

Effect of sitagliptin on lipid profile, Inflammation and Oxidative Stress in high cholesterol –fed male rabbits

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الخلاصة:

يعتبر تصلب الشرايين المرض القاتل الرئيس على مستوى العالم. إن من أهم عوامل الخطورة لهذا المرض هي ارتفاع مستوى الدهون بالدم ومرض السكري وعوامل أخرى مثل الالتهابات المزمنة
هدف الدراسة: لتقييم تأثير السيتاكلبتين على تقليل مستوى الدهون بالدم والالتهابات وعوامل التاكسد وتأثيرها على سُمك الطبقة الوسطية والداخلية للشريان الأبهري.

الطريقة: أدخلت في هذه الدراسة ١٨ أرنباً محلياً تم توزيع هذه الأرانب بصورة عشوائية إلى أربعة مجاميع المجموعة الأولى هي مجموعة السيطرة الطبيعية والمجموعة الثانية أعطيت غذاء عالي الدسم يحتوي ١% كولسترول. المجموعة الثالثة أعطيت غذاء عالي الدسم لمدة ستة أسابيع، بعد ذلك أعطيت عقار السيتاكلبتين ٢٥ مل/كغم لكل يوم عن طريق الفم.

النتائج: سبب تغذية الأرانب بالغذاء عالي الدسم الذي يحتوي ١% كولسترول لمدة اثني عشر أسبوعاً في زيادة معنوية ($P < 0.05$) في مستوى الكولسترول الكلي والكليسيريدات الثلاثية ومستوى البروتين المتفاعل نوع C وعامل الالتهاب $TNF-\alpha$ في الدم و مقياس درجة التصلب وزيادة في عامل التاكسد مالونيلداي الديهايد (MDA) وانخفاضاً في عامل التاكسد كلوتاتايون (GSH) في الانسجة. سبب معالجة الارانب بعقار السيتاكلبتين لمدة ستة أسابيع انخفاضاً معنوياً ($P < 0.05$) في الكولسترول الكلي، الكليسيريدات الثلاثية، مستوى البروتين المتفاعل نوع C وعامل الالتهاب $TNF-\alpha$ في الدم ومقياس درجة التصلب. بينما سبب زيادة معنوية ($P < 0.05$) في مستوى الكولسترول عالي الكثافة في الدم وانخفاض في عامل التاكسد (MDA) وزيادة في عامل التاكسد (GSH) و سبب انخفاضاً معنوياً ($P < 0.05$) في سمك الطبقة الداخلية والوسطى للشريان الأبهري.

Abstract

Back ground: Atherosclerosis is the major world wide killing disease. The most common risk factors are hyperlipidemia, diabetes, and other factors like chronic infection and inflammation.

Objective: This study was undertaken to assess the effect of sitagliptin on atherosclerosis via interfering with lipid parameter, inflammatory and oxidative pathways

Method: 18 local domestic male rabbits were included in this study. The animals were randomly divided into three groups: Group I rabbits fed normal chow (oxid) diet for 12 weeks. Group II rabbits fed 1% cholesterol enriched diet. Group III rabbits fed with cholesterol enriched diet for 6 weeks, and then continued on cholesterol enriched diet and treated with Sitagliptin 125mg/kg/day orally for the next 6 weeks.

Results: Treatment of rabbits with sitagliptin for 6 weeks results in a significant reduction ($P < 0.05$) in serum level of TC, TG, hsCRP and $TNF\alpha$ 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. sitagliptin treatment cause increment in aortic GSH in comparison to induced untreated group.

Keywords: sitagliptin, atherosclerosis, oxidative stress, inflammation

Introduction:

The endothelium is a dynamic autocrine and paracrine organ that regulates anti-inflammatory, mitogenic, and contractile activities of the vessel wall as well as the homeostatic process within the vessel lumen.

Atherosclerosis is likely initiated when endothelial cells over-express adhesion molecules in response to turbulent flow in the setting of an unfavorable serum lipid profile. Increased cellular adhesion and associated endothelial dysfunction then "sets the stage" for the recruitment of inflammatory cells, release of cytokines and recruitment of lipids into the atherosclerotic plaque. Atherothrombosis are mediated, in large part, by the inflammatory cascade⁽¹⁾. Recruited macrophages both release additional cytokines and begin to migrate through the endothelial surface into media of the vessel. This process is further enhanced by the local release of monocyte-colony stimulating factor (M-CSF), which causes monocytic proliferation; local activation of monocytes leads to both cytokine-mediated progression of atherosclerosis, and oxidation of low-density lipoprotein (LDL).

Once initiated, many mediators of inflammation have been described to influence the development of the atherosclerotic plaque⁽²⁾. Inflammatory mediators expressed by smooth cells within the atherosclerotic plaque include, but are not limited to, interleukin (IL)-1 β , tumor necrosis factor (TNF) α and β , IL-6, M-CSF, MCP-1, IL-18 and CD-40L. The impact of these mediators is diverse and includes mitogenesis, intracellular matrix proliferation, and angiogenesis and foam cell development.⁽³⁾

Gliptins are a novel class of oral anti-diabetic agents that enhance and prolong the physiological actions of incretin hormones which increase insulin secretion

Sitagliptin, an orally available Dipeptidyl peptidase -IV inhibitor developed to be used as a once daily treatment for T2DM, has shown beneficial effects on glycaemic control, reducing HbA1c, and preventing hypoglycemia, as well as on islet mass and function, with no relevant adverse effects^(4,5) Considering the vast physiological actions promoted by the incretins, not only related with the control of glucose by insulin and glucagon regulation, but also with the peripheral insulin sensitization, cardiac and neuronal protection and beta-cell preservation, the use of an incretin enhancer (such as sitagliptin) might present beneficial effects on diabetes pathophysiology and on prevention of its serious complications like atherosclerosis

Materials and methods:

Animals. 18 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 III rabbits fed with cholesterol enriched diet for 6 weeks, and then continued on cholesterol enriched diet and treated with Sitagliptin 125mg/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. Serum lipids profile [(TC), (TG), (HDL)) and hsCRP, TNF α at the end of the study the aorta were removed for measurement of aortic MDA & glutathione. Aortic intima- media thickness and sectioning for histopathology.

Drugs

Sitagliptin: It was used in a dose of 125 mg/kg⁽⁶⁾, 100 mg tablet supplied by (Merck) was suspended in distilled water and the dose was given according to body weight, once daily through stomach tube.

Cholesterol powder: Pure cholesterol powder ($C_{27}H_{46}O$) M. wt = 386.67 supplied by BDH Chemicals Ltd Poole England was used. The cholesterol enriched diet was prepared by mixing 10 gram of pure cholesterol powder with 1 kg of oxiod diet (to produce 1% cholesterol enriched diet).

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 , hs.C-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 Na_2EDTA . The homogenates were centrifuged at 20,000 $\times g$ at 4 C for 30 min and the supernatants were used for biochemical measurements of GSH &MDA level **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 sitagliptin 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 sitagliptin 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 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.

For sitagliptin treated group(III) After 12 wks 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)

Effect of sitagliptin on TNF- α and hsCRP

Before the study, the baseline levels of serum hsCRP and TNF- α were statistically not significant among all groups. After 6 wks of high cholesterol diet, the TNF- α and hsCRP level significantly increased ($P < 0.05$) in all group except normal group.

After 12 weeks, the hsCRP and TNF- α level significantly decreased in sitagliptin treated groups ($P < 0.05$) as compared with induced untreated group. As shown in table(3)

Effect on aortic intima-media thickness

At the end of 12 weeks of high cholesterol diet. The level of aortic intima-media thickness (measured 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 sitagliptin treated (III) was significantly ($P < 0.05$) lower than that of induced untreated group (II). As shown in table(4)

Table 1: Effect of cholesterol enriched diet, sitagliptin 125mg/kg/day, vildagliptin 50mg/kg/day on serum VLDL level in mg/dl. The data expressed as mean \pm SEM

| Group | | | TC mg/dl | TG (mg/dl) | HDL (mg/dl) |
|------------|----------------------------------|-----------|-----------------|------------------|------------------|
| I | Normal group | Zero time | 54.3 \pm 2.00 | 44.5 \pm 0.6 | 16.2 \pm 0.25 |
| | | 6 weeks | 56 \pm 1.9 | 44.7 \pm 0.9 | 15.8 \pm 0.50 |
| | | 12 weeks | 57.2 \pm 1.5 | 46.50 \pm 1.0 | 16.4 \pm 0.30 |
| II | Induced untreated group | Zero time | 52.5 \pm 2.26 | 45.5 \pm 1.8 | 16.7 \pm 0.34 |
| | | 6 weeks | 596 \pm 4.5* | 159.3 \pm 5.0* | 20.0 \pm 0.30* |
| | | 12 weeks | 720 \pm 9.5† | 187.8 \pm 8.0† | 18.9 \pm 0.75† |
| III | Sitagliptin treated group | Zero time | 56.5 \pm 2.00 | 47.5 \pm 1.5 | 16.9 \pm 0.55 |
| | | 6 weeks | 605 \pm 4.9* | 162.8 \pm 3.8* | 17.9 \pm 0.40* |
| | | 12 weeks | 380 \pm 3.2† | 86.0 \pm 1.90† | 20.4 \pm 0.90† |

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

† $P < 0.05$ (means at 12 weeks versus means at 6 weeks)

Table2: Changes in aortic oxidative stress (GSH in nmole/mg and MDA in μ mole/gm)at the end of study. The data expressed as mean \pm SEM

| groups | | Aortic MDA level (μ mole/gm) | Aortic GSH level (nmole/mg) |
|------------|----------------------------------|-----------------------------------|-----------------------------|
| I | Normal group | 1.9 \pm 0.22 | 40.3 \pm 2.4 |
| II | Induced untreated group | 9.0 \pm 0.56* | 20.9 \pm 1.9* |
| III | Sitagliptin treated group | 2.9 \pm 0.42* | 30.3 \pm 2.7* |

* $P < 0.05$ (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, sitagliptin 125mg/kg/day on serum inflammatory marker (TNF- α level in pg/ml and hsCRP in mg/l) the data expressed as mean \pm SEM

| Group | | | TNF- α (pg/ml) | hsCRP(mg/l) |
|------------|----------------------------------|-----------|-----------------------|---------------|
| I | Normal group | Zero time | 0.60 \pm 0.09 | 3.0 \pm 0.0 |
| | | 6 weeks | 1.08 \pm 0.11 | 3.0 \pm 0.0 |
| | | 12 weeks | 1.05 \pm 0.06 | 3.0 \pm 0.0 |
| II | Induced untreated group | Zero time | 0.77 \pm 0.10 | 3.0 \pm 0.0 |
| | | 6 weeks | 4.70 \pm 0.54* | 43 \pm 1.8* |
| | | 12 weeks | 7.05 \pm 0.44† | 57 \pm 3.0† |
| III | Sitagliptin treated group | Zero time | 0.90 \pm 0.10 | 3.0 \pm 0.0 |
| | | 6 weeks | 5.52 \pm 0.15* | 42 \pm 1.6* |
| | | 12 weeks | 2.50 \pm 0.20† | 18 \pm 1.6† |

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

| groups | | Aortic intima-media thickness (μ m) |
|------------|----------------------------------|--|
| I | Normal group | 47.0 \pm 2.7 |
| II | Induced untreated group | 289 \pm 53.7* |
| III | Sitagliptin treated group | 204 \pm 22.35* |

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

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

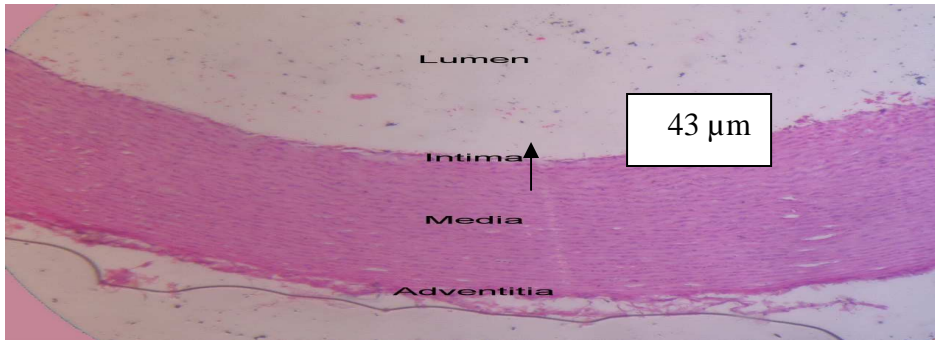
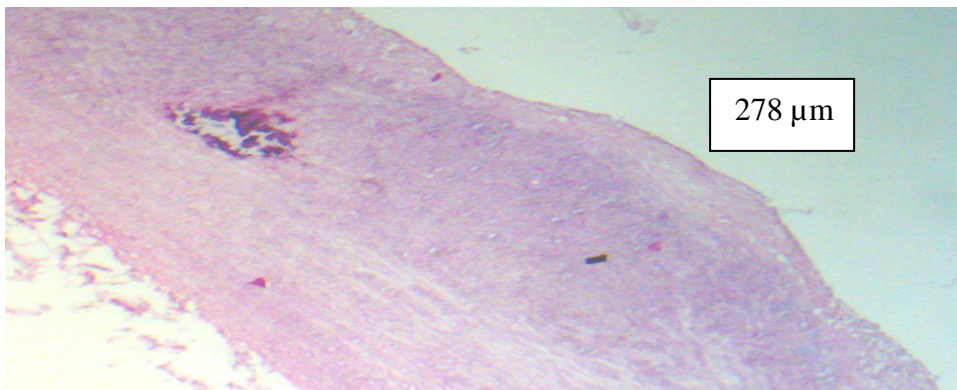


Figure (1): photomicrograph of histomorphometric section in aortic arch shows the normal appearance of arterial wall layers. The section stained with haematoxylin and eosin ($\times 4$)



Figure(2) photomicrograph of histomorphometric section in aortic hyperlipidemic rabbits shows a fibro-atheromatous plaque with thick layers of fibrous connective tissue overlying a largely necrotic, fatty mass. Advance atherosclerotic lesion (Type IV). The section The section stained with haematoxylin and eosin ($\times 40$)

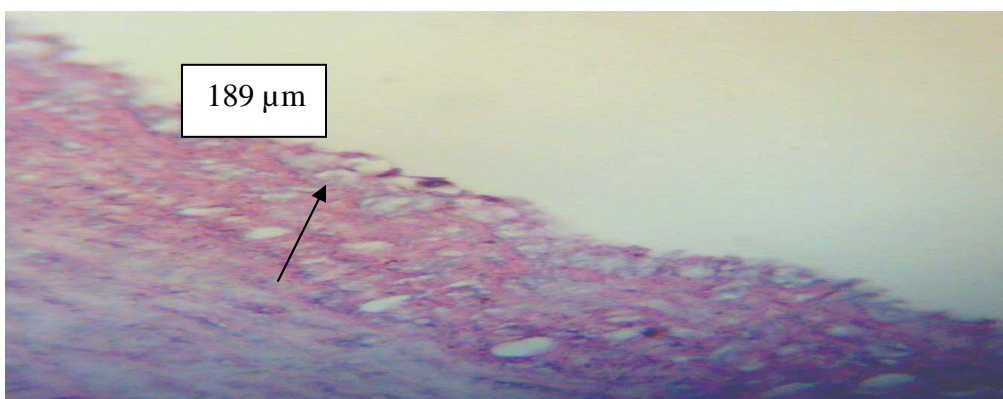


Figure (3) photomicrograph of histomorphometric section in aortic sitagliptin hyperlipidemic rabbits Show significant decrease in the aortic intima thickness as compare to induced untreated. section stained with haematoxylin and Eosin($\times 100$).

Discussion

In this study we demonstrate that high atherogenic diet cause significant increment in lipid parameter^(7, 8) (TC,TG,atherogenic index) in comparison with control group. Treatment with sitagliptin cause significant reduction in (TC,TG,atherogenic index) in comparison with induced untreated group⁽⁹⁾. In the present study sitagliptin treatment significantly reduce the elevation of inflammatory markers (hs.CRP, TNF α) in atherosclerosis model of hyper cholesterolemic rabbit^(10, 11) suggesting that sitagliptin inhibit vascular inflammation induced by high atherogenic diet⁽¹²⁾

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 status⁽¹³⁾. MDA level is widely utilized as a marker of lipid peroxidation and its measurement gives adirect evidence for LDL oxidation and is important 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 ROS through stimulation of polymorph- nuclear leukocytes (PMNLs) and dysfunction of endothelial cells^(14, 15).

Furthermore hypercholesterolemia, especially if prolonged, results in vascular oxidant burden^(16, 17), which could favor GSH depletion because of enhanced oxidation of the tripeptide or its consumption by electrophilic compounds like lipoperoxidation aldehydes^(18,19).

Sitagliptin treatment had significantly reduced aortic MDA level suggesting decrease in ROS and subsequent lipid peroxidation. Also sitagliptin had significant effect on aortic GSH levels where prevents GSH depletion in hypercholesterolemic rabbit, and thus, maintain antioxidant reserve which is important for vascular protection against lipid peroxide⁽¹²⁾

In rabbits treated with sitagliptin there was a significant reduction in the severity of atherosclerotic lesions in comparison with rabbits in the induced untreated group also there is significant decrement in aortic intima media thickness (P<0.05) of sitagliptin treated group compared with that of the induced untreated group. In our study we found that sitagliptin exert anti inflammatory effect by reducing (hsCRP, &TNF- α) and had antioxidant effect by reducing lipid peroxide (MDA) and enhancing GsH . So these findings may provide answers how sitagliptin reduce aortic intima-media thickness via suppression of systemic inflammatory response and oxidative stress.

Conclusion

The results of present study reveal that sitagliptin possess antihypertensive effects in experimentally induced atherosclerosis via interfering with inflammatory and oxidative pathways.

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