DE GRUYTER OPEN

ENDOCRINE REGULATIONS, Vol. 52, No. 2, 69–75, 2018

doi:10.2478/enr-2018-0008

Darapladib inhibits atherosclerosis development in type 2 diabetes mellitus Sprague-Dawley rat model

Titin Andri Wihastuti[1], Teuku Heriansyah[2], Hanifa Hanifa[1], Sri Andarini[1], Zuhrotus Sholichah[1], Yuni Hendrati Sulfia[1], Aditya Angela Adam[1], Jeki Refialdinata[1], Nurul Cholifah Lutfiana[1]

[1]Faculty of Medicine, Brawijaya University, Malang, East Java, Indonesia;
[2]Faculty of Medicine, Syiah Kuala University, Aceh, Sumatera, Indonesia
E-mail: teuku_hery@unsyiah.ac.id

Objective. Increase in the low-density lipoprotein (LDL) level in diabetes mellitus and atherosclerosis is related to lipoprotein associated phospholipase A2 (Lp-PLA2). Lp-PLA2 is an enzyme that produces lysophosphatidylcholine (LysoPC) and oxidized nonesterified fatty acids (oxNEFA). LysoPC regulates inflammation mediators, including intra-cellular adhesion molecule-1 (ICAM-1). Darapladib is known as a Lp-PLA2 specific inhibitor. The aim of this study was to reveal the effect of darapladib on the foam cell number, inducible nitric oxide synthase (iNOS), and ICAM-1 expression in aorta at early stages of the atherosclerosis in type 2 diabetes mellitus Sprague-Dawley rat model.

Methods. Thirty Sprague-Dawley male rats were divided into 3 main groups: control, rats with type 2 diabetes mellitus (T2DM), and T2DM rats treated with darapladib (T2DM-DP). Each group was divided into 2 subgroups according the time of treatment: 8-week and 16-week treatment group. Fasting blood glucose, insulin resistance, and lipid profile were measured and analyzed to ensure T2DM model. The foam cells number were detected using hematoxylin-eosin (HE) staining and the expression of iNOS and ICAM-1 was analyzed using double immunofluorescence staining.

Results. Induction of T2DM in male Sprague-Dawley rats after high fat diet and streptozotocin injection was confirmed by elevated levels of total cholesterol and LDL and increased fasting glucose and insulin levels compared to controls after both times of treatment. Moreover, T2DM in rats induced a significant increase ($p<0.05$) in the foam cells number and iNOS and ICAM-1 expression in aorta compared to controls after both treatment times. Darapladib treatment significantly reduced ($p<0.05$) foam cells number as well as iNOS expression in aorta in rats with T2DM after both treatment times. A significant decrease ($p<0.05$) in ICAM-1 expression in aorta was observed after darapladib treatment in rats with T2DM only after 8 weeks of treatment.

Conclusion. Our data indicate that darapladib can decrease the foam cells number, iNOS, and ICAM-1 expression in aorta at the early stages of atherosclerosis in T2DM rat model.

Key words: darapladib, atherosclerosis, type 2 diabetes mellitus rat model

Diabetes mellitus is a metabolic disease characterized by hyperglycemia (American Diabetes Association 2011; Silvana and Rifa'i 2015). International Diabetes Federation (2013) have stated that there are 382 million adults (8.3%) suffering from diabetes and this number is expected to be increasing until 592 million within 25 years. The high prevalence of diabetes can increase atherosclerosis prevalence because it is a major risk factor for atherosclerosis development and clinical manifestations, including coronary artery disease, stroke, and peripheral vascular disease (Chait and Bornfeldt 2009; Heriansyah et al. 2015).

Corresponding author: Teuku Heriansyah, Garot 15-57 Aceh, Sumatera, Indonesia; phone: +628116618282; fax: (0651) 43878; e-mail: teuku_hery@unsyiah.ac.id.

Hyperglycemia induces most of the changes at cellular level in vascular tissue that potentially accelerate atherosclerosis process (Aronson and Rayfield 2002). Hyperglycemia also leads to an impairment in nitric oxide (NO) production (Avogaro et al. 2011). Inducible NOS (iNOS) is one of NO synthase isoform. Atherosclerosis is associated with iNOS expression increase (Fukuchi and Giaid 1999) influencing the cellular adhesion molecules (CAM) interaction (Galkina and Ley 2007). In atherosclerosis, high low-density lipoprotein (LDL) level can increase free radical that is vulnerable to various oxidative process and triggered pro-inflammatory cells recruitment and adherence (Wihastuti et al. 2016). Atherosclerosis affects discrete portion of the blood vessels, such as vessel curvature and bifurcation. These sections are marked by disturbed oscillatory flow, which induces upregulation of the adhesion molecules pro-inflammatory, such as intercellular adhesion molecule 1 (ICAM-1) (Galkina and Ley 2007).

Low density lipoprotein (LDL), oxidized LDL (oxLDL), and free fatty acid (FFA) level have been shown to be increased in type 2 diabetes mellitus (T2DM) (Turan and Dhalla 2014). LDL cholesterol is harmful because of lipid peroxidation process (auto-oxidation) (Wihastuti et al. 2014). LDL is closely related to enzyme lipoprotein-associated phospholipase A2 (Lp-PLA_2). This enzyme is originally known as a platelet-activating factor acetylhydrolase (PAF-AH), which has two biological activities such as inactivation of PAF-AH pro-inflammatory mediators and hydrolyzing the oxidative modified polyunsaturated fatty acids that produce lysophosphatidylcholine (LysoPC) and oxidized nonesterified fatty acids (OxNEFA). OxNEFA has chemotactic activity of monocytes and LysoPC is involved in the regulation of inflammatory mediators, including cytokines, adhesion molecules, and MCP-1 (Carlquist et al. 2007).

Darapladib was known as a specific inhibitor of Lp-PLA_2 and has been already developed as a drug for atherosclerosis (Thompson et al. 2013). The Lp-PLA_2 role in the atherogenesis is still ambiguous (Heriansyah et al. 2016). There are few studies on darapladib effects in atherosclerosis. One of them is the research conducted by Heriansyah et al. (2016). These authors have shown that darapladib administration can reduce oxLDL and foam cells amount.

Therefore, the aim of this study was to investigate the effect of darapladib on foam cells number, and iNOS, and ICAM-1 expression in aorta in early stages of atherosclerosis in Sprague-Dawley T2DM rat model.

Materials and Methods

Animals and treatment. Four-week-old male Sprague-Dawley rats with 150–200 g of body weight (b.w.) were used in this study. Animals were obtained from Bogor Agricultural University, Bogor, Indonesia. Ethical approval for the animal treatment and experimental processes in this study were obtained from the Animal Care and Use Committee Brawijaya University (400/EC/KEPK/10/2016). Rats were divided into 3 main groups: control, type 2 diabetes mellitus (T2DM), and T2DM with darapladib administration (T2DM-DP). Each group was exposed to 2 serials time treatments: 8-week and 16-week. Two T2DM groups were fed with high fat diet (HFD) and one of them also intraperitoneally (i.p.) injected with low dose of streptozotocin (STZ; 35 mg/kg b.w.). STZ was diluted with 1 ml of citrate buffer as vehicle/injection. Darapladib was obtained from Glaxo Smith Kline. Darapladib (20 mg/kg b.w.) was administered orally once a day. Darabadib was diluted with 5 ml of PZ Solution (0.9% NaCl) as vehicle per rat per daily oral administration. Normal rats' food contained 3.43 kcal/g total energy calories, while the HFD contained 5.29 kcal/g total calories. Thirty grams of food were given for each rat every day. Body weights of rats were measured before and after DM induction. The measurement of biochemical parameters was performed at the Central Laboratory of Biological Sciences, Brawijaya University (Malang, East Java, Indonesia).

Blood glucose measurement. STZ (35 mg/kg b.w.) was administered before the first blood glucose measurement to induce T2DM. T2DM was diagnosed after blood glucose level measurement using GlucoDR blood glucose test meter (All Medicus Co. Ltd, Dongan-gu, Anyang-si, Korea). T2DM was diagnosed after obtaining fasting blood glucose levels >126 mg/dL in rats.

Insulin resistance measurement. Plasma insulin levels were measured using Rat INS (insulin) ELISA kit (Cat. No. E-EL-R2466) from ElabScience. Plasma insulin levels were converted into IU/L. WHO formula was used by dividing the result with 0.0347, as 1 IU is equivalent with 0.0347 mg/L (Burns et al. 2010). Insulin resistance can be measured with HOMA-IR (homeostatic model assessment-insulin resistance) formula especially in rats, which required some data, such as fasting glucose and plasma insulin levels by the following formula (Van Dijk et al. 2013):

$$\text{HOMA-IR} = (\text{FBS} \times \text{FINS})/14.1$$

HOMA-IR: Homeostatic Model Assessment-Insulin Resistance
FBG: Fasting Blood Glucose (mmol/L)
FINS: Fasting Insulin Plasma (μU/L).

Interpretation of HOMA-IR calculation in rats is if the result >1.716 when it can be categorized as insulin resistance with 83.87% sensitivity and 80.56% specificity (95% confidence interval) (Cacho et al. 2008).

Lipid profile measurement. Lipid profiles [(total cholesterol, high-density lipoprotein (HDL) and low-density lipoprotein (LDL)] were measured in rat blood serum using EnzyChrom™ kit (Cat. No EHDL-100) from BioAssay System Inc.

Foam cell measurement. The foam cells observation started with a slide preparation. The aortic tissues were stained using Oil Red O. Aortic tissues were embedded in paraffin and cut into slices of 5 µm thickness with Sakura Accu-cut SRM 200 Rotary Microtome. Then hematoxylin-eosin (HE) staining was performed. The study started with scanning of histopathological slides using a microscope at 400× magnification, viewed, and then counted using dotslide software.

iNOS and ICAM-1 expression measurement using immunofluorescence. The expression of iNOS and ICAM-1 in aortic tissue was determined by immunofluorescence. The samples were fixed with a 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 processed by immunofluorescence labeling with anti-rat antibody ICAM-1 using rhodamin secondary antibody (BIOS Inc., Boston, MA, USA) and anti-rabbit antibody iNOS using FITC secondary antibody. The samples that have been labeled with antibodies were incubated for 10 min at room temperature. This parameter was observed under confocal laser scanning microscopy (Olympus Corporation, Tokyo, Japan) and were quantitatively analyzed using Olympus FluoView software (version 1.7A; Olympus Corporation).

Statistical Analysis. Data were analyzed using one-way analysis of variance (ANOVA) followed by Duncan Multiple Range Test to compare each group. All the analyses were performed using SPSS statistical software, version 16.0. Value $p<0.05$ was considered to be statistically significant.

Results

Biochemical parameters, foam cells number, expression of iNOS and ICAM-1 in aortic tissues determined in all treatment groups are summarized in Table 1. Induction of T2DM in Sprague-Dawley male rats after high fat diet and streptozotocin administration was confirmed by increased fasting blood glucose (>126 mg/dL), plasma insulin levels, and insulin resistance (using HOMA-IR >1.716) compared to controls after both times of treatment. Moreover, elevated levels of total cholesterol and LDL, and decreased HDL levels were measured compared to controls after both times of treatment (Table1). Darapladib administration induced a significant inhibitory effect ($p<0.05$) on investigated elevated biochemical parameters compared to T2DM group after both times of treatment (except LDL levels where reduced levels in T2DM rats were observed after 16-week treatment) indicating a beneficial effect of darapladib on animals

Table 1
Biochemical parameters, foam cell number, iNOS, and ICAM-1 expression in aorta of Sprague-Dawley rats.

Parameter	Group					
	8 Weeks			16 Weeks		
	Normal	T2DM	T2DM-DP	Normal	T2DM	T2DM-DP
Fasting blood glucose (mmol/L)	5.24±0.23^{abcef}	8.45±0.90^{acef}	5.45±0.62^{abcef}	4.69±0.56^{abef}	7.86±0.28^{d}	5.44±0.60^{abcef}
Plasma insulin (µIU/mL)	1.36±0.10^{be}	3.48±0.50^{cdf}	1.63±0.16^{be}	1.44±0.06^{be}	11.59±1.19^{acdf}	2.42±0.24^{be}
Insulin resistance	0.523±0.092^{ab}	2.002±0.072^{c}	1.746±0.674^{e}	0.514±0.126^{ab}	6.456±0.603^{d}	3.062±2.183^{f}
Cholesterol total (mg/dL)	72.78±4.05^{a}	123.02±2.85^{c}	97.97±1.70^{ef}	56.57±5.42^{b}	111.71±7.28^{d}	98.84±3.26^{ef}
HDL level (mmol/L)	1.92±0.46^{ab}	0.27±0.002^{c}	0.88 ± 0.06^{def}	1.99±0.009^{ab}	0.77±0.04^{def}	1.15±0.19^{def}
LDL level (mmol/L)	2.76±0.28^{af}	5.30±0.48^{cde}	4.77±0.38^{cde}	1.07±3.47^{b}	4.89±0.34^{cde}	3.41±0.77^{af}
Foam cell number	72±3.53^{abf}	107.8±6.91^{c}	82.2±3.11^{e}	70.5±4.82^{abf}	120.8±11.19^{d}	95.66±18.2^{abf}
iNOS expression	355.65±75.52^{ab}	725.53±122.28^{c}	595.89±128.31^{e}	500.13±54.13^{ab}	878.93±297.92^{d}	672.54±174.51^{f}
ICAM-1 expression	442.51±178.82^{ab}	991.28±255.69^{cd}	573.93±237.14^{abef}	542.02±40.61^{ab}	1010.73±186.98^{cd}	874.85±215.06^{bcdf}

Abbreviations: T2DM – type 2 diabetes mellitus; T2DM-DP – type 2 diabetes mellitus with darapladib; HDL – high-density cholesterol; LDL – low-density cholesterol; iNOS – inducible nitric oxide synthase; ICAM-1 – intra cellular adhesion molecule-1. Data were analyzed by one-way ANOVA and following Duncan multiple range post-test; different superscripts indicate a statistical significance.

with T2DM (Table 1). Darapladib induced a beneficial effect on HDL levels ($p<0.05$) compared to T2DM group only after 8 weeks of treatment (Table 1).

T2DM induced a significant increase ($p<0.05$) in the foam cells number (Figure 3), iNOS (Figure 1) and ICAM-1 (Figure 2) expression in aortic tissues of male rats compared to controls after both times of treatment (Table1). The highest values of foam cells number, iNOS and ICAM-1 expression were seen in T2DM 16-week group (Table1). Treatment with darapladib significantly reduced ($p<0.05$) foam cells number (Figure 3) as well as iNOS expression (Figure 1) in aortic tissue of rats with T2DM after both times of treatment (Table 1). A significant decrease ($p<0.05$) in ICAM-1 expression in aortic tissue was observed after darapladib treatment in rats with T2DM only after 8 weeks of the treatment (Figure 2, Table 1).

Discussion

High fat diet consumption lead to an abnormal fat accumulation in muscle and liver. Lipid accumulation is related to insulin resistance. Insulin resistance in muscle leads to hepatic lipid accumulation through de novo lipogenesis. Hepatic lipid accumulation can cause hepatic insulin resistance by increasing glycogen synthesis and gluconeogenesis (Samuel and Shulman 2012). In this study, insulin resistance and hyperglycemia were seen.

Sumagin et al. (2008) have studies a possible role of ICAM-1 and leukocyte-endothelial interactions on the vascular permeability regulation. During inflammation development, there are two major events. Leukocytes interact with endothelium leading to the cell passing through the barrier created by the en-

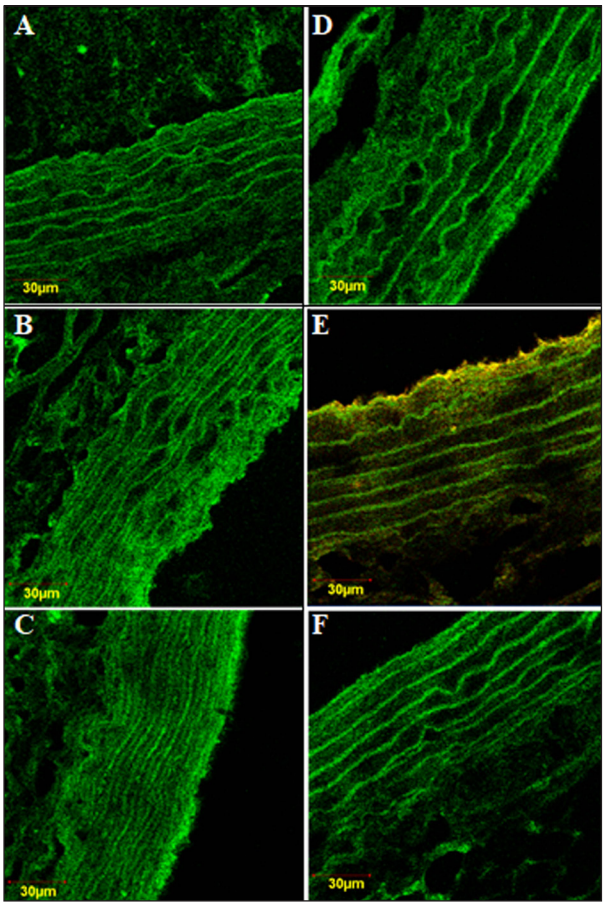

Figure 1. Representative pictures of inducible nitric oxide synthase (iNOS) expression in aortic tissue of Sprague-Dawley male rats. A – normal 8-week group; B – T2DM 8-week group; C – T2DM-DP 8-week group; D – normal 16-week group; E – T2DM 16-week group; F – T2DM-DP 16-week group

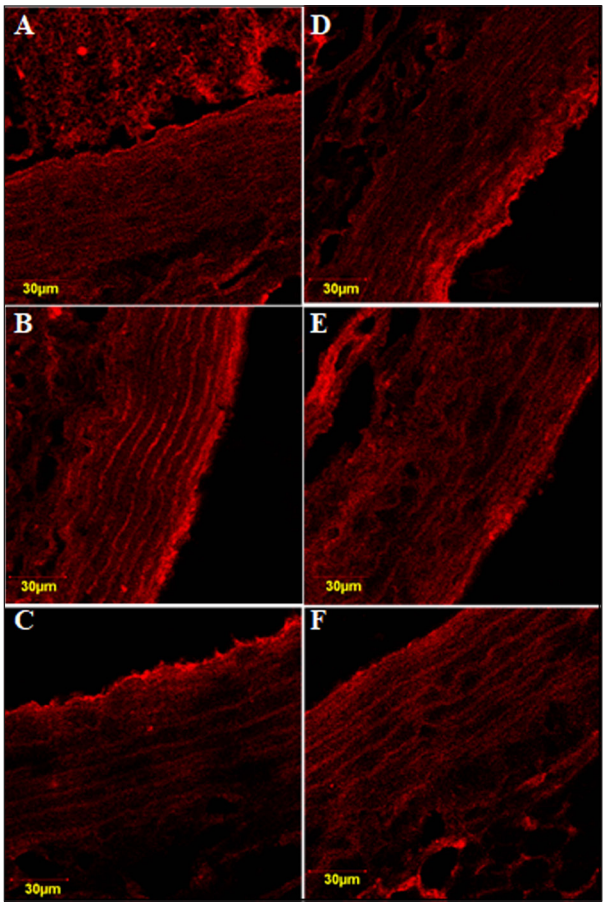

Figure 2. Representative pictures of intracellular adhesion molecule-1 (ICAM-1) expression in aortic tissue of Sprague-Dawley male rats. A – normal 8-week group; B – T2DM 8-week group; C – T2DM-DP 8-week group; D – normal 16-week group; E – T2DM 16-week group; F – T2DM-DP 16-week group

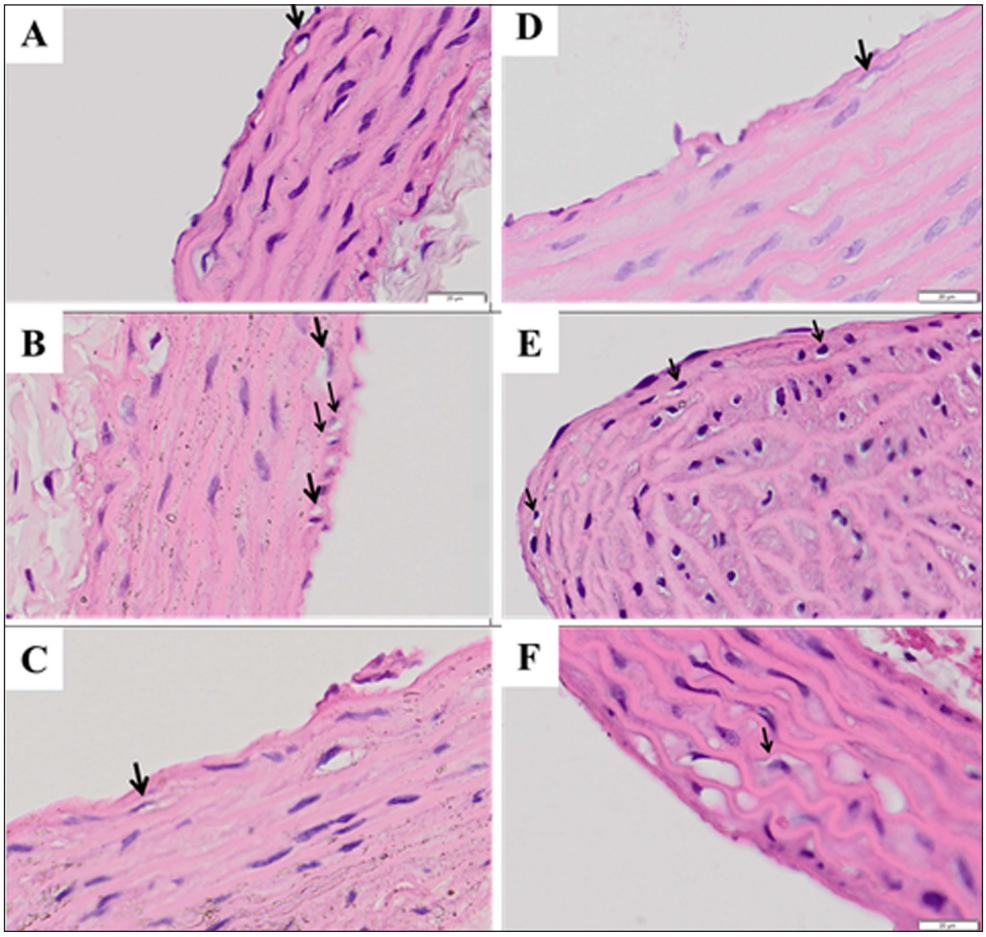

Figure 3. Foam cells in aortic tissue of Sprague-Dawley male rats. A – normal 8-week group; B – T2DM 8-week group; C – T2DM-DP 8-week group; D – normal 16-week group; E – T2DM 16-week group; F – T2DM-DP 16-week group

dothelial cells. Migration of these cells will lead to vascular permeability modification, which cause a transfer of solutes into peripheral tissues. Sumagin et al. (2008) have shown that both arterioles and venules can respond to pro-inflammatory stimuli. Under basal conditions, changes in vascular permeability were associated with the expression of ICAM-1 on PKC.

Recent study has shown a significant increase in adhesion molecule in T2DM group compared to normal (control) group. This may be caused by hyperglycemia in diabetes mellitus. Hyperglycemia can enhance the monocyte adhesion to endothelial cells (Navale and Paranjape 2013). Meanwhile, we found a decrease in ICAM-1 expression between T2DM 8-week and T2DM-DP 8-week groups ($p<0.05$). There was no significant decrease in T2DM 16-week and T2DM-DP 16-week groups ($p>0.05$), although the trend was decreasing. Decreased expression of ICAM-1 after darapladib administration is still controversial. Hu et al. (2011) have shown that darapladib only decreased the expression of VCAM-1 but not the expression of ICAM-1. Meanwhile, Wang et al. (2011) have shown that the expression of ICAM-1 in the darapladib group was lower in comparison with the positive control group, which correlates with our finding. Wang et al. (2011) have also reported that oxLDL can activate inflammation signaling pathways, such as NF-KB, MAPK, or PPAR. There is also a positive relationship between Lyso-PC in activating inflammation genes, such as iNOS, IL-1β or cyclooxygenase-2 (Wang et al. 2011). Darapladib administration can inhibit hydrolysis of oxLDL to LysoPC and oxNEFA and this can inhibit inflammation genes, such as iNOS.

In atherosclerosis development, LDL, macrophage and T lymphocyte can easily migrate through intima. LDL is a small and dense particle, what makes it vul-

nerable to oxidation and the antioxidant decrease can cause a high production of free radicals and modified the oxidative LDL. Lp-PLA_2 is then activated by oxidized phospholipid to oxLDL. The enzyme minimalizes oxLDL modification. During this process, lysophospholipids and oxNEFA are highly produced and cause adhesion macrophage pulled to the arterial intima. Activated macrophage slowly changes into foam cells (Silva et al. 2010). This may be a cause of the increasing foam cells number in this study.

Darapladib, a carbon-based small molecule taken orally specifically inhibits Lp-PLA_2 at certain doses. The inhibition of Lp-PLA_2 is expected to inhibit oxidation of phospholipids in the vascular space and to reduce expression of adhesion molecules and other factors that may lead to atherosclerosis. Darapladib has been shown to reduce the content of lysophosphatidylcholine and the expression of 24 genes related to macrophage and T lymphocyte function, with a decrease them in plaque and necrotic areas (Wilensky et al. 2008). The results of the present study showed that darapladib can decrease the foam cells number as well as iNOS and ICAM-1 expression, the markers of the atherosclerosis progression.

Conclusions

The results of the present study showed that darapladib can decrease the foam cells number, iNOS and ICAM-1 expression in aorta in early stages of the atherosclerosis in Sprague-Dawley T2DM rat model. These results indicate for a potential beneficial effect of darapladib on atherosclerosis.

The future biomedical implications our research findings may be in a form of biomarker helping detect the early stages of atherosclerosis.

Acknowledgement

International Publication Assistance for Lecturer's Scientific Article of Brawijaya University. This study was supported by Indonesia Ministry of Research, Technology, and Higher Education with grant No. 530.2/ UN10.21/PG/2015.

References

American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care 34 Suppl 1, S62–S69, 2011.

Aronson D, Rayfield EJ. How hyperglycemia promotes atherosclerosis: molecular mechanisms. Cardiovasc Diabetol 1, 1–10, 2002.

Avogaro A, Albiero M, Menegazzo L, de Kreutzenberg S, Fadini GP. Endothelial dysfunction in diabetes: the role of reparatory mechanisms. Diabetes Care 34, S285–290, 2011.

Burns TA, Geor RJ, Mudge MC, McCutcheon LJ, Hinchcliffe KW, Belknap JK. Proinflammatory cytokine and chemokine gene expression profiles in subcutaneous and visceral adipose tissue depots of insulin-resistant and insulin-sensitive light breed horses. J Vet Intern Med 24, 932–939, 2010.

Cacho J, Sevillano J, de Castro J, Herrera E, Ramos MP. Validation of simple indexes to assess insulin sensitivity during pregnancy in Wistar and Sprague Dawley rats. Am J Physiol Endocrinol Metab 295, E1269–E1276, 2008.

Carlquist JF, Muhlestein JB, Anderson JL. Lipoprotein-associated phospholipase A_2: a new biomarker for cardiovascular risk assessment and potential therapeutic target. Expert Mol Diagn 7, 511–517, 2007.

Chait A, Bornfeldt KE. Diabetes and atherosclerosis: is there a role for hyperglycemia? J Lipid Res 50, S335–S339, 2009.

Fukuchi M, Giaid A. Endothelial expression of endothelial nitric oxide synthase and endothelin-1 in human coronary artery disease: specific reference to underlying lesion. Journal of Technical Methods and Pathology 79, 659–670, 1999.

Galkina E, Ley K. Vascular adhesion molecules in atherosclerosis. Arterioscler Thromb Vasc Biol 27, 2292–2301, 2007.

Heriansyah T, Wihastuti TA, Anita KW, Iskandar A, Suhendra RB, Setiabudi PA, Sishartami LW. Atherogenesis inhibition by darapladib administration in dyslipidemia model sprague-dawley rats. National Journal of Physiology, Pharmacy and Pharmacology 6, 52–58, 2015.

Heriansyah T, Adam AA, Wihastuti TA, Rohman MS. Elaborate evaluation of serum and tissue oxidized LDL level with darapladib therapy: a feasible diagnostic marker for early atherogenesis. Asian Pacific Journal of Tropical Biomedicine 7, 134–138, 2016.

Hu MM, Zhang J, Wang WY, Wu WY, Ma YL, Chen WH, Wang YP. The inhibition of lipoprotein-associated phospholipase A2 exerts beneficial effects against atherosclerosis in LDLR-deficient mice. Acta Pharmacol Sin 32, 1253–1258, 2011.

International Diabetes Federation: IDF Diabetes Atlas (6nd ed.), 2013.
Navale AM, Paranjape AN. Role of inflammation in development of diabetic complications and commonly used inflammatory markers with respect to diabetic complications. Int J Pharm Pharm Sci 5, 1–5, 2013.
Samuel VT, Shulman GI. Mechanisms for insulin resistance: common threads and missing links. Cell 148, 852–871, 2012.
Silva IT, Mello APQ, Damasceno NRT. Antioxidant and inflammatory aspects of lipoprotein-associated phospholipase A2 (Lp-PLA2): a review. Lipids in Health and Disease 10, 170, 2010.
Silvana GT, Rifa'i M. Activity test of VipAlbumin against blood glucose levels and lymphocytes profiles in Balb/c mice as type 2 diabetes mellitus model. Jurnal Biotropika 3, 17–20, 2015.
Sumagin R, Lomakina E, Sarelius IH. Leukocyte-endothelial cell interactions are linked to vascular permeability via ICAM-1-mediated signaling. Am J Physiol Heart Circ Physiol 295, H969–H977, 2008.
Thompson PL, Nidorf SM, Eikelboom J. Targeting the unstable plaque in acute coronary syndromes. Clin Ther 35, 1099–1107, 2013.
Turan B, Dhalla NS. Diabetic cardiomyopathy: biochemical and molecular mechanism. Springer, New York, 2014.
Van Dijk JW, Venema M, van Mechelen W, Stehouwer CD, Hartgens F, van Loon LJ. Effect of moderate-intensity exercise versus activities of daily living on 24-hour blood glucose homeostasis in male patients with type 2 diabetes. Diabetes Care 36, 3448–3453, 2013.
Wang W-y, Zhang J, Wu W-y, Li J, Ma Y-l, Chen W-h, Yan H, Wang K, Xu WW, Shen JH, Wang YP. Inhibition of lipoprotein-associated phospholipase A2 ameliorates inflammation and decreases atherosclerotic plaque formation in ApoE-deficient mice. PLoS ONE 6, e23425, 2011.
Wihastuti TA, Sargowo D, Tjokroprawiro A, Permatasari N, Widodo MA, Soeharto S. Vasa vasorum anti-angiogenesis through H2O2, HIF-1α, NF-κB, and iNOS inhibition by mangosteen pericarp ethanolic extract (garcinia mangostana linn) in hypercholesterol-diet-given rattus norvegicus wistar strain. Vasc Health Risk Manag 10, 523–531, 2014.
Wihastuti TA, Heriansyah H, Soraya M, Wijayanti M, Firani NK, Iskandar A, Anita KW, Agustin D, Pujiana L, Sandika N, Ardyanto E, Puji A, Wiji Y, Febriato A, Nuraminudin A, Quirino F, Rachmawati S, Candra Y. Inhibition of oxidative stress in hypercholesterolemic rats by soy milk. Journal of Cardiovascular Disease Research 7, 74–82, 2016.
Wilensky RL, Shi Y, Mohler ER 3rd, Hamamdzic D, Burgert ME, Li J, Postle A, Fenning RS, Bollinger JG, Pelchovitz DJ, Yang J, Mirabile RC, Webb CL, Zhang L, Zhang P, Gelb MH, Walker MC, Zalewski A, Macphee CH. Inhibition of lipoprotein-associated phospholipase A2 reduces complex coronary atherosclerotic plaque development. Nat Med 14, 1059–66, 2008.