Original Research Article

Effect of Dorsomorphin Homolog 1 (DMH1) against Diabetic Dyslipidemia in Streptozotocin-induced Diabetic Rats

Abstract

Dyslipidemia is usually observed in both types of diabetes and, particularly, “atherogenic dyslipidemic triad” is strongly linked to a higher risk of adverse cardiovascular outcome. On the other hand, bone morphogenetic proteins (BMP) are a group of wide variety of proteins which were found overexpressed and implicated in contribution and acceleration of atherosclerotic calcification. So, the present study aimed to assess effect of DMH1, a selective BMP inhibitor, in a rat model of diabetic-induced dyslipidemia.

Methods: STZ-induced diabetes in Wistar rats was used as a model to assess the antihyperlipidemic effect of DMH1(5mg/kg) for a period of 8 weeks. Rats were divided intonormal control (C=10), diabetic control (DC=10), diabetic+vehicle (DV=10) and diabetic DMH1-treated rats (DT=10). Fasting blood glucose (FBG) level was measured on weekly bases. Then, at the end of the experiment, rats were anesthetized and blood samples were collected for the determination of total cholesterol (TC), triglyceride (TG), LDL and HDL levels using the appropriate ELISA assay.

Results: FBG levels for all diabetic groups were significantly high, during the experiment period, compared to the control ($P< 0.001$). While dyslipidemia was remarkable in the diabetic non-treated groups, DMH1 treatment showed a significant decrease in TC ($P< 0.001$), TG ($P< 0.05$) and LDL levels ($P< 0.001$) compared to the non-treated groups (DC & DV). Concurrently, HDL levels for DT group were significantly increased compared to DC or DV groups ($P< 0.01$).

Conclusion: The present experiment showed that DMH1 possessed encouraging activityagainst dyslipidemia in STZ-induced diabetic rats. Our results are promoting for more interest and investigation regarding antihyperlipidemic effect of DMH1 and BMP/Smad pathway in further experimental studies.

Keywords:Dorsomorphin homolog 1, DMH1, Diabetic Dyslipidemia, bone morphogenetic protein, BMP.

1. INTRODUCTION

Cardiovascular disease (CVD) is a definitehealth concern which commonly occurred in both types of diabetes. Despite the substantial advances in diabetic care, both absolute and relative risks ofCVD among the insulin-dependent diabetic (T1DM) patients remained extremely high. Although CVD risk may considerably vary based on age, sex or duration of diabetes, however, patients with early-onset (<10 years of age) type 1 diabetes are carryingabout 30-fold increased risks of CVD or coronary heart disease (CHD) and approximately 7.4-times more risk of cardiovascular-related mortality [1].

On the other hand, dyslipidemia is usually observedin poorly controlled T1DM and obese T2DM patients.Particularly,“atherogenic dyslipidemic triad” of raised triglyceride levels, low levels of high-density lipoprotein cholesterol (HDL-C) andincrease in small and dense low-density lipoprotein (LDL) particles is more tightlylinkedto higherCVDrisk and maybe useful biomarker for risk assessment [2].Also, the association of hypertriglyceridemia with CHD was stronger in patients with diabetes than in the general population, whilelipid lowering therapy has significantly decreased CHD risks in diabetic patients as well as non- diabetics [3]. Unfortunately, underscreening and undertreatment of dyslipidemia as a major modifiable risk factor for CHD in diabetic patients was numerously reported [4-6].

The most common lipid abnormality in diabetic populations is hypertriglyceridemia which occurred due toeither increasedhepatic secretion in T2DM ordefect in the removal in absolute insulin deficient state (T1DM). Moreover, chylomicron removal by lipoprotein lipase (LPL) is also majorly regulated by insulin. Additionally, diabetic kidney disease (DKD) isa common microvascular complication andsubstantially involved in both dyslipidemia and CVD [7]. On the other hand, bone morphogenetic proteins (BMP) are group of wide variety of proteins forming a subset of larger superfamily called transforming growth factor-β (TGF-β). Previous work has outlined theinvolvement of BMP signaling in vascular injury and calcification and, therefore, it was experimentally investigated. *In vitro* study of human coronary artery smooth muscle cells, BMP was overexpressed and implicated in contribution and acceleration of atherosclerotic calcification [8]. Furthermore,*Derwall et al*, provided further confirmation

of activation of BMP in early atherosclerotic lesions, besides the remarkable reduction of vascular calcification achieved by inhibition of BMP signaling in atherogenic animals [9]. Additionally, hyperglycemia and diabetes were found to stronglyactivate BMP signaling in endothelial cellsand aortic wall with consequent increase in expression of osteogenic markers and medial calcification [10].

Dorsomorphin Homolog 1 (DMH1) is a small molecule with a promising pharmacological property which almost exclusively inhibit BMP with no off-target effects [11]. DMH1 was used as BMP pharmacological inhibitor in many experiments and displayed positive results with low toxicity andeffective inhibition of BMP/Smad pathway, which make it a good choice to be tested in purpose of BMP inhibition [12-14]. Therefore, the present study is the preliminary attempt to explore effect of the selective BMP inhibitor (DMH1) in experimental animal with diabetic dyslipidemia.

2. MATERIAL AND METHODS

2.1. Chemicals and kits

Streptozocin (STZ) (Cat. No. 1621) was purchased from Tocris Bioscience® (Bristol, UK) and supplied as crystalline solid to be dissolved in 0.1 M sodium citrate buffer (pH 4.5) just prior use. DMH1 (Dorsomorphin Homolog 1) (Cat. No. 4126) was also purchased from Tocris Bioscience® (Bristol, UK) supplied as a yellow crystalline solid. (2-Hydroxypropyl)-β-cyclodextrin (HPβCD) powder (Cat. No. OH05393) was acquired from BiosynthCarbosynth® (Compton, UK) and freshly prepared as 12.5% solution to be used as vehicle for DMH1. QuickDetectTM Total Cholesterol (Rat) ELISA kit (Cat No.MBS846775), rat triglyceride (TG) ELISA kit (Cat No. MBS726298), rat HDL ELISA kit (Cat No.MBS2505957), rat LDL ELISA kit (Cat No.MBS702165) were all purchased from MyBioSource (San Diego, USA).

2.2. Induction of diabetes and experimental design

A total of forty male adult Wistar rats which weighed (180-220) g were used for the current study. The animals were obtained from pharmaceutical consultation and research unit, Faculty of Pharmacy, King Abdulaziz University (KAU), Jeddah, Saudi Arabia. Feed and tap water *ad libitum* were provided. Additionally, standard animal room temperature (29-30 ºC) and 12 hours of light/dark cycle were maintained during the whole study period. The current experiment was carried out based on the Good Clinical Practice (GCP) Guidelines and granted the ethical approval from Research Ethics Committee (REC) at KAU (Reference No 546-19). Following one week of acclimating to the facilities, ten random rats were marked as the control (C=10), and the remaining 30 rats were prepared for diabetes induction by intraperitoneal (i.p.) injection with Streptozotocin (STZ) 60 mg/kg. Three days later, rats with blood glucose level of ≥ 300

mg/dl were considered as diabetics [15] and were randomly divided into three different groups with ten rats each, as the following: Diabetic non-treated group (DC, n = 10) supplemented by regular food and water, Diabetic non-treated group + vehicle (DV, n = 10) injected (i.p.) with equivalent amount of vehicle (12.5% 2-hydroxypropyl-β-cyclodextrin [HPβCD]) for 8 weeks, Diabetic treated group (DT, n = 10) injected (i.p.) with 5 mg/kg DMH1 each other day (q.o.d) for 8 weeks [12,16]. HPβCD was selected based on its property of increasing drug solubility and bioavailability [17], as well as being previously and successfully used as vehicle for DMH1 [12].

Fasting blood glucose (FBG) readings were recorded using *ACCU-CHEK*® *Active* (Roche Diagnostics GmbH, Mannheim, Germany) at the baseline andon weekly bases thereafter. At the end of experiment, blood samples were withdrawn and centrifuged at 3000g for 10 min to obtain clear sera which were used for assessment total cholesterol (TC), triglyceride (TG), high-density lipoproteins (HDL) and low-density lipoproteins (LDL) using appropriate enzyme-linked immunosorbent assay (rat ELISA kit). The rats were sacrificed after being anesthetized with diethyl ether.

2.3. Statistics

One-way analysis of variance (ANOVA) was used to examine the gathered results using SPSS statistics software package (version 23) followed by Tukey's HSD multiple comparison post hoc test. Differences if P-values < 0.05 were considered statistically significant.

3. RESULTS

By the end of the experiment, a total of nine rats were dead; three rats were from the DC group, two rats were from DV group, while the other four rats were in the treated group. However, fasting blood glucose (FBG) levels were constantly and significantly high during the study period (8 weeks) for all diabetic groups (DC, DV& DT) in comparison to the control group (P< 0.001). Moreover, comparing FBG levels between all the three diabetic groups (DC, DV & DT) revealed no significant difference (***Figure 1***).

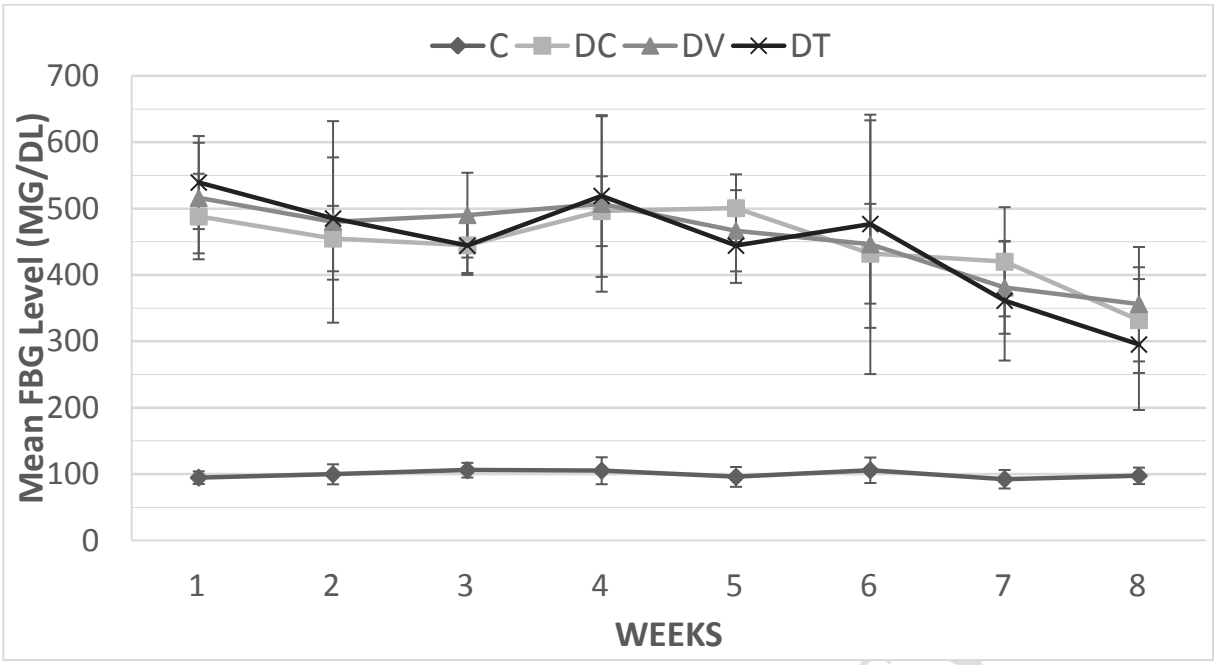

Figure1: Averages of weekly FBG levels ± SD (mg/dl) for all groups of rats during the study period (8 weeks)

C = Control rats (n=10), DC= diabetic control group (n=7), DV= diabetic+vehicle group (n=8), DT= diabetic-DMH1 treated group [5mg/kg q.o.d] (n=6).

There were remarkable differences in lipid profiles between the four different groups. Total cholesterol (TC) & LDL levels were significantly increased for diabetic non-treated groups (DC & DV), while treated group showed no difference versus (C) group and appeared significantly lower than DC or DV groups ($P< 0.001$). Similar results were obtained for triglycerides (TG) levels for the tested groups (increased for DC & DV groups and lower levels for DT group) ($P< 0.05$) (***Figure 1***). Furthermore, for the HDL levels, DT group has significantly increased levels compared to DC or DV groups ($P< 0.01$), while both DC & DV were significantly lower than the control group ($P< 0.05$). Although it was statistically insignificant ($P> 0.05$), mean HDL for DT group was superior to that of the control group (***Figure 2***).

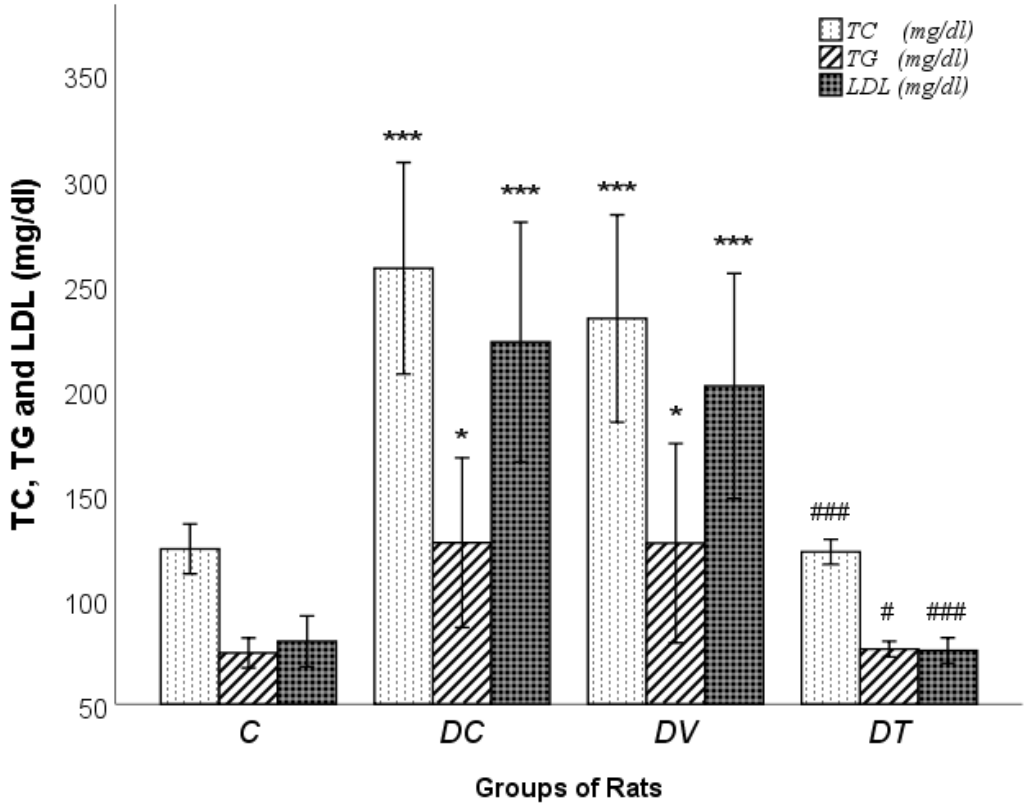

Figure 1: Averages of TC, TG & LDL readings for all tested groups of rats after 8 weeks of the study

Data were presented as mean ± SD. TC (Total cholesterol mg/dl), TG (Triglyceride mg/dl), LDL (low-density lipoproteins) mg/dl. C = Control rats (n=10), DC= Diabetic Control group (n=7), DV= Diabetic + Vehicle group (n=8), DT= Diabetic-DMH1 Treated group [5mg/kg q.o.d] (n=6). *significant (P< 0.05) against control group. *** very highly significant (P< 0.001) against control group. $^{\#}$significant (P< 0.05) against DC or DV group. $^{\#\#\#}$ very highly significant (P< 0.001) in comparison to Diabetic non-treated group (DC or DV).

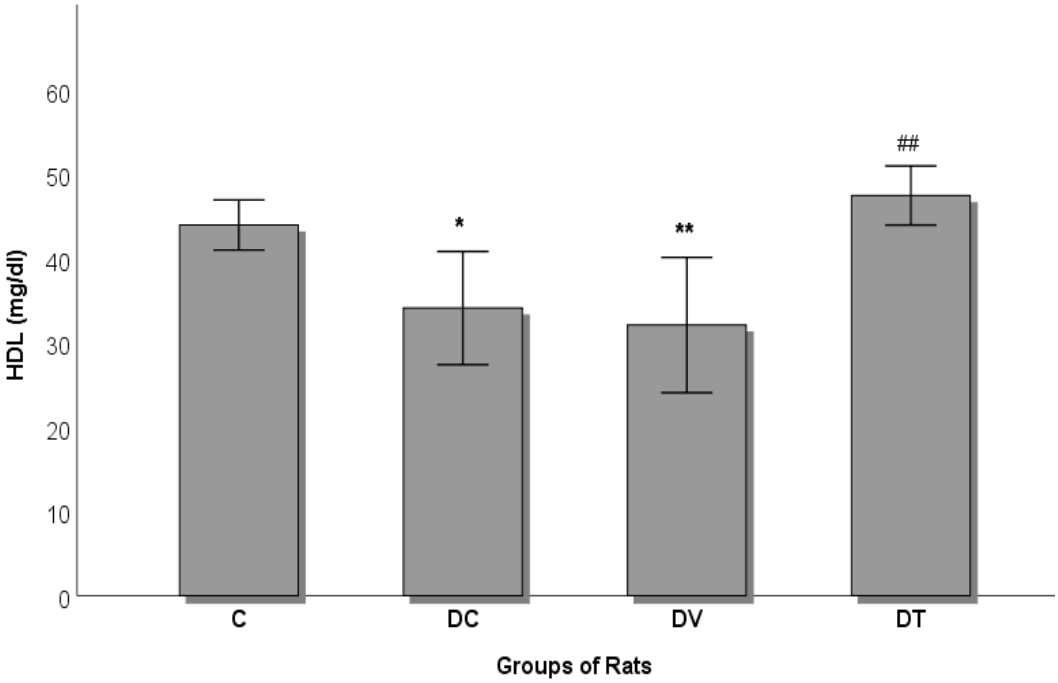

Figure 2: Mean HDL for rats after 8 weeks of the study

*Data were presented as mean ± SD. HDL (high-density lipoproteins) mg/dl, VLDL (Very-low-density lipoprotein) mg/dl. C = Control rats (n=10), DC= Diabetic Control group (n=7), DV= Diabetic + Vehicle group (n=8), DT= Diabetic-DMH1 Treated group [5mg/kg q.o.d] (n=6). * significant (P< 0.05) against control group.** highly significant (P< 0.01) against control group. ## highly significant (P<0.01) compared to DC or DV group.*

4. DISCUSSION

Dyslipidemia is prevalent in diabetic patients and commonly linked to premature atherosclerotic cardiovascular outcomes. Additionally, there is a substantial evidence addressingbenefit of lipid lowering drugs in reducing risk of CHD in patients with or without pre-existing cardiovascular condition [3].However, more options to manage dyslipidemia and further clarification of potential mechanisms are critically needed. In the current study, effect of BMP inhibition using intraperitoneal administration of DMH1 was tested against diabetic-induceddyslipidemia in experimental rats.

DMH1 is a small, potent and highly selective molecule. Originally, DMH1 was the designed analog of non-selective BMP receptor inhibitor (Dorsomorphin). However, unlike Dorsomorphin, DMH1 demonstrated higher stability, lower toxicity and advantage of specifically inhibiting BMP-2 and BMP-4induced Smad1/5/8 activation through Activin receptor-like kinase-1(ALK1), ALK2, and ALK3 receptors with negligible effect on ALK6 receptor and no effect on p38/MAPK signaling or vascular endothelial growth factor (VEGF) pathway [11].

On the other hand, BMP family isa component of transforming growth factors beta (TGF-β) superfamily. It contained many members with pleiotropic effects on cellular development and fate, including adipogenesis. Notably, BMP-2 and BMP-4 was reported to activate the expression and phosphorylation of downstream signaling to initiate adipogenic commitment from cell line of mesenchymal stem cells (MSCs), while knockdown of coregulator of BMP/Smad signaling (Smad4) resulted in disruption of this commitment [18]. Furthermore, a later study carried out to evaluate the association of BMP-4 levels with metabolic disorders. Interestingly, researchers found levels of BMP-4 were significantly correlated with waist circumference, body mass index, triglyceride (TG) and high-density lipoproteins (HDL) cholesterol [19].

In fact, dyslipidemia is known to associate diabetes. In diabetic patients, the activation of triacylglycerol lipase enzyme led to more free fatty acids (FFA) mobilization from the adipose tissue with a consequent hepatic TG overproduction. Also, further increases in TG production and remnant cholesterol are due to suppressed activity of lipoprotein lipase (LPL) [20]. In the current study, dyslipidemia was found remarkable in both diabetic non-treated groups; total cholesterol, triglyceride, LDL showed significant increased levels in DC & DV groups when compared to the control group. On the other hand, DMH1-treated group showed readings within similar ranges to that of the normal rats. The current results are indicating a promising effect of DMH1 against dyslipidemia. Simultaneously, DMH1 administration resulted in a significant increase in HDL compared to the non-treated groups. So, both HDL and LDL cholesterol as well as triglyceride and TC levels were significantly and positively affected by DMH1.

Arguably, β-cyclodextrin (βCD), which (its derivative [HPβCD]) was used as vehicle in the current study, was reported to decrease plasma lipid in the experimental rats [21]. βCD was claimed, due to its chemical structure (ring), to act as sequestrant and slowly hydrolyzed in the large intestine and excreted intact in the feces when administered orally [21]. Contrarily, the present study, HPβCD was given intraperitoneally (DV group) and did not demonstrate any lipid lowering effect. Other reports of sustained parenteral administration ofβCD in animals were showing either increased [22] or unaffected plasma cholesterol [23]. Moreover, the administered dose in our study was much lower than the reported oral and parenteral doses. Furthermore, the cholesterol lowering effect of oral diet containing βCD was not uniformly in addition to a significant decrease in HDL cholesterol [21]. By contrast, the treated group in the current study showed a uniformly antihyperlipidemic effect with increase in HDL cholesterol, an effect which is more plausible to be credited to DMH1.

In fact, our results are consistent with others who reported that inhibition of BMP would increase lipid efflux and reduce intracellular lipid accumulation. Almost ten years ago, LDN-193189, which is one of dorsomorphin structural analogs who has similar DMH1 inhibitory property against BMP/Smad pathway, was investigated to explore its pharmacological effect in treating atherosclerosis. Results of the treated mice proved increasing lipid efflux and reduction in formation of foam cells which have important role in occurrence and development of atherosclerosis [24]. Almost within the same year, another study has outlined BMP role in atherosclerosis using the same BMP inhibitor (LDN-193189) for 20 weeks with high-fat diet fed to LDL receptor-deficient mice ($LDLR^{-/-}$), a commonly used model for atherosclerosis. Treatment with LDN-193189 has reduced cholesterol and LDL levels, but not HDL or triglyceride levels. Furthermore, the pharmacological BMP inhibition effectively decreased SMAD1/5/8 activation as well as inflammation, oxidative stress, atherosclerosis, vascular calcification and hepatic steatosis. Interestingly, authors reported that early atherosclerotic lesions were distinguished by noticeable activation of the BMP signaling. Moreover, the cholesterol lowering effect was appeared irrelevant to HMG CoA reductase or its hepatic gene expression, suggesting novel therapeutic strategy for dyslipidemia [9].

In the current study, DMH1 lipid lowering effects were significant and remarkable in total cholesterol, triglyceride and LDL levels. Furthermore, the average, as well as majority, of HDL cholesterol readings for the treated group showed mild increase in comparison to the control group, however, this increase was not statistically significant. Although the current findings were based on the serum biomarkers solely, however, it is the first, as far as we know, to mark the antihyperlipidemic effect of DMH1. Also,the results can be useful to add to the existing evidence regarding involvement of BMP in

dyslipidemia and atherosclerosis as well as encouraging future investigations regarding DMH1 and its potential therapeutic applications.

5. CONCLUSION

In the current study, DMH1 revealed antihyperlipidemic effect on animal model of diabetic dyslipidemia. This promising effect was manifested by significant decreases in TC, LDL, TG levels with simultaneous increases in HDL levels. The current results can also support the previous establishment of BMP/Smad involvement in dyslipidemia and atherosclerosis.It also encourages further investigations for downstream signaling as well asthe potential applicability and utilization of DMH1 in a wider experimentalcontext.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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