Effect of lipoprotein-associated phospholipase A2 inhibitor on insulin resistance in streptozotocin-induced diabetic pregnant rats

Guo-Hua Wang\textsuperscript{1,2}, Jun Jin\textsuperscript{3} and Li-Zhou Sun\textsuperscript{1}

\textsuperscript{1) Department of Obstetrics and Gynecology, The First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, Jiangsu, China}
\textsuperscript{2) Department of Obstetrics and Gynecology, The First People’s Hospital of Lianyungang City, Affiliated Hospital of Xuzhou Medical University, Lianyungang 222000, China}
\textsuperscript{3) Department of Clinical Laboratory, The First People’s Hospital of Lianyungang City, Affiliated Hospital of Xuzhou Medical University, Lianyungang 222000, China}

Abstract. This paper aims to investigate the influence of lipoprotein-associated phospholipase A2 (Lp-PLA2) inhibitor, darapladib, on insulin resistance (IR) in streptozotocin (STZ)-induced diabetic pregnant rats. The rat models were divided into Control (normal pregnancy), STZ + saline (STZ-induced diabetic pregnant rats), STZ + Low-dose and STZ + High-dose darapladib (STZ-induced diabetic pregnant rats treated with low-/high-dose darapladib) groups. Pathological changes were observed by Hematoxylin-eosin (HE) and Immunohistochemistry staining. Lp-PLA2 levels were determined by enzyme-linked immunosorbent assay (ELISA). An automatic biochemical analyzer was used to measure the serum levels of biochemical indicators, and homeostatic model assessment for insulin resistance (HOMA-IR) and insulin sensitivity index (ISI) were calculated. Western blot was applied to determine levels of inflammatory cytokines. Compared with Control group, rats in the STZ + saline group were significantly decreased in body weight, the number of embryo implantation, the number of insulin positive cells and pancreatic islet size as well as the islet endocrine cells, and high-density lipoprotein (HDL-C) level, but substantially increased in Lp-PLA2, low-density lipoprotein (LDL-C), fatty acids (FFA), serum total cholesterol (TC), triglyceride (TG) levels. Moreover, the increased fasting plasma glucose (FPG) and HOMA-IR and inflammatory cytokines but decreased fasting insulin (FINS) and ISI were also found in diabetic pregnant rats. On the contrary, rats in the darapladib-treated groups were just opposite to the STZ + saline group, and STZ + High-dose group improved better than STZ + Low-dose group. Thus, darapladib can improve lipid metabolism, and enhance insulin sensitivity of diabetic pregnant rats by regulating inflammatory cytokines.

Key words: Lipoprotein-associated phospholipase A2, Insulin resistance, Darapladib

MATERNAL DIABETES is one of the most common medical complication and a metabolic disorder that occurs during pregnancy, which seriously affecting the health of mother and infant [1]. Even though the pathogenesis of maternal diabetes is complex and has not been elucidated so far, it may involve many a wide range of different factors, such as genetic factors, environmental factors, and lifestyles, and in particular, it often linked to disorders owing to its close association with insulin resistance (IR) [2, 3]. Previous evidence pointed out that IR, as well as the dysfunction of pancreatic beta cells, might be one important step to the pathogenic mechanism of maternal diabetes [4]. During the course of pregnancy, insulin sensitivity decreased dramatically but IR exacerbated, thereby inducing the onset of maternal diabetes [5]. Meanwhile, the abnormal glucose metabolism during pregnancy has come to be viewed as potentially dangerous to affect health outcomes in both mother and infant. As reported, pregnant women with maternal diabetes were prone to complicate with pregnancy-induced hypertension, excessive amniotic fluid, premature birth,
macrosomia, fetal macrosomia, and so on [6-8]. On the other hand, the offspring of maternal diabetes mothers have an increased risk of obesity, type 2 diabetes mellitus, and metabolic syndrome [9, 10]. Therefore, it is of importantly significance to develop new drugs which can improve IR for prevention and treatment of maternal diabetes and its complications, eventually improving the outcomes of pregnant women and infants.

Lipoprotein-associated phospholipase A2 (Lp-PLA2), encoded by \( PLA2G7 \), is also known as platelet-activating factor-acetylhydridase (PAF-AH) belonging to the phospholipase A2 family [11, 12]. To our knowledge, Lp-PLA2, produced by monocyte-derived macrophages, could mostly bound to low-density lipoprotein (LDL) particles to be responsible for the generation of pro-inflammatory mediators, such as lysophosphatidylcholine and oxidized fatty acids, which is often abnormally up-regulated during various inflammation-associated diseases [13-15]. To date, there is considerable in vitro and in vivo evidence to support a causative agent of Lp-PLA2 in promoting atherosclerotic plaque development, and inhibition the activity of Lp-PLA2 may thus induce beneficial effects for the prevention and treatment of cardiovascular diseases [16, 17]. For instance, an animal study conducted by Robert L Wilensky et al. utilizing pigs revealed that selective inhibition the activity of Lp-PLA2 with darapladib, an oral Lp-PLA2 specific inhibitor, can suppress the progression of advanced coronary atherosclerosis in diabetes mellitus and hypercholesterolemia pigs [18]. In addition, the inflammatory burden and the plaque area were significantly reduced in the low density lipoprotein receptor-deficient mice with darapladib treatment [19]. Fortunately, darapladib, has been tested in a number of basic researches, as well as the preclinical trials, with quite striking outcomes [20]. However, it is worth to mention that IR is the common pathophysiological basis of coronary heart disease, as well as diabetes [20]. More importantly, Nelson TL et al. demonstrated that Lp-PLA2 activity had a positive relation with IR, which was an independent risk factor for the prediction of the presence of type 2 diabetes [21]. Along these lines, inhibiting the activity of Lp-PLA2 may also have the therapeutic effects on maternal diabetes.

Given the above, this study aims to investigate the effect of the Lp-PLA2 inhibitor, darapladib on IR in diabetic pregnant rats, thereby providing a new perspective for developing new drugs for the clinical prevention and treatment of maternal diabetes.

### Materials and Methods

#### Ethics statement

The study was approved by the Ethics Committee of The First Affiliated Hospital of Nanjing Medical University and all animal procedures in this study are in line with the local principles for the care and use of laboratory animals and follow the Guide for the Care and Use of Laboratory Animals by National Institutes of Health of the U.S.A.

#### Animals and experimental grouping

Female and male Sprague-Dawley (SD) rats (weighing 260–300 g, purchased from the Laboratory Animal Center of China Medical University) were separately kept in the open environment at room temperature of 18–28°C with a relative humidity of 40%–70%. During the estrus cycle, the male and female rats were kept in one cage with the ratio of 1:1, and mating was checked at 7 o’clock every morning by the presence of spermatozoa in the vaginal smear under a light microscope. The date when both the vaginal plug and active sperms were seen was set as the first day of pregnancy. The 40 pregnant rats were randomly divided into four groups (10 rats per group): Control group (normal pregnancy), STZ + saline group (streptozotocin-induced diabetic pregnant rats), STZ + Low-dose darapladib group, and STZ + High-dose darapladib group. After fasting for 12 h since the 6th day of pregnancy, rats in the STZ group and the two darapladib intervention groups were administered by intraperitoneal injection with streptozotocin (STZ) dissolved in citric acid-sodium citrate buffer (45 mg/kg) [22]. Meanwhile, rats in the Control group were injected with the same volume of citric acid-sodium citrate buffer. After injection for 72 h, fasting blood was taken from the tail tip of pregnant rats. The serum obtained after centrifugal separation was determined for fasting blood glucose by a glucose assay kit and the glucose ≥ 6.7 mmol/L indicated the successful establishment of model rats [23]. After that, rats in the Low-dose darapladib group were administered by gavage with 25 mg·kg\(^{-1}\)·d\(^{-1}\) darapladib and those in the High-dose darapladib were administered with 50 mg·kg\(^{-1}\)·d\(^{-1}\) darapladib [24]. At the same time, rats in the STZ group were given with same volume of physiological saline for 1 week. The serum samples were collected from the tail tip of pregnant rats and fasting plasma glucose (FPG) levels were measured on days 9, 11, 13, 15, 17, 19, fasting insulin (FINS) levels were measured on days 6, 9, 12, 15 and 19. On the
19th day of pregnancy, all rats were killed and laparotomy was performed to examine the number of implanted embryos and the abortion rate. Animal grouping and experiment procedures were shown in Fig. 1. Then, endometrial tissues were stripped out from rats’ uterus and preserved in a refrigerator at –80°C, while rats’ pancreases were fixed in 4% paraformaldehyde.

**Hematoxylin-eosin (HE) staining**

The pancreatic tissues of rats in each group were routinely dehydrated, transparentized, immersed in and embedded with paraffin. Ten serial sections of 3-μm thickness were obtained by using a paraffin sectioning machine (Shandon325, UK) and baked for 1 h at 50°C. Then, sections were stained for 3 min in Hematoxylin (Xi’an Chemical Reagent Factory) and for 1 min in Eosin (Xi’an Chemical Reagent Factory), sealed with neutral resin, and observed for morphological changes of pancreatic tissues under a microscope (LEICADMLB2, Germany).

**Immunohistochemistry**

Samples of pancreata were fixed in 4% buffered formaldehyde solution for 24 h at room temperature and embedded in paraffin of 57°C melting point. Sections of 5-μm-thick were immunoperoxidase stained for insulin. After blocking the endogenous peroxidase activity, CAS Block (Zymed Laboratories, San Francisco, CA, USA) serum was applied to reduce non-specific background staining. Sections were incubated overnight in the guinea-pig anti-insulin (diluted 1:100, Dako Corporation, Carpinteria, CA, USA) primary antibody at 4°C. After the application of the secondary antibody, the sections were incubated with streptavidin peroxidase complex (Zymed Laboratories), and the subsequent development was performed with diaminobenzidine to yield a brown color reaction. Insulin within pancreatic islets was examined by measuring the cumulative signal strength of images, a quantitative immunohistochemistry technique established by Matkowskyj and colleagues [25, 26]. Besides, differences in islet size were accounted according to previous studies [27].
**Determination of biochemical indicators**

Venous blood was collected from rats’ tails on day 19 of pregnancy, and fasting plasma glucose (FPG) and fasting insulin (FINS) levels in each group were determined in order to calculate the HOMA-IR and ISI by One Touch glucose meter (LifeScan, Inc, Johnson & Johnson, USA) and an insulin radioimmunooassay kit (Beijing Furui Bio-Tech Co. Ltd, Beijing, China) respectively. IR was evaluated by homeostasis model assessment (HOMA): HOMA - IR = FINS (mU/L) × FPG (mmol/L)/22.5 [28]. Meanwhile, Insulin Sensitivity Index (ISI) = ln1/(FPG × FINS) [29]. Lp-PLA2 ELISA Kit (Beijing CWBIOTECH Co., Ltd.) was used to determine the serum Lp-PLA2 levels in rats of each group. An automatic biochemical analyzer (Olympus AU 2700, Japan) was used to detect the levels of serum levels of fatty acids (FFA), serum total cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL-C) and low-density lipoprotein (LDL-C) in rats of each group.

**Detection of inflammatory factors by Western blot**

The endometrial tissues of rats in each group were added with RIPA lysis buffer (Beyotime, Haimen, China). After homogenization in a glass homogenizer, centrifugation was conducted for 15 min at the rate of 12,000 rpm, and the supernatant was collected for SDS-PAGE electrophoresis. The isolated protein after electrophoresis was transfer to nitrocellulose membrane by electric transfer, closed at room temperature for 1 h in 5% skim milk-PBS solution, and incubated overnight at 4°C with primary antibodies MCP-1 (ab151538, Abcam), ICAM-1 (ab33894, Abcam), IL-6 (ab208113, Abcam), and TNF-α (ab8348, Abcam). After washing the membrane with PBS buffer for three times, secondary antibodies were added for 1 h of incubation at room temperature. Then, the membrane was washed for another three times with PBS buffer and developed by enhanced chemiluminescence. With GAPDH as the internal reference gene, the gray value ratio of target band to reference band was regarded as the relative expression level of proteins.

**Statistical method**

Data analysis was conducted by using the statistical software SPSS 21.0. Measurement data were presented by mean ± standard deviation (X ± s). Difference between groups was analyzed by Student’s t-test and comparison among multiple groups was made by One-Way ANOVA. Analysis of variance with repeated measures was used to determine the differences in FPG across time points during pregnancy. Besides, inter-group difference of enumeration data was tested by Chi-Square (χ²) Test. p < 0.05 was considered statistically different.

**Results**

**General information of rats in each group**

On the 3rd day after injection with STZ, the rats in the STZ + saline group showed a gradually increase in food and water intake, as well as urine volume, and had manifestations such as body weight loss and lusterless, dry or messy hair, as compared with the Control group. However, the symptoms of rats in the STZ + Low-dose and STZ + High-dose darapladib groups were somewhat relieved after administered with darapladib for one week, and the improvements of high-dose group were the most obvious. Meanwhile, rats in the Control group had no observable abnormal behaviors or manifestations. As shown in Table 1, on day 0 of pregnancy, rats in the Control group were heavier than those in the other three groups (p < 0.05) with no significant difference among the three groups (all p > 0.05). On day 19 of pregnancy, the body weight and fetal weight of rats in the three groups was still lower than that in the Control group. Besides, compared to the STZ + saline group, those rats with the STZ + Low-dose and STZ + High-dose darapladib treated demonstrated a substantially elevation in body weight and fetal weight, and rats in the STZ + High-dose darapladib group were even heavier than those in the STZ + Low-dose darapladib group (all p < 0.05). In addition, rats in the STZ + saline group were remarkably lower in the number of embryo implantation and live birth number than those in the Control group. However, rats in the two treated groups had the increased number of embryo implantation and live birth number when compared with the STZ + saline group, and rats of the STZ + High-dose group increased more obvious (all p < 0.05).

**Morphological changes of pancreatic tissues**

In the Control group, the islets of rats were normal in shape (round or oval), well arranged, uniform in size, and rich in cytoplasm, with observable nuclear chromatin as well as abundant islets and endocrine cells. By contrast, a large number of vacuoles could be observed in rats from STZ + saline group, with disordered and irregular arrangement. On the other hand, rats in the STZ + Low-dose and STZ + High-dose darapladib groups were...
significantly reduced cell vacuoles, and had a relatively regular morphology (Fig. 2A). Immunohistochemical examination confirmed vigorous islet β-cell regeneration, with larger and more abundant islets evident in rats from Low-dose and STZ + High-dose darapladib group, in contrast to rare and scattered small islets in rats from STZ + saline group (Fig. 2B). Besides, rats in STZ + saline group led to a significant reduction in insulin positive cells and pancreatic islet size (both \( p < 0.05 \)). When compared with rats in the STZ + saline group, the insulin positive cells and pancreatic islet size were markedly increased in darapladib treated groups (all \( p < 0.05 \), Fig. 2C, D). Further, these changes of High-dose group were more remarkable.

### Table 1  Comparison of fetal outcomes of rats in each group (\( \bar{x} \pm s \))

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>STZ + saline</th>
<th>STZ + Low-dose darapladib</th>
<th>STZ + High-dose darapladib</th>
</tr>
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<tbody>
<tr>
<td><strong>Body weight (g)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 9 of pregnancy</td>
<td>278.16 ± 14.23</td>
<td>257.42 ± 10.12*</td>
<td>258.36 ± 11.23*</td>
<td>260.54 ± 12.31*</td>
</tr>
<tr>
<td>Day 19 of pregnancy</td>
<td>376.28 ± 16.47</td>
<td>307.16 ± 12.35*</td>
<td>326.14 ± 13.69**</td>
<td>351.12 ± 13.82**</td>
</tr>
<tr>
<td><strong>Embryo implantation (number/rat)</strong></td>
<td>17.52 ± 1.23</td>
<td>12.13 ± 1.48*</td>
<td>14.12 ± 1.56**</td>
<td>15.99 ± 1.72**</td>
</tr>
<tr>
<td><strong>Fetal weight (g)</strong></td>
<td>4.54 ± 0.36</td>
<td>3.16 ± 0.28*</td>
<td>3.65 ± 0.32**</td>
<td>4.11 ± 0.34**</td>
</tr>
<tr>
<td><strong>Live birth number</strong></td>
<td>16.98 ± 1.78</td>
<td>10.29 ± 1.69*</td>
<td>12.56 ± 1.58**</td>
<td>14.85 ± 1.66**</td>
</tr>
</tbody>
</table>

Note: STZ, streptozotocin; *, \( p < 0.05 \) compared with the Control group; **, \( p < 0.05 \) compared with the STZ group; @, \( p < 0.05 \) compared with the Low-dose darapladib group.

**Fig. 2** Morphological changes of pancreatic tissues in rats of each group (× 400, Scale bar = 50 µm)

Note: A, The morphological changes in pancreatic tissues of rats in each group observed by HE staining; B–C, Representative images of pancreatic islet insulin staining, (B) and insulin quantification (C); D, Comparison of pancreatic islet area. The same letter meant that \( p \) value was greater than 0.05, and the different letter indicated that \( p \) value was less than 0.05.

**Comparison of biochemical indicators of rats in each group**

As illustrated in Fig. 3, the levels of Lp-PLA2, LDL-C, FFA, TC and TG were significantly up-regulated and HDL-C was statistically down-regulated in the rats from the STZ + saline group by comparison with the rats from the Control group (all \( p < 0.05 \)). On the contrary, rats in the darapladib treated groups had the reduced levels of Lp-PLA2, LDL-C, FFA, TC and TG, and the elevated level of HDL-C when compared to the STZ + saline group; and more importantly, the improvement was more obvious in the rats from the STZ + High-dose darapladib group (all \( p < 0.05 \)).
Comparison of FPG, FINS, HOMA-IR and ISI in rats of each group

Rats in the STZ + saline group had higher levels of FPG and HOMA-IR, and an apparently decreased FINS and ISI level than those in the Control group (all \( p < 0.05 \), Table 2, Fig. 4). When compared with rats in the STZ + saline group, the FPG and HOMA-IR levels were markedly declined while the FINS and ISI level was highly increased in darapladib treated groups (all \( p < 0.05 \)). Besides, the changes were more obvious in the rats with the STZ + High-dose darapladib treatment, and there was statistical difference regarding FPG, HOMA-IR, and ISI between the STZ + High-dose and STZ + Low-dose darapladib groups (all \( p < 0.05 \)).

Protein expressions of inflammatory factors in rats of each group

As displayed in Fig. 5, rats in the STZ + saline group showed substantial up regulations of MCP-1, ICAM-1, IL-6 and TNF-α in the endometrial tissues when compared with those in the Control group (all \( p < 0.05 \)). In addition, the protein expression levels of MCP-1, ICAM-1, IL-6 and TNF-α were markedly lowered in rats with the darapladib treatment groups, as compared to the rats in the STZ + saline group (all \( p < 0.05 \)). Furthermore, the above protein levels were more lowered in the STZ + High-dose group than in the Low-dose group (all \( p < 0.05 \)).
The onset of maternal diabetes poses a great threat to the health of mothers and infants [30]. Nowadays, the establishment of maternal diabetes rat models has been utilized for the further study of the etiology and treatment methods of maternal diabetes as well as the evaluation of new drugs for maternal diabetes [31]. Streptozotocin (STZ), a glucosamine-nitrosourea compound, has been widely used to induce diabetes in experimental animals, since it has highly selective toxic effects on pancreatic β-cells [32]. Therefore, in this study, the body weight and the number of embryo implantation, as well as the fetal weight and live birth number were examined in the diabetic pregnant rats, and found that all of the above factors in the STZ-induced diabetic pregnant rats were reduced with certain pathological changes of pancreatic β-cells when compared to

**Discussion**

The onset of maternal diabetes poses a great threat to the health of mothers and infants [30]. Nowadays, the establishment of maternal diabetes rat models has been utilized for the further study of the etiology and treatment methods of maternal diabetes as well as the evaluation of new drugs for maternal diabetes [31]. Streptozotocin (STZ), a glucosamine-nitrosourea compound, has been widely used to induce diabetes in experimental animals, since it has highly selective toxic effects on pancreatic β-cells [32]. Therefore, in this study, the body weight and the number of embryo implantation, as well as the fetal weight and live birth number were examined in the diabetic pregnant rats, and found that all of the above factors in the STZ-induced diabetic pregnant rats were reduced with certain pathological changes of pancreatic β-cells when compared to

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**Fig. 4** Comparison of FPG (A) and FINS (B) in rats of each group at different time points

**Fig. 5** The protein expressions of inflammatory factors in rats of each group

Note: The same letter meant that $p$ value was greater than 0.05, and the different letter indicated that $p$ value was less than 0.05.
the rats of normal pregnancy, which was consistent with the findings in previous studies [22, 33].

In these STZ-induced diabetic pregnant rats, Lp-PLA2 was significantly up-regulated, which could be consequence of a change in the metabolic milieu due to medical intervention, such as STZ, to directly modulate the activity of LpPLA2. Meanwhile, the serum levels of LDL-C, FFA, TC and TG were increased, but HDL-C level was obviously down-regulated when compared with rats in the Control group, highlighting the abnormally expressed Lp-PLA2 and obvious disorders of blood lipid metabolism in STZ-induced diabetic pregnant rats. Lp-PLA2, as a famous inflammation marker, was in agreement with another previous study reported by Noto et al. to be clearly higher in patients with type 2 diabetes, which was positively relevant to TG, and LDL-C, but was negatively associated with HDL-C [34]. As recorded, Lp-PLA2 is mainly produced by macrophages, and in particular, macrophage infiltration was observed to be enhanced in the arterial wall of patients with diabetic mellitus, which could induce the increased production of LpPLA2 [35, 36]. It is well-known that diabetes is an irreversible disorder characterized by the elevated blood glucose levels, due to the lack of sensitivity to insulin and impaired insulin secretion, which can be compensated by increased functional pancreatic β-cell mass [37]. There was evidence pointed out a positive relation between LpPLA2 activity and fasting glucose in diabetes, which suggested that hyperglycaemia may also affect the activity and the biological function of this enzyme [38]. On the other hand, the free radical resulted from oxidation of excess glucose could elevate the oxidation of lipoproteins, which also increase the substrate for LpPLA2, further contributing to the up-regulation in circulating Lp-PLA2 activity [39]. Besides, significant alterations in TG, TC, LDL-C, and HDL-C were also detected by H Shen et al. between GDM patients and normal controls [33]. As for IR, a state of reduced responsiveness to normal circulating levels of insulin, which is a predictor of diabetes, has a close relation with the abnormalities of lipids and glucose metabolism [40], and in particular, the rate of detection of hyperglycemia increased with an increased incidence of IR [41]. To be specific, IR, mainly manifested by an excessive rate of lipolysis, could result in chronic increase in the serum level of FFA, and thereby leading to the elevation of TG and very low density lipoproteins, thus participating in HDL formation and metabolism, which in turn reduced HDL particle remodeling owing to the disruption of LPL as the consequence of IR [44, 45]. After diabetic pregnant rats with darapladib treatment in Low-dose and High-dose respectively, the levels of Lp-PLA2 were remarkably down-regulated, as well as LDL-C, FFA, TC, and TG, and these changes were more prominent in rats with High-dose darapladib, indicating that the potent darapladib could inhibit the elevated LpPLA2, and reduce the serum levels of LDL-C, FFA, TC, and TG in a dose-dependent manner. In agreement, Zhang J and his group also performed the strategy with the different doses of darapladib in male rats models of atherosclerosis and found the similar trend of TC, LDL-C, and Lp-PLA2 after darapladib treatment as our results [24]. Moreover, the elevation of FFA and the formation of concomitant advanced glycation end products (AGE) during prolonged hyperglycemia could cause pancreatic β-cell damage, as indicated by Han et al., owing to the insufficient leptin action and the induction of receptor for AGE in type 2 diabetes [46]. Our data discovered the decreased levels of FFA and improved pancreatic damage after treated with darapladib, which raised the possibility that Lp-PLA2 inhibitor could improve pancreatic function via reducing the levels of FFA.

In addition, the pronounced situation of IR was observed among diabetic pregnant rats in our research, with the markedly increased FPG and HOMA-IR and the dramatically decreased FINS and ISI. HOMA-IR, a mathematical model to quantify IR and beta-cell function from basal (fasting) glucose and insulin, has been largely utilized as an independent predictor in clinical practice [47]. More importantly, diabetic pregnant rats were significantly decreased in FPG and HOMA-IR, but appreciably enhanced in FINS and ISI level with the administration of darapladib, which suggested that the specific Lp-PLA2 inhibitor, darapladib, may improve insulin sensitivity via reducing fasting blood glucose and insulin, thus repairing the metabolic abnormalities of diabetic pregnant rats to some extent. As mentioned before, high glucose concentration might also be a risk element for the subclinical infection, resulting in chorioamnionitis and preterm birth [48]. In addition, intrauterine growth restriction has been suggested to be associated with the maternal vascular lesions, which was the most common placental lesion in GDM [49]. Moreover, Lp-PLA2 upregulation in the inflamed vascular tissue tended to indicate a potential role of Lp-PLA2 in the
development and progression of related-vascular diseases and endothelial dysfunction, whereas inhibition of its activity could provide the vascular protection [50]. Specifically, with regard to endothelial dysfunction, it also triggered the increased vascular permeability and activation of the maternal coagulation system, which affected the exchange of nutrients between the mother and the fetus, and ultimately influencing the growth and development of the fetus [51]. Similarly, in our study, we also found that diabetic pregnant rats treated with darapladib had the increased number of embryo implantation and live birth number, further indicating that darapladib could improve fetal outcome via reducing fasting blood glucose and regulating the function of blood vessel endothelial cells.

Furthermore, another important finding in our study was that the expressions of MCP-1, ICAM-1, IL-6 and TNF-α in the endometrial tissues from diabetic pregnant rats administered by darapladib, were appreciably down-regulated in a dose-dependent manner. Similarly, in the study by Wang WY et al., the ApoE-deficient mice treated with darapladib, also had a lowered expression of specific inflammatory genes, such as MCP-1, VCAM-1 and TNF-α, to ameliorate inflammation and alleviate atherosclerosis [52]. As mentioned before, Lp-PLA2, a marker of inflammation and atherosclerosis, which is at the crossroads of lipid metabolism and the inflammatory response [53]. Specifically, it could produce lysophosphatidylethanolamine and a series of complex biochemical and pathophysiologic reactions to trigger an inflammatory response with the interaction between monocytes/macrophages [54], with the up-regulation of the adhesion molecules, like VCAM-1 and ICAM-1, and induction of chemokine secretions due to the endothelial dysfunction, as well as inflammatory cytokine expressions, such as IL-6 and TNF-α, ultimately leading to pancreatic β-cell failure and IR [55-57]. MCP-1 is a potent chemokine expressed in atherosclerosis, whose release could be attributed to Lp-PLA2 production [16]. In this sense, darapladib could modulate the expression of inflammatory cytokines to ameliorate hyperglycemia and IR, and metabolic alterations.

In summary, this study advances our understanding of the impact that inhibition of Lp-PLA2 by darapladib, may decrease the expression of inflammatory cytokines to reduce HOMA-IR, enhance ISI, increase insulin sensitivity, contributing to improving blood lipids metabolism in diabetic pregnant rats in a dose-dependent manner.

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Disclosure of Conflict of Interest

None.

References


