EFFECT OF AN ANTI-INFLAMMATORY DRUG (PIROXICAM) ON SOME BIOCHEMICAL FACTORS AND DEVELOPMENT OF EXPERIMENTAL ATHEROSCLEROSIS IN RABBITS

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ABSTRACT

Introduction: To investigate the effect of an anti-inflammatory drug (Piroxicam) on the development of fatty streak in rabbits.

Methods: Male rabbits were fed with four types of regimen: a) normal rabbit chow, b) 1% cholesterol diet c) cholesterol diet plus Piroxicam, d) normal rabbit chow plus Piroxicam. After 12 weeks, the animals were sacrificed and the aorta, as well as the right and left branches of the coronary artery were dissected, and histological processing was carried out. Samples were observed under light microscope with different magnifications. Some biochemical tests were measured before and after the treatment.

Results: A significant difference was found between the two groups receiving Piroxicam-supplemented diet and the two others ($p < 0.05$) in respect of the mean grade of fatty streaks in the right and left coronary arteries, however, progression of the lesion in aorta was not statistically significant.

Conclusion: Piroxicam was found to reduce C-reactive protein (CRP), triglyceride (TG) and LDL-C; it also led to an increase in antioxidant capacity and HDL-C. It is suggested that the anti-inflammatory drug, Piroxicam, has beneficial effects in preventing the development of fatty streaks.

Key words: Fatty streak, piroxicam, rabbit, inflammation.

C ardiovascular disease (CVD) remains a major cause of mortality worldwide in spite of the recent advances in medical and surgical treatments[1]. High serum cholesterol; especially the low-density lipoprotein fraction appears to play a major role in this process[2]. The true mechanisms of CVD stretch far beyond the role of known major risk factors. This is born out by the fact that despite lifestyle changes and the use of various pharmacological approaches to reduce plasma cholesterol, CVD are still the leading causes of mortality in developed and developing countries[3,4].

In fact, atherosclerosis damages are a sign of a series of very specialized cellular and molecular responses that overall can best be described as an inflammatory disease[5-8]. The earliest damage (fatty streak) that is prevalent in infants and young children is a pure inflammatory lesion including monocyte derived macrophages and T lymphocytes, and its formation in atherosclerosis seems to be the consequence of an increase in lipoprotein in some parts of the intima[7, 9]. The lipoproteins go through chemical changes and are absorbed by macrophages[10]. The macrophages then discharge inflammatory mediators, which help further cellular changes and more damage[1]. Given these facts, evaluation of the effect of anti-inflammatory drugs on the development of fatty streaks and biochemical factors of blood have special importance.

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METHODS

Animals and diets: Twenty New Zealand white male rabbits weighing between 2000-2500 g were housed in stainless steel mesh-bottomed cages and accustomed to a whole-grain commercial diet for two weeks.

Male rabbits were used to avoid any effect of the female hormonal cycle. Fasting blood samples were collected from the marginal ear vein of all rabbits before the intervention.

Five rabbits were assigned randomly to four groups and one was kept in each cage. These four groups of rabbits were fed by: a) normal rabbit chow, b) 1% cholesterol diet c) 1% cholesterol diet supplemented with Piroxicam, and d) normal diet supplemented with Piroxicam. Cholesterol-rich diet was used to provoke an atherosclerotic process and piroxicam used 6 mg daily. Cholesterol and peroxide-free ether from Sigma Inc were used. The cholesterol-rich diets were prepared by dissolving cholesterol in peroxide-free ether, spraying it over the diet and allowing the ether to evaporate in closed hood under nitrogen at room temperature. The cholesterol-free control diet was sprayed with an equal volume of peroxide-free ether. The diets were prepared weekly and stored at 4°C until use. Food and water were provided ad libitum.

Histological analysis: After 12 weeks, the rabbits were deprived of food for an overnight. After inducing anesthesia, their chests were opened, blood was withdrawn through heart puncture, and the aortas were removed immediately and washed with 0.9% ice-cold saline solution[10]. Fixed aortas were stained with Sudan IV stain to visualize areas of atherosclerotic plaques. Tissue for histological analysis was taken from the aortic arch where the most prominent plaques were found in both the right and left coronary arteries. Histological sections were stained with Hematoxylineosin. Samples were examined blindly to evaluate the presence of the fatty streaks, medical calcifications and development of fibrous plaque[11].

Lesions were scored on a four-point intensity semi-quantitative scale as follows: 1) absence, 2) mild, 3) moderate, and 4) intense for each type of damage presents (fatty streak, medical calcification and fibrous plaque).

Biochemical measurements: Before initiation of the diet and after 12 weeks of intervention, blood samples were collected from animals in each group. Total cholesterol, LDL-C, HDL-C, fasting blood sugar (FBS) and TG were measured enzymatically using standardized PARS-Azmun kits. Quantitative CRP was measured using the spectrophotometric method. Anti-oxidant capacity and malondia-ldehyde (MDA) were measured according to standard methods[12, 13].

Statistical analysis was performed using SPSS software. Comparison between the mean of biochemical data was performed with the Student's t-test and Mann- Whitney analysis. P-values below 0.05 were considered to be significant.

RESULTS

The severity of aortic atherosclerosis, as judged by gross grading was more marked in the group fed only with cholesterol. The control group did not show any evidence of atherosclerosis (Table 1). Histological findings showed significant decrease in the fatty streak formation in the group receiving cholesterol-rich diet supplemented Piroxicam, compared with the group fed cholesterol-rich diet, both on the left and right coronary arteries (P=0.016 and p=0.042, respectively) (Table 1).

Statistically significant differences were found between some biochemical factors of the control group and those of the cholesterol-rich group (Table 2).

There was no significant difference between the means of biochemical factors and weights of experimental groups in the beginning of the study.

Table 1: Comparing histological results of aorta and right and left coronary according to the diet group

	Control	Control +piroxicam	Cholesterol rich	Cholesterol rich + piroxicam
Aorta	0±0	0±0	3±0.70	2.2±1.30
Right coronary artery	0±0	0±0	2.4±1.14	1.6±1.81
Left coronary artery	0±0	0±0	2.8±0.83	0.8±0.83

The Mean±SD of fatty streak is compared according to the diet group. Using Piroxicam significantly decrease in the fatty streak formation in the group receiving cholesterol-rich diet.

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Table 2: Comparison of the Mean±SD of biochemical factors between the control group and cholesterol-rich group at the end of experimental period

	Anti-O %	Cht mg/dl	CRP mg/dl	FBS mg/dl	HDL-C mg/dl	LDL-C mg/dl	MDA mol/l	TG mg/dl
Control diet	76.25±8.24	40.75±11.58	2.88±2.24	97.75±6.65	19.75±7.13	10.65±7.59	1.1±0.22	80.5±15.67
Cholesterol rich diet	48.86±18.36	2806±884.2	5.42±1.69	83.4±12.4	17.2±1.3	2740±858.89	1.23±0.43	221±149.12
P	0.02*	<0.0001*	0.1	0.07	0.5	<0.0001*	0.6	0.01*

Anti-O: Anti-oxidant capacity   Cht: Cholesterol   CRP: C - reactive protein   FBS: Fasting blood sugar
MDA: Malondialdehyde   TG: Triglyceride

Table 3: Comparison of the Mean±SD of biochemical factors between the control group and control group supplemented piroxicam at the end of experimental period

	Anti-O %	Cht mg/dl	CRP mg/dl	FBS mg/dl	HDL-C mg/dl	LDL-C mg/dl	MDA mol/l	TG mg/dl
Control diet	76.25±8.42	40.75±11.58	2.8±2.24	97.57±6.65	19.75±7.13	10.65±7.59	1.1±0.22	80.5±15.67
Control diet +piroxicam	68.2±13.36	50±11.51	1.8±1.23	86.2±11.77	26.6±5.03	11.76±9.22	1.1±0.321	58.2±14.06
P	0.3	0.27	0.04*	0.1	0.1	0.8	0.9	0.05*

Anti-O: Anti-oxidant capacity   Cht: Cholesterol   CRP: C - reactive protein   FBS: Fasting blood sugar
MDA: Malondialdehyde   TG: Triglyceride

Table 4: Comparison of the Mean±SD of biochemical factors between the cholesterol-rich group and cholesterol-rich group supplemented with piroxicam the end of experimental period

	Anti-O %	Cht mg/dl	CRP mg/dl	FBS mg/dl	HDL-C mg/dl	LDL-C mg/dl	MDA mol/l	TG mg/dl
Cholesterol rich	48.8±18.36	2806±884.24	5.42±1.6	83.4±12.42	17.2±1.3	2740±858.899	1.23±0.43	221±149.12
Cholesterol rich + piroxicam	56.6±16.47	1824±882.83	2.68±1.59	83.6±16.04	21.8±2.28	1311.66±849.62	1.07±0.116	349.2±384.5
P	0.5	0.11	0.8	0.9	0.004*	0.06	0.4	0.5

Anti-O: Anti-oxidant capacity   Cht: Cholesterol   CRP: C - reactive protein   FBS: Fasting blood sugar
MDA: Malondialdehyde   TG: Triglyceride

At the end of the experimental period, significant decrease was observed in the amount of TG and CRP in the control group supplemented with Piroxicam, compared with the control group (Table 3).

HDL-C was significantly higher in the group fed cholesterol-rich diet supplemented with piroxicam, compared with the group fed cholesterol-rich diet (Table 4).

The severity of aortic atherosclerosis was more prominent in the group fed cholesterol alone compared with the group fed cholesterol supplemented with Piroxicam, but the difference was not significant (P=0.34).

DISCUSSION

Based on the injury hypothesis and according to Rayer, atherosclerosis has an inflammatory nature. This is confirmed by Virchow who suggests that minor injuries to the coronary artery are a result of inflammation, itself causing an increase in the plasma components of the coronary intima[14]. Gore and Sapphire have pointed to evidences suggesting the existence of an inflammatory response to injury in atherosclerosis disease[14]. In fact, every injury had a different stage of a deep inflammatory process in the artery which if not seen to with care, will cause a progressive and complicated damage[6, 15].

In light of laboratory findings in studies on atherosclerosis involving animals and humans, it can be concluded that fatty streaks are the first damage in the atherosclerotic process, and their formation seems to be highly due to the increase in lipoprotein in certain areas of the intima. Such lipoproteins may go through biochemical changes such as oxidation or non-enzymatic

glycosylation and cause pathogenic variations in the development of atherosclerosis[1].

Macrophages absorb such altered lipoproteins and change them to foam cell and discharge inflammatory mediators which in turn will make more cellular changes and damage. Therefore, the above-mentioned inflammatory process plays a key role in making anyone prone to atherosclerosis[16]. Autopsy of plaque parts has shown the presence of active inflammation at the site of macrophages[17].

Severe coronary syndromes usually occur as a result of the mechanical relation of atherosclerosis plaques and the process of inflammation[18]. These are all in conformity with the main finding of this study, i.e. reduction of fatty streak formation in rabbits fed high-cholesterol diet supplemented with the anti-inflammatory drug, Piroxicam, compared to those fed solely high-cholesterol diet.

High-cholesterol diet causes an increase in LDL-C and a decrease in HDL-C and antioxidant capacity, all of which are in favor of fatty streak formation and the increase in CVD risk.

By contrast, Piroxicam in high-cholesterol diet groups decreased total and LDL cholesterol, and increased HDL-C, in comparison to high-cholesterol diet groups receiving no Piroxicam. Piroxicam also reduced CRP, which is an inflammation marker and predict coronary heart disease [19-20]. Moreover, piroxicam reduced TG in the group receiving a usual diet.

This study demonstrates that the use of the anti-inflammatory drug, Piroxicam, slows down the atherosclerosis process by increasing the antioxidant capacity and HDL-C levels, and decreasing CRP, LDL-C and TG.

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