

Anti-inflammatory, anti-oxidative and Athero-protective effects of irbesartan in rabbits with atherogenic diet

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Abstract: Atherosclerosis is a dysfunction of endothelium due to inflammation of the blood vessel wall. In primary phases it characterized by activation and recruitment of macrophages. Irbesartan, an angiotensin receptor blocker, has ability to activate a peroxisome proliferator-activated receptor gamma. Numerous studies have been showed that treatment with angiotensin II receptor blockers (ARBs) can attenuate atherosclerotic plaque formation, however the underlining mechanism still obscure.

Objectives: The present study is to evaluate the athero-protective effects of irbesartan via reduction of inflammation and oxidative stress.

Methods: This study enrolled twenty four male rabbits which were randomly divided into three groups (each group 8 rabbits). All groups were fed for 12 weeks with normal chow (oxiod) diet (**group I**), normal chow (oxiod) diet with 0.05 % high cholesterol diet (**Group II**) and normal chow (oxiod) diet with 0.05 % high cholesterol diet together with irbesartan (0.5 mg/kg once daily at morning) (**Group III**).

Blood samples were analyzed at (zero time) and every four weeks of study to assess serum endothelin-1 (ED-1), intracellular adhesion molecule-1 (ICAM-1) and HDL-Cholesterol, triglycerides (TG), total cholesterol (TC). At the end the study (12 weeks) the aorta was collected to evaluate the aortic intimal thickness and the levels of aortic Malondialdehyde (MDA) and aortic reduced glutathione (GSH).

Results: Irbesartan treated group revealed insignificant change of lipid parameters of when compared with induced untreated group ($P > 0.05$). However Irbesartan significantly reduced the elevated ED-1, ICAM-1, aortic MDA and aortic intimal thickness and restored aortic GSH level compared with induced untreated group ($P < 0.05$).

Conclusions: Irbesartan could participate in reduction of atherosclerosis progression in rabbit fed with rich cholesterol diet by interfering with inflammatory and oxidative pathways.

Key words: Irbesartan, atherosclerosis , inflammatory markers, oxidative stress.

INTRODUCTION

Atherosclerosis is the main cause of mortality and morbidity in the most developing countries⁽¹⁾. It is the common cause of peripheral artery disease, strokes and heart attacks. It is a complex process, and it is maybe caused via sedentary life style and high-fat diet⁽²⁾. is One of the most important risk factors for cardiovascular disorders and atherosclerosis is Hypercholesterolemia. Atherosclerosis is a progressive functional and structural vascular disorder that initiates cellular and molecular episode triggered via endothelial dysfunction, leading to reduced nitric oxide production, raised production of endothelin-1 [ET-1] and activity of cyclooxygenase and inflammation⁽³⁾. Atherosclerosis incidence is 3–4 times larger in diabetics than non-diabetics patients at equivalent plasma total cholesterol levels⁽⁴⁾.

Angiotensin II through its type1 receptor promotes endothelial dysfunction, stimulates the oxidation of plasma lipoproteins and encourages inflammation in atherosclerotic plaques^(5,6). As the dysfunction of endothelium signify the initiation of atherosclerosis, enhanced inflammation supports the growth of weak plaques, and reactive oxygen species exert destructive effects such as the instruction of the apoptosis of smooth muscle cells and macrophage^(7,8).

The blockade of the Angiotensin II type1 receptor may suppress progression of atherosclerosis and stabilize vulnerable plaques⁽⁹⁾. Numerous studies showed that treatment with AT1 receptor blockers can satisfy atherosclerotic plaque development and decrease inflammation levels and cytokine expression⁽¹⁰⁾. It has been usually known that adhering of monocytes to vascular endothelial cells and converting into macrophage is a important position at the atherosclerosis prophase⁽¹¹⁾. Endothelial cell selectin (E-selectin), vascular cellular adhesion molecule-1 (VCAM-1) and intracellular adhesion molecule-1 (ICAM-1) play essential roles in this development⁽¹²⁾. In patients with coronary heart disorders, ICAM-1, VCAM-1, and E-selectin have been considered as biomarkers for the revealing of endothelial dysfunction. Thus, it may be an excellent method to inhibit the improvement of atherosclerosis by stopping the expression of ICAM-1, VCAM-1 and E-selectin⁽¹³⁾.

It has been extensively established that inflammation plays a vital role in atherogenesis and mediating all steps of this disorder from beginning through progression and the thrombotic obstacles of atherosclerosis⁽¹⁴⁾. The formation and progression of atherosclerosis contributes by inflammation and the therapeutic potential of some anti-inflammatory agents have been estimated for possible anti-atherosclerotic action. Recent findings propose that some drugs with anti-inflammatory actions appear to have useful effects on atherosclerosis or consequent risk for cardiovascular disorders. In Addition to that,

enhanced expression of vascular adhesion molecules like VCAM-1 play a significant role in the pathogenesis of atherosclerotic disorders⁽¹⁵⁾. Therefore, the ability drugs to interfering with oxidative stress and inflammation is measured as the therapeutic potential for in assessment of their anti-atherosclerotic activity⁽¹⁶⁾.

Irbesartan, one of the most widely used angiotensin receptor blockers, in addition to its role in the blockade of the AT1 receptor it has been suggested as a peroxisome proliferator-activated receptor gamma (PPARc) ligand⁽¹⁸⁾. Since PPARc activation also exerts anti-inflammatory effects and decrease the ROS production⁽¹⁹⁾, irbesartan may further suppress inflammatory chemokine expression level and reduce apoptotic cell death in atherosclerotic plaque. The antiatherogenic effects of irbesartan, however, have not been totally investigated, and the mechanisms underlying the therapeutic actions stay not clear⁽¹⁹⁾.

MATERIALS AND METHODS

Animals

In the present study twenty-four male rabbits (weighing 1.2-1.8 kg) were used. Rabbits were placed in the animal house of faculty of Pharmacy in Al Kufa-university. They were kept in cages in air conditioned room with 60–65% humidity, 25°C ± 2 temperature and a 12 hr light:12hr dark cycle. There was adaptation period of seven days before the experiment. The study was conducted according in the direction of the national guidelines for the Care and Use of Laboratory Animals. The study procedure was accepted by the High Committee for Review and Approval of Research Proposals in University of Kufa \ Faculty of pharmacy.

Study design

The animals were randomly grouped into three groups:

- 1. Control group (n=8):** rabbits were fed normal chow diet and tap water for 12 weeks.
- 2. Induced untreated group (n=8);** rabbits were fed high cholesterol diet (a 0.05% cholesterol) and tap water for 12 weeks
- 3.Irbesartan treated group (n=8):** All rabbits of this group were fed same high cholesterol diet (0.05%) in group II plus irbesartan (0.5 mg/kg) once daily at morning for 12 week.

Serum and tissue preparations

The blood samples were analyzed at zero time and every four weeks on experimental diets for assesment of serum HDL-Cholesterol, triglycerides (TG), total cholesterol (TC) ,endothelin-1(ED-1) and intracellular adhesion molecule-1(ICAM-1) level. At the end of the study, aorta was collected after scarification of animals and use to prepare homogenate of tissue which was made in media of phosphate- buffered (pH 7.4) , saline (0.1 M) that contain1% Triton-100 and protease inhibitor cocktail via using a high intensity ultrasonic liquid processor. The homogenates were centrifuged at 4 C^o and supernatants were used for evaluate the aortic intimal thickness and the levels of aortic Malondialdehyde (MDA) and aortic reduced glutathione (GSH).

Statistical analysis

Statistical analysis was done by SPSS 21.0 for windows Inc. Data were expressed as mean \pm SEM.; the means at different time for the same group was compared by paired t-test. Analysis of Variance (ANOVA) was used for multiple comparisons among all groups. Level of significance was $P < 0.05$ for statistical decision

RESULTS

There was a slight insignificant decrease in body weight of irbesartan receiving group suggesting that food consumption probably was similar in all the groups and cholesterol or irbesartan had no effect on body weight. Compared with the control group, levels of TC, TG, HDL-C,ED-1, ICAM-1, aortic MDA and aortic intimal thickness were significantly increased and aortic GSH were significantly decreased in the rabbits with atherogenic diet(induced untreated group) ($P < 0.05$).

Irbesartan treatment don't show significant effect on lipid parameters compared with induced untreated group ($P > 0.05$). Irbesartan was reduced the elevation in ED-1, ICAM-1, aortic MDA and aortic intimal thickness compared with induced untreated group ($P < 0.05$). Also it restored aortic GSH level ($P < 0.05$) .as shown in table (1), table (2) and table (3).

Table 1: Changes in the serum lipid profile (TC, TG and HDL-C) of the study groups. The data was expressed as Mean \pm SEM. Using paired T-test.

Group		TC(mg/dl)	TG(mg/dl)	HDL(mg/dl)
1. Control group (n=8)	Zero time	83\pm2.42	36.9\pm5.16	12.9\pm2.8
	12 weeks	88\pm3.66	34.9\pm3.2	13.5\pm3.1
2.Induced untreated Group (n=8)	Zero time	92\pm3.35	36\pm5.1	13.2\pm1.4
	12 weeks	895\pm23*	355\pm13.3*	35\pm4.8*
3.Irbesartan treated Group (n=8)	Zero time	86\pm5.4	33.8\pm5.1	13.4\pm3.9
	12 weeks	911\pm10.22*	322\pm12.1*	41.1\pm4.9*

***p<0.05**

Table 2: Changes in the serum inflammatory markers (ED-1 and ICAM- 1) in all experimental groups. The data was expressed as Mean \pm SEM .Using paired T-test.

Group		ED-1(pg/ml)	ICAM-1(pg/ml)
1.Control group (n=8)	Zero time	0.39\pm0.26	6.9\pm1.2
	12 weeks	0.44\pm0.15	7.2\pm0.8
2.Induced Untreated Group (n=8)	Zero time	0.41\pm0.32	7.1\pm0.7
	12weeks	1.9\pm0.59*	24.6\pm1.9*
3.Irbesaran treated Group (n=8)	Zero time	0.48\pm0.17	7.6\pm0.27
	12weeks	1.1\pm1.12*	17.3\pm1.8*

***p<0.05**

Table 3: The aortic oxidative stress parameters (MDA and GTH) and aortic intimal thickness of the three experimental groups at the end of the study .The data was expressed as Mean \pm SEM. Using paired T-test.

Group	Aortic MDA μ mole/gm aorta	Aortic GTH nmole/mg aorta	Aortic intima thickness (μ m)
1.Control_group (n=8)	3.1 \pm 0.44	35.9 \pm 2.9	27.4 \pm 2.9
2.Induced untreated Group (n=8)	9.7 \pm 0.71*	17.8 \pm 2.5*	288.2 \pm 22.8*
3.Irbesartan treated Group (n=8)	4.1 \pm 0.58**	31.3 \pm 2.2**	197.3 \pm 25.9**

*p<0.05

** p<0.05 as compare to induced untreated

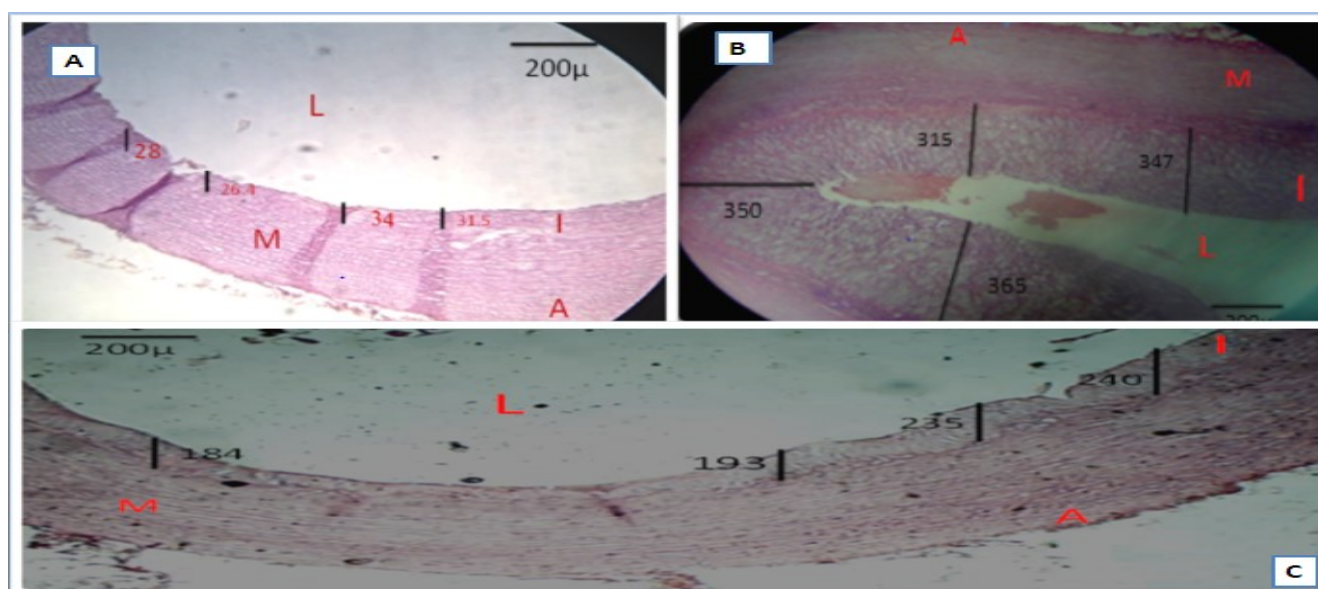


Figure (1) : Photomicrographs of rabbit aorta section taken 100X magnification. (L) lumen. (I) intima, (M) media, (A) adventitia & section stained with haematoxylin and Eosin ($\times 10$). (A) showed normal arterial wall; (B) aortic arch of rabbits on atherogenic diet for 10 weeks(induced untreated) show diffuse intima thickening & confluence of lipid collections creates an extracellular dense accumulation of fat in a well determined area (C) aortic arche of Irbesartan treated groups . Show significant decrease in the aortic intima thickness as compare to induced untreated

Discussion

In the current study, feeding of a cholesterol rich diet to animals for 12 weeks resulted in marked elevation in lipid profile parameters compared to zero time and control group. Interestingly, this study established that irbesartan treatment was significantly reduced total cholesterol and triglyceride while increased HDL-Cholesterol serum level compared to induced untreated group. Also, the results of present study demonstrated that atherogenic diet induced inflammatory response described via significant elevation in the serum level of ICAM-1 and ED-1 in comparison with that of control group. This is in agreement with that **(Zhang et al., 2006)** who indicated that hypercholesterolemia may lead to the elevation of CRP secretions by decreasing PPAR [gamma] expressions in adipocytes. Moreover, **(Bohm F., et al 2002)** showed that atherosclerosis cause reduction in nitric oxide production and elevation in endothelin-1 production, cyclooxygenase activity and inflammation^(20,21).

Interestingly, these inflammatory responses induced by atherogenic diet suppressed by irbesartan treatment to notable extent as revealed by reducing serum level of ICAM-1 and ED-1 than that in induced untreated groups. This is in agreement with **(Y. Jiang et al 2015)** who showed that irbesartan satisfy TNF α - induced ICAM-1, VCAM-1 and MCP-1 expression via the reduction of NF- κ B pathways. These findings suggested that irbesartan would be of large benefit to delaying the progression of inflammatory disorders, like atherosclerosis. Angiotensin II (which blocked by irbesartan) plays an vital role in the cardiovascular disorders like myocardial infarction and atherosclerosis⁽²²⁾. It causes the pro-atherosclerotic action. Reviewing recent studies, they have suggested that angiotensin II enhances ICAM-1, VCAM-1, and E-selectin expression through NF- κ B pathways⁽²³⁾.

In addition to that, the results of present study illustrated that diet rich with cholesterol lead to increment in lipid peroxidation and oxidative stress , which was marked via distinct elevations in aortic MDA level and remarkable reduction in aortic GSH level. These effects were predictable since the previous studies in rabbits have demonstrated that high cholesterol diet associated with enhancement of lipid peroxidation and reduction of antioxidant molecules^(24,25). In this study irbesartan was significantly inhibited the increase of aortic MDA level induced in induced untreated group suggesting decrease in ROS and consequent lipid peroxidation. Also irbesartan significantly elevate aortic GSH level so it prevented GSH reduction in hyper-cholesterolemic rabbit, and thus, preserved antioxidant reserve which is important for vascular defense against lipid peroxide. Therefore, irbesartan

has protective activity in cardiovascular and cerebro-vascular disorders associated with elevation of free radical production. Oxidative stress involves in many processes of atherogenesis, like LDL oxidation, endothelial cell injury and expression of adhesion molecules⁽²⁶⁾. In addition to that, inflammation plays an essential role in atherogenesis and mediating all levels of this disorder from initiation to progression and the thrombotic complications of atherosclerosis⁽¹¹⁾.

The current study established that irbesartan treatment significantly reduce the elevation in intimal thickness induced via atherogenic diet in rabbits as compared with induced untreated group.

Collectively, In this study we establish that irbesartan treatment exert anti inflammatory action by reducing (ED-1 and ICAM-1) and antioxidant action via decreasing lipid peroxide (MDA) and enhancing GSH. So these findings may give mechanistic answers how irbesartan decrease aortic intima thickness via number of pathways, including the inhibition of systemic inflammatory response and oxidative stress.

Conclusions

Irbesartan treatment effectively prevents atherosclerosis progression through interfering with inflammation pathways and oxidative stress.

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