

Research Article

Effect of Vildagliptin on Atherosclerosis Progression in High Cholesterol – Fed Male Rabbits

Najah R Hadi*, Hussain Abdulkadhim, Ahmad Almudhafer and Sahar A Majeed

Department of Pharmacology and Therapeutics, Kufa College of Medicine, Kufa, po: 21 Iraq

Abstract

Background: Atherosclerosis is a progressive disease of large and medium-sized arteries characterized by the accumulation of lipids and fibrous elements in the large arteries.

Aim of the study: This study was undertaken to assess the effect of vildagliptin on the progression of atherosclerosis via interfering with inflammatory and oxidative pathways.

Materials and Methods: 18 local domestic male rabbits were included in this study. The animals were randomly divided into three groups (6 rabbits for each group): Group I rabbits fed normal chow (oxiod) diet for 12 weeks. Group II rabbits fed 1% cholesterol enriched diet for 12 weeks. Group III rabbits fed 1% cholesterol enriched diet for 12 weeks. Group III rabbits fed with cholesterol enriched diet for 6 weeks, and then continued on cholesterol-enriched diet and treated with vildaglipin 50 mg/kg/day orally for the next 6 weeks. Blood samples were collected at the start of the study, at 6weeks of the study and then at the end of treatment course to measure Serum lipids profile [(TC), (TG), (HDL)], hsCRP and TNFα. At the end of the study the aorta were removed for measurement of aortic MDA, glutathione, sectioning for histopathology and measuring aortic intima- media thickness.

Results: Treatment of rabbits with vildagliptin for 6 weeks results in a significant reduction (P<0.05) in serum level of TC, TG, hsCRP and TNF α and a significant increase (P<0.05) in serum HDL level. There was a significant reduction (P<0.05) in aortic MDA and intima-media thickness, in comparison to the rabbits in the induced untreated control group. vildagliptin treatment cause significant increment (P<0.05) in aortic GSH in comparison to induced untreated group. Regarding the histopathological results, vildagliptin treatment for 6 weeks results in a significant reduction (P<0.05) in atherosclerotic lesions in comparison to the induced untreated group and significant reduction in aortic intima-media thickness (P<0.05).

Conclusions: Vildagliptin reduced atherosclerosis progression in hyperlipidemic rabbit via its effect on lipid parameters and interfering with inflammatory and oxidative stress pathway.

Keywords: Vildagliptin; Atherosclerosis; Oxidative stress; Inflammation

Background

Atherosclerosis is a multifactorial, multistep disease that involves chronic inflammatory at every stage, from initiation to progression and, eventually, plaque rupture [1]. Cardiovascular disease (CVD) is the principal cause of death and disability in developed countries and is increasing rapidly in the developing world [1,2]. Endothelial dysfunction is the early step that allows dissemination of lipids and inflammatory cells into the endothelial and sub-endothelial spaces. Secretion of cytokines and growth factors promote SMC proliferation, accumulation of collagen matrix, monocytes infiltration, and other white blood cells, forming an atheroma [3].

Incretin hormones

The role of the gastrointestinal tract in regulating the secretion of insulin is demonstrated by the thought that insulin secretion is substantially increased in response to oral glucose load, compared to intravenous glucose administration. This difference is known as the incretin effect; these peptides are secreted from endocrine cells (L-cells) in the gastrointestinal tract, and are released in response to ingestion of food and subsequent oral glucose load. The two main incretin hormones are glucagon-like peptide-1 (GLP-1) and glucosedependent insulinotropic polypeptide (GIP). Dipeptidyl peptidase-IV (DDP-IV) is proteolytic enzyme that is widely expressed in many tissues, including the capillary bed of the gut mucosa and is responsible for rapid inactivation of (GLP-1) [4]. In cretin-like agents and DPP- 4 inhibitors show the need to act on the underlying disease rather than on its symptoms. They enhance glucose-dependent insulin secretion by pancreatic beta cells, and consequently there is less risk of hypoglycemia. They also suppress elevated glucagon secretion and increase feeling of satiety. It has been theorized that they may reestablish beta-cell sensitivity to glucose, which could mean that they may be able to delay the onset of type 2 diabetes, slow its progression, and reduce its cardiovascular and metabolic complications [5]. Vildagliptin is a potent, reversible, competitive inhibitor of DPP-4, with high selectivity for DPP-4 over other peptidases enzymes [6].

Materials and Methods

Eighteen local domestic male rabbits were included in this study. The animals were randomly divided into three groups (6rabbits in each group): *Group I* rabbits fed normal chow (oxiod) diet for 12 weeks. *Group II* rabbits fed 1% cholesterol enriched diet for 12 weeks. *Group*

*Corresponding author: Najah R Hadi, Department of Pharmacology and Therapeutics, Kufa College of Medicine, Kufa, po: 21, Iraq, Tel: 009647801241456; E-mail: drnajahhadi@yahoo.com

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III rabbits fed with cholesterol-enriched diet for 6 weeks, and then continued on cholesterol-enriched diet and treated with vildaglipin 50mg/kg/day orally for the next 6 weeks. Blood samples were collected at the start of the study, at 6weeks of the study and then at the end of treatment course for measurement of Serum lipids profile [total cholesterol (TC), serum triglyceride (TG) and high density lipoprotein (HDL)], high sensitive C-reactive protein (hsCRP), tumor necrosis factor (TNF α) at the end of the study the aorta were removed for measurement of aortic malonyl dehydrogenase (MDA), glutathione (GSH) and Aortic intima- media thickness and sectioning for histopathology.

Preparation of samples

From each rabbit, about 3 ml of blood was collected from the central ear artery without use of heparin after an overnight fasting. The blood sampling was done firstly at the start of the study i.e. at zero time and after 6 weeks of the induction period, and then at end of treatment course (12week). The blood samples were allowed to clot at 37°C and centrifuged at 3000 rpm for 15 min. Sera were taken, and analyzed for determination of serum total cholesterol, triglycerides, HDL-C , hsC-reactive protein and TNF- α .

Tissue preparation for oxidative stress measurement

20% homogenates of tissues were prepared in phosphate buffer at pH 7.5 containing 1 mmol/l Na₂EDTA. The homogenates were centrifuged at 20,000 X g at 4°C for 30 min and the supernatants were used for biochemical measurements of GSH & MDA level.

Histopathological procedure

Autopsy of aortic (abdominal and thoracic aorta) sectioning were done at the end of the study (after 12 weeks), the histopathology is used to confirm the anti-atherogenic effect of vildagliptin in comparison with the control groups (normal control and induced untreated control). The sections were examined by microscope under magnification power of (x4, x10 and x40) then the histological changes were determined according to the American Heart Association classification of atherosclerosis which divides atherosclerotic lesions into six types [7].

Statistical analysis

Data were expressed as mean \pm SEM; by using SPSS version 17, unpaired t-test was used to compare the mean values between different groups.

Results

Effect of vildagliptin on serum lipid profile

There was a statistically significant increase in serum TC, TG level (P<0.05) in all groups fed with cholesterol enriched diet for 6 weeks. At the end of 12 weeks, there was a statistically significant increase in serum TC level (P <0.05) in the induced untreated group continued to have cholesterol enriched diet for another 6 weeks, whereas groups treated with vildagliptin for 6 weeks showed a significant decrease in serum TC, TG level and significant increment in HDL level (P<0.05). As shown in Table 1.

Effect on aortic tissue reduced glutathione level (GSH) and malonyl dehydrogenase (MDA)

At the end of study 12 weeks of high cholesterol diet the aortic GSH level was significantly decreased in induced untreated group (II) and significant increment in MDA level (P<0.05) in compared with normal

control group. Regarding vildagliptin treaded group (III), after 12 weeks of high cholesterol diet, there was significant increment in the GSH level (P < 0.05) associated with significant decrement of MDA level (P<0.05) as shown in Table 2.

Page 2 of 4

Effect of vildagliptin on TNF-a and hsCRP

Before the study, the baseline levels of serum hsCRP and TNF- α were statistically not significant among all groups. After6 weeks of using high cholesterol diet, the TNF- α and hsCRP levels significantly increased (P < 0.05) in all groups except the normal group. After 12 weeks, the hsCRP and TNF- α level significantly decreased in vildagliptin treated groups (P < 0.05) as compared with induced untreated group as shown in Table 3.

Effect of vildagliptin on atherosclerosis and aortic intimamedia thickness

At the end of 12 weeks of high cholesterol diet rabbits treated with vildagliptin had a significant reduction in the severity of atherosclerotic lesions in comparison with rabbits in the induced untreated group as in Figures 1 and 2. The level of aortic intima-media thickness (measured

Group		TC mg/dl	TG(mg/dl)	HDL (mg/dl)	
I	Normal group	Zero time	54.3 ± 2.00	44.5 ± 0.6	16.2 ± 0.25
		6 weeks	56 ± 1.9	44.7 ± 0.9	15.8 ± 0.50
		12 weeks	57.2 ± 1.5	46.50 ± 1.0	16.4 ± 0.30
11	Induced untreated group	Zero time	52.5 ± 2.26	45.5 ± 1.8	15.9 ± 0.25
		6 weeks	596 ± 4.5*	159.3 ± 5.0*	20.0 ± 0.30*
		12 weeks	720 ± 9.5†	187.8 ± 8.0†	18.9 ± 0.75†
111	vildagliptin treated group	Zero time	54.7 ± 2.20	45.7 ± 1.5	16.9 ± 0.55
		6 weeks	598 ± 4.0*	159.5 ± 4.5*	18.2 ± 0.89*
		12 weeks	375 ± 2.7†	80.0 ± 2.50†	21.7 ± 1.10†

^{*} P < 0.05(means at 6 weeks versus means at zero time) + P < 0.05(means at 12 weeks versus means at 6 weeks)

 Table 1: Effect of cholesterol enriched diet, vildagliptin 50mg/kg/day on serum lipid

 profile level in mg/dl. The data expressed as mean ± SEM.

Groups		Aortic MDA level (µmole/gm)	Aortic GSH level (nmole/mg)
I	Normal group	1.9 ± 0.22	40.3 ± 2.4
II	Induced untreated group	9.0 ± 0.56*	20.9 ± 1.9*
Ш	vildagliptin treated group	3.3 ± 0.39	33.7 ± 2.2*

* P < 0.05(means at 6 weeks versus means at zero time)

† P < 0.05(means at 12 weeks versus means at 6 weeks)</p>

Table2: Changes in aortic oxidative stress (GSH in nmol/mg and MDA*in* µmole/gm)at the end of study. The data expressed as mean ± SEM.

	Group	TNF-α(pg/ml)	hsCRP(mg/l)	
I		Zero time	0.60 ± 0.09	3.0 ± 0.0
	Normal group	6 weeks	1.08 ± 0.11	3.0 ± 0.0
		12 weeks	1.05 ± 0.06	3.0 ± 0.0
11		Zero time	0.77 ± 0.10	3.0 ± 0.0
	Induced untreated group	6 weeks	4.70 ± 0.54*	43 ± 1.8*
		12 weeks	7.05 ± 0.44†	57 ± 3.0†
111		Zero time	0.85 ± 0.05	3.0 ± 0.0
	vildagliptin treated group	6 weeks	4.60 ± 0.15*	41 ± 1.8*
		12 weeks	2.70 ± 0.17†	19 ± 1.0†

* P < 0.05(means at 6 weeks versus means at zero time) + P < 0.05(means at 12 weeks versus means at 6 weeks

Table 3: Effect of cholesterol enriched diet, vildagliptin 50mg/kg/day on serum inflammatory marker (TNF- α level in pg/ml and hsCRP in mg/l) the data expressed as mean ± SEM.

by histomorphometry) was significantly increased in induced untreated group (II), in compared with normal control (P<0.05). The aortic intima-media thickness level of vildagliptin treated (III) was significantly lower than that of induced untreated group (II) as shown in Table 4 and the Figure 2.

Discussion

In this study we demonstrate that high atherogenic diet cause significant rise in lipid parameter (TC, TG, atherogenic index) in comparison with control group [8,9]. Treatment with vildaglitin cause significant reduction in (TC, TG, and atherogenic index) in comparison with induced untreated group. This result is consistent with those reported by Matikanianin et al. [10] and Monami et al. [11]. A significant increase inflammatory markers (hs.CRP, TNFa) level was found in rabbits fed with cholesterol enriched diet as compared with that in the normal control group. This result is in agreement with that reported by Howard and Culley [12], Rajamannan et al. [13] and Sun et al. [14]. The increase in serum CRP level is due to the fact that CRP is acute phase reactant that increases many folds during the inflammatory response to tissue injury, so it is increased by cholesterol enriched diet because cholesterol enriched diet causes the development of atherosclerosis which is a chronic inflammatory disease [15]. In this study, we used histopathological sectioning and histological examination to evaluate atherosclerotic changes. While Trivedi et al. and Korosoglou et al. used non-invasive techniques including multisequence MRI of atherosclerotic plaques in major arteries before and after injection of ultrasmall superparamagnetic particles of iron oxide (USPIOs) [16,17]. These particles are accumulated in macrophages in 80% of the atherosclerotic plaques. Furthermore, detection of number of macrophages in the atherosclerotic plaques can



Figure 1: A cross section from aorta shows a narrowing of the arterial lumine by bulging of atherosclerotic plaque. (Type-5 atherosclerosis). The section stained with haematoxylin and eosin x40.



Figure 2: Photomicrograph of histomorphometric section in aorta of vildagliptin hyperlipidimic rabbit shows significant decrease in the aortic intima thickness as compared to induced untreated group. Section stained with haematoxylin and Eosin (x40).

Groups		Aortic intima-media thickness (µm)	
I	Normal group	47.0 ± 2.7	
II	Induced untreated group	289 ± 53.7*	
III	vildagliptin treated group	204 ± 0.33†	

* P < 0.05(means at 12 weeks versus means at zero time) † P < 0.05(means at 12 weeks versus means at 6 weeks)

Table 4: Changes in aortic intima-media thickness in ($\mu m)$ at the end of the study the data expressed as mean \pm SEM.

predict risk of its rupture. USPIOs-enhanced MRI can identify plaque inflammation in vivo by accumulation of USPIOs within macrophages in large artery plaques [16,17]. Vildagliptin treatment significantly reduces the elevation of inflammatory markers (hs.CRP, TNFa) in atherosclerosis model of hypercholesterolemic rabbit suggesting that vildagliptin inhibit vascular inflammation induced by high atherogenic diet these results constant with those reported by Bolli et al. [18] and Rizzo et al. [19]. In our study, atherosclerosis was associated with increases in the levels of the lipid peroxidation product MDA, and decrease in the level of GSH in aortic tissue suggesting an increase in the levels or activity of oxygen radicals. MDA and GSH have been considered as specific indicators of oxidative stress [20]. MDA level can be used as a marker of lipid peroxidation and its measurement gives a direct evidence for LDL oxidation and is leading in predicting free radical-induced injury, therefore, the observed elevation in tissue MDA may be attributed to hyperlipidemia that enhances the processes of lipid peroxidation. . Hypercholesterolemia could increase the levels of reactive oxygen species (ROS) through stimulation of polymorphnuclear leukocytes (PMNLs) and dysfunction of endothelial cells [21,22]. Furthermore, hypercholesterolemia, especially for long time, results in vascular oxidant problem [23,24], which could favor GSH depletion because of enhanced oxidation of the tripeptide or consumption by compounds like lipo-peroxidation aldehydes [25,26]. Vildagliptin treatment had significantly reduced aortic MDA level suggesting decrease in ROS and subsequent lipid peroxidation. In addition, vildagliptin had significant effect on aortic GSH levels where prevents GSH depletion in hypercholesterolemic rabbits; therefore, maintain antioxidant reserve which is important for vascular protection against lipid peroxide [20]. In rabbits treated with vildaglipin there was a significant reduction in the severity of atherosclerotic lesions [19,27] in comparison with rabbits in the induced untreated group also there is significant decrement in aortic intima media thickness (P<0.05) of vildagliptin treated group compared with that of the induced untreated group. In our study, we found that vildagliptin has anti-inflammatory effects by reducing (hsCRP, &TNF-a) and had antioxidant effect by reducing lipid peroxide (MDA) and enhancing GSH. Therefore, these findings may provide answers how vildagliptin reduce aortic intimamedia thickness via suppression of systemic inflammatory response and oxidative stress. This anti-inflammatory and anti-atherosclerotic effect of vildagliptin may be due to increase incretin level as the latter has anti-inflammatory and protective effects against atherosclerosis [28]. We recommend further studies using the watanabe hereditary hyperlipidemic (WHHL) rabbit model where, atherosclerosis is already happened and no time needed for induction of atherosclerosis as compared with cholesterol fed model of atherosclerosis. Furthermore, lipoprotein metabolism, atherosclerotic plaques and coronary artery disease in WHHL rabbit model resemble that happened in human [29]. Additionally, non-invasive imaging studies like IRON-MRI contrast imaging may be used which precisely highlight macrophage -rich plaques [17] to further clarify the effect of vildagliptin on atherosclerosis.

Page 3 of 4

Page 4 of 4

Conclusion

Vildagliptin reduced atherosclerosis progression in hyperlipidemic rabbit via its effect on lipid parameters and interfering with inflammatory and oxidative stress pathway.

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