Comparative Study Between Carnosine and Fluvastatin in Hypercholesterolemic Rabbits

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Abstract: The present study was directed to evaluate carnosine as a drug against hypercholesterolemia. The parameters under investigation were lipid profile, phospholipids composition and some elements; iron (Fe), magnesium (Mg) and calcium (Ca). Hypercholesterolemia in rabbits induced increased level of serum total cholesterol (TC), triglycerides (TG), low density lipoprotein (LDL) while serum high density lipoprotein (HDL) was decreased. Both ratios (TC: HDL and LDL: HDL) were increased pointing to serious prognosis of atherosclerosis development. Although fluvastatin treatment of hypercholesterolemic (HC) animals showed decrease in serum TC, TG and LDL, and normalization of serum HDL, both ratios tested were still higher than normal. Carnosine as an antioxidant induced a decrease in serum lipid profile parameters except HDL level which showed no change in HC rabbits. Four individual phospholipids of liver tissue, phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatic acid (PA) and phosphatidylinositol (PI) content were reduced in HC rabbits compared to normal animals. Fluvastatin treatment affected the content of the individual PLs; PC and PE, were highly below normal while, the other two, PI and PA were increased. Unfortunately, carnosine induced a great reduction in all PL contents compared to normal animals. Although cornonine as an antioxidant as reported in several studies it doesn’t prevent the oxidation of iPLs measured. Whereas it improved the lipid profile, so it can be used as drug for the treatment of coronary heart diseases (CHD). Also cornosine is preferable since it did not repel the calcium from the bone like fluvastatin did as found in this study.

Key words: Hypercholesterolemia, lipoproteins, phospholipids, carnosine, fluvastatin

INTRODUCTION

Coronary Heart Disease (CHD) is considered the most serious disease all over the world in the present decades. There is a positive relation ship between CHD and elevation of LDL-C level. Hypercholesterolemia is known to cause alterations in lipid composition of plasma membrane lipoproteins due to its induction of oxidative stress in different organisms (Balkan et al., 2002). A cholesterol rich diet induces other abnormal decreases in membrane fluidity, permeability and activities of some membrane-bound enzymes (LeBlanc et al., 2003). These were the causes of an imbalance in rabbit heart antioxidant defense mechanisms, some of which are increased whereas others are decreased, eventually resulting in enhanced myocardial lipid peroxidation (Lapenna et al., 1992; Mahfouz and Kummerow, 2000).

Barnes and Weinberg (1999) and Buchanan et al. (2003) proved that rabbits were susceptible to development of atherosclerosis when fed on a high cholesterol diet. During the last years, a developing set of evidences demonstrated that oxidized low density lipoprotein (LDL-C) had an important role in the pathogenesis of atherosclerosis (Gofman et al., 1999, Shuji Shikato et al., 2005).

Atherogenesis is not only due to the increase in LDL-C level, but other factors share in this process (Tomonari et al., 2005; Tavridou et al., 2006). Oxidized LDL particles forming foam cells in the vessel intima lead to the beginning of the pathogenesis of atherosclerosis (Robert et al., 2002). This process is a company by a flux of free radicals (Fukai et al., 2002).

Statin therapy is known to reduce the incidence of cardiovascular events and death, probably by functional changes of atherosclerotic lesions through inhibition of hydroxymethyl glutaryl Co-A (Brass et al., 2006). These benefits are mainly ascribed to the strong lipid-lowering properties of this class of drugs.

Carnosine is a natural dipeptide found in different tissues; olfactorry nerve, saphenous vein, mitral/tufted cells and skeletal muscles (Gonzalez-Estrada and Freeman, 1980; Bonfanti et al., 1999; Babizhayev, 2006). It has an
excitatory action on mitral/tufted cells and rabbit saphenous vein rings with greater efficacy than noradrenaline. The maximum carnosine induced tension is enhanced by zinc ions (Mihal and O’Dowd, 2000).

Carnosine exhibits antioxidative properties against different oxygen-derived free radicals and also lipoperoxyl radicals (Babizhayev et al., 1998). Due to the potent activity of carnosine, it was of interest in the present study to investigate its action on serum Total Cholesterol (TC), triglycerides (TG), low density lipoprotein cholesterol LDL-C and high density lipoprotein cholesterol HDL-C levels. More over four classes of phospholipide (PLs): Phosphatidylycholine (PC), Phosphatidylethanolamine (PE), Phosphatidic Acid (PA) and phosphatidylinsitol (PI) were measured in liver of hypercholesterolemic and treated rabbits with either fluvastatin or carnosine. Some element concentrations in serum, iron (Fe), magnesium (Mg) and calcium (Ca) serum levels were also measured.

**MATERIALS AND METHODS**

**Animals:** Twenty adult male New-Zealand white rabbits of similar age and mass (2.5-3 kg) were used for this study. The animals were housed individually in galvanized cages with wire bottoms in a room maintained at 21-24°C with a 12-h light: dark cycle.

**Basal diet:** The basal diet contained 11.38% moisture, 8.12% crude ash, 16.1% protein, 3.4% crude fat, 13.83% cellulose and 47% carbohydrates. The food was stored at 4°C.

**Experimental design:** Animals were divided into four groups, each of 5 rabbits. Group 1 (served as normal healthy control), the animals were kept on a basal diet. Group 2 (high cholesterol diet-fed group), the animals were fed basal diet supplemented with 1% cholesterol for 12 weeks. Group 3 (carnosine treated group), the animals were fed high cholesterol diet, then each animal received oral dose of 50 mg carnosine kg⁻¹ day⁻¹ at the last 6 weeks. Group 4 (fluvastatin-treated group), the animals were fed high cholesterol diet, then each animal received oral dose of 2 mg kg⁻¹ day⁻¹ at the last 6 weeks. The animals were allowed free access to food and water. At the end of the feeding period (12 weeks), the animals were fasted overnight (12-14 h) and the rabbits were sacrificed. The blood samples were taken from the sublingual vein. The livers and blood serum were served at -80°C until used for biochemical analysis.

**Chemicals:** Chemicals were of annular quality products Merk, Germany.

**Biochemical analysis**

**Serum determinations:** The following parameters were assessed in fasted blood serum of each animal in all groups; cholesterol by cholesterol enzymatic endpoint method (Roeschlaub et al., 1974), triglycerides (Trinder, 1969), LDL-C (Friedwald et al., 1972) formula and HDL-C (Randox precipitant kit). Iron, magnesium and calcium were measured spectrophotometrically according to Artiss et al. (1981), Bohuon (1962) and Tietz (1970), respectively.

**Liver determinations**

**Lipid extract:** Total lipids were estimated in rabbits liver tissues by the method of Duncan et al. (1987).

**Analysis of phospholipids composition:** HPTLC of PC, PE, PA and PI on silica gel by fourfold automated multiple developments with chloroform-methanol-2-propanol-triethylamine-0.25% aqueous KCI 60:18:50:36:9, chamber precondition with 0.1 N NH₄OH. Visualization by spraying with (1) 1, 6-diphenyl-1, 3,5-hexatriene and after intermediate drying with (2) molybdenum blue reagent according to Dittmer and Lester. Quantification by densitometry at 370/=460 nm. Visual detection limits of down to 10 ng (Muthing and Radloff, 1998).

**Statistical analysis:** Data are expressed as mean±SD and statistically analyzed using analysis of variance (ANOVA) and Least Significant Difference (LSD) post hoc test.

**RESULTS AND DISCUSSION**

The present study of serum levels in hypercholesterolemic rabbits showed an increase in TC, TG and LDL-C (Table 1). Similarly, the same parameters were increased in heart muscle and in erythrocyte membrane as a result of high fat intake by several authors (Lapenna et al., 1992; Deepa and Varalakshmi, 2003; Galle et al., 2003). In support, many dyslipidemic states were reported to be associated with abnormal metabolism of serum and membranous lipoproteins (Rodriguez et al., 1997). Earlier studies attributed disturbed levels to hypercholesterolemia inducing oxidative stress (Yasuhara et al., 2000; Balkan et al., 2002; Cicero et al., 2003).

The concurrent decreased serum HDL and individual phospholipids (iPLs); Phosphatidylycholine
(PC), Phosphatidylethanolamine (PE), phosphatidic acid (PA) and phosphatidylinosit (PI) could be supported by Donchenko et al. (2002). The authors found that incubating cardiomyocytes plasma membrane (CPM), of intact animals in a medium containing excess fat, PLs decreased. They explained the decrease to excess ROS liberation and increased peroxidation of unsaturated FA. In addition hypercholesterolemic oxidative stress was reported to be accompanied by disturbance of PL compositions (Mitani et al., 1996).

The disturbed level of any of the tested parameters was doomed untoward by National Cholesterol Education Program Expert Panel (1994) and Temme et al. (2002). The decreased serum HDL and tissue iPLs were labeled harmful to cardio-vascular function by Benjamin et al. (2005). Hypercholesterolemic disturbed PLs basically involved compositions (Mitani et al., 2003). These disturbed iPLs composition changed their physicochemical and even membrane location properties leading to further metabolic affections (Shvedova et al., 2002).

In the present study fluvastatin was used as a reference drug to compare the effect of carnosine on TC, TG, HDL, LDL and some elements in serum and phospholipid compositions in liver tissue of hypercholesterolemic rabbits with its effect. The common factors which lead us to use fluvastain as a reference drug its antioxidant effect like carnosine (Babizhayev et al., 1994; Kurusu et al., 2000). Guliaeva et al. (1989) found that carnosine injected to albino rats (20 g kg⁻¹ body weight) before stress prevents LPO activation but during stress it decreases the LPO, due to its antioxidant activity.

Administration of carnosine lowered TC, TG and LDL-C in serum. It also decreased phospholipids composition levels in hypercholesterolemic rabbit's liver which is in accordance with Nijima et al. (2002) who showed that L-carnosine is an endogenous factor controlling the blood pressure in a manner possibly antagonistic to the obesity. The concurrent results were also in parallel with those of Lee et al. (2005) who found that when histidine and carnosine were added into drinking water to diabetic Balb/CA mice, they significantly decreased TC and TG levels in heart and liver.

An increase in HDL-C is associated with a decrease in coronary risk (Harrison et al., 2003) and most of the drug which decrease TC also decrease HDL-C, this is an advantage in the treatment with carnosine and fluvastatin.

The decrease of serum TG levels is supported by EL-Hazmi and Warys (2001). They referred the decrease of TG levels is related to the increase in the endothelium bound lipoprotein lipase which hydrolyses TG into fatty acids. Another explanation is that carnosine stimulate sympathetic nervous system for secretion of noradrenaline (Catecholamines) from adrenal medulla and enhanced TG metabolism (Goncharenko and Antonova, 1995). Total cholesterol: LDL and LDL: HDL cholesterol ratios are also predictors of coronary risk (National Cholesterol Education Program Expert Panel, 1994). In this study these ratios are markedly reduced by carnosine Table 1.

Oxidative stress affects PL; mainly PC and PE (Fukai et al., 2005). Although carnosine and fluvastatin are good antioxidants (Yilmaz et al., 2004, Lee et al., 2005), they induced an alteration in phospholipids composition and concentration in liver tissue of high cholesterol treated rabbits (Table 2).

The same results were obtained with Gereken and Trotz (1983) and Kurusu et al. (2000), respectively. This may be due to the untoward effect of carnosine when used in long duration. In this case carnosine acts as pro-oxidant. Its pro-oxidant effect was showed by Boldrey and Abe (1999) and Soliman et al. (2004).

The pro-oxidant effect of long term use of carnosine is comparable to other antioxidants turning to be pro-oxidant when used in big dosages. This was proved for vitamin E given to intact animals in big dosages. Hence, the pro-oxidant effect of the assumed antioxidant compound is so induce endothelial membrane dysfunction as reported by Donchenko et al. (2002).

The percent change in individual PLs was found to be variable (Table 3). This effect was proved to induce different chemical and physical properties as loss of membrane asymmetry and permeability (Shvedova et al., 2002). The disturbance in membrane permeability causes variable changes in cell constituents including elements.

It was found that Fe²⁺ level was increased in hypercholesterolicim rabbits (Table 4) than control one which is coincided with Ren Mingin et al. (2003) and Yilmaz et al. (2004). In carnosine treated HC rabbits, the iron (Fe) level was normalized to near its normal level. Normalization of iron can be explained on the fact that carnosine interacts with Fe²⁺ as reported by Klebanov et al. (1998). In contrast to the effect of carnosine, fluvastatin treatment increased Fe level which is supported by Obata et al. (2000) who studied the effect of fluvastatin on hydroxyl radical generation in rat myocardium through administration of Cu and Fe. Their results showed that Fe, agonist LDL-C oxidation, may be insensitive to fluvastatin treatment compared with Cu. Magnesium (Mg) and calcium (Ca) also showed an increase in sera of hypercholesterolim rabbits (Table 4). The increase in Ca concentration is in accordance with Batrukova et al. (1992) who studied the effect of
Table 1: Lipid profile in control and treated groups

<table>
<thead>
<tr>
<th>Sample</th>
<th>Cholesterol (mg dl⁻¹)</th>
<th>Triglyceride (mg dl⁻¹)</th>
<th>Low density lipid LDL-C (mg dl⁻¹)</th>
<th>High density lipid HDL-C (mg dl⁻¹)</th>
<th>TC: HDL</th>
<th>LDL: HDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-Control</td>
<td>234.83±10.30</td>
<td>70.02±1.04</td>
<td>155.36±9.82</td>
<td>65.47±6.00</td>
<td>3.59</td>
<td>3.37</td>
</tr>
<tr>
<td>LSD</td>
<td>(2)</td>
<td>(2)</td>
<td>(2)</td>
<td>(2)</td>
<td>(2)</td>
<td>(2)</td>
</tr>
<tr>
<td>2-Hypercholesteremic</td>
<td>720.48±24.49</td>
<td>163.35±15.22</td>
<td>656.29±36.28</td>
<td>20.14±1.70</td>
<td>35.77</td>
<td>32.58</td>
</tr>
<tr>
<td>LSD</td>
<td>(1, 3, 4)</td>
<td>(1, 3, 4)</td>
<td>(1, 3)</td>
<td>(1, 3)</td>
<td>(1, 3)</td>
<td>(1, 3)</td>
</tr>
<tr>
<td>3-Hypercholesteremic</td>
<td>317.58±39.37</td>
<td>71.2±13.16</td>
<td>280.21±17.89</td>
<td>69.65±11.48</td>
<td>4.56</td>
<td>4.02</td>
</tr>
<tr>
<td>Treated with flavusatin LSD</td>
<td>(2)</td>
<td>(2)</td>
<td>(2)</td>
<td>(2)</td>
<td>(2)</td>
<td>(2, 4)</td>
</tr>
<tr>
<td>4-Hypercholesteremic</td>
<td>264.57±22.11</td>
<td>48.58±5.52</td>
<td>272.08±53.75</td>
<td>21.90±2.36</td>
<td>12.08</td>
<td>12.42</td>
</tr>
<tr>
<td>Treated with carnosine LSD</td>
<td>(2)</td>
<td>(2)</td>
<td>(2)</td>
<td>(1, 3)</td>
<td>(1, 3)</td>
<td>(1, 3)</td>
</tr>
</tbody>
</table>

The results are expressed as mean±SE

Table 2: Phospholipid profile in liver of control and treated groups

<table>
<thead>
<tr>
<th>Samples</th>
<th>PC (mg g⁻¹)</th>
<th>PE (mg g⁻¹)</th>
<th>PA (mg g⁻¹)</th>
<th>PI (mg g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-Control</td>
<td>0.199±0.06</td>
<td>0.084±0.038</td>
<td>0.091±0.006</td>
<td>0.120±0.012</td>
</tr>
<tr>
<td>LSD</td>
<td>(2, 3, 4)</td>
<td>(2, 3, 4)</td>
<td>(2, 3, 4)</td>
<td>(2, 3, 4)</td>
</tr>
<tr>
<td>2-Hypercholesteremic</td>
<td>0.157±0.04</td>
<td>0.065±0.01</td>
<td>0.071±0.014</td>
<td>0.116±0.038</td>
</tr>
<tr>
<td>LSD</td>
<td>(1, 3, 4)</td>
<td>(1, 3, 4)</td>
<td>(1, 3, 4)</td>
<td>(1, 3, 4)</td>
</tr>
<tr>
<td>3-Hypercholesteremic</td>
<td>0.08±0.01</td>
<td>0.054±0.01</td>
<td>0.111±0.007</td>
<td>0.681±0.005</td>
</tr>
<tr>
<td>Treated with flavusatin LSD</td>
<td>(1, 2)</td>
<td>(1)</td>
<td>(2, 4)</td>
<td>(1, 2, 4)</td>
</tr>
<tr>
<td>4-Hypercholesteremic</td>
<td>0.087±0.04</td>
<td>0.046±0.025</td>
<td>0.059±0.016</td>
<td>0.1±0.03</td>
</tr>
<tr>
<td>Treated with carnosine LSD</td>
<td>(1, 2)</td>
<td>(1, 2)</td>
<td>(1, 3)</td>
<td>(1, 3)</td>
</tr>
</tbody>
</table>

The results are expressed as mean±SE

Table 3: Percent ratio of the four phospholipid in liver of hypercholesteremic and treated rabbits

<table>
<thead>
<tr>
<th>Sample</th>
<th>PC</th>
<th>PE</th>
<th>PA</th>
<th>PI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>37.81</td>
<td>15.96</td>
<td>17.29</td>
<td>28.88</td>
</tr>
<tr>
<td>Hypercholesteremic</td>
<td>38.39</td>
<td>15.89</td>
<td>17.36</td>
<td>28.36</td>
</tr>
<tr>
<td>Hypercholesteremic treated</td>
<td>29.59</td>
<td>16.33</td>
<td>20.07</td>
<td>34.01</td>
</tr>
<tr>
<td>with carnosine</td>
<td>8.44</td>
<td>5.84</td>
<td>12.01</td>
<td>73.7</td>
</tr>
</tbody>
</table>

Table 4: Effect of carnosine and flavusatin on some elements in rabbit sera of control and treated groups

<table>
<thead>
<tr>
<th>Sample</th>
<th>Fe (mM L⁻¹)</th>
<th>Mg (mM L⁻¹)</th>
<th>Ca (mM L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-Control</td>
<td>32.38±2.44</td>
<td>10.27±0.91</td>
<td>42.15±5.60</td>
</tr>
<tr>
<td>LSD</td>
<td>(2, 3)</td>
<td>(3, 4)</td>
<td>(3)</td>
</tr>
<tr>
<td>2-Hypercholesteremic</td>
<td>135.10±6.00</td>
<td>9.43±1.11</td>
<td>44.55±8.43</td>
</tr>
<tr>
<td>LSD</td>
<td>(1, 3)</td>
<td>(3)</td>
<td>(3)</td>
</tr>
<tr>
<td>3-Hypercholesteremic</td>
<td>338.48±28.77</td>
<td>93.74±4.71</td>
<td>173.22±9.08</td>
</tr>
<tr>
<td>Treated with flavusatin LSD</td>
<td>(1, 2, 4)</td>
<td>(1, 2, 4)</td>
<td>(1, 2, 4)</td>
</tr>
<tr>
<td>4-Hypercholesteremic</td>
<td>70.35±17.33</td>
<td>28.97±3.49</td>
<td>64.21±11.02</td>
</tr>
<tr>
<td>Treated with carnosine</td>
<td>(2, 3)</td>
<td>(1, 2, 3)</td>
<td>(3)</td>
</tr>
</tbody>
</table>

The results are expressed as mean±SE

Carnosine on Ca-channels in rabbit skeletal muscle sarcoplasmic reticulum. They found that the effect of carnosine is dose dependent which indicate the presence of saturable carnosine-binding sites in the Ca-release channel molecule. So, it improves contraction in the isolated rat heart and increases free intracellular calcium levels. Increase of calcium stimulated reverse cholesterol transport by promoting a 30 fold increase in the rate of clearance of free cholesterol from the circulation (Stamler et al., 2000).

The results of the present study revealed that carnosine possess a hypocholesteremic effect this is emphasized by decreasing LDL/HDL level and also decreases iron load compared by hypercholesteremic group. So, carnosine can be advised for patients of CHD.

REFERENCES


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