The In 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 factors1-3. 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 placebo4. This drug inhibits cholesterol absorption up to 96% in animals and approximately 50% in hypercholesterolemic patient5. 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 hypertension6. 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.  

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, C/HDL-C. Activity of serum super oxide dismutase (SOD) was determined according the Aebi et al. and Beutler E, et al. methods. 

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

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-2). 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

<table>
<thead>
<tr>
<th>Biochemical factors</th>
<th>Hypercholesterolemic</th>
<th>Garlic</th>
<th>Ezetimibe</th>
<th>Garlic/Ezetimibe</th>
<th>Chow</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>37.5 ± 1.5&lt;sup&gt;f&lt;/sup&gt;</td>
<td>35.7 ± 0.8</td>
<td>35.9 ± 0.9</td>
<td>36.6 ± 1.2</td>
<td>30.7 ± 0.7</td>
</tr>
<tr>
<td>FBS (mg/dl)</td>
<td>160.1 ± 5.6</td>
<td>152.7 ± 4.6</td>
<td>148.2 ± 12.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>124.2 ± 3.4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>138.1 ± 9.7</td>
</tr>
<tr>
<td>TC(mg/dl)</td>
<td>230.1 ± 4.5&lt;sup&gt;f&lt;/sup&gt;</td>
<td>191.4 ± 5.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>180.2 ± 6.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>167.5 ± 9.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>129 ± 13.4</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>160.5 ± 7.5&lt;sup&gt;d&lt;/sup&gt;</td>
<td>137.4 ± 4.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>144.2 ± 4.1</td>
<td>135.2 ± 2.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>133.5 ± 4.0</td>
</tr>
<tr>
<td>VLDL-C (mg/dl)</td>
<td>32.1 ± 1.5&lt;sup&gt;d&lt;/sup&gt;</td>
<td>27.5 ± 0.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>28.5 ± 0.8</td>
<td>27.1 ± 0.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>26.7 ± 0.8</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>110.2 ± 8.1</td>
<td>106.2 ± 3.8</td>
<td>95.0 ± 8.4</td>
<td>104.0 ± 8.0</td>
<td>87.4 ± 9.7</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>92.8 ± 9.8&lt;sup&gt;f&lt;/sup&gt;</td>
<td>57.6 ± 7.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>52.6 ± 10.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>36.4 ± 7.8&lt;sup&gt;c&lt;/sup&gt;</td>
<td>24.9 ± 5.5</td>
</tr>
<tr>
<td>AI</td>
<td>1.13 ± 0.09&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.8 ± 0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.85 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.61 ± 0.07&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.59 ± 0.05</td>
</tr>
<tr>
<td>LDL/HDL ratio</td>
<td>0.84 ± 0.07&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.54 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.55 ± 0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.35 ± 0.03&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.28 ± 0.05</td>
</tr>
<tr>
<td>non-HDL-C</td>
<td>119.9 ± 6.6&lt;sup&gt;f&lt;/sup&gt;</td>
<td>85.2 ± 5.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>85 ± 6.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>63.5 ± 3.8&lt;sup&gt;c&lt;/sup&gt;</td>
<td>41.6 ± 2.7</td>
</tr>
</tbody>
</table>

Data represent as mean ± SEM (n = 8), ap < 0.05, bp < 0.01 and cp < 0.001 considered as significant compared with Hypercholesterolemic. 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.
have examined the hypolipidemic effects of garlic. Previously, medicine for the treatment of numerous diseases. Many studies have examined the hypolipidemic effects of garlic. Previously, medicine for the treatment of numerous diseases. Many studies have shown that administration of ezetimibe with dose of 1mg/kg in rat which fed atherogenic diet, inhibited cholesterol absorption by 92-96%. Studies have shown that ezetimibe decrease 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. 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. 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 protected body from oxidative stress. On the other word, antioxidants are able to neutralize and stabilize free radicals before they attack cells.

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. 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 $O_2$. 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.

### Discussion:
Garlic is broadly used as a potent herbal medicine for the treatment of numerous diseases. Many studies have shown that administration of ezetimibe with dose of 1mg/kg in rat which fed atherogenic diet, inhibited cholesterol absorption by 92-96%.

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 adding 8% raw garlic to atherogenic diet (diet containing 2% cholesterol), declined serum total levels, compared with hypercholesterolemic mice. El Mahdi B, et al. also, showed that addition of 10% fresh garlic to atherogenic diet containing 2% of cholesterol, decreased 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. 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. 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.

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### Table-2
Comparison of oxidative factors among different treated animals

<table>
<thead>
<tr>
<th></th>
<th>Hypercholesterolemic</th>
<th>Garlic</th>
<th>Ezetimibe</th>
<th>Garlic/Ezetimibe</th>
<th>Chow</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD (units/ml)</td>
<td>90.1 ± 6.2</td>
<td>119.2 ± 5.0</td>
<td>114.6 ± 6.6</td>
<td>133.7 ± 7.3</td>
<td>112.4 ± 5.8</td>
</tr>
<tr>
<td>GSH (µmol/ml)</td>
<td>3.1 ± 0.6</td>
<td>5.7 ± 0.5</td>
<td>5.1 ± 0.4</td>
<td>6.6 ± 0.4</td>
<td>5.4 ± 0.6</td>
</tr>
<tr>
<td>Catalase (units/ml)</td>
<td>34.2 ± 4.4</td>
<td>47.2 ± 4.8</td>
<td>48.5 ± 5.0</td>
<td>64.5 ± 6.3</td>
<td>61.5 ± 5.3</td>
</tr>
</tbody>
</table>

Data of this experiment are expressed as mean ± SEM. ap < 0.05, bp < 0.01 and cp < 0.001 compared to Hypercholesterolemic.
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.

Acknowledgment

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References

10. Abbasi Oshaghi E., Sorkhani A.N. and Rezaei A., Effects of Walnut on Lipid Profile as Well as the Expression of Sterol-Regulatory Element Binding Protein-1c(SREBP-1c) and Peroxisome Proliferator Activated Receptors α (PPARα) in Diabetic Rat, Food and Nutrition Sciences, 3, 255-259 (2012)


