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The Effect of Itraconazole on Aortic Histophatology in Hyperlipidemic Rabbits

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Atherosclerosis is a chronic inflammatory disease and begins with the dysfunction of the endothelial layer of the vessels and continues to change the nature of the area beneath Intima and accumulation of lipoproteins in this area. Itraconazole, with a wide range of antifungal features, has important attributes such as anti-inflammatory activity, its inhibitory effect on the activity of 5-lipoxygenase and effect of the chemotaxis of T-Cell. 40 rabbits were divided into 4 equal groups. The first group were fed normal diet, second group was fed high-cholesterol diet, the third group was fed with normal food and itraconazole drug and the fourth group was fed high-cholesterol diet and itraconazole and at the end of the twelfth week, the animals were anesthetized and aortic samples were taken, and information was studied after coloring. The mean size of the inner, middle and the adventitia of the aorta layers at the end of the twelfth week in the fourth and second groups were increased compared to control group, this increase was more obvious in the second group while it was reduced in the third group. In the second group at the end of the twelfth week a mass of foamy cells in the Intima layer was observed,while in the fourth group the amount was little and dispersed. Layers reduction of the aorta in the third group and also the fourth group compared to the second group can be caused by anti-inflammatory feature and antioxidant effects of itraconazole that avoid transient increase in blood fats after meal, which is highly atherogenic. Itraconazole drug affects the endothelial layer performance.

Key words: Itraconazole , Aorta, Histophatology, Hyperlipidemic, Rabbits

Atherosclerosis is a chronic inflammatory disease that begins with dysfunction of the endothelial layer of the vessels and continues to change the nature of the area beneath Intima and accumulation of lipoproteins in this area and, by erosion, rupture of the Intima layer and creation of thrombosis,the process of Atherosclerosis is complete (Higashi.2010). The initial step in the pathogenesis of atherosclerosis is endothelial dysfunction layer (Higashi. 2009). Moreover, activation of endothelial cells of vessels by vascular endothelial growth factor (VEGF) which is an angiogenesis factor may also increase the mechanisms of evolution pathophysiology and plaque instability of atherosclerosis (Holm. 2009). Inflammation plays an important role in the development of Atheroma. Inflammatory mechanisms cause Dyslipidemia and this disorder in fat accumulation causes the formation of Atheroma (Libby P. 2002). Leukocytes migrate

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beneath endothelial and the activation of T-Cells is considered among the most important steps in beginning atherosclerosis and its pathophysiology and plaque formation (Bogdanski P. 2007; Libby P. 2002). It is well documented that from the onset of initial damage to plaque formation, several molecular and cellular inflammatory cytokines are involved in the disease process (Kleemann R. 2008).

One of these proinflammatory cytokines is Interleukin 8 (Gustafson B. 2010). Recently, Han et al have reported wide anti-atherogenic role for interleukin in macrophages and include increased excretion of harmful lipoproteins, inhibition of inflammatory molecules and apoptosis reduction (Han X. 2009). In recent years it has been found that the 5-lipoxygenase pathway may play an important role in the pathogenesis of atherosclerosis. This information significantly increases the probability of effective diet treatment in atherosclerosis by Anti-leukotriene drugs (JawieD J. 2009). It is thought that increased serum levels of LDL-cholesterol areone of the major risk factors of atherosclerosis and cardiovascular illnesses while the opposite can be seen in patients with HDL-cholesterol (Jain KS 2009; Schneider B. 2007). Numerous studies show that diets rich in carbohydrates and fats increase harmful lipid and lipoproteins of plasma and will increase serum level of triglycerides, total and bad cholesterol,reduce the level of good cholesterol and increase the risk of cardiovascular disease (Albrink, 1986).

Itraconazole, with a wide range of antifungal attributes has the important feature of being anti-inflammatory (Vlckova-laskoska, 2009), specifically,this drug is a potent inhibitor of the activity of 5-lipoxygenase and by the formation of leukotriene prevent chemotaxis of neutrophil and interleukin eight production (Jaschonek K.1989). After 6 months of treatment with Itraconazole an increase in Interleukin 10 was observed (Gimenes VM. 2006). Han et al have reported that Interleukin 10 increases cholesterol removal by macrophages to protect against poisoning of the cholesterol accumulation in the cells (Han X. 2009). Itraconazole in particular prevented the progression of endothelial cell cycle in G1 phase in the laboratory and stops the vascular endothelial growth factor or dependent base of fibroblasts growth factor to angiogenesis (Chong CR.2007) In addition, Itraconazole has an inhibitory effect on the synthesis of cholesterol (Lütjohann D. 2009). With respect to the following items: 1-anti-inflammatory activity of Itraconazole 2- inhibition effect on 5-lipoxygenase activity 3- its inhibitory effect on chemotaxi of T-cell 4- inhibitory effect on the production of leukotriene 5- its effect on stopping the VEGF 6- its reducing effect on the (LDL-cholesterol) level 7- its increasing effect on the (HDL-cholesterol) level 8- its increasing effect on the IL-10 level, the study was conducted.

METHODS

In this study, 40 white male New Zealand rabbits weighing 2.2±0.5kg were purchased and transferred to Experimental Animal Center of Jahrom Medical University and were kept separately in individual cages and standard conditions (standard food, adequate water, temperature of 2±23 and humidity of 5±55 per cent). A week after the animals adaptation to the new environment, the rabbits were randomly divided into 4 groups of 10 rabbits. Then, while the rabbits were kept 12-15 hours in fasting condition, they were first weighed and then the blood was taken from the artery of rabbit ear, (day zero) and, in laboratory after the clotting of blood samples, the serum was separated by centrifugation at 3000 rpm for 15 min and until the pathology tests, serums were stored at -20° C. Then, using a triglyceride kit (Zist Shimi Company) and cholesterol (Pars Azmoon Company), levels of total cholesterol, triglycerides, LDL-C, HDL-C were determined. The next step was performed one day after blood sampling. The first group was considered as control group and received 130 g of standard food and enough water for 12 weeks. The second group was considered as a high cholesterol group and daily received 130 g of high fat diet and enough water for 12 weeks. 2 g of cholesterol was dissolved in 20 ml of sunflower oil and then mixed with 100 g of standard powdered rabbit food and poured in a case. . Prepared meals daily intake of 130 g of each rabbit was. The third group was determined as the group which received Itraconazole and for 12 weeks received standard food and enough water each day in addition to 80 mg oral capsules of Itraconazole of each kg body weight in which every ten milligrams was dissolved in one milliliter

of distilled water by gavage feeding (Jehangir K., 2000; Perfect JR. 1986). The fourth group, like the second group received a high-fat and adequate water diet and, furthermore, received itraconazole like the third group. At the end of the twelfth week all rabbits were injected by Intraperitoneal injection of Ketamine plus Xylazine and were anaesthetized respectively 50 mg and 20 mg per kg body weight and then the heart and aorta of each rabbit were severed and removed from the body, cleaned from the tissue section and fixed in ten percent buffered formalin. After the tissue fixation, the tissue sections were removed for staining with Hematoxlin-eosin (two lateral sections of three millimeters of chest aorta and two sections of abdominal aorta).

From the mentioned sections for staining with Hematoxlin-eosin after tissue processing, 5 micro-meter sections are prepared and stained with Hematoxlin-eosin and then randomly five painted sections from each sample were selected and evaluated microscopically and Intima layer tunica compared to the media layer tunica and the degree of plaque formation in the Intima layer was determined (Madhumathi BG .2006) and the degree of plaque formation were determined according to the report of Chekanov 2003 (Chekanov 2003).

Statistical analysis

Results were expressed as mean ± standard deviation. The method of statistical analysis of histological part of the design was completely random. To compare the means, SPSS software and one-way ANOVA test and Duncan post hoc test was used. Significant differences were considered in the level of P>/05.

Findings

Morphmetric results of aorta

Compared the mean and standard deviation (Std.Error) of studied variables of aortic cross of New Zealand white rabbits (based on micro meter μm) in high-cholesterol and itraconazole drug group (Group 4), normal diet plus itraconazole drug group (Group 3) high-cholesterol group (group 2) compared to the group with normal diet (control group or group 1) and also to compare the groups with each other at the end of the twelfth week (Table 1).

A: Compared the mean of the inner layer of the aorta (Intima) at the end of the twelfth week in the fourth group compared to the other groups and in the second group compared to the other groups and in the third group compared to the groups 2 and 4,significant difference was observed with P <0 / 05 , but in the third group compared to the control group significant difference was not observed with P <0 / 05.

mean size of the inner layer of the aorta is not significant in the fourth group which is about 1/22 times, in the third group it is about 94% and in the second group is about 3/8 times more than the control group. The inner layer of the fourth group of the aorta is about 1/31 times more than group 3 and is about 33% of second group. In the third group, its size is about 25% that of the second group.

B The mean size of the Media layer of aorta at the end of the twelfth week, in the second and third groups compared to the other groups and in the fourth group compared to the second and third groups, significant difference was observed with P <0 / 05 . But in the fourth group compared to the control group no significant difference was observed with P <0 / 05.

- The mean size of the Media layer of aorta is not significant in the fourth group which is about 1/08 times, in the third group it is about 75% and in the second group, about 2/2 times more than the control group. The size of the Media layer of the fourth group is about 1/44 times more than group 3 and about 50% of the second group. In the third group, its size is about 35% of the second group.

C In the mean size of the adventitia layer of aorta at the end of the twelfth week, in all four groups compared to each other significant differences were observed with P <0 / 05.

The mean size of the adventitia layer of aorta in the fourth group is about 1/19 times, in the third group it is about 83% and in the second group is about 2 times more than the control group. The mean size of the layer of the fourth group is about 1/43 times more than group 3 and is about 60% of the second group. In the third group, the mean size is about 42% that of the second group.

Table 1. Comparison of mean and standard deviations of studied parameters related to the aorta in white New Zealand rabbits (based on micro meter µm) in group with high-cholesterol diet (group 2), group with normal diet and itraconazole drug (Group 3) and group with high-cholesterol diet plus itraconazole drug (Group 4) compared to the same parameter in control group (group 1) and compared to each other at the end of the experiment

Parameters and Groups	Tunica Intima	Tunica Media	Tunica Adventit	The ratio of Tunica Media
Groups1	94/30 49/1 *	0/470 58/18 ☆	44/429 79/19 ★	065/0 002/0 ★
Groups2	89/113 77/6 ★	83/1016 93/1 ★	67/856 52/18 ★	1046/0 009/0 ☆
Groups3	17/29 17/2 *	00/355 68/19 ★	33/358 19/21 ★	085/0 007/0 ☆
Groups4	25/38 21/2 ☆	00/510 99/43 ✦	08/512 60/32 ★	086/0 0074/0 ☆

In parameters that have the number of exponent, the number of exponent indicating that this parameter has significant difference with the same parameter in another group that his number is written as a exponent number (P <0 / 05).
This symbol ★ indicates that this parameter is significantly different with the same parameter in the other three groups (P <0/05).
This symbol ☆ indicates that this parameter is significantly different with the same parameter in the second and third groups (P <0/05).
This symbol * indicates that this parameter is significantly different with the same parameter in the second and fourth groups (P <0/05).

D In comparing the mean size of the inner layer with the Media layer of the aorta (Intima / Media) at the end time of the experiment the fourth, third and second group toward the control group has significant difference with P <0 / 05 . But, a significant difference with P <0 / 05 was not observed between these three groups.
the mean size of the inner layer with the Media layer of the aorta in the fourth group is about 33%, in the third group it is about 32% and in the second group is about 62%, which indicates an increase compared to the control group.

Histopathology results of the aorta

1) In groups 1 and 3 there was no trace of atherosclerotic plaques at the end of the twelfth week. (Figures 1 and 3)
2) In the second group mass of foamy cells in the Intima layer was observed at the end of the twelfth week (Figure 2).
3) In the group of high-cholesterol diet plus itraconazole drug, fewer foamy cells compared to group 2 were observed in the Intima layer at the end of the twelfth week (Fig. 4).

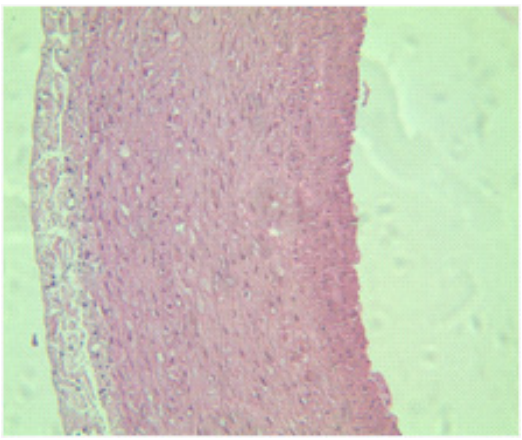

Fig. 1. A part of aorta of the control group (there is no trace of Atherosclerotic plaques in the control group at the end of the twelfth week)

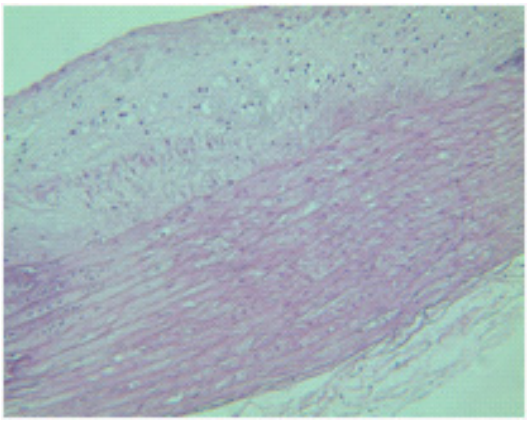

Fig. 2. A part of aorta of the second group (mass of foamy cells in the Intima layer can be observed in the high-cholesterol diet group at the end of the twelfth week that also increasing the tunica of the layer

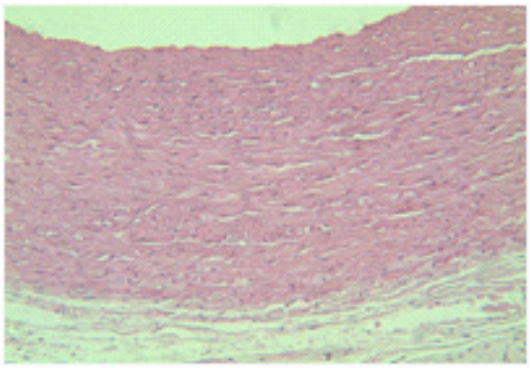

Fig. 3. A part of aorta of the third group (in the group who only received Itraconazole drug, there is no trace of Atherosclerotic plaques at the end of the twelfth week)

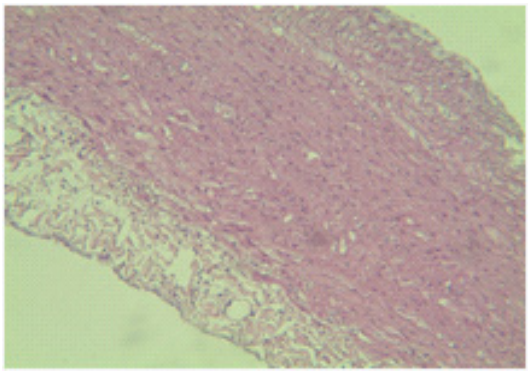

Fig. 4. A part of aorta of the fourthgroup (in the group receiving Itraconazole plus high-cholesterol diet ,scattered foamy cells in the Intima layer is observed at the end of the twelfth week of

DISCUSSION

high-cholesterol diet can affect the mechanisms of atherosclerosis initiation, because of acute changes in vascular endothelial cell function (Slyper, 1992) ,research has shown that high-cholesterol meals fairly increase blood fat , lipid peroxides and exacerbated of the destruction of endothelial function and this effect can be inhibited by antioxidants (Ross R, 2002). So itraconazole drug with high cholesterol diet which in this study significantly decreases cholesterol and triglycerides and bad cholesterol and 76% reduction of Indisaterogenic and significant reduction in the layers of the aorta compared with the high-cholesterol diet also can be caused by anti-inflammatory feature and probable effect of antioxidant effect of these drugs in which prevent transient increase of blood fats after meal that is highly atherogenic. According to Prasad K reports in 2003, increase of cholesterol and triglycerides and bad cholesterol and lowering good cholesterol through effects on Arachidonic acid metabolism and stimulates leukocytes increased production of free radicals and oxidative stress which leading to exacerbation of atherosclerosis followed by vascular damage (Prasad K, 2003). Quoted from Kraml P in 2004 ,increased production of free radicals and oxidative stress cause reduction of Nitric oxide synthesis in endothelial cells and reduction of Nitric oxide synthesis cause relaxation dependent to endothelium of flat muscle which ready the vessel for plaque formation. Antioxidants have been proposed as an effective factor in preventing the progression of the disease. Due to the effect of itraconazole drug on cholesterol lowering and liver enzymes in group 4 , its antioxidant effect is not unexpected (Kraml P, 2004).

Conclusions: in this study the itraconazole drug reduces aortic wall tunica so it is suggested that after further investigation and research on humans and determining the dosage, introduce this drug as an anti-atherosclerosis drug.

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