The Effect of Atherogenic Diet with or without Enzyme Inhibitors on the Incidence and Progress of Atherosclerosis in Rabbits

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ABSTRACT

PON1 (Paraoxonase – 1) is an esterase enzyme which is associated with high density lipoprotein (HDL). The enzyme prevents the peroxidation of low density lipoprotein (LDL). The susceptibility of LDL for oxidation is the proven risk factor for coronary artery diseases. The aim of this study is to survey the effect of atherogenic diet with or without enzyme inhibitors on the incidence and progression of atherosclerosis in rabbits. Twenty four New Zealand white rabbits divided into three groups (control, under the atherogenic diet, atherogenic diet and nandrolone decanoate - paraoxonase inhibitor therapy) and were treated for two months. At the beginning and end of the treatment, 5 mL of blood was obtained to determine the levels of total antioxidant capacity (TAC), HDL, malon dialdehyde (MDA) and PON1. After sixty days rabbits anesthetized under standard conditions, and sampling carried out from heart arteries for pathological examinations. Data were analyzed using statistical software SPSS/15 and one way ANOVA and paired t-test statistical tests. The results showed that the plasma levels of TAC, HDL, MDA and PON1 had significant changes in this study (P<0.05). The pathological study showed that in the presence of PON1, the formation and progression of atheroma is diminished. The results of this study showed that cholesterol-rich diet decreased serum level of PON1 which in turn led to a reduction in formation and progression of atheroma. It was shown that the enzyme inhibitor helps accelerating the development of atheroma.

1. Introduction

It has been shown that increased levels of LDL leads to the oxidation of endothelial cells due to free radicals and the structural changes that ultimately accelerate the process of atherosclerosis.¹ Paraoxonase (PON1) is an esterase enzyme which is carried on HDL-C. This enzyme prevents from LDL-C peroxidation.² It has been proven that the susceptibility of LDL to oxidation is a risk factor in coronary disease while HDL is considered a protective factor because of the reverse cholesterol...
transport from tissues to the liver and its antioxidant affect due to paraoxonase enzyme. Studies showed that the biological factors involved in atheroma production somehow affect the levels and the activity of paraoxonase. On the other hand, it is reported that PON1 levels increase in artery walls with atheroma. However, whether the presence of increased PON1 levels has a protective role or not remains controversial. Although some studies report a role of reduced enzyme in atherosclerosis, but are not clear whether atheroma formation is reduced with paraoxonase, or decreased paraoxonase activity is caused by progression of atherosclerosis. This study investigated the impact of atherogenic diet with or without enzyme inhibitors on the incidence and progression of atherosclerosis in rabbits.

2. Materials and Methods

Twenty four New Zealand rabbits were divided randomly into three groups of eight rabbits (Control, Atherogenic and Atherogenic plus inhibitor). 2% Cholesterol were added to animals’ diet for two months to induce hypercholesterolemia In order to do this, 32 grams of MERK cholesterol powder mixed with some corn oil was used for preparing the food and was impregnated with 1600 g normal rabbit food (Chow). The prescribed amount was 100 g per rabbit per 24 hours. At first, 5 mL blood was collected from all animals to evaluate the total antioxidant capacity (TAC), malondialdehyde (MDA) HDL and PON1. Then plasma was separated and stored in -76°C to measure the described factors. The rabbits in both groups were kept in similar conditions. They were placed under Dekanvat diet and subjected to nandrolone injection. After 60 days blood sampling were repeated. Then the rabbits were anesthetized under standard conditions and samples were taken from their coronary arteries for pathological examination.

2.1. Measurement of blood biochemical factors

2.1.1. Measuring metabolic parameters

Cholesterol, triglycerides, HDL and LDL in plasma were measured by the Abbott autoanalyzer using enzymatic methods with standardized kits (Pars test).

2.1.2. Measurement of PON-1 enzyme activity

Ariel esterase enzyme activity of PON-1 was measured using phenyl acetate substrate (Fluka). Initial rate of phenyl acetate hydrolysis was determined with a spectrophotometer at 37 °C and 270 nm wavelength (with extinction coefficient equal to 1310). The results were expressed as unit per millilitre (U / mL). Each unit of Ariel esterase activity is the amount of enzyme that can hydrolyze one micro mole of phenyl acetate per minute in each milliliter of serum.

2.1.3. Measurement of MDA levels

Plasma malondialdehyde was measured based on reaction with thiobarbituric acid (TBA). The extraction was performed with normal butanol, and measurement was done using a spectrophotometer and absorption were compared with standard curve.

2.1.4. Measurement of TAC level by colorimetric method

In this method, ABST is incubated with methemoglobin (as peroxidase) and hydrogen peroxide to produce cation radicals ABST⁺. This radical is greenish blue color, relatively stable, and measurable at 600 nm wavelength. Antioxidants in samples are reduced. Color intensity was formed which is proportional to its concentration in the sample.

2.2. Pathologic review of vessels

Rabbits were anesthetized under standard conditions and samples were taken from heart for pathological examinations. Samples were fixed and after cutting and staining Atroma progress in heart arteries, they were identified and evaluated according to the rating of American pathologists.

2.3. Statistical analysis

Recorded data were analyzed using SPSS-15, descriptive statistics (percentage, mean and standard deviation), one way ANOVA tests, and paired t-test.

3. Results

3.1. Formation and Progression of Atheroma

Atheroma formation was present in 80% of rabbits in the atherogenic diet group, while 20% of this group did not have evidence of atheroma formation. Among rabbits receiving enzyme inhibitor regimens, 14.3% of the rabbits did not develop atheroma, 28.6% developed type I atheroma, and 57.1% developed type II atheroma. None of the control group developed atheroma. Square test showed significant difference between the three groups in terms of formation and progression of atheroma (P=0.007) (Figure 1).

![Comparison of the formation and progression of atheroma in three groups of rabbits receiving atherogenic diet with or without enzyme inhibitors and controls after the sixty days of treatment.](image-url)

The average amount of cholesterol in sixtieth day was 198 ± 14 mg in atherogenic diet group, 251± 69 mg in
diet group plus inhibitors, and 172±8 mg in the control group. The ANOVA showed significant difference (P=0.045) among the three groups. Similarly, according to one way ANOVA there was a significant difference (P=0.001) among the three groups in triglyceride level. On the contrary, significant differences were not observed (P=0.779) among the three groups in serum levels of LDL in the sixthtieth day. The average level of HDL in serum after 60 days treatment was 24.0±4.1 in atherogenic diet, 18.0±2.3 in enzyme inhibitor regimens, and 32.0±3.2 mg/100mL in control group. Based on the ANOVA there was a significant difference (P=0.022) between the three groups (Table 1). According to Table 1, in the sixthtieth day, the average of TAC was 0.70±0.06 in atherogenic diet group, 0.76±0.03 in enzyme inhibitor regimens, and 0.77±0.04 mmol/L in the control group. The difference among these three groups was not statistically significant. The average level of MDA as an antioxidant parameter in the sixthtieth day was 14.50±3.0 in atherogenic diet group, 16.30±2.10 in enzyme inhibitor, and 6.90±1 µM/min/mL in the control group. Based on one-way ANOVA there was a significant difference (P=0.000) between the three groups in terms of changes of MDA.

**Table 1**: Comparison of lipid factors, total antioxidant lipid and lipid peroxidation quantities in three groups of rabbits receiving atherogenic diet with or without enzyme inhibitors and controls after the sixty days of treatment.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Atherogen</th>
<th>Atherogen + Inhibitor</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chol (mg/dL)</td>
<td>172±8</td>
<td>198±14</td>
<td>251±32</td>
<td>0.045</td>
</tr>
<tr>
<td>TG (mg/dL)</td>
<td>105±5</td>
<td>179±13</td>
<td>297±42</td>
<td>0.001</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>32±2</td>
<td>24±1</td>
<td>18±3.2</td>
<td>0.022</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>122±13</td>
<td>160±18</td>
<td>141±36</td>
<td>0.700</td>
</tr>
<tr>
<td>TAC (Mm/L)</td>
<td>0.77±0.01</td>
<td>0.70±0.06</td>
<td>0.67±0.03</td>
<td>0.160</td>
</tr>
<tr>
<td>MDA (nM/mL)</td>
<td>6.9±1.2</td>
<td>14.5±3.0</td>
<td>16.3±2.1</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Mean levels of serum PON1 activity was found to be 2.50±0.29 in sixty days in atherogenic diet group, 2.80±0.50 in enzyme inhibitor regimens and 3.37±0.26 µM/min/mL in the control group. Based on one-way ANOVA this difference was statistically significant (Figure 2).

**4. Discussion**

Most studies have considered a major role for antiatherogenic enzyme PON1 in cardiovascular disease and suggested that this effect is due to the decrease in oxidative stress. PON1 can hydrolyze cholesteryl esters and oxidize special phospholipids that reside in oxidized lipoproteins. Malin and his colleagues showed that there is a inverse relationship between level of serum PON1 activity and lipid peroxidation quantities. Also marked aggregation of peroxidized lipids decrease in LDL in the presence of PON1 was reported. HDL isolated from mice lacking the gene PON1 is not able to prevent oxidation of LDL and on the other hand HDL isolated from control rats have such a function. Also Aviram and colleagues have shown that oxidized lipids in oxidized LDL and HDL may have the capacity to disable the PON1. Decreased quantities of PON1, HDL and increased levels of cholesterol and triglyceride indicate the role of the enzyme inhibitor and atherogenic diet particularly in experimental groups. In this study, we measured the quantity of malondialdehyde and TAC in order to assess the level of oxidative stress. TAC level was not significantly different in group receiving atherogenic diet and atherogenic diet plus inhibitors compared with the control group. However MDA level is an indicator of lipid peroxidation and is remarkably increased compared to the control group. Our results, the changes in TAC and MDA level, in this study are consistent with findings of other studies. Gotto and colleagues showed that in their study the levels of TAC decrease in the patients with CAD (Coronary Artery Disease) compared with the control group. Furthermore Azarsiz and colleagues reported in their reports that MDA level is increased in patients with CAD compared with the control group. The survey of the relationship of paraoxonase and atherosclerosis in the presence of enzyme inhibitor shows that the level of PON1 is significantly reduced in sixtieth day. As expected atherogenic diet as an accelerating factor of progression of atheroma lead to this condition in 80% rabbits. Also, enzyme inhibitors lead to acceleration in formation of atheroma by reducing blood levels of enzyme PON1. In general we can conclude that it will increase atheroma formation in the absence of Paraoxonase. Our findings are consistent with other studies. Gotto and colleagues showed that paraoxonase activity significantly decreases with the increase in the number of involved vessels. Serdar and his colleagues reported that paraoxonase activity is decreased with the increase in the number of blocked vessels.
Ethical issues

The study was approved by the Ethical Committee of the University.

Conflict of interests

No conflict of interest to be declared.

References