Cholesterol lowering effect of SG-GN3, the extract of salted and fermented small shrimps, *Acetes japonicus*, in Triton WR-1339 or high cholesterol-diet induced hypercholesterolemic rats

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Received 20 March 2003; received in revised form 10 December 2003; accepted 23 December 2003

Abstract

The cholesterol lowering effect of SG-GN3, the extract of salted and fermented small shrimps, *Acetes japonicus*, was investigated in hypercholesterolemic animal models. Hypercholesterolemia was induced with Triton WR-1339 (nonionic detergent) or high cholesterol (HC)-diet. SG-GN3 significantly decreased total cholesterol (TC) in Triton WR-1339 model at 30 post-treatment hour (549.80 ± 152.46 mg/dl) compared to the control which induced by only Triton WR-1339 (798.84 ± 94.98 mg/dl), whereas high-density lipoprotein (HDL) content did not decrease (P<0.05). In HC-diet model, TC content significantly decreased by SG-GN3 treatment at 3 post-treatment day (P<0.05). These results suggest that SG-GN3 effectively decreased serum TC level in hypercholesterolemic animal models.

Keywords: SG-GN3; *Acetes japonicus*; Hypercholesterolemia; Total cholesterol (TC); High-density lipoprotein (HDL)

1. Introduction

Salted and fermented small shrimps are most commonly used in Kimchi (Korean fermented vegetable product) (Cheigh and Park, 1994), an important sp. food of the Korean food culture. A recent study revealed that the serum cholesterol concentration was significantly lower in mice fed shrimp and squid with 0.1% cholesterol diet when compared with that in mice fed a commercial diet (Tanaka et al., 1998). The shrimps, *Acetes japonicus*, used for salted and fermented small shrimps are mainly caught in the Yellow Sea in February, May, June, and October. They are abundant in nitrogen sources including protein and amino acids, which accelerate the fermentation process; in particular, nitrogen sources in baby shrimps speed up the fermentation process. In this study we sought to verify the usefulness of the hypercholesterolemia rat models induced by Triton WR-1339 (nonionic detergent) and high cholesterol (HC)-diet, using lovastatin, a well-known cholesterol lowering drug. Secondly, the cholesterol lowering effect (reduction in both plasma triglycerides and cholesterol) of SG-GN3, the extract of salted small shrimps, was evaluated using the same models.

2. Materials and methods

2.1. Preparation of salted and fermented small shrimps extract

One hundred grams of SG-GN3 (Korean patents No. 2001-37965) extract was obtained by extracting the salted and fermented small shrimps, *Acetes japonicus* (1.5 kg), three times with ethanol (6 l). It was dried up and then suspended in H2O and centrifugated at 12,000 × g for 10 min. The pellets were resuspended in H2O at 10% (w/v), homogenized using a glass homogenizer (Wheaton, USA), and ultracentrifugated (Bachmann, USA; SW41Ti rotar) at 124,100 × g for 2 h. The pellets were used in this study.

2.2. Animals and diets

All animal experiments were performed under protocols approved by Institutional Animal Care and Use Committee of Seoul National University. All efforts were made to minimize animal suffering. Sprague–Dawley male rats were purchased from Slc Ltd. (Japan), with mean body weight.
Table 1
Descriptive data of the number and weight of each group

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number</th>
<th>W(g)1</th>
<th>W(g)2</th>
<th>W(g)3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triton WR-1339 model</td>
<td>5</td>
<td>80.50 ± 2.92</td>
<td>117.80 ± 5.69</td>
<td>121.20 ± 7.77</td>
</tr>
<tr>
<td>SG-GN3</td>
<td>5</td>
<td>82.90 ± 5.35</td>
<td>118.40 ± 5.97</td>
<td>121.10 ± 5.80</td>
</tr>
<tr>
<td>Lovastatin</td>
<td>5</td>
<td>84.90 ± 1.85</td>
<td>125.30 ± 2.59</td>
<td>131.20 ± 2.84</td>
</tr>
<tr>
<td>Triton/HC-diet (positive control)</td>
<td>5</td>
<td>51.60 ± 2.77</td>
<td>94.90 ± 5.46</td>
<td>116.60 ± 5.81</td>
</tr>
<tr>
<td>Saline (control)</td>
<td>5</td>
<td>82.10 ± 2.41</td>
<td>117.90 ± 4.92</td>
<td>134.40 ± 5.98</td>
</tr>
</tbody>
</table>

Values are mean ± S.D. Abbreviations: W(g)1, weight (g) at the acclimation period; W(g)2, weight (g) at the beginning of study period; W(g)3, weight (g) at the end of study period.

of 51.8 ± 4.37 g (HC-diet model) and 82.6 ± 3.50 g (Triton WR-1339 model) at the start of the study period. The animals were kept at a constant temperature (20–22 °C) and humidity (50–55%), and were fed with commercial diet (Samyang, Korea). Water was allowed ad libitum.

2.3. Inducing hypercholesterolemic rats

2.3.1. Triton WR-1339 model

Twenty rats (5-week-old) were randomly assigned to four groups. After 1 week of acclimation, all groups except the fourth one were injected i.p. with a 10% aqueous solution of Triton WR-1339 (Sigma, USA) at 60 mg/100 g BW. Food was withdrawn 10 h prior to the blood sampling just before Triton WR-1339 injection. To the first group, SG-GN3 (0.2 g) in saline (1 ml) was administered by means of a stomach tube 2 h before Triton WR-1339 injection. Using the same method, lovastatin (Sigma; 40 mg/kg BW) was administered to the second group. The third group (Triton positive control) was only administered with Triton WR-1339. The fourth group was injected with only normal saline i.p. Blood was collected from the retro-orbital sinus just before and every 10 h after the administration of Triton WR-1339.

Fig. 1. Total cholesterol (TC) of the rats treated with Triton WR-1339 or HC-diet. Results are expressed as mean ± S.D. *P < 0.05, comparison of values at different times with respect to time 0 in the respective groups. (A) TC of Triton-inducing model. *P < 0.05, SG-GN3 vs. Triton positive control; bP < 0.05, lovastatin vs. Triton positive control, at 30 post-treatment hour. (B) TC of HC-diet-inducing model. Abbreviations: Lov, lovastatin; Triton, Triton WR-1339; HC-diet, high cholesterol-diet; Con, saline control.
2.3.2. High cholesterol (HC)-diet model

Four-week-old rats were given 1.5% cholesterol (Sigma) and 0.5% cholic acid (Sigma) with standard equilibrated diet (Samyang, Korea) for 6 days. Fifteen hypercholesterolemic rats were selected and were separated into three groups. HC-diet was withdrawn 10 h prior to blood sampling on the seventh day of experiment period, at the same time, the first group was administered with SG-GN3 (0.1 g) dissolved in saline (1 ml) by means of a stomach tube. The standard diet was withdrawn 10 h prior to every blood sampling, but water was allowed ad libitum. Using the same method, lovastatin (40 mg/kg BW) was administered to the second group. The third group was HC-diet positive control, and the fourth group was administered with only normal saline. Blood was collected from the retro-orbital sinus once daily after drug administration.

2.4. Determination of serum lipoproteins

Serum samples were assayed for total cholesterol, high-density lipoprotein and triglyceride using standard enzymatic assay kits: total cholesterol Kit, HDL-Cholestase Kit, Cleantech TG-S Kit (Asan Pharmacy Ltd., Korea).

2.5. Statistical analysis of data

Data were expressed as mean ± S.D. Repeated-measures Statistical Analysis System (SAS) was used for statistical analysis of TC, HDL, TC/HDL ratio, TG, and body weight. The significant difference between each group was analyzed through the Duncan’s multiple range test at a level of $P < 0.05$.

3. Results

3.1. Clinical signs and weight change

During the experimental period, no peculiar clinical signs nor significant changes in body weight were observed among the groups (Table 1).

3.2. Effect of the extract, SG-GN3 on serum TC, HDL and TG

3.2.1. Triton WR-1339 model

Initial values for serum TC were similar among all groups. In the group administered with only Triton WR-1339,
the TC value gradually increased up to 30 post-treatment hour (798.84 ± 94.98 mg/dl). The groups administered with SG-GN3 and lovastatin showed significant decreases in TC compared with the Triton WR-1339 administered group (SG-GN3: 549.80 ± 152.46 mg/dl; lovastatin group: 512.18 ± 42.97 mg/dl) at 30 post-treatment hour (Fig. 1A).

The HDL value increased in SG-GN3 group at 50 post-treatment hour (Triton only: 19.18 ± 2.34 mg/dl; SG-GN3 30.28 ± 4.87 mg/dl) (Fig. 2A). Initial TC/HDL ratio was not different among groups, but significantly decreased in SG-GN3 group, compared with Triton WR-1339-treated group from 30 to 50 post-treatment hours (at 30 post-treatment hour: Triton only TC/HDL ratio: 39.80 ± 7.82; SG-GN3: 28.40 ± 6.17, at 40 post-treatment hour: Triton only: 33.82 ± 4.75; SG-GN3: 21.74 ± 6.15, at 50 post-treatment hour: Triton only: 31.36 ± 5.45; SG-GN3: 18.18 ± 3.77) (Fig. 3A).

TG value increases were too high in the Triton WR-1339 Model to be calculated into the data (the value of TG was over 3000 mg/dl, data not shown).

### 3.2.2. HC-diet model

The TC value of HC-diet treatment group (283.80 ± 147.97 mg/dl) was two times higher than that of the saline-treated control group (106.22 ± 29.72 mg/dl) at 3 post-treatment day. Furthermore, TC values of SG-GN3 (182.22 ± 22.74 mg/dl) and lovastatin groups (220.78 ± 28.46 mg/dl) decreased at 3 post-treatment day (Fig. 1B).

No significant changes were observed in HDL values among all groups (Fig. 2B). Although not significantly, TC/HDL ratio decreased in SG-GN3 and lovastatin groups compared with the control group treated with only HC-diet at 3 post-treatment day (Fig. 3B).

Initial TG values were different among groups; however, from 3 post-treatment day, no significant differences were observed. At 5 post-treatment day, significant increases in TG values were observed in SG-GN3 treatment groups (only HC-diet control: 78.38 ± 5.49 mg/dl versus SG-GN3: 116.63 ± 9.71 mg/dl) (data not shown).

### 4. Discussion

Triton WR-1339 has been reported to block the removal of TG from plasma (Catanozi et al., 2001). Injection of Triton WR-1339 into rabbits resulted in the increased plasma cholesterol and TG concentrations for up to 36–48 h (Mitropoulos et al., 1994). Triton WR-1339 was also reported to interact preferentially with HDL, changing the size and density of lipoprotein. When Triton-treated HDL parti-
cles were used as substrates for the enzyme LCAT (Lecithin: cholesterol acyltransferase), enzyme activity decreased in parallel to the displacement of apo A-1 (Yamamoto et al., 1984). In our study, the HDL values decreased slightly in Triton WR-1339-treated group compared with the control group (Fig. 2A). On the other hand, the HDL value of SG-GN3 group significantly increased at 50 post-treatment hour. In other studies, Triton WR-1339 suppressed intravascular lipolysis (Chirieac et al., 2000), and lipoprotein lipase activity was significantly inhibited 2 h after the injection of Triton (Borensztajn et al., 1976). The TG value in our Triton WR-1339 Model greatly increased up to 3000 mg/dl, an indication that TG was not hydrolyzed by the lipoprotein lipase. TC values increased in Triton WR-1339-treated groups up to 40 post-treatment hour, and decreased thereafter. Therefore, Triton WR-1339 experiment was performed within 40–50 post-treatment hour, as confirmed through the effectiveness of Triton on TC in the course of time.

The TC/HDL ratio is a better indicator of coronary heart disease risk than individual lipoprotein concentration (Stampfer et al., 1991; Kinosian et al., 1995; Kailash, 1999). Therefore, it may be more appropriate to study the effect of cholesterol on the TC/HDL than on individual lipoprotein concentration. In this study, the TC/HDL ratios of SG-GN3-treated groups steeply decreased in both hypercholesterolemic models compared with the TC values of Triton WR-1339 and HC-diet Models at 50 post-treatment hour and 5 post-treatment day, respectively, an indication that the HDL values of SG-GN3-treated groups did not decrease in both hypercholesterolemic models. Altogether the experiments show that the cholesterol lowering effect of SG-GN3 and it would then provide the opportunity to develop new drugs and food adducts for the prevention and treatment of hypercholesterolemia.

In conclusion, our results confirmed Triton WR-1339 model, except for the high TG values observed and HC-diet model, as effective hypercholesterolemia rat models. Secondly, our investigations demonstrated that SG-GN3, significantly decreased serum TC but did not decrease HDL in Triton WR-1339 model. The serum TC-lowering effect was observed in the HC-diet model. Further studies related to the actions of SG-GN3 on the mechanism may provide fresh insights into new cholesterol lowering materials.

Acknowledgements

This work was supported by the Brain Korea 21 Project, Republic of Korea.

References