Journal of Applied Sciences

ISSN 1812-5654

science alert

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Journal of Applied Sciences 6 (8): 1725-1730, 2006
ISSN 1812-5654

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), phosphatidic 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 cornonise is 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 peroxidaiton (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 oxidated low density lipoprotein (LDL-C) had an important role in the pathogenesis of atherosclerosis (Gofman *et al*., 1999; Shuji Shakuto *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 intimae lead to the beginning of the pathogenesis of atherosclerosis (Roberta *et al*., 2002). This process is a companied 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 glutryl 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; olfactory nerve, saphenaus 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 (Milelr and O'Dowd, 2000).

Carnosine exhibits antioxidative properties against different oxygen-derived free radicals and also lipoperoxyl radicals (Babizhayvev *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); Phosphatidylcholine (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 newzealand 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 scarified. 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 (Roeschlau *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 KCl 60:18:50:36:9, chamber precondition with 0.1 N NH_4OH. 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 hock 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 Varalakshim, 2003; Galle *et al*., 2003). In support, many dyslipidemic states were reported to be associated with abnormal metabolism of serum and membranous lipoproteins (Rodrigueza *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); Phosphatidylcholine

(PC), Phosphatidylethanolamine (PE); phosphatidic acid (PA) and phosphatidylinsitol (PI) could be supported by Donchenko *et al.* (2002). The authors found that incubating cardiomyocytes plasma membrance (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-cannosine 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 Warsy (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 noradrenalin (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 Exert 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 Gercken and Trotz (1983) and Kurusu *et al.* (2000), respectivily. 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 Boldyrev 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 Fe^{2+} level was increased in hypercholesterolimic rabbits (Table 4) than control one which is coincided with Ren Minqin *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 Fe^{2+} 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 hypercholesterol 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

Sample	Cholesterol (mg dL^{-1})	Triglycrides (mg dL^{-1})	Low density lipid LDL-C (mg dL^{-1})	High density lipid HDL-C (mg dL^{-1})	TC: HDL	LDL: HDL
1-Control	234.83±10.30	70.02±1.04	155.36±9.62	65.47±6.90	3.59	3.37
LSD	(2)	(2)	(2)	(2,4)		
2- Hypercholesterolemic	720.48±42.49	163.35±19.22	656.26±36.28	20.14±1.70	35.77	32.58
LSD	(1, 3, 4)	(1, 3, 4)	(1, 3, 4)	(1, 3)		
3-Hypercholesterolemic	317.58±39.37	71.2±13.16	280.21±78.19	69.65± 11.48	4.56	4.02
Treated with fluvastatin LSD	(2)	(2)	(2)	(2, 4)		
4-Hypercholesterolemic	264.57±42.11	48.58±5.2	272.08±53.75	21.90± 2.36	12.08	12.42
Treated with carnosine LSD	(2)	(2)	(2)	(1, 3)		

The results are expressed as mean±SE

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

Samples	PC (mg gt^{-1})	PE (mg gt^{-1})	PA (mg gt^{-1})	PI (mg gt^{-1})
1-Control	0.199±0.06	0.084±0.038	0.091±0.008	0.152±0.012
LSD	(2, 3, 4)	(2, 3, 4)	(2, 4)	(2, 3,4)
2-Hypercholesterolemic	0.157±0.04	0.065±0.01	0.071±0.014	0.116±0.038
LSD	(1, 3, 4)	(1, 4)	(1, 3)	(1, 3)
3-Hypercholesterolemic	0.08±0.01	0.054±0.01	0.111±0.007	0.681±0.005
Treated with fluvastatin LSD	(1, 2)	(1)	(2,4)	(1, 2, 4)
4-Hypercholesterolemic	0.087±0.04	0.048±0.025	0.059±0.016	0.1±0.03
Treated with carnosine LSD	(1, 2)	(1,2)	(1, 3)	(1, 3)

The results are expressed as mean±SE

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

Sample	PC	PE	PA	PI
Control	37.81	15.96	17.29	28.88
Hypercholesterolemic	38.39	15.89	17.36	28.36
Hypercholesterolemic treated with fluvastatin	29.59	16.33	20.07	34.01
Hypercholesterolemic treated with carnosine	8.44	5.84	12.01	73.7

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

Sample	Fe (mM L^{-1})	Mg (mM L^{-1})	Ca (mM L^{-1})
1-Control	52.38±2.44	10.27±0.91	42.15±5.60
LSD	(2, 3)	(3, 4)	(3)
2-Hypercholesterolemic	135.10±6.00	9.43±1.11	44.55±8.43
LSD	(1, 3)	(3, 4)	(3)
3-Hypercholesterolemic Treated with fluvastatin	338.48±28.77	93.74±6.71	173.22±9.08
LSD	(1, 2, 4)	(1, 2, 4)	(1, 2, 4)
4-Hypercholesterolemic Treated with carnosine	70.35±17.33	28.97±3.49	64.21±11.02
LSD	(2, 3)	(1, 2, 3)	(3)

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 posses a hypocholestermic effect this is emphasized by decreasing LDL/HDL level and also decreases iron load compared by hypercholestermic group. So, carnosine can be advised for patients of CHD.

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