Experimental Models Regarding Cardiovascular Diseases Prevention through Guided Diet

Marian MIHAIU1), Viorel MICLAUS1), Ovidiu ROTARU1), Sorin Daniel DAN1), Romolica MIHAIU2), Alexandra LAPUSAN1), Raluca Florina GHERMAN1), Crina CORBEANU1), Carmen JECAN1)

1)University of Agricultural Sciences and Veterinary Medicine, Faculty of Veterinary Medicine, 3-5 Calea Manastur Street, Cluj-Napoca, Romania; m.mihaiufmv@yahoo.com
2)Babes Bolyai University, Faculty of Economics and Business Administration, 57-58 Teodor Mihaly Street, Cluj-Napoca, Romania; mromi19@yahoo.com

Abstract. Consumers are becoming more aware of the impact on their health of the food they eat. One of the possible ways to reduce the risk of cardiovascular disease is consuming more polyunsaturated fatty acids (PUFA), particularly n-3 fatty acids. This study was undertaken to determine whether omega-3 fatty acids have effects on the serum level of cholesterol and triglycerides and anatomopathologic changes. Twenty-one Guinea pigs (12 weeks old) were randomly assigned to and fed a normal, high fat (0.5 % cholesterol) without (CD) or with Omega-3 (COD) diet for 12 weeks. The CD diet increased serum triglycerides, total and LDL cholesterol levels by 50.28%, 50.51% and 40.98 % respectively. Adding Omega-3 (0.6 mg/kg) in diet reduced the serum level of total cholesterol (59mg/dl vs. 39 mg/dl), triglycerides (94 mg/dl vs. 41 mg/dl) and LDL-C (45 mg/dl vs. 31 mg/dl). The animals fed with CD diet developed fibrosis of myocardium and artery besides those fed with COD diet where it was a marked reduction in coronary degenerative lesions from myocardium. We conclude that in our experimental model, dietary n-3 PUFAs decrease the development of coronary artery diseases, through changes in serum levels and anatomopathologic aspects.

Keywords: diet, coronary diseases, fatty acids, serum level, Omega-3

INTRODUCTION

Reducing intake of saturated fat and dietary cholesterol and avoiding excess calories, remain the cornerstone of the dietary approach to decreasing risk of atherosclerotic vascular disease. During the past 20 years, however, there has been renewed interest in other dietary components that might favorably improve lipid profiles and reduce risk of coronary heart disease (CHD). (Stone, 1996). Dietary intake of n-3 polyunsaturated fatty acids (PUFA), mostly derived from fish or as pharmacological supplements, is associated epidemiologically with cardiovascular protection. Randomized intervention trials have shown that n-3 PUFA reduce mortality endpoints, specifically sudden death and fatal myocardial infarction. (Albert et al, 2002; Kris-Etherton et al, 2002) Many studies have reported a negative relation between intake of omega-3 fatty acids (FAs) and CVD incidence and/or mortality (Osler et al., 2003; Ervin et al., 2004; Psota et al., 2006). Omega-3 PUFAs generally exert their cardioprotective effects through changes in lipids and lipoproteins. In addition, Omega-3 FAs especially EPA and DHA contribute benefits through their antiarrhythmic, anti-inflammatory, antithrombotic effects. Moreover, EPA and DHA also improve vascular endothelial function and help lower blood pressure, platelet sensitivity (Wijendran and Hayes, 2004).
The aim of this study was therefore to prove from an anatomopathologic, histologic and biochemical point of view the supposed protective role of the Omega 3 fatty acids in the prevention of the cardiovascular diseases induced by a nutrition high in cholesterol, using experimental models, based on a controlled diet.

MATERIALS AND METHODS

Materials
Concentrated fish oil was extracted from Omacor capsules (Pronova, Norway), cholesterol was obtained from sheep wool powder, ~95% (GC) (Sigma-Aldrich), and the standard chow for Guinea pigs was obtained from Pannonmill Takarmany Kft., Hungary.

Animals and Diets
Twenty-one Guinea pigs were used for this study, which were randomized to three different dietary regimens: a standard chow diet, a high fat (0.5 % cholesterol) diet (CD) and high fat (0.5 % cholesterol) with Omega-3 (0.6 mg/kg) diet (COD). Guinea pigs were chosen because they are excellent models to evaluate the mechanisms by which diet interventions and drug treatments alter plasma lipids and lipoprotein metabolism (Fernandez and Volek, 2006).

Guinea pigs were housed five per cage, in a temperature-controlled environment with a 12-h light–dark cycle, and allowed free access to food and water. Body weight was recorded once a week. The animals were sacrificed after 12 weeks on the experimental diet.

Serum determinations
Fasting serum samples were obtained for cholesterol and triglyceride determinations at baseline and after 4, 8 and 12 weeks on the experimental diet. The blood was collected from the retro orbicular sinus in serum separator tubes, and centrifuged immediately. Serum was then stored at 0-4 °C until analysis. The samples were analyzed with Konelab 20i clinical chemistry analyzer.

Anatomopathological aspects
After 12 weeks on experimental diets, Guinea pigs were sacrificed. The heart, including the aortic root was removed and prepared for histological procedures. Olympus CX41 microscope with camera was used for microscopic exam.

RESULTS AND DISCUSSION

Serum determinations
In the case of the control group all values of the parameters analyzed were according to the standard limits characteristic to this specie and didn’t present any significant changes during the entire experiment. (tab.1.) Therefore, the diet with an optimal lipid concentration (the combined diet administrated to this group contained 2.39% fat) does not determine an overstepping of the superior limits in the biochemical parameters.

After 6 weeks, during which the guinea pigs were administrated a high fat diet with 0.5% cholesterol, the values of the parameters studied in the case of the positive group raised as following: total cholesterol from 48mg/dl to 89 mg/dl, LDL cholesterol from 36 mg/dl to 47 mg/dl, HDL cholesterol from 4 mg/dl to 9 mg/dl, and triglycerides from 88 mg/dl to 129 mg/dl. Although their values have risen compared to the initial ones, HDL cholesterol and LDL cholesterol values are in between the normal limits. The total cholesterol and triglyceride values moderately overstep the standard limits. These results are in accordance with the other data from studies done in this field, Lin et al. (1994), obtaining similar results. After 12 weeks, the values of the parameters studied are higher also from baseline as well as
from day 45. It can be noticed that the cholesterol and triglyceride values have doubled compared to the initial ones, and the HDL cholesterol value has overstepped the superior limit. It is known as a well fact that the successive administration of cholesterol over a long period is directly proportional to the raising of the biochemical values in time. Therefore, we can observe that at 6 weeks from the diet’s administration with an add of 0.5% cholesterol the values are slightly higher, at 12 weeks, these values have doubled compared to the initial ones. The atherogen dyslipidemia includes the triad: high triglyceride level, a lower HDL cholesterol value, and the increase of the LDL cholesterol level. The triglyceride value is a risk factor independent for cardiovascular diseases. The raising of this value can be due to an endothelial dysfunction. (Shekelle et al, 1989).

In the case of the Omega-3 group it can be noticed that the administration of a diet with an add of 0.5% cholesterol at the same time with the diet supplement Omacor has led to a decrease in the majority of the parameters studied. At 6 weeks it can be seen the decrease in the total cholesterol value from 59 mg/dl at 56 mg/dl, LDL cholesterol from 45 mg/dl to 39mg/dl, and triglycerides from 94 mg/dl to 73 mg/dl. The exception remains in the HDL value which remains constant. The parameters values from the 12th week are lower compared to the values obtained in the 6th week as well as baseline, exception making the HDL cholesterol which presents a growing from 11 mg/dl to 15 mg/dl. This fact is explained by the benefic action that the polyunsaturated fatty acids with long chain have in the organism: they enrich the lipid rapport from blood decreasing the total cholesterol, triglycerides and VLDL, and at the same time increasing the HDL (benefic cholesterol). It can be noticed that the unsaturated fatty acids with long chain Omega – 3 types, acts especially on the serum level of the triglycerides, their value reducing to half. This fact can be explained by the step by step accumulation of these acids in the organism.

<table>
<thead>
<tr>
<th>Diet</th>
<th>Total cholesterol</th>
<th>Tryglicerides</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>6 weeks</td>
<td>12 weeks</td>
<td>Baseline</td>
<td>6 weeks</td>
<td>12 weeks</td>
</tr>
<tr>
<td>Standard diet</td>
<td>51 mg/dl</td>
<td>58 mg/dl</td>
<td>65 mg/dl</td>
<td>82 mg/dl</td>
<td>90 mg/dl</td>
<td>120 mg/dl</td>
</tr>
<tr>
<td>CD diet</td>
<td>48 mg/dl</td>
<td>89 mg/dl</td>
<td>97 mg/dl</td>
<td>88 mg/dl</td>
<td>129 mg/dl</td>
<td>177 mg/dl</td>
</tr>
<tr>
<td>COD diet</td>
<td>59 mg/dl</td>
<td>56 mg/dl</td>
<td>39 mg/dl</td>
<td>94 mg/dl</td>
<td>73 mg/dl</td>
<td>41 mg/dl</td>
</tr>
<tr>
<td>Diet</td>
<td>LDL cholesterol</td>
<td>HDL cholesterol</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Baseline</td>
<td>6 weeks</td>
<td>12 weeks</td>
<td>Baseline</td>
<td>6 weeks</td>
<td>12 weeks</td>
</tr>
<tr>
<td>Standard diet</td>
<td>48 mg/dl</td>
<td>42 mg/dl</td>
<td>52 mg/dl</td>
<td>6 mg/dl</td>
<td>17 mg/dl</td>
<td>19 mg/dl</td>
</tr>
<tr>
<td>CD diet</td>
<td>36 mg/dl</td>
<td>47 mg/dl</td>
<td>61 mg/dl</td>
<td>4 mg/dl</td>
<td>9 mg/dl</td>
<td>9 mg/dl</td>
</tr>
<tr>
<td>COD diet</td>
<td>45 mg/dl</td>
<td>39 mg/dl</td>
<td>31 mg/dl</td>
<td>11 mg/dl</td>
<td>11 mg/dl</td>
<td>15 mg/dl</td>
</tr>
</tbody>
</table>

* Values are given as mean, in mg/dl

Anatomopathological lesions

In the case of the histological slides made from the samples taken from the control group, it can be noticed the normal histological structure in the arterial level. Therefore, the intima’s endothelium is smooth, without any disconnections of the elastic limitant and without any modifications in the muscular layer (fig. 1).
The slides sampled from the positive group presented significant histological modifications. In the myocardium (fig. 3) it can be seen the forming of the subendocardic fibrous nodules (dark red arrow), the rapport between the muscular tissue and the conjunctive one is in favor of the second one. Also we can see the presence of the diffuse interfascicular edema. The fibromatous proliferation (orange arrow) can also be seen in muscular layer of artery and subendothelial (fig. 2).
The histological slides made from the organs of the guinea pigs from Omega-3 group presented microscopic alterations in a less advanced stadium compared to the positive control lot. In figure 4 and 5 it can be seen the histological alterations in the myocardium, of a reduce intensity compared to the positive group, because of the protection given by the Omega-3 fatty acids. Therefore, it can be seen the intima’s thickness, infiltrated by the fatty cells beneath the vascular endothelium (blue arrow) and edema in the muscular layer (red arrow). It can be seen the enlarged macrophages (fatty cells) aspect and the nucleus pushed toward the periphery.

CONCLUSIONS

1. The cholesterol’s sanguine concentration grows in direct proportion to the period of the high fat diet’s administration. (0.5% cholesterol adding).
2. Dietary cholesterol in high percents determines the increase of the biochemical parameters involved in the cardiovascular pathogenesis, especially the total cholesterol (with 50.51%) and the triglycerides (with 50.28%).

3. In what concerns the biochemical parameters, the Omega – 3 influences clearly in the most obvious way the level of the triglycerides and total cholesterol, in decreasing their levels with 56.38%, and respectively 33.33%.

4. Omega-3 intervenes in the modification of the lipoprotein fraction values, increasing the HDL- cholesterol and decreasing the LDL- cholesterol.

5. The administration of 0.5% cholesterol in the diet on a long term determines morphologic alterations in the arteries expressed through fibromatous proliferation and interfibrilar edema.

6. The arterial protection in the case of supplementation the diet high in cholesterol with Omega -3 is not totally assured, fact proved by the presence of fatty cells in the vascular endothelium.

Acknowledgments. This study has been financed by the CNMP, Project 52-135/01.10.2008.

REFERENCES