Acute Myocardial Infarction (AMI) (Tsimikas et al., 2009).

![Fig. 1: Lp-PLA2 and GAPDH Amplification](image)

**METHODS**

**Study group**

This study used 50 4-weeks male Sprague-Dawley and weighted around 150-200 grams. Samples were obtained from Bogor Agricultural University, Bogor, Indonesia. These rats were divided into five groups; normal group (N); Dyslipidemia group which fed with High Fat Diet (DL); Type 2 DM Model groups (T2DM) which fed with High Fat Diet and injected with Streptozotocin (STZ) intraperitoneal low dose 35 mg/KgBW, Dyslipidemia with Darapladib administration group (DLDP) and type 2 DM model with Darapladib administration group (DMDP). Each group was divided into two serial times (8 weeks and 16 weeks) and consist of 5 rats. Darapladib was obtained from Glaxo Smith Kline. Samples were given Darapladib orally 20mg/KgBW once a day according to the time-serial groups given. Normal rats’ food contained 3.43 kcal/g total energy calories (67% carbohydrate, 21% protein and 12% fat), while the HFD contained 5.29 kcal/g total calorie energy (58% fat, 17% carbohydrate and 25% protein). 30 grams of food were given for each rat every day. Parameters measurement were done at the Central Laboratory of Biological Sciences, Brawijaya University.
**Blood glucose level measurement**

Mouse blood glucose level was measured using enzymatic method after fasting for 16 hours, after 4 weeks of HFD administration in T2DM mouse group before low dose STZ injection, 7 days after STZ injection, and before dissection. A blood sample was collected from the top of mouse tail (vena lateralis). Blood glucose level measurement using glucometer (GlucoDrCo.Ltd Korea). Using eligible measurement scale mg/dl.

**Plasma insulin level measurement and insulin resistance**

Insulin level was measured in T2DM groups before 30 mg/kgBW of STZ injection to confirm insulin resistance. With enzyme-linked immunoabsorbent assay (ELISA) method. Plasma insulin level normal value is <2500 pg/mL. Insulin resistance (IR) in the mouse was measured with HOMA-IR (Homeostatic Model Assessment-Insulin Resistance) formula as described below:

$$[HOMA - IR] = \frac{FBG \times FINS}{14.1}$$

**Information:**

HOMA-IR: Homeostatic Model Assessment-Insulin Resistance

FBG: Fasting Blood Glucose (mmol/mL)

FINS: Fasting blood insulin (μU/mL)

Insulin resistance is determined if HOMA-IR value >1.716.

**Measurement of mRNA expression lipoprotein-associated phospholipase A2 of aortic tissue and blood**

Messenger Ribonucleic acid (mRNA) from blood (whole blood sample) and aortic tissue extraction were prepared for quantitative examination with polymerase chain reaction (PCR) using primer and probe from Applied Biosystems 7300 Real-Time PCR system, Foster City, California (Goncalves et al., 2012; van Dijk et al., 2013).

![Fig. 2: Aorta with Rhodamin Secondary Antibody on N8 Groups, N16, DM8, DL16, DMDP8, and DLDP 16. (A) Normal mouse groups of 8 weeks (N8); (B) Normal Mouse Groups of 16 weeks (N16); (C) DMT2 Mouse Groups of 8 weeks (DM8); (D) Dyslipidemia Mouse Group of 16 weeks (DL16); (E) DMT2 Mouse groups given DP administration 30 mg/kg body weight for 16 weeks (DMDP16); (F) Dyslipidemia mouse groups given DP administration 30 mg/Kg body weight for 16 weeks (DLDP16).](image-url)
Aortic tissue that has been separated from animal carcass and was stored in a cooler (−70°C) until used. mRNA from early-stage atherosclerosis plaque that was stored in an isolated was using Fast Track mRNA isolation kit (Invitrogen) and cDNA synthesis kit (Invitrogen) following a factory published procedure. Meanwhile, extracted RNA from blood was isolated using Trizol reagent (GIBCO BRL) and reverse transcriptase from DNase I (GIPCO BRL)-treated total RNA using Superscript II and random hexamer primer (GIPCO BRL) following factory published procedure (Hatoum et al., 2010).

cDNA samples (approximately 5 μL each sample) were analyzed to see Lp-PLA expression and Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) housekeeping genes using real-time quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) with fluorescent TaqMan 5′ nuclease assay. Taqman oligonucleotide primers and probes examination were designed using Primer Express software version 1.0 (PE Biosystems). Each TaqMan hydrolyzed probe consists of fluorescent reporter dye 6-carboxyfluorescein (FAM) that have a covalent association with 5′ end of the oligonucleotide I and also the quencher dye 6-carboxytetramethylrhodamine (TAMRA), which is attached on the 3′ end using a group linker (PE Biosystems).

PCR (5′ > 3′ nuclease assay) were performed in microamp optical 96-well reaction plates with ABI PRISM 7300 sequence detection system for thermal cycle and real time fluorescence measurement (PE Biosystems). Each 25 μL reaction consist of 1× 1× TaqMan Universal PCR Master Mix (10 mmol/L Tris HCL (pH 8.3) 50 mmol/L KCl, 10 mmol/L EDTA, 60 mmol/L passive reference dye 1 (6-carboxy-X-rhodamine), 0.2 mmol/L dATP, 0.2 mmol/L dCTP, 0.2 mmol/L dGTP, 0.4 mmol/L dUTP, 5.5 mmol/L MgCl₂, 8% glycerol, 0.625 U AmpliTaq Gold DNA polymerase and 0.25 U AmpErase uracilN-glycosylase), 300 nmol/L forward primer, 300 nmol/L reverse primer, 100 μL TaqMan Quantification probe and 5 μL template with 20 μL mineral oil (Promega).

**Primary Sequence**

Lp-PLA: F: 5′-CCACCCAAAATGTC-ATGTGC-3′
R: 5′-GCCAGTCAAAAGGATACACAG-3′
GAPDH: F: 5′-GCAGGTGTCCTATCGAACA-3′
R: 5′-GAGACCTATGGATCCGAC-3′

TaqMan Probe (FAM-5′ > 3′-TAMRA)

Lp-PLA: 5′-TTCTGCTCTGCGGTCTCCTG-3′
GAPDH: 5′-CTCATGACCACA-GTCCATGCCATCACT-3′

Certain circumstances for the reaction are described as follow: 50°C for 2 minutes, 95°C for 10 minutes, and continued for 40 cycles at 95°C for 15 seconds and 60°C for 1 minute.

Continued analyses were performed from external data of sequence detector software using Microsoft Excel software. Lp-PLA expression quantitative value that obtained from sequence detector will be divided with each GADPH sample quantitative value to achieve a normal value of Lp-PLA for each sample. This value will be divided with the lowest value that is achieved to get the increased value of each sample. The obtained results were analyzed using 2−ΔΔCt method (Hatoum et al., 2010; Goncalves et al., 2012).

**Expression of Lp-PLA enzyme measurement in form of protein in aortic tissue**

Lp-PLA in aortic tissue was measured by immunofluorescence that was previously fixed with PHEMO buffer (68 mM PIPES, 25 mM, HEPES, pH 6.9, 15 mM EGTA, 3 mM MgCl₂, 10% [v/v] dimethyl sulfoxide containing 3.7% formaldehyde and 0.05% glutaraldehyde) and were processed by immunofluorescence labelling with anti-rat antibody Lp-PLA using rhodamine secondary antibody (BIOS Inc., Boston, MA, USA). This parameter was observed with confocal laser scanning microscopy (Olympus Corporation, Tokyo, Japan) and was quantitatively analyzed using Olympus FluoView software (version 1.7A; Olympus Corporation).

**Ethics**

We obtained ethical approval for the animal treatment and experimental processes in this study from the Animal Care and Use Committee Brawijaya University Number 229-KEP-UB.

**RESULT**

**Lipid profile and fasting blood glucose level**

Total cholesterol level and non-HDL cholesterol in 8 weeks normal groups are lower than experimental group. This result was also shown in 16 weeks normal groups, there is a lower trend compare to the experimental group. Total cholesterol and non-HDL cholesterol level in dyslipidemia and type 2 diabetes mellitus is decreased with darapladib administration. The decrease in total cholesterol level with darapladib administration on 16 weeks is lower than 8 weeks. Non-HDL cholesterol level is lower parallel with administration period. Treatments with darapladib administration for 8 weeks and 16 weeks have total cholesterol and non-HDL cholesterol level near normal group level.

HDL level on 8 weeks normal group is higher compared with experimental groups. Moreover, 16 weeks normal group tends to increase compared with the experimental group. Dyslipidemia and type II DM are affecting the increment of HDL level in experimental groups with darapladib administration. Darapladib administration for 8 weeks and 16 weeks have HDL level near normal group level.

Fasting blood glucose level on 8 weeks normal groups is lower than experimental group. Moreover, 16 weeks normal groups tend to be lower than the experimental groups. Mouse with dyslipidemia and type II DM have a decrease fasting blood glucose level in the experimental group with darapladib administration. The experimental group with darapladib administration for 8 weeks and 16 weeks have fasting blood glucose level near normal group level.

Insulin resistance measurement using HOMA-IR formula is conducted after 4 weeks in either standard feeding or HFD feeding. Interpretation result from HOMA-IR calculation on the mouse as the experimental animal model is if the result > 1.710 then insulin resistance is diagnosed with sensitivity 83.87% and specificity 80.56% (95% confidence interval). HFD feeding mouse groups are experiencing insulin resistance compared with standardized feeding groups (normal diet) that does not experience
insulin resistance. According to the calculation of groups with diabetes mellitus experience insulin resistance before DO administration. Insulin resistance is an indication for T2DM. This show that T2DM model in these trials are what we are expected. All of the results above can be seen in Table 1.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Groups</th>
<th>8 weeks Mean ± SD</th>
<th>16 weeks Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Cholesterol</td>
<td>Normal</td>
<td>72.80 ± 4.05*</td>
<td>56.56 ± 5.43*</td>
</tr>
<tr>
<td></td>
<td>DL</td>
<td>109.56 ± 12.25</td>
<td>100.57 ± 22.64</td>
</tr>
<tr>
<td></td>
<td>DLDP</td>
<td>79.57 ± 10.54*</td>
<td>96.96 ± 18.97*</td>
</tr>
<tr>
<td></td>
<td>DM</td>
<td>123.00 ± 2.86*</td>
<td>111.72 ± 7.30*</td>
</tr>
<tr>
<td></td>
<td>DMDP</td>
<td>97.96 ± 1.70*</td>
<td>98.85 ± 3.25*</td>
</tr>
<tr>
<td>HDL</td>
<td>Normal</td>
<td>34.74 ± 8.31*</td>
<td>35.77 ± 1.68*</td>
</tr>
<tr>
<td></td>
<td>DL</td>
<td>19.16 ± 0.30*</td>
<td>17.55 ± 0.85*</td>
</tr>
<tr>
<td></td>
<td>DLDP</td>
<td>20.62 ± 0.20*</td>
<td>13.00 ± 1.82*</td>
</tr>
<tr>
<td></td>
<td>DM</td>
<td>4.96 ± 0.41*</td>
<td>13.96 ± 0.87*</td>
</tr>
<tr>
<td></td>
<td>DMDP</td>
<td>15.94 ± 1.21*</td>
<td>20.79 ± 2.76*</td>
</tr>
<tr>
<td>Non HDL</td>
<td>Normal</td>
<td>49.83 ± 5.06*</td>
<td>19.24 ± 3.67*</td>
</tr>
<tr>
<td></td>
<td>DL</td>
<td>88.20 ± 3.08*</td>
<td>98.14 ± 11.43*</td>
</tr>
<tr>
<td></td>
<td>DLDP</td>
<td>60.34 ± 2.64*</td>
<td>96.51 ± 17.06*</td>
</tr>
<tr>
<td></td>
<td>DM</td>
<td>95.53 ± 8.66*</td>
<td>88.25 ± 6.23*</td>
</tr>
<tr>
<td></td>
<td>DMDP</td>
<td>85.92 ± 6.84*</td>
<td>61.52 ± 6.03*</td>
</tr>
<tr>
<td>Fasting Blood Glucose</td>
<td>Normal</td>
<td>91.60 ± 7.16*</td>
<td>79.60 ± 14.64*</td>
</tr>
<tr>
<td></td>
<td>DL</td>
<td>114.60 ± 7.73*</td>
<td>118.60 ± 15.65*</td>
</tr>
<tr>
<td></td>
<td>DLDP</td>
<td>110.20 ± 6.18*</td>
<td>99.60 ± 19.03*</td>
</tr>
<tr>
<td></td>
<td>DM</td>
<td>128.00 ± 15.02*</td>
<td>147.80 ± 58.23*</td>
</tr>
<tr>
<td></td>
<td>DMDP</td>
<td>103.60 ± 13.72*</td>
<td>101.80 ± 19.07*</td>
</tr>
</tbody>
</table>

Analysis of two mean different groups with Duncan Multiple Range Test (DMRT), there is a similar letter notation, it means that the difference between two mean values is not significant. *α = 0.05.

Aortic tissue and blood mRNA Lp-PLA₂ and aortic tissue protein Lp-PLA₂

In 8 weeks group, mRNA Lp-PLA₂ blood expression in experimental groups are always lower compare with normal groups. However, mRNA Lp-PLA₂ blood expression in the experimental group are always higher compare with the normal group in 16 weeks groups.

mRNA Lp-PLA₂ tissue expression in experimental groups tends to be lower in 8 weeks normal group. A different result is found in 16 weeks normal groups, mRNA Lp-PLA₂ tissue expression in the experimental group always higher than normal groups.

In 8 weeks normal groups, Lp-PLA₂ protein level is lower than the experimental group. 16 Weeks normal groups also tend to be lower than experimental group. Descriptively, in dyslipidemia and type II DM mouse, 8 weeks of Lp-PLA₂ protein level tissue were decreased with darapladib administration, and in 16 weeks diabetes mellitus group were experiencing a higher level. 16 weeks of Lp-PLA₂ protein level tissue were lower if we compared it with 8 weeks group. Darapladib administration in 8 and 16 weeks diabetes mellitus groups has Lp-PLA₂ protein level near normal level.

DISCUSSION

The newest information from this study is that we able to achieve a more complete and integrated data for mRNA Lp-PLA₂ aortic tissue and blood expression pattern in two risk factors model of cardiovascular disease, which are dyslipidemia and type II diabetes mellitus by following chronicity theory of atherosclerotic process using time series experimental design (animal model). The animal model used in this experiment is using the combination of low doses of STZ and high-fat diet could induce metabolic syndrome mimicking human criteria (Rohman et al., 2017). This experimental has Lp-PLA₂ activity data in aortic tissue with protein/enzyme form. Which is far more different with a previous study that its goal is to only identify Lp-PLA₂ expression’s degree only on one side (either only on aortic tissue or blood) and also without time series, and in turn Lp-PLA₂ expression pattern data cannot be achieved.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Groups</th>
<th>8 weeks Mean ± SD</th>
<th>16 weeks Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>mRNA Lp-PLA₂</td>
<td>Normal</td>
<td>1/1.00 ± 0.00</td>
<td>1/1.00 ± 0.00</td>
</tr>
<tr>
<td>Aortic tissue</td>
<td>DL</td>
<td>1/1.13 ± 0.00</td>
<td>1/0.62 ± 0.00</td>
</tr>
<tr>
<td></td>
<td>DLDP</td>
<td>1/0.37 ± 0.00</td>
<td>1/0.48 ± 0.00</td>
</tr>
<tr>
<td></td>
<td>DM</td>
<td>1/1.08 ± 0.00</td>
<td>1/0.72 ± 0.00</td>
</tr>
<tr>
<td></td>
<td>DMDP</td>
<td>1/2.00 ± 0.00</td>
<td>1/0.48 ± 0.00</td>
</tr>
<tr>
<td>mRNA Lp-PLA₂</td>
<td>Normal</td>
<td>1/1.00 ± 0.00</td>
<td>1/1.00 ± 0.00</td>
</tr>
<tr>
<td>Blood</td>
<td>DL</td>
<td>1/0.37 ± 0.00</td>
<td>1/2.27 ± 0.00</td>
</tr>
<tr>
<td></td>
<td>DLDP</td>
<td>1/0.17 ± 0.00</td>
<td>5/1.12 ± 0.00</td>
</tr>
<tr>
<td></td>
<td>DM</td>
<td>1/0.59 ± 0.00</td>
<td>5/1.69 ± 0.00</td>
</tr>
<tr>
<td></td>
<td>DMDP</td>
<td>1/0.42 ± 0.00</td>
<td>1/1.83 ± 0.00</td>
</tr>
<tr>
<td>Lp-PLA₂ Protein</td>
<td>Normal</td>
<td>788.96 ± 49.73*</td>
<td>752.59 ± 153.47*</td>
</tr>
<tr>
<td>Aortic tissue</td>
<td>DL</td>
<td>1220.52 ± 200.61*</td>
<td>804.61 ± 116.52*</td>
</tr>
<tr>
<td></td>
<td>DLDP</td>
<td>1096.05 ± 93.59*</td>
<td>599.63 ± 32.30*</td>
</tr>
<tr>
<td></td>
<td>DM</td>
<td>925.13 ± 405.28*</td>
<td>751.65 ± 240.29*</td>
</tr>
<tr>
<td></td>
<td>DMDP</td>
<td>820.86 ± 152.10*</td>
<td>798.5 ± 108.67*</td>
</tr>
</tbody>
</table>

Trials result of two mean value with Duncan’s Multiple Range Test (DMRT), there are two similar notations, which means the difference between two mean value are not significant *α = 0.05.

This study found that aortic tissue and plasma mRNA Lp-PLA₂ expression profile is different between dyslipidemia and type II diabetes mellitus conditions. Lp-PLA₂ protein expression also showed an activity which tends to be different in such conditions. Much more surprising findings is aortic tissue mRNA Lp-PLA₂ expression pattern is similar with aortic tissue Lp-PLA₂ protein expression, which showed that Lp-PLA₂ activity either on both transcriptase level and on enzymatic activity in tissue are much more dominant on the early phase of atherogenesis (in this study shows a higher level of expression on 8 weeks, experimental groups). This expression pattern was seen on both dyslipidemia and type II diabetes mellitus condition. This study supports a
previous scientific proof (in vitro study) that found Lp-PLA$_2$ formation is dramatically increased in an arterial blood vessel at the time of monocyte differentiation into macrophage which occurs in a much early (Cao et al., 1998; Ferguson et al., 2012).

Contradictory expression patterns are found in blood mRNA Lp-PLA$_2$ expression pattern. mRNA Lp-PLA$_2$ expression pattern is significantly increased in 16 weeks experimental groups (both dyslipidemia and Type II diabetes mellitus conditions). This findings support previous an in vitro study findings which found that the early phase of inflammation process (monocyte to macrophage pre-transformation phase), mRNA Lp-PLA$_2$ expression level are so low to be detected in the blood, mRNA Lp-PLA$_2$ expression will significantly increase in blood after macrophage maturation phase (longer observation period phase) (Ferguson et al., 2012). Even though there is a possibility that Lp-PLA$_2$ is produced by macrophage locally in aortic tissue can also contribute to half of circulating Lp-PLA$_2$. It is not right to use the circulating Lp-PLA$_2$ level as an independent biomarker which represents Lp-PLA$_2$ action in atherosclerotic tissue. In other words, mRNA expression and Lp-PLA$_2$ protein activity in the blood is a poor predictor of PLA2G7 action on the arterial plaque (Ferguson et al., 2012). This result showed a supporting evidence for that in vitro study and support the pro-inflammatory pro-atherogenic role of Lp-PLA$_2$.

This study results showed that DP administration therapy tends to consistently decrease enzymatic activity of Lp-PLA$_2$ in aortic tissue either on both dyslipidemia or Type II diabetes mellitus. This finding is consistent with previous in vivo study result, that reported a significant decrease in enzymatic activity of Lp-PLA$_2$, on mouse serum (Wang et al., 2011). Other in vivo study also reported that DP administration can decrease Lp-PLA$_2$ enzymatic activity in plasma and aortic blood vessel of animal’s trial (Wilenisky et al., 2008). A different report is seen in a human study that showed DP therapy does not decrease Lp-PLA$_2$ plasmic activity significantly but change atheroma plaque characteristic from fragile plaque with wide necrotic lipid core into much more stable plaque with the smaller necrotic core (Serruys et al., 2008).

Different with suppression of Lp-PLA$_2$ protein activity as mentioned above, DP administration therapy in this study is giving an interesting result in terms of mRNA Lp-PLA$_2$ expression in aortic tissue and blood. DP administration has the ability to decrease Lp-PLA$_2$, expression in blood, in type II Diabetes Mellitus conditions with 16 weeks observation period. Meanwhile, in dyslipidemia condition DP administration therapy tend to increased mRNA Lp-PLA$_2$, expression in blood. Different conditions are seen in tissue mRNA Lp-PLA$_2$ expression, decrease expression of mRNA Lp-PLA$_2$ only found in dyslipidemia conditions with 8 weeks observation period. In type II diabetes mellitus conditions with DP therapy, mRNA Lp-PLA$_2$ expression of aortic tissue increased significantly in 8 weeks observation period groups. This result phenomenon shows that both conditions (dyslipidemia and type II diabetes mellitus) are not a role for a different pattern on both groups, mRNA Lp-PLA$_2$, expression pattern in treatment group with DP administration in this study support previous in vitro result that showed with administration of Lp-PLA$_2$ inhibitor (SB35495) to oxLDL results in undetected enzymatic activity of Lp-PLA$_2$, however there is an increased of mRNA Lp-PLA$_2$, expression as an effect of oxPC that are hydrolysed in oxLDL which then cause an up-regulatory effect of oxLDL to stimulate much mRNA Lp-PLA$_2$, expression (because of SB-oxLDL consist of much more oxPC as the result of less hydrolysed oxPC in those complex) (Wang et al., 2010). Another study in an in vivo model (another animal model) also reported that with the administration of DP does not decrease mRNA Lp-PLA$_2$, expression significantly, however with the administration of RNA Lp-PLA$_2$, inhibitors (Lp-PLA$_2$, RNAi) using lentivirus were a success to significantly decreasing mRNA Lp-PLA$_2$, expression. Enzymatic activity of Lp-PLA$_2$, degree that was decreased in plasma is not different between DP groups and Lp-PLA$_2$, RNAi. Usable explanation to analyze this in vivo trial and previous two clinical trials is that DP works on such level as a reversible inhibitor between ox LDL with Lp-PLA$_2$, interactions, not on genetic transcription level. Inhibitions with DP can increase the number of mRNA Lp-PLA$_2$, expression level as a result of much more un-hydrolyzed oxPC. Our in vivo study result can also explain as an indirect answer to all three major clinical trials (STABILITY, SOLID-TIMI 52 and CIBIS 2) to observed DP effectivty in lowering inflammation response and cardiovascular morbidity and mortality. Inflammation response that involved Lp-PLA$_2$, is much more complex in nature and is not linear and not easy to predict.

CONCLUSION

Lp-PLA$_2$, protein expression in aortic tissue was consistently increased in both dyslipemias and type 2 diabetes models. It was significantly seen in 8 weeks group compared to 16 weeks group. Similar phenomenon confirmed by the treatment using a selective blocker of Lp-PLA$_2$, (darapladib/DP). The expression of Lp-PLA$_2$, enzymatic was significantly suppressed (p < 0.05) with the administration of darapladib especially in 8 weeks groups in both dyslipidemia and type 2 diabetes models. This happened due to the high inflammatory response that appears at the beginning of the period that followed by high production of Lp-PLA$_2$, by its cellular source, namely macrophages. The administration of selective blockers Lp-PLA$_2$, (darapladib) in vivo models of dyslipidemia and type 2 diabetes in this study did not significantly suppress the relative expression of mRNA Lp-PLA$_2$, in both blood and aortic tissue. The failure in suppressing the genetic expression of Lp-PLA$_2$, was found in both 8 weeks and 16 weeks groups. The expression of Lp-PLA$_2$, protein also showed an inclined different in activity between dyslipidemia and type 2 diabetes. The surprising finding was the pattern of mRNA Lp-PLA$_2$, expression of aortic tissue was similar to the pattern of Lp-PLA$_2$, protein expression of aortic tissue which shows that the activity of Lp-PLA$_2$, in both transcription and protein level in tissues are more dominant in the phase before atherosclerosis process (in this study, it shows the level of expression was higher in the 8 weeks group). This expression pattern was shown in both dyslipemias and type 2 diabetes models. This study supports the previous in vitro research that found that the formation of Lp-PLA$_2$, increased dramatically in arterial blood vessel walls tissue during the process of differentiation of monocytes into macrophages that occurs in an early phase.

ACKNOWLEDGMENTS AND NOTICE OF GRANT SUPPORT

Ministry of Research, Technology and High Education.
CONFLICTS OF INTEREST
None.

REFERENCE


