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Effect of the monascus pigment threonine derivative on regulation of the cholesterol level in mice

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Abstract

Different amino acid derivatives were synthesized during cultivation of a *Monascus* species. Derivatives exhibiting an inhibitory activity against HMG-CoA reductase were screened by *in vitro* tests. The threonine derivative had a high inhibitory activity of 38% while four other derivatives showed a greater than 23% activity. The orange monascus pigment showed a high activity of 36%. *In vivo* tests using female C57BL/6 mice were performed with the threonine derivative and orange pigment. Changes in the cholesterol and lipid levels in mice due to addition of the pigments were investigated. The total cholesterol (TC) level of mouse serum was reduced by 8–9% with the threonine derivative and by 16% with orange pigment. Supplementation with the threonine derivative and orange pigment decreased the LDL cholesterol level by 18–26% and increased the HDL cholesterol level by 1–9%. The atherogenic index (AI) value was reduced by 23–27% with pigment supplementation. The anti-atherosclerosis effect of monascus pigments can be induced by control of the lipid content in the serum rather than in the liver of mice.

Keywords: Monascus pigment; Threonine derivative; HMG-CoA reductase inhibitor; Atherogenic index (AI); Mice *in vivo* test

1. Introduction

Atherosclerosis is a process in which fat, cholesterol, cellular waste products, calcium, and other substances are deposited inside the artery (Ross, 1993). As the disease progresses, the inside diameter of the artery narrows so that blockage of large and medium-sized arteries can occur. Atherosclerosis leads to an inflammatory disease via a sequential process of initiation, progression, and rupture of lipid-rich atherosclerotic plaques (Libby, 2002; Ross, 1999). Blood clots can be induced by ruptured plaques resulting in travel to another part of the body and blockage of blood flow leading to a heart attack. Heart diseases are responsible for approximately 50% of all deaths in Western countries. Cholesterol is a key substance causing coronary artery disease (CAD). The cholesterol level in the blood can be expressed by the atherogenic index (AI) and is a negative risk factor for atherosclerosis and heart disease.

Cholesterol is synthesized by transfer of acetyl-CoA from the mitochondrion to the cytosol. In cholesterol metabolism, acetyl-CoA can be sequentially converted to HMG-CoA, mevalonate, squalene, lanosterol, and cholesterol. The conversion reaction of HMG-CoA to mevalonate, which is catalyzed by HMG-CoA reductase, is known to be a key rate-limiting step in cholesterol biosynthesis. Therefore, activity regulation of HMG-CoA reductase can control the cholesterol content in the body. Lovastatin, a well known inhibitor of HMG-CoA reductase, is produced by *Aspergillus terreus* and other fungi. Presently, it is widely used as a hypercholesterolemia drug for reduction of plasma cholesterol levels in humans (Albets et al., 1980; Endo, 1980).

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Monascus pigments are produced by the fungus *Monascus* species. Six major components of monascus pigment are known, including yellows of monascin (Brich et al., 1962; Chen, Manchand, & Whalley, 1971; Fielding et al., 1961) and ankaflavin (Manchand, Whalley, & Chen, 1973), oranges of monascorubrin (Hadfield, Holder, & Stanway, 1967; Kurono, Nakanishi, Shindo, & Tada, 1963) and rubropunctatin (Haws & Holker, 1961), and reds of monascorubramine (Hiroi, Shima, Isobe, & Kimura, 1975) and rubropunctamine (Fowell, Robertson, & Whalley, 1956). The oxygen part of orange pigments can be replaced by nitrogen compounds, amino acids, peptides, amino sugars, amino alcohols, chitosan, and nucleic acids (Moll & Farr, 1976; Nakawa, Watanabe, & Kobayashi, 1980). Monascus pigments have various biological activities, including antioxidant (Yang, Tseng, Lee, & Mau, 2006), lipid reduction (Sumioka, Hayama, Shimokawa, Shiraishi, & Tokunaga, 2006), hypo-lipidemic (Lee, Tsai, Wang, & Pan, 2006), and anti-mutagenicity (Izawa et al., 1997).

We have reported that various amino acid derivatives of monascus pigments can be produced by addition of amino acids during *Monascus* cultivation (Jung, Kim, Kim, & Shin, 2003). Some derivatives showed antimicrobial activities (Kim, Jung, Kim, & Shin, 2006a; Kim, Jung, Kim, & Shin, 2006b) and lipase inhibitory activities (Kim et al., 2007). The derivatives are based on monascus orange pigment (Fig. 1) and the reaction mechanism was proposed in another report (Lin, Yakushijin, Büchi, & Demain, 1992).

Wei et al. (2003) reported the anti-artherogenic effects of monascus fermented rice. However, the effects of monascus pigment derivatives on atherosclerosis have not been extensively investigated. In this paper, various amino acid derivatives of monascus pigment were produced by cultivation of a *Monascus* species and substances having an anti-atherogenic activities were determined. *In vitro* inhibition tests of the pigment derivatives against HMG-CoA reductase were performed, followed by *in vivo* tests using mice. Changes in the cholesterol (HDL and LDL) and triglyceride levels in mouse serum and liver were determined.

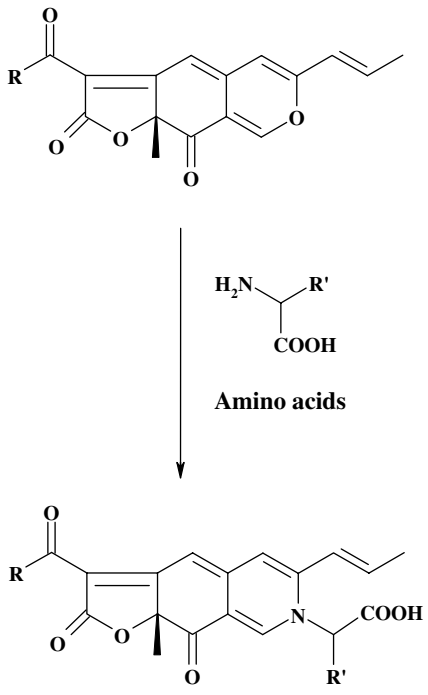

Fig. 1. A scheme for amino acid derivation of monascus pigments. R = C_5H_{11} or C_7H_{15}; R′ = functional group of amino acids.

2. Materials and methods

2.1. Reagents

NADPH and HMG-CoA, and 13 different amino acids (leucine, glycine, tyrosine, phenylalanine, histidine, arginine, threonine, lysine, glutamic acid, aspartic acid, glutamine, asparagine and methionine) were purchased from Sigma–Aldrich Co. Lovastatin was purchased from Chong Kun Dang Pharm (Seoul, Korea). The glucose–peptone medium was obtained from Difco Co. Chloroform and methanol was purchased from Tedia Co. All other chemicals were products of Duksan Pure Chemical Co.

2.2. Enzyme source

Rat liver S-9, which was used as an HMG-CoA reductase solution, was purchased from Moltox Inc. (Mol, Belgium). This enzyme solution contained 200 μg/ml of protein in a phosphate buffer (pH 7.0).

2.3. Microorganism and media

Monascus sp. KCCM 10093 (KCCM: Korea Culture Center for Microorganisms) was used for production of monascus pigments. The strain was preserved on a slant of Hiroi agar medium, which consisted of 10% sucrose, 0.5% casamino acid, 0.3% yeast extract, 0.2% $NaNO_3$, 0.1% KH_2PO_4, 0.05% $MgSO_4 \cdot 7H_2O$, 0.05% KCl, 0.001% $FeSO_4 \cdot 7H_2O$, and 2% agar powder in distilled water (w/v) (Hiroi, Shima, Suzuki, Tsukioka, & Ogasawara, 1979). The seed culture medium consisted of 5% glucose, 2% bacto peptone, 0.8% KH_2PO_4, 0.2% CH_3COOH, 0.1% NaCl, and 0.05% $MgSO_4 \cdot 7H_2O$ in distilled water (w/v) (Moll & Farr, 1976). The fermentation culture medium for production of monascus pigments consisted of 5% glucose, 0.3% NH_4NO_3, 0.1% KH_2PO_4, 0.05% $MgSO_4 \cdot 7H_2O$, 0.05% KCl, and 0.001% $FeSO_4 \cdot 7H_2O$ in distilled water (w/v) (Jung et al., 2003). The medium pH was adjusted to 6.6 prior to sterilization.

2.4. Cultivation and pigment extraction

After 5 ml of distilled water was put into a *Monascus* slant, the mixture was shaken on a vortex. Then, the spore solution was collected. For seed cultures, 5 ml of the spore solution was added to 500 ml baffled flasks containing

75 ml of the glucose–peptone medium. Flasks were then incubated for 36 h on an HM-90R rotary shaker (Human Science Co., Seoul, Korea) at 30 °C and 200 rpm. For fermentation cultures, after 7% seed cultures were added to 500 ml baffled flasks containing 75 ml of the fermentation medium, the cultures were incubated for 5 days on a rotary shaker at 30 °C and 200 rpm. Thus, the control red, orange, and yellow pigments were produced.

For synthesis of amino acid derivatives of monascus pigments, 13 different amino acids were added at 7% to the fermentation culture broths at 48 h, followed by 3 days of further cultivation. All other procedures were the same as for the previous fermentation culture.

For extraction of the control red and amino acid derivatives, 100 ml of 95% ethyl alcohol was added to 500 ml flasks containing 100 ml of the fermented culture broth. The mixtures were incubated on a reciprocal shaker (International Science Co., Seoul, Korea) at 30 °C and 180 rpm. Solutions were then filtered to obtain crude pigment extracts. After 100 ml of pigment extract in separating funnels was mixed with an equal amount of hexane, the hexane layers containing yellow pigments were removed. This procedure was twice repeated. The pigment extracts were concentrated under a reduced pressure. For extraction of the orange pigment, ethyl acetate was used instead of ethanol and all other procedures were the same as for the control red and amino acid derivatives.

Pigments were purified via TLC (silica gel 60 F_{254} plates, Merck, Darmstadt, Germany) and identified by HPLC (HP-1100) using an ODS C_{18} column (250 × 4.6 mm, 5 μm and Hypersil; Kleinostheim, Germany). LC–MS spectrometric analysis of the threonine derivative was performed. The LC–MS spectrometer (Micromass Quattro-LC triple quadrupole; Micromass, Manchester, UK) was equipped with an ESI probe and a Z-spray interface. ESI was performed in a positive mode with the desolvation gas set at 580 l/h and the probe temperature set at 200 °C.

2.5. In vitro inhibitory activity assay against HMG-CoA reductase

Inhibitory activities of monascus amino acid derivatives against HMG-CoA reductase were measured using a modified method of Kleinsek, Dugan, Baker, and Porter (1981). Tubes containing a mixture of 200 μM NADPH, 20 mM dithiothreitol, and 0.1 ml of the enzyme solution were preincubated for 10 min in a water bath at 37 °C. Reactions were started by adding HMG-CoA at 100 μM to the mixture. For addition of monascus pigments, the pigments were first dissolved in dimethylsulfoxide (DMSO), then added at 400 μM. Reactions were carried out for 5 min, then terminated by 20 s of heating at 100 °C. The reacted solutions were centrifuged (Micro 17R, Hanil Science Industrial Co., Ltd., Korea) for 15 min at 25,000*g* for removal of denatured proteins. The enzyme activity based on NADPH oxidation was determined by measuring absorbance values at 340 nm using a spectrophotometer (UV-1201, SHIMADZU, Japan).

Reactions were performed in the presence (sample) or absence (control) of inhibitor pigments or lovastatin. In the case of a blank, no HMG-CoA was added. The inhibitory activities of samples against HMG-CoA reductase were calculated as

$$\text{Inhibitory activity } (\%) = [1 - (\Delta OD_{\text{sample}} - \Delta OD_{\text{sample-blank}}) / (\Delta OD_{\text{control}} - \Delta OD_{\text{control-blank}})] \times 100$$

2.6. Determination of the protein content

The protein content of the HMG-CoA reductase solution was measured by the method of Lowry, Rosebrough, Farr, and Randall (1951) using bovine serum albumin as a standard.

2.7. In vivo experiments using mice

Thirty female five week-old C57BL/6 mice with a weight range of 17.8–18.9 g were obtained from Samtako Co. (Kyunggido, Korea). After all mice were fed orally with a pellitized commercial chow diet (diet medium) for 7 days, they were randomly divided into six groups with five mice each. As shown in Tables 1 and 2 (Reeves, 1997), the control group (D) was fed orally with the diet medium for 10 weeks. The diet–cholesterol (DC) group, a negative control group, was fed with the diet medium plus 2% cholesterol. For the diet–cholesterol–lovastatin (DCL) group, a positive control, the diet–cholesterol medium was supplemented with 0.2 mg of lovastatin per kg of mouse. The DCO_{100}, DCT_{100}, and DCT_{200} groups were fed with the diet–cholesterol medium supplemented with 100 mg of monascus orange pigment, and 100 mg and 200 mg of monascus threonine derivative per kg of mouse, respectively. The mice were housed in an air-conditioned room at 25 °C under light exposure from 8 AM to 8 PM. Every 2 days, the food medium was replaced and mouse body weight and feed intake were measured.

Table 1
Composition of the diet medium

Ingredient	Content (g/kg)	Calorie (kcal)
Casein, lactic	200	800
L-Cystine	3	12
Corn starch	397.486	1590
Sucrose	100	400
Maltodextrin	132	528
Cellulose	50	0
Soybean oil	70	630
t-Butylhydroquinone	0.014	0
AIN-93G mineral mix	35	0
AIN-93G vitamin mix	10	40
Choline bitartrate	2.5	0
Total	1000	4000

Table 2
Mice groups fed with different media

Group	Feeding medium
D	AIN-93G diet medium
DC	AIN-93G + 2% cholesterol
DCL	AIN-93G + 2% cholesterol + 0.2 mg lovastatin/kg mouse/day
DCO_{100}	AIN-93G + 2% cholesterol + 100 mg monascus orange pigment/kg mouse/day
DCT_{100}	AIN-93G + 2% cholesterol + 100 mg monascus threonine derivative/kg mouse/day
DCT_{200}	AIN-93G + 2% cholesterol + 200 mg monascus threonine derivative/kg mouse/day

Orange monascus pigment, threonine derivative, and lovastatin were supplemented after being dissolved in polyethylene glycol (PEG). The D and DC groups were supplemented only with PEG (Table 2).

2.8. Determination of cholesterol and lipid levels in mouse serum and the liver

At the end of the feeding period all mice were fasted for 14 h. Mice were sacrificed by suffocation with CO_2 gas. Blood samples were collected in an EDTA-containing syringe, then stored at −80 °C prior to analysis. Livers were immediately removed from each mouse, washed with a saline solution, weighed, then stored in a liquid nitrogen container or deep freezer (−80 °C) for subsequent cholesterol and triglyceride determinations.

The total cholesterol (TC), triglyceride (TG), and HDL-cholesterol levels were measured using a commercial enzyme kit (Asan Pharmaceutical, Seoul, Korea) and a clinical chemical analyzer (Prime, BPC). The LDL-cholesterol content was calculated using the following Friedewald formula (Friedewald, Levy, & Fedrickson, 1985):

$$\text{LDL-cholesterol} = \text{Total cholesterol} - (\text{HDL-cholesterol} + \text{Triglyceride}/5)$$

The atherogenic index (AI) was calculated from the ratio of total cholesterol to HDL-cholesterol:

$$\text{AI} = (\text{Total cholesterol} - \text{HDL-cholesterol})/\text{HDL-cholesterol}$$

In order to determine the cholesterol and triglyceride levels in the mouse liver, the hepatic lipids were extracted using the procedure developed by Folch, Lee, and Sloan-Stanley (1957). First, liver fractions were homogenized in a chloroform–methanol mixture (2:1, v/v) and centrifuged for 10 min at 1000*g*. The residues were incubated with 0.05% $CaCl_2$, then centrifuged. A chloroform–methanol–$CaCl_2$ mixture (3:48:47, v/v/v) was then added. After the mixtures were centrifuged, residues were obtained and dried with N_2 gas. The lipid residues were dissolved in ethanol for assay of total cholesterol and triglyceride. All assays were performed using commercial kits in triplicate and mean values were calculated.

2.9. Statistical analysis

All data were expressed as mean ± SEM. Data were analyzed with SAS (statistical analysis system) software and differences between the means were assessed using Duncan's multiple-range test. Statistical significance was considered to be $P < 0.05$.

3. Results and discussion

3.1. Screening of an HMG-CoA reductase inhibitor from derivatives of monascus pigment

Thirteen amino acid derivatives of monascus pigment were produced by adding the corresponding amino acids during cultivation of *Monascus* sp. KCCM10093. The inhibitory activities of these derivatives against HMG-CoA reductase were measured via an *in vitro* enzymatic assay. In addition to the control red and orange monascus pigments, lovastatin was also tested.

Among the derivatives, ten substances showed some inhibitory activity (Fig. 2). The threonine derivative had the highest inhibitory activity of 38%, followed by the leucine derivative with 35%. Derivatives of glycine, tyrosine, and histidine exhibited a considerable inhibitory level of 23–25%. Low levels of 10–14% were observed for derivatives of phenylalanine, lysine, glutamate, asparagines, and methionine. No activity was detected for derivatives of arginine, aspartic acid, and glutamine. The orange pigment, control red pigment, and lovastatin exhibited inhibitory levels of 36%, 15%, and 98%, respectively.

The orange pigment is known to be highly reactive because, as seen in the mechanism of derivative synthesis, its oxygen moiety can be easily replaced with the nitrogen moiety of an amino acid (Moll & Farr, 1976; Nakawa et al., 1980). Threonine and tyrosine derivatives exhibiting relatively high activities have a hydroxyl group in their

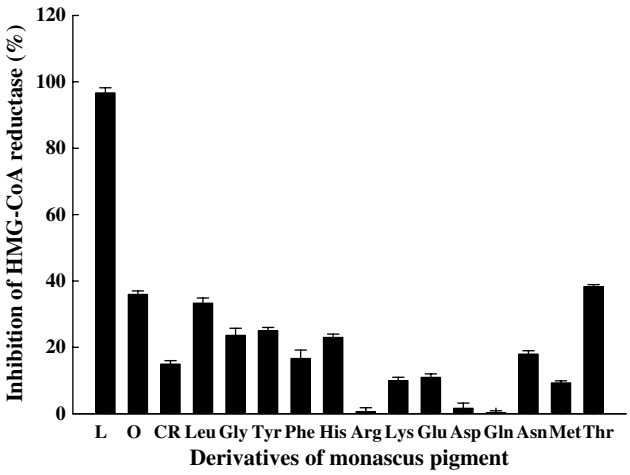

Fig. 2. Inhibitory effect of monascus pigment amino acid derivatives on HMG-CoA reductase. L: lovastatin; O: monascus orange pigment; CR: monascus control red.

amino acid structure. Both the orange pigment and the derivatives have a reactive oxygen moiety that can bind strongly with proteins, such as enzymes, leading to enzyme inhibition. Derivatives that contain hydrophobic polar-uncharged, non-polar aliphatic, and aromatic amino acids showed a relatively high activity. Thus, both an oxygen moiety and hydrophobicity are considered to be important for the inhibitory activities of monascus pigments. However, the derivatives of amino acids with amine or a carbonyl group did not show a significant inhibitory effect. In other words, the derivatives of negatively or positively charged amino acids showed little or no inhibitory activity. Similar phenomena were also observed by Kim et al. (2007) for the lipase inhibitory activity of monascus amino acid derivatives.

3.2. Diet effect of the pigments for mice

The threonine derivative and orange pigment, having exhibited the highest *in vitro* inhibitory activity, were chosen for *in vivo* tests with mice. The threonine derivative was purified and identified via analysis of LC–MS (data not shown). The effect of the pigments on body weight gain was analyzed every 2 days for 10 weeks.

All mice groups exhibited normal growth over the feeding period. Mice initially weighing approximately 18 g each were used. After 10 weeks, as shown in Fig. 3, the mice group D fed solely with the AIN-93G diet medium exhibited a weight gain of 3.08 g per mouse, whereas mice in groups fed with both diet medium and cholesterol showed weight gains of 3.92–4.64 g per mouse, regardless of pigment addition. Cholesterol added to the diet medium increased mouse weight by 27–51%. Mouse weight was not significantly changed in the case of supplementation with the HMG-CoA reductase inhibitor, the threonine derivative, orange pigment, or lovastatin ($P > 0.05$).

3.3. Effect of the pigments on mouse liver weight

The liver weights of mice resulting from different feed supplements were measured. Liver weights after sacrifice at the end of the feeding schedule are shown in Table 3. The average liver weight of mice fed with the diet medium was 1.33 g, whereas weights for mice fed with a cholesterol supplement (the diet–cholesterol medium) were 1.59–1.70 g, corresponding to a 20–28% increase. The weights per mouse were converted into values per 10 g of body weight. The values of the diet–cholesterol medium group were increased by 15–23% compared to the diet medium group. However, there were no significant differences among members of the cholesterol group. Apparently, the liver weight was not affected by the pigment derivative, orange pigment, or lovastatin probably because pigments and lovastatin are easily metabolized in the liver.

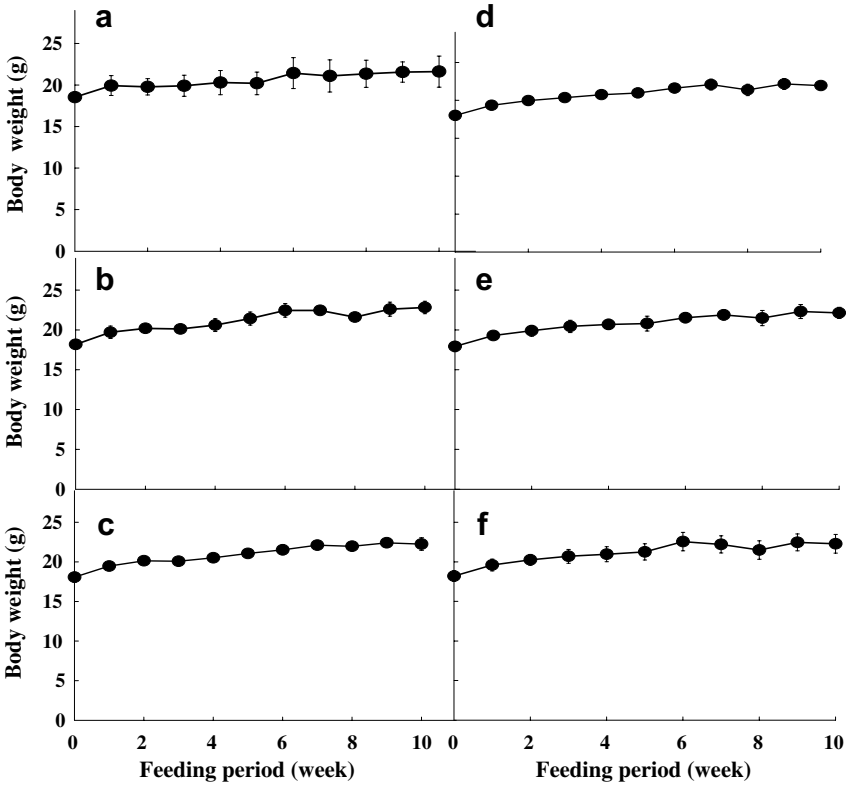

Fig. 3. Changes in body weight due to feeding of different supplements: (a) D; (b) DC; (c) DCL; (d) DCO_{100}; (e) DCT_{100}; (f) DCT_{200}.

Table 3
Changes in mouse liver weight depending on different supplements

Group	Liver weight (g)	Liver weight/10 g/mouse
D	1.33 ± 0.18[a]	0.62 ± 0.05
DC	1.62 ± 0.09	0.71 ± 0.04
DCL	1.70 ± 0.10	0.76 ± 0.04
DCO_{100}	1.64 ± 0.15	0.75 ± 0.06
DCT_{100}	1.59 ± 0.14	0.74 ± 0.03
DCT_{200}	1.70 ± 0.14	0.76 ± 0.08

[a] Values are expressed as mean ± SD ($n = 5$). Mean values were calculated at $P < 0.05$ by Duncan's multiple-range test.

3.4. Effect of the pigments on mouse plasma cholesterol and triglyceride levels

The effects of different feeding media on the levels of cholesterol and triglycerides (TG) in mouse serum were investigated. As shown in Table 4, the average total cholesterol (TC) level of the diet medium group (D) was 91.6 mg/dl. When cholesterol was added to the diet medium (DC), the value increased to 159.6 mg/dl. However, when the threonine derivative or orange pigment was added to the diet–cholesterol medium (DCT_{100} and DCT_{200}, and DCO_{100}), the total cholesterol levels in serum were 145.8–147.2 and 134.6 mg/dl, respectively, corresponding to decreases of 8–9% and 16% compared with the DC group. The total cholesterol level for lovastatin was similar to the level for the threonine derivative.

The triglyceride (TG) level in mouse serum increased from 24.0 mg/dl for the diet medium group to 36.2 mg/dl for the diet–cholesterol medium (DC) group. When the threonine derivative was added to the DC group feed, the TG content increased to 38.6–41.2 mg/dl. However, the addition of lovastatin reduced the TG level by 19% (29.2 mg/dl), similar to the results reported by Krause and Newton (1995).

The effect of monascus pigments on the HDL and LDL cholesterol levels in mouse serum was investigated. Addition of cholesterol to the diet medium increased the HDL and LDL cholesterol levels from 38.6 and 47.2 mg/dl to 54.5 and 99.2 mg/dl, respectively, corresponding to 41% and 110% increases. The r-HDL ratio was estimated to be 33.3%. However, when the threonine derivative and orange pigment were added to the diet–cholesterol medium, the HDL cholesterol level increased by 1–9% (55.2–59.6 mg/dl) while the LDL cholesterol level decreased by 18–26% (73.9–81.7 mg/dl). The r-HDL ratios increased by 18–23% compared to the diet–cholesterol medium group. On the other hand, addition of lovastatin increased the HDL cholesterol level by 42% and decreased the LDL cholesterol level by 28%, resulting in a 47% increase in the r-HDL ratio. Witztum (1996) also reported that lovastatin can reduce the LDL-cholesterol content.

The r-HDL ratio increased significantly due to addition of the threonine derivative, orange pigment and lovastatin. An increase in the HDL cholesterol level and a decrease in the LDL cholesterol level were observed with lovastatin. However, while the pigments slightly increased the HDL cholesterol level, they remarkably decreased the LDL cholesterol level. There were no differences in HDL and LDL cholesterol levels among the pigment-added groups. These results indicate that food supplementation with either the theonine derivative or orange pigment is effective for prevention of hyperlipidemia and atherosclerosis.

The values of HDL-cholesterol/LDL-cholesterol, TG/HDL-cholesterol, and the atherogenic index (AI) were determined (Table 4). The ratio of HDL-cholesterol to LDL-cholesterol was the highest at 1.04 for lovastatin, indicating a strong positive effect. The ratios for the pigments were 0.72–0.80, indicating considerable effects. The ratio of TG to HDL-cholesterol was the lowest at 0.42 for lovastatin, and 0.68–0.73 for the pigments, indicating that lovastatin exhibited a significant effect while the pigments did not. The AI value, a coronary heart disease risk factor, was estimated to be 2.05 for the diet–cholesterol (DC) medium group. When the pigments and lovastatin were added to the DC medium, the AI value decreased to 1.50–1.57 for the pigments and 1.24 for lovastatin, corresponding to

Table 4
Effect of supplements on the cholesterol, triglyceride levels and lipid ratios in mice

Item	D	DC	DCL	DCO_{100}	DCT_{100}	DCT_{200}
TC (mg/dl)[a]	91.6 ± 8.8[g]	159.6 ± 5.5	147.6 ± 5.8	134.6 ± 3.7	145.8 ± 3.1	147.2 ± 5.7
HDL-C (mg/dl)[b]	38.6 ± 6.1	54.5 ± 8.2	77.1 ± 6.6	55.2 ± 4.4	59.6 ± 9.4	57.8 ± 5.8
LDL-C (mg/dl)[c]	47.2 ± 9.2	99.2 ± 7.6	71.9 ± 15.0	73.9 ± 8.0	78.0 ± 12.2	81.7 ± 9.4
r-HDL (%)[d]	43.5 ± 7.4	33.3 ± 4.5	47.2 ± 10.7	39.4 ± 4.8	40.9 ± 7.0	39.4 ± 5.0
TG (mg/dl)[e]	24.0 ± 3.7	36.2 ± 4.0	29.2 ± 7.0	38.4 ± 8.7	41.2 ± 9.9	38.6 ± 4.0
HDL-C/LDL-C	0.88 ± 0.3	0.54 ± 0.1	1.04 ± 0.4	0.73 ± 0.2	0.80 ± 0.3	0.72 ± 0.2
TG/HDL-C	0.61 ± 0.1	0.69 ± 0.2	0.42 ± 0.1	0.73 ± 0.2	0.69 ± 0.2	0.68 ± 0.1
Atherogenic index[f]	1.35 ± 0.4	2.05 ± 0.4	1.24 ± 0.7	1.56 ± 0.3	1.50 ± 0.4	1.57 ± 0.3

[a] TC: total cholesterol.
[b] HDL-C: HDL-cholesterol.
[c] LDL-C: LDL- cholesterol.
[d] r-HDL: (HDL-C/TC) related HDL-cholesterol.
[e] TG: triglyceride.
[f] Atherogenic index = (total cholesterol − HDL-cholesterol)/HDL-cholesterol.
[g] Values are expressed as mean ± SD ($n = 5$). Mean values were calculated at $P < 0.05$ by Duncan's multiple-range test.

Table 5
Effect of supplements on the total cholesterol, triglyceride, and TBARS levels in mice liver

Group	TC[a] (mg/g)	TG[b] (mg/g)	TBARS[c] (mg malonaldehyde/kg)
D	38.00 ± 9.43[d]	181.0 ± 2.0	0.53 ± 0.09
DC	247.2 ± 22.2	735.5 ± 5.0	0.33 ± 0.01
DCL	194.8 ± 4.7	737.7 ± 25.2	0.32 ± 0.04
DCO_{100}	232.3 ± 35.6	548.7 ± 104.0	0.33 ± 0.02
DCT_{100}	276.3 ± 21.4	741.3 ± 23.4	0.37 ± 0.02
DCT_{200}	249.0 ± 25.9	700.8 ± 5.7	0.37 ± 0.03

[a] TC: total cholesterol.
[b] TG: triglyceride.
[c] TBARS: thiobarbituric acid reactive substance.
[d] Values are expressed as mean ± SD ($n = 5$). Mean values were calculated at $P < 0.05$ by Duncan's multiple-range test.

reductions of 23–27%, and 40%. These results indicate that the threonine derivative and orange pigment can significantly reduce the atherogenic index.

The threonine derivative and orange pigment may prevent the progression of atherosclerosis by decreasing the LDL-cholesterol level in mouse serum. Decreases in TC and the atherogenic index caused by addition of the pigments also indicate an anti-atherosclerosis effect. Considering that LDL-cholesterol negatively affects blood flow and the heart (Sniderman et al., 2003), the monascus threonine derivative and orange pigment should be considered as anti-atherogenic substances.

3.5. Effect of the pigments on the cholesterol, triglyceride, and TBARS levels in mouse liver

The effects of the threonine derivative and orange pigment on the total cholesterol (TC), triglyceride, and TBARS (thiobarbituric acid reactive substances) levels in mouse liver were investigated. As shown in Table 5, the total cholesterol level in mouse liver increased from 38 to 247 mg/g due to cholesterol added to the diet medium. Lovastatin added to the diet–cholesterol medium caused the TC content to decrease to 195 mg/g, corresponding to a 21% reduction. However, neither the threonine derivative nor the orange pigment reduced the TC content.

The triglyceride (TG) level in mouse liver was increased four times by addition of cholesterol to the diet medium. Addition of either the threonine derivative or lovastatin did not cause significant changes in the TG level. However, the orange pigment reduced the triglyceride (TG) level by 25%. Added cholesterol caused the TBARS value to decrease from 0.53 to 0.32–0.37, corresponding to a 30–40% reduction. There were no significant differences among the diet–cholesterol medium groups.

4. Conclusions

Among amino acid derivatives of monascus pigments, the threonine derivative exhibited the highest inhibitory activity against HMG-CoA reductase. The orange monascus pigment also showed a high activity. The oxygen moiety and hydrophobicity of pigments are considered to be related to inhibition. The threonine derivative, orange pigment, and lovastatin significantly reduced the total cholesterol in serum. The atherogenic effect of the pigments was evidenced by a significant decrease in the LDL-cholesterol level in mouse serum. Both the r-HDL value and the ratio of HDL-cholesterol to LDL-cholesterol were increased by pigments. Apparently, the lipid content of mouse liver was not regulated by the pigments. The positive effect of the threonine derivative and orange pigment on atherosclerotic effects apparently relates to regulation of the lipid concentration of the serum.

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