Glycoprotein (90 kDa) Isolated from *Opuntia ficus-indica* var. saboten MAKINO Lowers Plasma Lipid Level through Scavenging of Intracellular Radicals in Triton WR-1339-Induced Mice

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The *Opuntia ficus-indica* var. saboten MAKINO (OFI) has been traditionally used as health food and herbal agent in folk medicine in Korea. In this study, we investigated whether the OFI glycoprotein has antioxidative activity and hypolipidemic effect on Triton WR-1339-induced A/J mice. The OFI glycoprotein inhibits the production of reactive oxygen species (ROS) generated by glucose/glucose oxidase (G/GO) in BNL CL.2 cells. With its antioxidative property, the mice were orally administered in the OFI glycoprotein [50 mg/kg body weight (BW)] for two weeks. Our finding resulted in a significant decrease of plasma lipid levels in Triton WR-1339-treated mice such as total cholesterol (TC), triglyceride (TG), and low-density lipoprotein (LDL). Indeed, mice which induced by Triton WR-1339 were significantly increased the levels of TC, TG and LDL, whereas the high-density lipoprotein (HDL) level obviously decreased. However, the values were reversed at pretreatment with OFI glycoprotein in Triton WR-1339-treated mice. The data also showed that pretreatment with OFI glycoprotein resulted in decrease of thiobarbituric acid-reactive substances (TBARS) level and in increase of nitric oxide (NO) amount in presence of Triton WR-1339-treated mice, while the activities of antioxidant enzyme [superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx)] were augmented. Therefore, we speculate that the OFI glycoprotein would be effective in lowering of plasma lipid levels.

Key words *Opuntia ficus-indica* (OFI) glycoprotein; antioxidant enzyme; plasma lipid

Cardiovascular disease including atherosclerosis is the most common cause of death in Korea where has changed to western food pattern. Especially, high levels of low-density lipoprotein (LDL) and triglycerides (TG) are mainly responsible for initiation in atherogenesis. It has reported that hypercholesterolemic or hyperlipidemic state leads to an increase in free radical production as well as high levels of plasma lipoprotein [LDL, TG and total cholesterol (TC)]. Once oxygen-derived radicals attack LDL, oxidatively modified LDL cannot only damage arterial tissue, but it can also attack white blood cells. An increase of reactive oxidants during hyperlipidemia can also cause the abnormal function of endothelial cells via reduced nitric oxide (NO) availability. In addition, there are changes in the enzymatic as well as non-enzymatic antioxidative defense systems such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), ascorbic acid and glutathione during hyperlipidemia, suggesting a reduced resistance to reactive oxidant-mediated damage. Thus, consumption of dietary source with antioxidative potential was closely associated to its beneficial effects on the overall disease processes.

Triton WR-1339, one of the well-known non-ionic detergents, induces the elevation of plasma cholesterol and triglyceride levels in fasted- or non-fasted mice. This mechanism has been proven to be a consequence of trapping of cholesterol and triglyceride in the blood, and subsequently to reduce the influx of plasma in the liver. Therefore, the Triton WR-1339 model has been examined not only as a screening method for hypolipidemic agents, but also as a means for elucidating lipid metabolism.

The *Opuntia ficus-indica* var. saboten MAKINO (OFI) is a traditional health food which has been used to improve nutritional status and to prevent various diseases in South Korea for a long time. Recently, it was isolated glycoprotein from OFI (OFI glycoprotein) with an approximate molecular mass of 90 kDa, and consists of carbohydrate contents (37.54%) and protein contents (62.46%). In previous study, our results showed that the OFI glycoprotein had scavenging activities against oxygen radicals in cell-free systems, and that it also had cytoprotective and anti-apoptotic activities in oxygen radical-induced NIH/3T3 cells. However, nobody has studied the biological effect of OFI glycoprotein on plasma lipid level in animal models.

Therefore, to understand property of OFI glycoprotein, we evaluated the level of TBARS, NO amounts, and the activities of antioxidant enzyme such as SOD, CAT and GPx. Also, we investigated whether the OFI glycoprotein modulates the plasma lipid levels (TC, TG, LDL and HDL) through its antioxidative property in Triton WR-1339-induced mice.

MATERIALS AND METHODS

Chemicals All the plastic materials were purchased from Falcon Labware (Becton-Dickinson, Franklin Lakes, NJ, U.S.A.). Dextran sulfate, β-nicotinamide adenine dinucleotide phosphate (β-NADPH), and triton WR-1339 were obtained from Sigma (St. Louis, MO, U.S.A.). The other chemicals and reagents were of the highest quality available.

Preparation of the OFI Glycoprotein The *Opuntia ficus-indica* var. saboten MAKINO were collected from Naju traditional market in the Chonnam province of South Korea, and OFI glycoprotein was isolated from it as described previously. The purity of OFI glycoprotein is more than 95%. The dried sample (4.5 mg, 0.05% from the original sample) was stored at −20°C during the experimental period. We used OFI glycoprotein with a molecular weight of 90 kDa in
this study.

**Cell Culture** BNL CL.2 cells (murine embryonic liver cell) were incubated in DMEM containing 10% FBS, 100 U/ml penicillin, and 100 µg/ml streptomycin at 37 °C, and 5% CO₂ atmosphere. The number of cells (1×10⁶ cells/ml) was divided into 96-well flat bottom plates. Oxidative stress was induced using the glucose/glucose oxidase (G/O) system, which involved the generation of hydrogen peroxide (H₂O₂) at a continuous rate by placing the cells in serum-free medium supplemented, and then treating the cells with 100 µM H₂O₂ for indicated time. Following the various treatments, medium was removed and the cells were incubated in 100 µl new medium containing 10 mg/ml neutral red for 90 min at 37 °C. After that, the cells viability for cytotoxicity of OFI glycoprotein was determined by neutral red assay, as previously. Briefly, the cells were treated with 40 mU/ml of glucose oxidase (GO), or co-treated with OFI glycoprotein (25—100 µg/ml) for indicated time. Following the various treatments, medium was removed and the cells were incubated in 100 µl new medium containing 10 mg/ml neutral red for 90 min at 37 °C. After that, the medium was removed, and the wells were washed three times with 100 µl PBS. One hundred micro liter 50% ethanol containing 50 mM sodium citrate (pH 4.2) was added into the each well on the 96-well multiple plate. After 20 min, the absorbance was measured at 510 nm using a SpectraCount® (Packard, Instrument Co., Meriden, U.S.A.) ELISA reader.

**Determination of Intracellular ROS** Amount of intracellular ROS was measured by using nonfluorescent 2',7'-dichlorodihydrofluorescein diacetate (H₂DCF-DA). H₂DCF-DA is a fluoresgenic freely permeable tracer specific for ROS assessment. It is deacetylated by intracellular esterases to the non-fluorescent 2',7'-dichlorofluorescein (DCF) by ROS. Cells were pre-incubated with 10 µM H₂DCF-DA for 30 min at 37 °C, and then washed twice with PBS to remove the excess of H₂DCF-DA. After that, the cells were treated with 40 mU/ml of glucose oxidase (GO), or co-treated with OFI glycoprotein (25—100 µg/ml) for indicated time. Finally, the fluorescence intensity was measured at excitation wavelength of ∼485 nm and emission wavelength of ∼530 nm using fluorescence microscopy reader (Dual Scanning SPECTRAmax, Molecular Devices Corporation, Sunnyvale, CA, U.S.A.). The values were calculated as relative intensity of DCF fluorescence, compared to the control.

**Experimental Design** Male mice (A/J), aged 5 weeks, were purchased from Daehan Lab. (Animal Research Center Co., Ltd, Dae Jeon, Korea) and housed according to the animal care guidelines approved by the Animal-care Committee of the American Society of Mammalogists at the Experimental Animal Room of Veterinary College of Chonnam National University (CNU). The mice were fed a commercial diet and water ad libitum during all experimental periods, and kept for at least 1 week prior to the experiments.

To investigate the hypolipidemic effect of the OFI glycoprotein, the mice were divided into three groups of six mice per group (the control and two OFI glycoprotein treatment groups). The OFI glycoprotein was orally administered at a dose [25 or 50 mg/body weight (kg)] once a day for 2 weeks, and the control group was administered with 100 µl of phosphate-buffered saline (PBS). During day 15, blood was collected by cardiac puncture.

The base of the experimentally induced hyperlipidemic animal model was the Triton WR-1339-induced model according to the method of Kusama et al. and Lee et al. The mice were divided into four groups of six mice per group (the control, Triton WR-1339 and two OFI glycoprotein treatment groups). OFI glycoprotein was administered orally once a day for 3 d. Triton WR-1339 was dissolved in PBS to a final concentration of 10%, and intraperitoneally injected at a dose of 400 mg/body weight (kg). 18 h after the Triton WR-1339 injection, 1 ml blood samples were drawn from the mice by cardiac puncture under diethyl ether anesthesia and centrifuged at 1500×g for 20 min at 4 °C, the supernatant was separated and stored at −70 °C. On the other hand, to determine the effects of OFI glycoprotein, the relative values of body and liver weights were calculated as shown the equations 1 and 2.

Relative value of BW:

\[
\frac{\text{BW}_{B} - \text{BW}_{A}}{\text{BW}_{A}} 
\]

Relative value of LW:

\[
\frac{\text{LW}_{B} - \text{LW}_{A}}{\text{LW}_{A}} 
\]

where BWB is the body weight before administration, BWA is the body weight after administration, LW is the liver weight and BW is the body weight.

**Determination of the Plasma Lipoprotein Levels** The amounts of plasma lipoprotein were measured according to the method of Warnick et al. and all experiments for fractionation and determination of the various lipoproteins in the plasma were conducted as previously described. To evaluate the amount of total cholesterol (TC), we separately determined VLDL, LDL and HDL. The fractions containing VLDL and LDL were determined by mixing 1 ml of plasma containing EDTA with 100 µl of reagent A [equal volumes of dextran sulfate (MW 50000 ± 5000; 20 g/l) and MgCl₂ (1.0 m)] for 10 min, and by centrifuging at 10000×g for 2 min. We used half of the supernatant for VLDL and LDL, and the rest for HDL. To assess the fraction containing HDL, 0.5 ml of the supernatant was further mixed with 50 µl of reagent B [1 volume of dextran sulfate (0.04 g/ml) with 3 volumes of MgCl₂ (2 m)] for 15 min, before being centrifuged at 100000×g for 15 min. The resulting supernatant was designated as the fraction containing HDL. The amounts of each fraction (those containing VLDL, LDL and HDL) were quantified by the colorimetric method described previously. Lipid peroxidation was estimated according to the presence of TBARS in plasma using the method of Buege and Aust. The result was expressed as relative percentages. One volume of a sample was thoroughly mixed with two volumes of a stock solution containing 15% (w/v) trichloroacetic acid, 0.375% (w/v) thiobarbituric acid and 0.25 N HCl. The mixture was heated for 30 min in a boiling water bath. After cooling, the flocculated precipitate was removed by centrifugation at 10000×g for 10 min, and the absorbance of the sample was measured at 535 nm. 1,1,1,3-Tetraethoxypropane was used as the standard. Each data value is expressed as a percentage of the control.

**Plasma NO Levels** The production of NO by the endothelium was indirectly assessed by measuring the nitrite levels in the plasma based on the Griess reaction. Plasma sample was diluted four times with distilled water and deproteinized by adding 1:20 volume of zinc sulfate (0.3 g/ml) to a final concentration of 0.15 g/ml. After centrifugation at...
10000×g for 5 min, the supernatant (100 μl) was mixed with 100 μl of the Griess reagent (1% sulfanilamide and 0.1% N-1-naphthylethylenediamine dihydrochloride in 2.5% polyphosphoric acid). After 10 min at room temperature, the absorbance was measured at 540 nm with a MicroReader (Hyperion, U.S.A.). Nitrite was quantified against a standard curve for sodium nitrate.

Measurement of the Antioxidant Enzyme Activities
The antioxidant enzyme activities in A/J mice were measured according to the methods of Beauchamp and Fridovich24) for SOD, Thomson et al.25) for CAT, and Paglia and Valentine26) for GPx. Liver tissues were homogenized with 5 ml of a 0.25 M sucrose buffer (pH 7.5) containing 10 mM EDTA. The homogenates were then centrifuged at 600×g for 10 min at 4 °C to remove nuclear fractions, and the remaining supernatant was re-centrifuged at 10000×g for 20 min at 4 °C to collect the mitochondrial fraction (pellet) for a CAT assay. The supernatant was ultra-centrifuged at 100000×g for 1 h at 4 °C to isolate the cytosolic fraction for an SOD and GPx assays. The amount of protein was measured using the Lowry method27) and the proteins were stored at −70 °C for further experimental use. One unit of antioxidant enzymes are defined as the amount of enzyme required to reduce the NBT by 50% for SOD at 560 nm, to reduce 1 μM of H₂O₂/min for CAT at 220 nm, and to oxidize 1 μM of NADPH/min for GPx at 340 nm, respectively. Results were represented as an unit/mg protein in each supernatant and the values were calculated as relative percentages to the control value.

Statistical Analysis Each value from in vivo (n=6) experiments is expressed as the mean±S.D. A one-way analysis of variance (ANOVA) and the Duncan test were used for multiple comparisons (SPSS program, ver. 11.0).

RESULTS

Scavenging Effect of ROS by OFI Glycoprotein in G/GO-Induced BNL CL.2 Cells We investigated the inhibitory effect of OFI glycoprotein on G/GO system-induced BNL CL.2 cells. As shown in Fig. 1A, the relative content of intracellular ROS was gradually increased in treatment with 40 mU/ml GO for indicated incubation time. In contrast, the treatment of OFI glycoprotein resulted in dose-dependent inhibition of G/GO system-induced ROS production in BNL CL.2 cells (Fig. 1B). For example, when the cells were treated with 40 mU/ml of GO for 4 h, the levels of ROS were significantly increased by 0.68, compared with the control. However, the treatment of OFI glycoprotein in presence of 40 mU/ml GO was significantly diminished by 0.24, 0.36 and 0.47 at 25, 50 and 100 μg/ml of OFI glycoprotein for 4 h, compared with the 40 mU/ml GO treatment alone. Furthermore, OFI glycoprotein has cytoprotective activity against radical generated by G/GO system (Fig. 1C). When the cells were exposed to 40 mU/ml GO for 4 h, the cell viability was decreased considerably. However, the addition of OFI glycoprotein prior to 40 mU/ml GO treatment induced a dose-dependent increase in the cell survival rate. For instance, the cell viability values were augmented by 6.2, 18.4 and 27.9% at 25, 50 and 100 μg/ml of OFI glycoprotein, respectively, with the 40 mU/ml GO treatment alone.

Inhibitory Effect of OFI Glycoprotein on Lipid Peroxidation As shown in Fig. 2, the administration of 50 mg/kg OFI glycoprotein in the absence of Triton WR-1339 did not change in the level of TBARS. In the Triton WR-1339-treated mice, the level of TBARS was significantly increased by 41.8%, compared with the control. However, the administration of OFI glycoprotein (25 or 50 mg/kg) prior to Triton WR-1339 treatment resulted in the decrease of TBARS levels by 13.3 and 30.9%, compared with the Triton WR-1339 treatment group, respectively.

Inhibitory Effect of OFI Glycoprotein on the Plasma Nitric Oxide (NO) Amounts When the mice were treated with Triton WR-1339 in the absence of the OFI glycoprotein, the amount of plasma nitric oxide was significantly decreased by 10.4 μM, compared with the control. However, the level of plasma nitric oxide was gradually increased by after the addition of the OFI glycoprotein (25 or 50 mg/kg) in the presence of Triton WR-1339, although the level of plasma NO in the presence of the OFI glycoprotein was not changed significantly without Triton WR-1339 (Fig. 3).

Effect of OFI Glycoprotein on the Antioxidant Enzyme Activities When the mice were administrated to the OFI
glycoprotein (50 mg/kg) alone, the activities of the hepatic antioxidant enzyme, SOD, CAT and GPx, were markedly higher than that of the control group. For instance, the activities of SOD, CAT and GPx were increased by 14.2, 11.3 and 16.5% at dose of 50 mg/kg OFI glycoprotein, compared with the control, respectively. In the Triton WR-1339-induced mice, the activities of the antioxidant enzyme were decreased. The reduced activities of the antioxidant enzyme, however, were all increased after the addition of the OFI glycoprotein in a dose-dependent manner. For instance, when the mice were pretreated with 50 mg/kg of the OFI glycoprotein prior to the Triton WR-1339 injection, the activities of SOD, CAT and GPx were respectively increased by 12.2, 19.8 and 28.9%, compared with the Triton WR-1339-treated group (Fig. 4).

**Effect of OFI Glycoprotein on the Plasma Lipid Levels**

The effect of OFI glycoprotein on the plasma lipid levels in mouse was calculated and presented in Table 1. There was no significant difference in the relative weight gain or liver weight between the treatment groups. When the mice were administered with 50 mg/kg of OFI glycoprotein, the amounts of TC, TG and LDL were significantly decreased in a dose-dependent manner, compared with the control. For instance, the amounts of TC, TG and LDL were diminished by 11.7, 9.5 and 14.8 mg/dl in the mice fed with 50 mg/kg of OFI glycoprotein in comparison with the control, respectively. However, the level of HDL showed no significant change in this group. In the hyperlipidemic mouse, the amounts of TC, TG and LDL after Triton WR-1339 injection were increased by 31.4, 54.6 and 31.6 mg/dl, respectively, whereas the level of HDL was reduced by 12.1 mg/dl, compared with the control (Table 2). However, pretreatment with the OFI glycoprotein (25 or 50 mg/kg) prior to the Triton WR-1339 injection significantly reduced the levels of TC, TG and LDL in a dose-dependent manner. For instance, when the mice were pretreated with 50 mg/kg of the OFI glycoprotein, the amounts of TC, TG and LDL were gradually reduced by 18.9, 37.1 and 13.1 mg/dl, respectively, compared with the Triton WR-1339 treatment group. The level of HDL in pretreatment of the OFI glycoprotein (50 mg/kg) was considerably increased by 10.6 mg/dl, compared with the Triton WR-1339-treated mice.

**Table 1. Effect of the OFI Glycoprotein on Plasma Lipid Levels**

<table>
<thead>
<tr>
<th>Control</th>
<th>OFI glycoprotein (mg/kg BW)</th>
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<tbody>
<tr>
<td></td>
<td>25</td>
</tr>
<tr>
<td>Changes</td>
<td></td>
</tr>
<tr>
<td>Food intake (g/day)</td>
<td>3.10±0.48</td>
</tr>
<tr>
<td>Relative body weight (%)</td>
<td>99.38±0.56</td>
</tr>
<tr>
<td>Relative liver weight (%)</td>
<td>5.1±0.18</td>
</tr>
<tr>
<td>Lipids</td>
<td></td>
</tr>
<tr>
<td>TC (mg/dl)</td>
<td>166.1±0.27</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>68.7±0.43</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>39.4±0.32</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>96.3±0.22</td>
</tr>
</tbody>
</table>

The OFI glycoprotein was orally administered at doses (25 and 50 mg/kg BW) once a day for 2 weeks and the control group was administered with 100 μl of PBS. The amount of plasma cholesterol was measured as described in Materials and Methods. Data represents the means±S.D. from triplicate experiments (n=6). * represents a significant difference compared with the control, p<0.05. ** represents a significant difference compared with the control, p<0.01. C: control; TC: total cholesterol; TG: triglyceride; HDL: high-density lipoprotein; LDL: low-density lipoprotein.
Completely understood. In the present study, we investigated the biological functions of glycoproteins are still not completely understood. In the present study, we investigated the biological functions of glycoproteins are still not completely understood. In the present study, we investigated the biological functions of glycoproteins.

Table 2. Plasma Lipid Levels of the OFI Glycoprotein in Triton WR-1339-Induced Mice

<table>
<thead>
<tr>
<th>Changes</th>
<th>Control</th>
<th>Triton WR-1339 + OFI glycoprotein (mg/kg BW)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Food intake (g/day)</td>
<td>3.10±0.48</td>
<td>3.03±0.36</td>
</tr>
<tr>
<td>Relative body weight (%)</td>
<td>99.38±0.56</td>
<td>99.78±0.48</td>
</tr>
<tr>
<td>Relative liver weight (%)</td>
<td>5.1±0.18</td>
<td>5.23±0.29</td>
</tr>
<tr>
<td>Lipids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TC (mg/dl)</td>
<td>164.1±0.23</td>
<td>195.5±0.65</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>67.7±0.11</td>
<td>122.3±0.24</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>38.0±0.45</td>
<td>25.9±0.17</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>90.3±0.13</td>
<td>121.9±0.63</td>
</tr>
</tbody>
</table>

The OFI glycoprotein was orally administered 1 h before the Triton WR-1339 injection once a day for 3 d. The amount of plasma cholesterol was measured as described in the Materials and Methods section. Data represents the means±S.D. from triplicate experiments (n=6). * represents a significant difference with the control, p<0.05. * represents a significant difference between treatment with Triton WR-1339 after pretreatment of OFI glycoprotein and Triton WR-1339 treatment alone, p<0.05. C: control; TC: total cholesterol; TG: triglyceride; HDL: high-density lipoprotein; LDL: low-density lipoprotein.

DISCUSSION

Abnormal lipid levels, particularly high levels of plasma lipoprotein (hyperlipidemia), contribute significantly to the risk of coronary heart disease, a major cardiovascular disease and a serious health problem in our society.1,2 Besides, oxidative stress caused by imbalance between generation of ROS and antioxidant defenses is critical role in the etiology of atherosclerosis.2,28) In recent, various dietary and pharmacologic treatments have been devised to reduce elevated blood cholesterol levels. Of these, many polysaccharide-protein complexes (glycoproteins) isolated from mushrooms, fungi, yeasts, algae, lichens, and plants have been evaluated its biological activities in terms of their clinical efficacy, such as their immunomodulatory, antioxidative, hypolipidemic and hypcholesterolemic effects, although the mechanisms for the biological functions of glycoproteins are still not completely understood.6,8,12) In the present study, we investigated that OFI glycoprotein has a hypolipidemic effect on plasma lipid levels in Triton WR-1339-induced mice. In order to verify the antioxidative property of OFI glycoprotein, we additionally tested the scavenging activity of OFI glycoprotein using G/GO system in BNL CL.2 cells. The results were showed that OFI glycoprotein did not any cytotoxic effect, but it was stead a protector of liver cells by its scavenging activity against G/GO-induced radical production (Fig. 1). These results indicate that OFI glycoprotein exerts, directly or indirectly, antioxidant and cytoprotective effect in BNL CL.2 cells. With respect to antioxidative property, the data from these experiments were understood that OFI glycoprotein showed a significant effect on the plasma lipid levels by reducing plasma TC, and TG and LDL (Table 1). Furthermore, the lowering effects of the OFI glycoprotein on plasma lipid levels were also revealed in Triton WR-1339-induced mice model. For instance, the plasma lipid levels (TC, TG and LDL) were increased after a treatment with Triton WR-1339, as similarly conducted in previous studies.10,12) However, the administration of OFI glycoprotein in the Triton WR-1339-induced mice significantly decreased the levels of TC, TG and LDL, whereas the level of HDL increased in a dose-dependent manner (Table 2). Interestingly, the data speculate that an increase in the HDL level by supplementation with the OFI glycoprotein may be responsible, in part, for the anti-atherosclerotic effect in mice.

The oxidative modification of lipids, particularly oxidation of LDL, has been reported as one of the possible mechanisms leading to cardiovascular disease. Namely, oxidatively modified LDL activates endothelial cells, leading to an alteration of the functional and structural integrity of the endothelial barrier, thus resulting in endothelial dysfunction.29) The superoxide produced by endothelium directly inactivates endothelium-derived NO and may also increase the subsequent oxidation of LDL particles by the formation of peroxynitrite. It has also been proposed that NO exhibits hypocholesterolemic activity due to its ability to interfere with the production of apoprotein B (apo B), the main constituent of LDLs.29) Endothelial-derived NO exerts vasodilatory, growth regulatory and anti-inflammatory effects, thus being an important regulator of cardiovascular homeostasis. The anti-atherogenic effect of antioxidants is closely associated to the inhibition of lipoprotein oxidation. It means that a decrease in lipid peroxidation leads to a reduction of atherosclerosis.

The administration of the OFI glycoprotein in this study suppressed the increased TBARS level and decreased NO amount in hyperlipidemic mice, considerably. It seems to be that the OFI glycoprotein had an antioxidative effect and counteractive effect against hyperlipidemia to some extent. In this point of view, our results exhibit that, in addition to lowering cholesterol, the OFI glycoprotein played a critical role in inhibiting lipid peroxidation and the oxidation of lipoproteins in Triton WR-1339-treated mice (Fig. 2 and 3). One of possible speculation of the inhibitory mechanism of oxidation is that supplement of OFI glycoprotein stimulates the activities of antioxidant enzymes in the liver. Indeed, administration of the OFI glycoprotein resulted in an increase in the activities of hepatic antioxidant enzyme. In the hyperlipidemia, several reports have shown that the antioxidant defense system is all reduced,30,31) thereby may lead to the production of toxic intermediates by elevating the lipid peroxide content.13 In other words, an increase of production of oxygen-free radicals in hypercholesterolemia exert plasma lipid levels by causing lipid peroxidation, decrease of antioxidant enzyme activities, and NO inactivation, although the precise mechanism is still not understood. In the present study, a supplement of the OFI glycoprotein all increased the activities of antioxidant enzyme in the Triton WR-1339-induced
mice. Interestingly, the OFI glycoprotein tended to more elevate the activities of CAT and GPx than that of the SOD (Fig. 4), indicating the inhibitory effect of OFI glycoprotein on lipid peroxidation during Triton WR-1339-induced hyperlipidemia. It is estimated that the radical scavenging property of OFI glycoprotein affects the antioxidant enzyme activities, and recovery of the function of endothelial cells via NO availability by blocking lipid peroxidation, and it could be changed plasma lipid levels. Taken together, our results showed that OFI glycoprotein has hypolipidemic and antioxidative effects on Triton WR-1339-induced hyperlipidemic mice. The possible reason is that OFI glycoprotein indirectly brought about the hypolipidemic effects through (via) either scavenging the intracellular ROS induced by Triton WR-1339 due to its antioxidative potential, or inactivation of HMG CoA synthase due to interact protein (OFI glycoprotein) and protein (HMG CoA synthase or LDL receptor). Still, it remains to elucidate the precise mechanism for the inhibitory effect on cholesterol biosynthesis by OFI glycoprotein.

In conclusion, our results showed that the OFI glycoprotein had a scavenging activity of radical production in G/GO-induced system. In the hyperlipidemic system, it has an ability to lower plasma lipids (TC, TG and LDL), to increase antioxidative enzyme activities, to block lipid peroxidation, to increase NO level. In other words, these results indicated that the OFI glycoprotein lowers levels of plasma lipid through its scavenging property of intracellular radical. Further research remains to elucidate the precise mechanism for the inhibitory effect on cholesterol biosynthesis by the OFI glycoprotein at the molecular and cellular levels.

Acknowledgments This study was financially supported by research funding from Chonnam National University in 2006.

REFERENCES