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Antihyperlipidemic effects of stilbenoids isolated from *Morus alba* in rats fed a high-cholesterol diet

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ABSTRACT

Mulberroside A (MUL) was purified from an ethanol extract of *Morus alba* root, and oxyresveratrol (OXY) was produced by enzymatic conversion of MUL. Normal rats, Triton WR-1339-induced hyperlipidemic rats, and high-cholesterol diet (HCD)-induced hyperlipidemic rats were orally treated with MUL or OXY (1–5 mg/kg/day). MUL and OXY were administered 1 h prior to concomitant treatment with Triton WR-1339 for a further 24 h, whereas the drugs were administered concurrently with HCD for 4 weeks. Oral MUL and OXY pre-treatment vs. water pre-treatment of Triton WR-1339-induced hyperlipidemic rats significantly ($p < 0.05$) reduced the levels of serum lipids in a dose-dependent manner, while high-density lipoprotein cholesterol (HDL-C, or "good" cholesterol) levels were increased. Oral MUL and OXY treatment of HCD-fed rats also showed a significant ($p < 0.05$) dose-dependent decrease in serum lipids, coronary artery risk index (CRI), and atherogenic index (AI), but not HDL-C. Furthermore, MUL and OXY treatment of HCD-induced hyperlipidemic rats demonstrated a significant dose-dependent improvement in the histological features of hepatic fatty degeneration. Aspartate aminotransferase and alanine aminotransferase values in OXY-treated normal rats were not significantly different from those in water-treated control rats. These results indicate that MUL and OXY might be developed as novel anti-hyperlipidemic agents.

1. Introduction

Hyperlipidemia is a major cause of cardiovascular disease (Fodor et al., 2000; Gotto, 2005). Treatment of hyperlipidemia ideally reduces the levels of low-density lipoprotein cholesterol (LDL-C, or "bad" cholesterol) in the blood and attenuates the risk of the disease (Ballantyne, 2007). Statin trials are mainstays for the treatment of cardiovascular disease; however, statins are associated with a number of adverse events. These include muscle-related complaints, such as rhabdomyolysis, myalgia, cramps, and muscle weakness (i.e., myopathy) (Parker and Thompson, 2012; Sewright et al., 2007). Statins also provoke liver dysfunction and renal failure (Fernandes et al., 2012; Beltowski et al., 2009). The number of patients with statin-related disturbances has recently increased, due to the widespread use of these drugs as blood cholesterol-lowering agents. Therefore, the development of promising cholesterol-lowering treatment alternatives to statins is of utmost importance.

Screening of phytochemicals as new drug candidates for managing hyperlipidemia is an encouraging endeavor. Many inhibitors of cholesterol synthesis (e.g., flavonoids (Raederstorff et al., 2003; Arai et al., 2000) and dietary fiber (Anderson et al., 2009)) are derived from natural sources and effectively lower blood cholesterol levels. Antioxidants are also reported to possess blood cholesterol-lowering activity (Hsu and Yen, 2007; Han et al., 2003). Stilbenoids, hydroxylated derivatives of stilbene that show antioxidative activity (Chao et al., 2008; Lorenz et al., 2003), may similarly exert anti-hyperlipidemic actions.

Mulberroside A (MUL) is a glycosylated stilbenoid and a predominant bioactive component of *Morus alba*, whereas oxyresveratrol (OXY) is an aglycone derivative of MUL. *M. alba* is widely used in traditional medicine, finding utility as a cough suppressant, anti-asthmatic, antibiotic, and anticancer agent (Venkatesh and Seema, 2008; Park et al., 2003). Of importance to the current study, an alcohol extract prepared from *M. alba* root bark demonstrated hypocholesterolemic and antioxidant effects in previous work (El-Beshbishy et al., 2006). In the current study, we purified MUL from the roots of *M. alba* and produced OXY by biotransformation (Kim et al., 2010). The antihyperlipidemic effects of MUL and OXY (1–5 mg/kg) were then investigated in normal rats and two experimental rat models of hyperlipidemia, and compared with a well-known lipid-lowering drug, simvastatin.

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2. Materials and methods

2.1. Chemicals

Mulberroside A (2,5′-dihydroxy-4,3′-bis(β-D-glucopyranosyloxy)-*trans*-stilbene, MUL) was prepared from an ethanol extract of *M. alba* roots, and oxyresveratrol (2,3′,4,5′-tetrahydroxy-*trans*-stilbene, OXY) was produced from MUL via enzymatic transformation by using Pectinex (Novozymes, Bagsvaerd, Denmark), as previously described (Kim et al., 2010). Triton WR-1339, simvastatin, and dimethyl sulfoxide (DMSO) were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

2.2. Experimental animals and diets

A total of 54 male Sprague Dawley rats, 5 weeks old and weighing around 100–110 g, were purchased from Koatect (Gyeonggi do, Korea) and adapted to a normal diet for 1 week. Rats were housed in cages under strict standard conditions (22 ± 1 °C; 55 ± 5% humidity; 12-h light and 12-h dark cycle). All animals had free access to water, as well as a normal diet or an experimental HCD purchased from Feedlab (Gyeonggi do, Korea). The composition of the diets is shown in Table 1. All experimental procedures were approved by the Korea University Institutional Animal Care and Use Committee (Approval No. KUIACUC-2012-100) and were in accordance with the Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, 1996).

2.3. Treatment of normal rats

Normal rats were randomly divided into five groups (six rats per group) and fed the normal diet described in Table 1. Group I rats were orally treated with 10 ml/kg/day distilled water for 4 weeks. Group II rats were orally treated with1 mg/kg simvastatin for 4 weeks. Groups III, IV, and V rats were orally treated with 1, 2.5, and 5 mg/kg/day OXY in 10 ml/kg DMSO and distilled water (1:9, v/v), respectively, for 4 weeks.

2.4. Induction of Triton WR-1339-induced hyperlipidemia

Normal rats fed the normal diet described in Table 1 were fasted overnight and then randomly divided into nine groups (six rats per group). Triton WR-1339 was treated to provoke acute hyperlipidemia. Group I rats served as the untreated negative control and were orally treated with 10 ml/kg distilled water. Group II (experimental model control) rats were pre-treated orally with 10 ml/kg distilled water for 1 h before receiving 200 mg/kg Triton WR-1339 intraperitoneally (Adeneye et al., 2010). Group III (positive control) rats were pre-treated orally with 1 mg/kg simvastatin dissolved in 10 ml/kg distilled water for 1 h before intraperitoneal injection of 200 mg/kg Triton WR-1339. Rats in Group IV, V, and VI were pre-treated orally with 1, 2.5, and 5 mg/kg MUL, respectively, for 1 h before intraperitoneal injection of 200 mg/kg Triton WR-1339. Group VII, VIII, and IX rats were pre-treated orally with 1, 2.5, and 5 mg/kg OXY, respectively, for 1 h before intraperitoneal injection of 200 mg/kg Triton WR-1339. After 24 h of Triton WR-1339 treatment, blood samples were collected from the heart chamber under inhaled diethyl ether anesthesia, and serum samples were obtained for analysis of TC, HDL-C, LDL-C, VLDL-C, and TG levels.

2.5. Induction of HCD-induced hyperlipidemia

To investigate the effect of mulberroside A and oxyresveratrol on the protection efficacy against high-cholesterol diet (HCD)-induced hyperlipidemia, we used a high-cholesterol diet (HCD)-induced hyperlipidemic rat model. Rats were randomly divided into nine groups (six rats per group) and treated with a daily oral injection of each test compound for 4 weeks. Group I rats (negative control) were concurrently fed a normal diet and treated with 10 ml/kg/day distilled water for 4 weeks. Group II (experimental model control) rats were fed the HCD instead of the normal diet (Table 1). Group III (positive control) rats were fed the HCD and treated with 1 mg/kg/day simvastatin. Rats in Groups IV, V, and VI were fed the HCD and treated with 1, 2.5, and 5 mg/kg MUL, respectively. Group VII, VIII, and IX rats were fed the HCD and treated with 1, 2.5, and 5 mg/kg OXY, respectively.

Table 1
Composition of experimental diets.

Components	Normal diet (g/100 g)	HCD (g/100 g)
Casein	18.00	18.00
Mineral mix[a]	4.00	4.00
Vitamin mix[a]	1.00	1.00
Corn oil	5.00	5.00
Sucrose	5.00	5.00
Starch	61.80	60.55
Sodium cholate	–	0.25
Methionine	0.20	0.20
Cholesterol	–	1.00
Cellulose	5.00	5.00

[a] The mineral mix and the vitamin mix are based on those provided by Daejung Chemical&Metals Co. Ltd. (Gyeonggi-do, Korea), and the diets were purchased from Feedlab Co. (Gyeonggi-do, Korea).

2.6. Measurement of weekly food intake, body weight, and relative liver weight

Daily food intake was assessed by subtracting the weight of any leftover food from the weight of the total amount of food provided, divided by six for each group. The body weight of each rat was measured every week. The absolute liver weight was measured, and the relative liver weight per 100 g of total body weight of each rat was calculated as follows:

$$\text{Relative liver weight} = \frac{\text{weight of rat liver (g)}}{\text{body weight on final experiment day (g)}} \times 100$$

2.7. Blood collection and biochemical assays

At the end of the 4-week treatment, blood samples were collected from the heart chamber of overnight fasted rats under inhaled diethyl ether anesthesia. Blood samples were allowed to clot at room temperature for 1 h, and were then centrifuged at 10,000*g* at 4 °C for 20 min to separate out the sera. Serum TG, TC, HDL-C, AST, and ALT levels were measured by using the FUSI DRI-CHEM SLIDE kit and a FUSI DRI-CHEM 4000 analyzer (Fujifilm, Tokyo, Japan).

Serum LDL-C levels were calculated by applying Frieldwann's equation:

$$\text{LDL-C} = \left[\text{TC} - (\text{HDL-C} + \left(\frac{TG}{5}\right)\right]$$

Serum VLDL-C levels were calculated by applying the following equation:

$$\text{VLDL-C} = \frac{\text{TG}}{5}$$

2.8. Determination of AI and CRI

The AI and the CRI were calculated as follows:

LDL-C (mg/dl)/HDL-C (mg/dl) (Abbott et al., 1988) and TC (mg/dl)/HDL-C (mg/dl) (Adeneye et al., 2010), respectively.

2.9. Hepatic morphology

For histological analysis, liver sections were fixed in 10% formalin and embedded in paraffin. Sections were prepared at a thickness of 5 μm, dyed with hematoxylin and eosin (H&E), and then mounted with Canada balsam. The slides were observed by using an optical microscope at 200× magnification (Hsu et al., 2009).

2.10. Statistical analysis

All data are presented as mean ± standard error (SE). The data were assessed by using the SPSS statistical analysis program (Chicago, IL, USA). Significant differences between the groups were determined by using one-way analysis of variance. Post hoc Duncan's multiple-range tests were performed when between-group differences were identified. Results were considered to be statistically significant at $p < 0.05$.

3. Results and discussion

3.1. Effect of OXY on body weight and serum lipid levels in normal rats

OXY or simvastatin (positive control) were orally administered to rats fed with a normal diet for 4 weeks (Table 1). OXY-treated vs. water-treated normal rats exhibited a significant ($p < 0.05$) decrease in the percentage change in body weight (% Δ body weight) in a dose-dependent manner (Table 2). Simvastatin also lowered the % Δ body weight. In addition, relative liver weight was significantly ($p < 0.01$) lower in rats fed a normal diet and given simvastatin or OXY than in negative control animals that received distilled water alone. However, the mean daily food consumption of all experimental rats was similar.

Serum lipid levels were next quantified. Serum total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), LDL-C, and very low-density lipoprotein cholesterol (VLDL-C) levels were 70.5 ± 19.0, 91.2 ± 28.0, 63.3 ± 23.3, 13.7 ± 7.6, and