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The I*n Vivo* Biochemical and Oxidative Change by Garlic and Ezetimibe Combination in Hypercholesterolemic Mice

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Abstract

Male mice were randomly divided to 5 groups (n=8): group 1: hypercholesterolemic diet, 2: garlic, 3: ezetimibe, 4: garlic plus ezetimibe, and 5: chow only. After one month mice were anesthetized and sacrificed. Lipid profiles and liver enzymes were measured enzymatically. Activity of serum super oxide dismutase (SOD) was determined by the Misra and Fridovich method. Activities of CAT and Reduced glutathione (GSH) were measured using the Aebi and Beutler E, methods respectively. Amount of fasting blood glucose significantly reduced in this combination ($p<0.001$), and ezetimibe group ($p<0.05$). Serum levels of LDL-C and total cholesterol significantly decreased in ezetimibe ($p<0.05$), garlic ($p<0.05$), and combination of garlic and ezetimibe groups ($p<0.001$). TG and VLDL-C markedly decreased in garlic and combination of garlic and ezetimibe groups ($p<0.05$). The atherogenic index (AI), non-HDL-C and LDL/HDL ratio markedly decreased in combination group compared with the hypercholesterolemic mice ($p<0.01$). Serum ALT ($p<0.05$), AST ($p < 0.05$), and GGT ($p < 0.01$) were significantly reduced in garlic ($p<0.05$), ezetimibe ($p<0.05$) and combination groups compared with the hypercholesterolemic mice ($p < 0.01$). The activity of SOD, Catalase and GSH levels were markedly increased in garlic ($p<0.05$) and ezetimibe ($p<0.05$) and combination of garlic and ezetimibe groups ($p<0.001$) compared with hypercholesterolemic mice. Coadministration of garlic and ezetimibe related with a noteworthy improvement in cardiovascular and diabetes risk factors. More experiment might be required to show the efficacy and safety of garlic and ezetimibe coadministration.

Keywords: Garlic, Ezetimibe, LDL-C, SOD, GSH, Catalase.

Introduction

High levels of glucose, total cholesterol, triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), and a low level of high-density lipoprotein cholesterol (HDL-C) have been known as diabetes and cardiovascular disease risk factors[1-3]. For control of dyslipidemia, patients receive different drugs. Statins is one of the lipid lowering medicines which inhibit cholesterol synthesis and suggested as the initial step in dyslipidemia treatment. For reach to target levels high dose of statins is necessary, so many patients cannot tolerate high doses, thus in these patients, substitute or combination therapy is necessary. Ezetimibe is a novel hypocholesterolemic agent that inhibits the cholesterol absorption in the small intestine. This medicine is well tolerated usually, and its side effects are similar to placebo[4]. This drug inhibits cholesterol absorption up to 96% in animals and approximately 50% in hypercholesterolemic patient[5]. On the other hand, garlic is an herbal medicine which has used for prevention and treatment many diseases such as diabetes, atherosclerosis, hyperlipidemia, thrombosis and hypertension[6]. This experiment has attempted to determine the useful effect of coadministration of garlic and ezetimibe on biochemical factors and antioxidant activity in hypercholesterolemic mice.

Material and Methods

Animals and treatments: Male N-Mary mice were kept on a 12h light/12 h dark cycle at a temperature of 22 ± 1 °C. After adapting for one week, mice were randomly divided into 5 groups (n=8): group 1 received chow + 2% cholesterol + 0.5% cholic acid (hypercholesterolemic), group 2: chow + 4% (w/w) garlic powder + 2% cholesterol + 0.5% cholic acid (garlic), group 3: chow + 0.005% (w/w) ezetimibe + 2% cholesterol +

0.5% cholic acid (ezetimibe), group 4: chow + 4% (w/w) garlic powder + 0.005% (w/w) ezetimibe +2% cholesterol + 0.5% cholic acid (combination), and group 5: chow only. The levels of fasting blood glucose, triglyceride and cholesterol were at the baseline before treatment and there were not different among groups. Garlic extract was dissolved in normal saline and was mix with animal diet; also equal volume of normal saline was added to the hypercholesterolemic control and chow group diets. Ezetimibe was dissolved in corn oil and mixed with diet. Equal volume of corn oil was added to the hypercholesterolemic control and chow group diets. After one month fasted mice were anesthetized and sacrificed. Blood was collected from heart, and then centrifuged for 10 minutes at 3000 *g* and serum was used for biochemical analysis[7-10].

Analysis of biochemical factors and antioxidant activity: Lipid profiles and liver enzymes were measured enzymatically. The levels of VLDL-C and LDL-C were calculated with Friedwald formula. Atherogenic index (AI): LDL-C + VLDL-C/HDL-C. Activity of serum super oxide dismutase (SOD) was determined by the Misra and Fridovich method (Misra HP, 1972)[9]. Activities of CAT and Reduced glutathione (GSH) were determined according the Aebi et al. and Beutler E, et al. methods[9-11].

Water soluble garlic extract: The extract was prepared according previous method[12].

Statistical analysis: All data of this experiment are presented as means ± S.E.M. Statistical analysis of the results was done with one-way analysis of variance with ANOVA (Tukey). Different were considered statistical significant when P was less than 0.05.

Results and Discussion

Body weight did not showed significant differences among garlic, ezetimibe and combination of garlic and ezetimibe groups. However, weight gain was markedly higher in hypercholesterolemic group as compared to chow. Amount of blood glucose significantly reduced in this combination (p<0.001), and ezetimibe group (p<0.05), while change of blood glucose in garlic-treated animals were not significant. Serum levels of total cholesterol and LDL-C markedly decreased in ezetimibe (p<0.05), garlic (p<0.05), and combination of garlic and ezetimibe group; these reductions were much more in combination group (p<0.001). The levels of TG and VLDL-C markedly decreased in garlic and combination groups (p<0.05), while change of TG and VLDL-C in ezetimibe-treated animals were not significant (table-1). The atherogenic index (AI), LDL/HDL ratio and non-HDL-C levels markedly decreased in garlic (p<0.05), ezetimibe (p<0.05) and combination groups compared with the hypercholesterolemic mice (p<0.01). Serum ALT (p<0.05), AST (p<0.05), and GGT (p<0.01), were significantly increased in the hypercholesterolemic mice when compared with control animals. These enzymes were significantly reduced in garlic (p<0.05), ezetimibe (p<0.05) and combination (p<0.01) groups compared with the hypercholesterolemic mice (table-1).

The activity of SOD was markedly increased in garlic (p<0.05), ezetimibe (p<0.05) and in combination of garlic and ezetimibe groups (p<0.001). Catalase and GSH levels were also significantly increased in garlic (p<0.05) and ezetimibe (p<0.05) and combination of garlic and ezetimibe groups (p<0.001) compared with hypercholesterolemic mice (table-2).

Table-1
Comparison of biochemical factors among different groups

Biochemical factors	Hypercholestrolemic	Garlic	Ezetimibe	Garlic/Ezetimibe	Chow
Body weight (g)	37.5 ± 1.5^{f}	35.7 ± 0.8	35.9 ± 0.9	36.6 ± 1.2	30.7 ± 0.7
FBS (mg/dl)	160.1 ± 5.6	152.7 ± 4.6	148.2 ± 12.1^{a}	124.2 ± 3.4^{c}	138.1 ± 9.7
TC(mg/dl)	230.1 ± 4.5^{f}	191.4 ± 5.4^{a}	180.2 ± 6.6^{b}	167.5 ± 9.1^{c}	129 ± 13.4
TG (mg/dl)	160.5 ± 7.5^{d}	137.4 ± 4.5^{a}	144.2 ± 4.1	135.2 ± 2.7^{a}	133.5 ± 4.0
VLDL-C (mg/dl)	32.1 ± 1.5^{d}	27.5 ± 0.9^{a}	28.5 ± 0.8	27.1 ± 0. 6^{a}	26.7 ± 0.8
HDL-C (mg/dl)	110.2 ± 8.1	106.2 ± 3.8	95.0 ± 8.4	104.0 ± 8.0	87.4 ± 9.7
LDL-C (mg/dl)	92.8 ± 9.8^{f}	57.6 ± 7.4^{a}	52.6 ± 10.1^{a}	36.4 ± 7.8^{c}	24.9 ± 5.5
AI	1.13 ± 0.09^{f}	0.8 ± 0.06^{a}	0.85 ± 0.04^{a}	0.61± 0.07^{c}	0.59 ± 0.05
LDL/HDL ratio	0.84 ± 0.07^{f}	0.54 ± 0.03^{a}	0.55 ± 0.07^{a}	0.35 ± 0.03^{c}	0.28 ± 0.05
non-HDL-C	119.9 ± 6.6^{f}	85.2 ± 5.5^{b}	85 ± 6.1^{b}	63.5 ± 3.8^{c}	41.6 ± 2.7

Data represent as mean ± SEM (n = 8), ap < 0.05, bp < 0.01 and cp < 0.001 considered as significant compared with Hypercholestrolemic. dp < 0.01 and fp < 0.001 considered as significant compared with chow. FBS: Fasting blood sugar, LDL-C: low-density lipoprotein cholesterol, TG: triglycerides, TC: cholesterol, VLDL-C: very low-density lipoprotein cholesterol, HDL-C: high-density lipoprotein cholesterol, AI: atherogenic index.

Table-2
Comparison of oxidative factors among different treated animals

	Hypercholestrolemic	Garlic	Ezetimibe	Garlic/Ezetimibe	Chow
SOD (units/ml)	90.1 ± 6.2	119. 2 ± 5.0[a]	114.6 ± 6.6[a]	133.7 ± 7.3[c]	112.4 ± 5.8
GSH (µmol/ml)	3.1 ± 0.6	5. 7 ± 0.5[a]	5.1 ± 0.4[a]	6.6 ± 0.4[c]	5.4 ± 0.6
Catalase (units/ml)	34.2 ± 4.4	47.2 ± 4.8[a]	48.5 ± 5.0 [a]	64.5 ± 6.3[c]	61.5 ± 5.3

Data of this experiment are expressed as mean ± SEM. ap < 0.05, bp < 0.01 and cp < 0.001 compared to Hypercholestrolemic. SOD: Serum super oxide dismutase, GSH: reduced glutathione.

Discussion: Garlic is broadly used as a potent herbal medicine for the treatment of numerous disease. Many studies have examined the hypolipidemic effects of garlic. Previously was proven that the maximum tolerable level of garlic extracted in animal diet is 10%[13]. In the present experiment, we used four percent of water garlic extract in order to be well tolerated by the mice. Yeh YY, et al. showed that hypocholesterolemic effect of water-soluble sulfur compounds of garlic is due to the inhibition of cholesterol synthesis pathway, while the inhibition by lipid-soluble extracts of garlic results from the strong toxic properties of this lipid-extract. They also proved that water-extract inhibited cholesterol synthesis more than methanol and petroleum extractable fraction[14]. In this experiment, water extract of garlic markedly reduced serum triglycerides (14.3%), VLDL-C (15.5%), cholesterol (16.9%) and LDL-C (30%) levels, compared with hypercholesterolemic mice. Elmahdi B, et al. showed that adding 8% raw garlic to rat atherogenic diet (diet containing 2% cholesterol), declined serum total cholesterol and LDL-C levels and increased HDL-c[15]. Aouadi et al. also, showed that addition of 10% fresh garlic to atherogenic diet (diet containing 2% cholesterol) led to significant reduction in LDL-C levels, and raised HDL-C levels in rat[16]. In our study, HDL-c significantly increased in garlic extract-treated animal compare with chow diet. Hypolipidemic effect by water extract may be due to inhibition of key enzymes in cholesterol and triglyceride metabolism such as hepatic 3-hydroxy-3-methylglutaryl-CoA reductase (HMG-CoA reductase), cholesterol 7α-hydroxylase, cholesteryl ester transfer protein activity, pentose-phosphate pathway activities[17], microsomal triglyceride transfer protein[18] and increase of bile acid excretion[19]. Our study also shows that the levels of fasting blood glucose do not decrease in garlic group. Compared with hypercholesterolemic mice, combination of ezetimibe and garlic significantly decreased blood glucose level (22.5%). Many human and animal studies reported that garlic has no effect on blood glucose level[6]. In agreement to Ali M, et al.[20] results in our study although body weight reduced in garlic and ezetimibe-treated animals but it was not significant.

Ezetimibe is novel cholesterol inhibitor which broadly used for the treatment of hypercholesterolemic patients. The results of many experiments have shown that ezetimibe decline LDL-C by about 20% when administered alone and decrease cholesterol by up to 60% when administered with one statin. In our study, ezetimibe declined the levels of LDL-C and total cholesterol by approximately 21.7 and 35% respectively. Van Heek M et al. showed that administration of ezetimibe with dose of 1mg/ kg in rat which fed atherogenic diet, inhibited cholesterol absorption by 92-96%[21].

Studies have shown that ezetimibe decrease the levels of serum triglyceride by 1.7 to 9.4%, but this reduction was not noticeable always. In our experiment, ezetimibe decrease triglyceride levels but it was not significant[22 ezetimibe]. Compared with hypercholesterolemic mice, combination of ezetimibe and garlic markedly decreased total cholesterol (27.4%), LDL-C (57%), triglyceride (15.6%), VLDL-C (16%). In Van Heek M et al. study combination of ezetimibe with atorvastatin led to 50-60%, simvastatin 44-57%, pravastatin 34-41% and lovastatin 33-45% reduction in LDL-C levels[22]. Coadministration of ezetimibe and garlic in hypercholesterolemic mice synergistically lead to decrease of cholesterol absorption and synthesis, because the efficient inhibition of cholesterol synthesis and the consequent decrease in serum.

A diversity of internal and external antioxidants[23-28] protected body from oxidative stress. On the other word, antioxidants are able to neutralize and stabilize free radicals before they attack to cells[29].

Glutathione (GSH) is an major antioxidant, which prevent damage to vital cellular components produced by reactive oxygen species including peroxides and free radicals. In our experiment, GSH significantly increased in combination group. Catalase is a heme-containing protein that converts H_2O_2 to water with at a high rate. The results of many studies have established that H_2O_2 is involved in the atherosclerosis pathogenesis by induce of lipid peroxidation. In oxidative stress situation, catalase activity is reduced. Studies have revealed that oxidative stress impair the function of endothelial cell[30]. In garlic, ezetimibe and combination of garlic, ezetimibe treated animals activity of this enzyme was significantly increased when compared with hypercholesterolemic mice. SOD is the chief defense against O_2 by catalyzing dismutation of this free radical to H_2O_2 and O_2. Consequently, SOD and catalase maintain cells from toxicity of oxidants by catalyzing the dismutation of O_2^- to H_2O_2 and the decomposition of H_2O_2 to H_2O and O_2[18]. There was a significant raise in SOD in garlic and ezetimibe treated groups, and there was more rise in the SOD activity in the garlic plus ezetimibe group when compared with the hypercholesterolemic animals.

Conclusion

Ezetimibe, when administrated with garlic, produces more decrease in serum LDL-C, cholesterol, triglyceride, VLDL-C, glucose levels, atherogenic index, non-HDL-C and LDL/HDL ratio as well as enhanced antioxidant capacity. This combination could be a useful treatment decision for diabetes and cardiovascular disease to reach their goals. More human studies should be conducted in the future for prove useful effect of this combination in human.

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