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Changes in hepatic gene expression associated with the hypocholesterolaemic activity of royal jelly

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Abstract

Royal jelly (RJ) has various pharmacological actions, including hypolipidaemic, hypocholesterolaemic and anti-atherosclerotic effects, in experimental animals but the molecular mechanisms involved remain unclear. Here, we investigated changes in the expression of lipid metabolism-associated genes in the liver of RJ-treated mice by means of a DNA microarray technique to obtain clues to the mechanism of the hypocholesterolaemic action of RJ. We compared the hepatic gene expression profiles in three groups of mice fed a diet containing 5% RJ, a diet containing 5% RJ stored at 40°C for 7 days (40–7d RJ) or a control diet which provided the same total energy as the other diets. RJ decreased gene expression of squalene epoxidase (SQLE), which is a key enzyme in cholesterol biosynthesis, and sterol regulatory element-binding protein (SREB)-1, which may be a transcriptional factor of SQLE. It increased gene expression of low-density lipoprotein receptor (LDLR), which is involved in cholesterol incorporation in liver. Thus, the hypocholesterolaemic action of RJ appears to be associated with a decrease of SQLE and an increase of LDLR in mice.

Introduction

Hyperlipidaemia and hypercholesterolaemia are major risk factors for atherosclerosis, which is a common feature of human arteriopathies, and results from abnormalities of serum lipid and cholesterol metabolism (Vitteck 1995). They are a consequence of both genetic factors and environmental factors, such as diet (Ross 1976, 1993; Seidel 1993). Evidence that various lipid-lowering therapies cause regression or stabilization of atherosclerotic diseases has been obtained from both animal studies and clinical experience (Armstrong 1976). Intensive lipid-lowering therapy has been reported to reduce the frequency of progression of coronary lesions, but to increase the frequency of regression of atherosclerotic diseases (Armstrong 1976; Loscalzo 1990; Mach et al 1993; O'Brien et al 1993). Decrease of cholesterol and systolic blood pressure also improves vascular function and stabilizes atheromatous vascular lesions (Brown et al 1990).

Several reports have suggested that royal jelly (RJ) is effective in preventing experimental atherosclerosis and is useful in the treatment of atherosclerosis in man (Matuszewski & Kaczor 1961; Zaicev & Poriadin 1962; Sitar & Cernochova 1967; Belong & Masek 1971; Nakajin et al 1982). RJ also significantly influences lipid metabolism in rats and prevents hypercholesterolaemia and development of atherosclerosis in rabbits fed a cholesterol-rich diet (Butcher & Baird 1968; Makarova 1969). It also has various other pharmacological actions, such as vasodilative and hypotensive activity (Shinoda et al 1978), activity to increase growth rate (Kawamura 1961) and disinfectant (Yatsunami & Echigo 1985), antitumour (Townsend et al 1959; Tamura et al 1987) and anti-inflammatory activity (Fujii et al 1990). However, the mechanisms involved in the pharmacological activity of RJ, and the constituents of RJ that are responsible, remain to be established.

RJ is secreted from the hypopharyngeal and mandibular glands of worker honey bees and is essential for the growth of the queen honey bee (*Apis mellifera*) larva (Patel et al 1960; Haydak 1970). RJ was reported to consist mainly of proteins, sugars, lipids and vitamins (Takenaka 1982; Howe et al 1985; Echigo et al 1986), together with a large number of bioactive substances, such as 10-hydroxy-2-decenoic acid (Blum et al 1959), antibacterial protein (Fujiwara et al 1990) and a stimulating factor for the development of the genital

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organs in male mice (Kato et al 1988). Storage of RJ at high temperature causes various changes, such as acceleration of the Maillard reaction and increase in viscosity, acidity and protein degradation, and also reduces the pharmacological activity of RJ (Takenaka et al 1986). We previously investigated the effects of RJ on gene expression in mouse liver by means of a DNA microarray technique to clarify the influence of the changes of composition during storage on the toxicity and safety of RJ (Kamakura et al 2005). The gene expression of various detoxifying enzymes, such as cytochrome P450 enzymes, glutathione S-transferase, UDP-glucuronosyltransferase, N-acetyltransferase and sulfotransferase, was not affected by RJ diet, but was increased by diet containing RJ stored at 40°C for 7 days. Thus, RJ does not include constituents that alter the gene expression levels of detoxifying enzymes, but toxicants that do have this effect might be generated in RJ during storage at high temperature.

In this study, we focused on the mechanism underlying the hypocholesterolaemic activity of RJ, and investigated the effects of RJ on the expression of lipid metabolism-associated genes in mouse liver by using a DNA microarray technique. Based on the results, we propose a mechanism for the hypocholesterolaemic activity of RJ.

Materials and Methods

Animals

Six-week-old male Std ddY mice (a closed colony from Japan Shizuoka Laboratory Center, Hamamatsu, Japan) were housed in wire-bottomed cages in a room on a 12-h light–dark cycle. Temperature and humidity were controlled at 23°C and 48–62%, respectively. All mice were treated in accordance with the guidelines established by the Japanese Society of Nutritional and Food Science.

Diet and experimental design

The mice were fed a commercial diet (CRF-1; Oriental Yeast, Tokyo, Japan) for 7 days before they were given experimental diets. Thirty mice were distributed randomly into three groups with the same average body weight in each group. Each group of 10 mice was given an experimental diet for 14 days, and water was freely available. Three experimental diets were prepared, as shown in Table 1. Royal jelly (RJ) was purchased from Saitama Yoho Co. Ltd (Saitama, Japan). RJ that had been stored at 40°C for 7 days (40–7 d RJ) was also used. RJ and 40–7d RJ were each lyophilized and added to the experimental diets at a rate of 5% (w/w). RJ used in this study consisted of 64% water, 14.5% protein (20 g/50 g dry RJ), 16.4% sugar (25 g/50 g dry RJ) and 3.8% lipid (5 g/50 g dry RJ). The sugar in RJ is composed of 42% glucose, 41% fructose and 10% other oligosaccharides (Takenaka 1982). Therefore, the control group was fed control diet including 20 g casein/1000 g diet, 10 g glucose/1000 g diet, 10 g fructose/1000 g diet, 5 g cornstarch/1000 g diet together with 5 g corn oil/1000 g diet instead of RJ, to provide the same total energy as RJ. The control diet was used to ensure that any observed effect of RJ was not simply due to the nutritive value of RJ as an energy source. Food intake and body weight were recorded daily. After a 14-day feeding period, the livers of all the mice were removed, and equal amounts of liver from 10 mice of each group were mixed, homogenized and used for DNA microarray assay.

Table 1 Composition of experimental diets

Ingredient	Control (g kg^{-1})	RJ (g kg^{-1})	40–7d RJ (g kg^{-1})
RJ	0	50	50
Casein[a]	200	180	180
Corn oil[b]	50	45	45
Cornstarch[a]	430	425	425
Fructose[b]	10	0	0
Glucose[b]	10	0	0
Sucrose[a]	200	200	200
Cellulose powder[a]	50	50	50
Mineral mixture[a]	35	35	35
Vitamin mixture[a]	10	10	10
L-Methionine[b]	3	3	3
Choline bitartrate[b]	2	2	2

[a]Oriental Yeast Co. (Tokyo, Japan). [b]Naclai Tesque (Tokyo, Japan). 40–7d RJ, RJ stored at 40°C for 7 days.

DNA microarray assay

DNA microarray assay was conducted as described previously by using CodeLink Uniset Mouse I Bioarrays (Amersham, Piscataway, USA) containing 10 000 cDNA spots (Kamakura et al 2005). The gene list used in this study can be found at (http://www.amershammedia.com/codelink_files/Gene_Lists_9.21.04/CodeLink_UniSet_Mouse_I.txt).

Real-time PCR

Total RNA was prepared from liver of each mouse by using the SV Total RNA Isolation System (Promega, Madison, WI). The reverse transcription reaction was performed using ExScript RT Reagent Kit (Takara, Tokyo, Japan). The primers used in this study were as follows: glyceraldehyde-3-phosphate dehydrogenase (GAPDH; forward primer, 5′-AAATGGTGAAGGTCGGTGTG-3′; reverse primer, 5′-TGAAGGGGTCGTTGATGG-3′), fatty acid transport protein 3 (FATP3; forward primer, 5′-GTCTGGGGACGTTTTCTTCA-3′; reverse primer, 5′-TCTCCAAGACCTCAGCCACT-3′), low-density lipoprotein receptor (LDLR; forward primer, 5′-GCTGCATCCTCGACATCTACCA-3′; reverse primer, 5′-TGCACAGGCCACTGTCACAC-3′), squalene epoxidase (SQLE; forward primer, 5′-TGTCAGAAACCAACCAAGTGCAG-3′; reverse primer, 5′-TCCTTGTATTGCACGCCGATTA-3′), 3-hydroxy-3-methylglutaryl-CoA reductase (HMG-CoA reductase; forward primer, 5′-CCAAGGTGGTGAGAGAGGTGTT-3′; reverse primer, 5′-CGTCAACCATAGCTTCCGTAGTT-3′), sterol regulatory element binding protein 1 (SREBP1; forward primer, 5′-ACAGAGCTTCCGGCCTGCTA-3′; reverse primer, 5′-CCGAGCTGTGGCCTCATGTA-3′), SREBP2 (forward primer, 5′-CAAGTCTGGCGTTCTGAGGAA-3′; reverse primer, 5′-ATGTTCTCCTGGCGCAGCT-3′). All assays were

performed using an ABI Prism 7500 Sequence Detection System (Applied Biosystems, Foster City, CA) Reactions were performed in a reaction mixture consisting of SYBR Premix Ex Taq (Takara, Tokyo, Japan) and primers with cDNA in a volume of 20 μL. For relative quantitative analysis a 4–5 log range standard curve was generated from 1:10 serial dilutions of total RNA. The PCR conditions were as follows: an initial step of 10 s at 95°C, followed by 40 cycles of 5 s at 95 °C and 35 s at 60°C. The average expression level relative to the control group was determined after normalization with respect to the GAPDH in each sample.

Quantitative analysis of several vitamins and 10-hydroxy-2-decenoic acid in RJ

The contents of vitamin B_1, vitamin B_2, vitamin B_6, folic acid, pantothenic acid, nicotinic acid, biotin and 10-hydroxy-2-decenoic acid (10-HDA) in RJ were quantitatively analysed as described previously (Kamakura et al 2001a).

Polyacrylamide gel electrophoresis (PAGE)

Native PAGE was run with 5–20% gradient polyacrylamide gel by the method of Davis (Davis 1964). SDS-PAGE was run with 5–20% gradient polyacrylamide gel by the method of Laemmli (1970).

Purification of 57-kDa protein

The purification of 57-kDa protein was conducted as described previously (Kamakura et al 2001a), with monitoring by the use of native PAGE. The purified 57-kDa protein was dialysed against distilled water and lyophilized.

Determination of the concentrations of proteins in RJ

For quantification of protein constituents in RJ, the results of native PAGE of RJ proteins were analysed by NIH Image Ver. 1.62 (NIH, USA), using purified 57-kDa protein as a standard.

Statistics

Values are expressed as mean ± s.e.m. All statistical analysis was done by using one-way analysis of variance with the Fisher method. Statistical calculations were performed with the Stat View 5 software for Macintosh (SAS Institute Inc., Cary, NC).

Results and Discussion

To investigate to what extent compositional changes in RJ occur during storage, the constituents of RJ stored at 4°C and at 40 °C for 7 days were analysed. We previously reported that 57-kDa protein in RJ was specifically degraded in proportion to both storage temperature and storage period, whereas the contents of other constituents, such as vitamin B_1, vitamin B_2, vitamin B_6, folic acid, pantothenic acid, nicotinic acid, biotin and 10-HDA, in RJ did not change during storage at 40 °C for 7 days (Kamakura et al 2001a, b, c). Similar results were obtained with the RJ samples used in this study (data not shown). The content of the 57-kDa protein in RJ was approximately 1.6%, and declined to 9.4% of the initial concentration during storage at 40°C for 7 days. Since the 57-kDa protein content in RJ was correlated with the improvement of exercise-induced physical fatigue by administration of RJ, the 57-kDa protein seems to be important for the physiological activity of RJ and its content is considered to be a measure of the quality of RJ (Kamakura et al 2001b, c). There were no significant differences in food intake or body weight among the three groups of mice fed control diet, RJ diet and 40–7d RJ diet (data not shown). Therefore, the difference in quality between RJ and 40–7d RJ did not affect food intake or body weight during the test period.

We previously examined RJ-related changes in gene expression by means of microarray assay, and found that the RJ diet altered the expression of many genes (Kamakura et al 2005). Comparison of the hybridization patterns of DNA from mice given the control diet and RJ diet showed that the RJ diet resulted in a 1.8-fold or greater upregulation of 148 genes and a 1.8-fold or greater downregulation of 119 genes, so that in total the expression of 2.7% of the 10 000 genes tested was altered. Serum biochemical parameters of mice after the feeding period showed no significant differences in total cholesterol, triglyceride, phospholipid, glucose, insulin or leptin concentration among the three groups (Kamakura et al 2005). However, serum cholesterol levels in the RJ diet group and 40–7d RJ diet group were slightly decreased as compared with the control diet group. Hypolipidaemic or hypocholesterolaemic effects of RJ were observed in animals fed a hyperlipidaemic diet or a high-cholesterol diet (Vitteck 1995). In our previous study, the oil content in diet was 5% by weight, and the lipid concentration in the experimental diets was equivalent to that in ordinary diet used for breeding animals. Cholesterol was not added to the experimental diet. These results indicate that RJ does not influence serum lipid or cholesterol levels in animals on an ordinary diet. Nevertheless, although RJ did not influence the serum biochemical parameters, the expression of genes in the liver showed striking alterations in response to RJ.

In this study, we examined changes in the expression of genes whose products have been implicated in lipid or cholesterol metabolism, in mice fed with RJ or 40–7d RJ. (The genes were chosen from the gene list shown at http://www.amershammedia.com/codelink_files/Gene_Lists_9.21.04/CodeLink_UniSet_Mouse_I.txt.) RJ increased the gene expression of fatty acid transport protein 3 (FATP3) and decreased that of sterol regulatory element binding protein (SREBP)-1 in liver (Table 2). However, the gene expression of other lipid metabolism-related factors was not influenced by RJ diet. Furthermore, the RJ diet group showed increased gene expression of low-density lipoprotein receptor (LDLR), which is involved in the uptake of cholesterol, and decreased gene expression of squalene epoxidase (SQLE), which is an enzyme involved in cholesterol biosynthesis, compared with control diet group (Table 3). However, RJ diet did not influence gene expression of 3-hydroxy-3-methylglutaryl (HMG)-CoA

Table 2 Changes in gene expression of lipid metabolism-related factors in mice fed a diet containing 5% royal jelly (RJ) or a diet containing 5% RJ stored at 40°C for 7 days (40–7d RJ) compared with a control diet

Gene	Fold (RJ/control)	Fold (40–7d RJ/control)	GeneBank accession No.
1-Acylglycerol-3-phosphate O-Acyltranferase 1	1.37	1.29	NM_018862
2–4-Dienoyl-CoA reductase 2	−1.15	1.25	NM_011933
2-Hydroxyphytanoyl-CoA lyase	−1.01	−1.01	NM_019975
3,2 Trans-enoyl-CoA isomerase	−1.14	−1.10	NM_010023
3-Ketoacyl-CoA thiolase	−1.17	−1.21	AK002555
Acyl CoA oxidase 1	1.14	1.16	NM_015729
Acyl-CoA oxidase 2	1.20	1.30	NM_053115
Apolipoprotein A-I	−1.03	−1.03	NM_009692
Apolipoprotein A-II	−1.01	−1.01	NM_013474
Apolipoprotein A-IV	1.11	−1.13	NM_007468
Apolipoprotein C2	1.10	1.11	NM_007385
Apolipoprotein CI	1.22	1.13	NM_007469
Apolipoprotein CII	−1.02	−1.04	NM_009695
Apolipoprotein CIII	1.02	−1.02	NM_023114
Apolipoprotein D	1.08	−1.12	NM_008062
Apolipoprotein E	−1.05	−1.03	NM_009696
Apoliporotein M	1.09	1.10	NM_018816
ATP citrate lyase	1.08	−1.17	AF332052
Carboxyl ester lipase	1.28	1.47	NM_009885
Carnitine palmitoyltransferase 1	−1.11	1.01	S82796
Carnitine palmitoyltransferase 2	−1.18	1.02	NM_009949
Cytosolic acyl-CoA thioesterase 1	−1.44	−1.06	NM_012006
Enoyl CoA hydratase 1	−1.12	−1.07	NM_016772
Fatty acid binding protien 1	−1.03	−1.08	NM_017399
Fatty acid binding protein 5	−1.33	−1.32	NM_010634
Fatty acid transport protein 3	2.28	1.72	AF072758
Glucose-6-phosphate dehydrogenase	−1.48	−1.20	NM_007470
Glycerol-3-phosphate acyltransferase	1.09	−1.00	NM_008149
Glyceronephosphate O-acyltransferase	−1.03	1.06	NM_010322
Glycolipid transfer protein	1.25	1.39	NM_019821
Lecithin cholesterol acyltransferase	−1.10	−1.06	NM_008490
Lipin 1	1.40	1.54	NM_015763
Lipopolysaccharide binding protein	−1.01	1.24	NM_008489
Lipoprotein lipase	1.26	1.23	NM_008509
Lipase, hepatic	−1.17	−1.24	NM_008280
Lysosomal acid lipase 1	−1.06	−1.09	NM_021460
Microsomal triglyceride transfer protein	−1.10	1.01	NM_008642
Peroxisomal delata3, delta2-enoyl-CoA isomerase	−1.13	−1.09	NM_011868
Peroxisome proliferator activated receptor alpha	1.26	1.45	NM_011144
Phosphatidylinositol transfer protein	1.11	1.11	NM_008850
Phospholipid transfer protein	−1.11	−1.36	NM_011125
Phosphate cytidyltransferase 1	−1.25	−1.42	NM_009981
Phosphatidylcholine-specific phospholipase D1B	−1.05	1.05	U87868
Phosphatidylethanolamine binding protein	1.03	−1.18	NM_018858
Phospholipase A2 group VII	−1.21	−1.18	NM_013737
Phospholipase A2, group IIA	1.26	−1.18	NM_011108
Phospholipase A2, group IIC	1.26	1.36	NM_008868
Phospholipase A2, group IID	1.01	1.15	NM_011109
Phospholipase A2, group IIF	1.05	−1.09	NM_012045
Phospholipase A2, group IVA	−1.09	−1.06	NM_008869
Phospholipase A2, group VI	−1.05	−1.01	NM_016915
Phospholipase C, beta 3	1.35	1.07	NM_008874
Phospholipase D2	1.01	−1.35	NM_008876
Phosphatidylinositol transfer protein beta	−1.01	−1.10	NM_019640
Pyruvate decarboxylase	−1.04	1.20	NM_008797
Pyruvate kinase 3	1.61	−1.15	NM_011099

(*cont.*)

Table 2 (*cont.*)

Gene	Fold (RJ/control)	Fold (40–7d RJ/control)	GeneBank accession No.
Solute carrier family 27 member 1	1.03	1.23	NM_011977
Solute carrier family 27 member 5	−1.06	1.02	NM_009512
Sterol regulatory element-binding protein-1	−1.70	−2.37	AB017337
Very-long-chain acyl-CoA dehydrogenase	−1.09	−1.06	Z71189

Table 3 Changes in gene expression of cholesterol metabolism-related factors in mice fed a diet containing 5% royal jelly (RJ) or a diet containing 5% RJ stored at 40 °C for 7 days (40–7d RJ) compared with a control diet

Gene	Fold (RJ/Control)	Fold 40–7d RJ/control)	GeneBank accession No.
24-Dehydrocholesterol reductase	1.08	−1.27	NM_053272
3-Hydroxy-3-methylglutaryl-CoA reductase	1.40	1.24	M62766
7-Dehydrocholesterol reductase	1.10	1.07	NM_007856
Diacylglycerol O-acyltransferase 1	−1.10	−1.15	NM_010046
Farnesyl diphosphate farnesyl transferase 1	−1.11	−1.04	NM_010191
Low-density lipoprotein receptor	2.08	1.19	AF247637
Mevalonate kinase	1.19	1.04	NM_023556
Pyrophosphate synthase	1.46	−1.27	AF309508
Squalene epoxidase	−1.84	−1.78	NM_009270
Sterol carrier protein 2	−1.16	−1.24	M62361
Sterol O-acyltransferase 1	1.67	1.39	NM_009230
Sterol O-acyltransferase 2	−1.20	−1.09	NM_011433
Sterol regulatory element binding protein 2	1.02	−1.35	AF374267
Sterol-C5-desaturase	−1.02	−1.21	AB016248
Steroyl-CoA desaturase 1	−1.08	−1.00	NM_009127

reductase, which is the rate-limiting enzyme of cholesterol biosynthesis (Table 3). On the other hand, the 40–7d RJ diet group showed decreased gene expression of SQLE, but there was no change in the gene expression of LDLR.

Next, real-time PCR was carried out to confirm the above changes. The mRNA level of FATP3 in the RJ diet group was significantly increased by 1.5 fold compared with the control diet group, whereas that in the 40–7d RJ was unchanged (Table 4). The mRNA level of LDLR in the RJ diet group was significantly increased by 1.8 fold compared with the control diet group, while that of SQLE in the RJ diet group and 40–7d RJ diet group was significantly decreased by 2.0 fold and 1.6 fold compared with the control, respectively (Table 4). There was no difference in mRNA levels of LDLR between the control and 40–7d RJ diet groups. The HMG-CoA reductase mRNA levels were not influenced by RJ diet or 40–7d RJ diet (data not shown). Furthermore, we found that the mRNA level of SREBP-1 was significantly increased by RJ diet and 40–7d RJ diet (Table 4), whereas that of SREBP-2 was unaffected (data not shown).

Fatty acid transport proteins (FATPs/solute carrier family 27) are integral transmembrane proteins that enhance the uptake of long-chain and very-long-chain fatty acids into cells (Stahl 2004). In man, FATPs comprise a family of six highly homologous proteins, hsFATP1–6, which are found in all fatty acid-utilizing tissues of the body (Stahl 2004). Therefore, the induction in gene expression of FATP3 by RJ may be associated with a hypolipidaemic effect. The RJ diet did not influence gene expression of HMG-CoA reductase, but decreased that of SQLE in liver. It also increased gene expression of SREBP-1, but not SREBP-2, in liver. SREBP-2 preferentially activates enzymes implicated in cholesterol

Table 4 Changes in gene expression of various factors involved in lipid and cholesterol metabolism in mice fed a diet containing 5% royal jelly (RJ) or a diet containing 5% RJ stored at 40°C for 7 days (40–7d RJ) compared with a control diet

Gene	Control	RJ	40–7d RJ
Fatty acid transport protein 3	100 ± 3.70	151 ± 8.00*	83.0 ± 13.2
Low-density lipoprotein receptor	100 ± 6.60	176 ± 2.30**	88.0 ± 11.8
Squalene epoxidase	100 ± 10.0	49.0 ± 3.20**	64.0 ± 8.20*
Sterol regulatory element-binding protein 1	100 ± 10.7	56.0 ± 4.70**	47.0 ± 10.8**

Total RNA was prepared from livers of the control, RJ and 40–7d RJ groups (10 mice each) and transcription was quantified by real-time PCR. GAPDH was chosen for internal normalization. Each value is the mean ± s.e.m. The value of gene expression in each group is shown as a relative value to control group. *$P < 0.05$ and **$P < 0.01$, compared with the control.

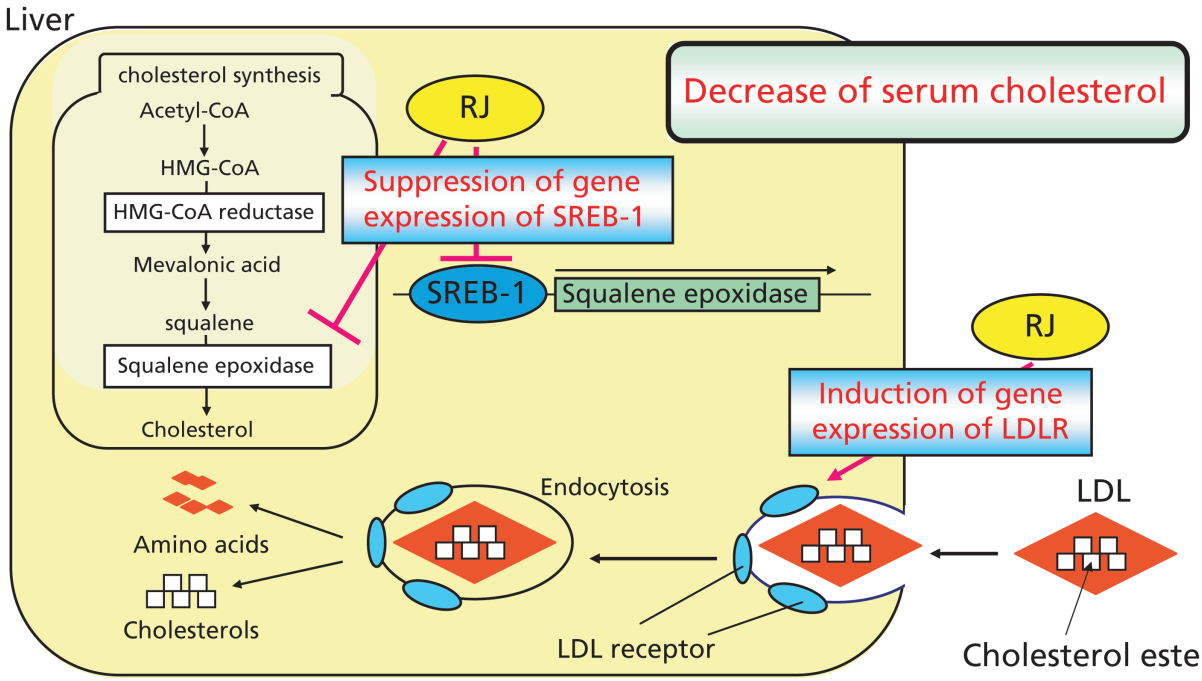

Figure 1 Proposed mechanism of the hypocholesterolaemic activity of royal jelly (RJ). RJ decreases gene expression of squalene epoxidase (SQLE), which is a key enzyme in cholesterol biosynthesis, and induces gene expression of low-density lipoprotein receptor (LDLR), which is involved in incorporation of cholesterol in liver. RJ also reduces gene expression of sterol regulatory element-binding protein-1, which may be a transcriptional factor of SQLE. Thus, RJ might decrease serum cholesterol in mice by decreasing SQLE and increasing LDLR.

production, such as HMG-CoA reductase, rather than those involved in fatty acid biosynthesis (Horton et al 1998, 2002), while SREBP-1 markedly increases the mRNA of SQLE (Sakakura et al 2001). These results suggest that the hypocholesterolaemic activity of RJ may be attributable to suppression of gene expression of SQLE through down-regulation of SREBP-1 by RJ. Further research is needed to identify the responsible component of RJ. Furthermore, RJ diet up-regulated gene expression of LDLR. It has recently been reported that activation of liver X receptor (LXR) induced a 2-fold increase in hepatic LDLR expression in wild-type mice (Masson et al 2004). Moreover, LXR agonist administration inhibited the development of atherosclerosis in LDLR-deficient and apolipoprotein E-deficient mouse models (Masson et al 2004). Therefore, RJ may induce gene expression of LDLR through activation of LXR. The up-regulation of gene expression of LDLR by RJ may also increase uptake of cholesterol into hepatocytes, induce hypocholesterolaemic action and inhibit atherosclerosis. However, RJ changed gene expression of lipid or cholesterol metabolism-related factors only by up to ±2 fold, and this may be the reason why RJ scarcely affected the serum cholesterol level in our experiments with non-high-fat diets. Since the SQLE mRNA level in both the RJ and 40–7d RJ diet groups were significantly decreased as compared with the control diet group, the effective constituents in RJ for down-regulation of SQLE appear to be heat-stable. On the other hand, the LDLR mRNA level in the RJ diet group was significantly increased as compared with that in both the control and 40–7d RJ diet groups, suggesting that the RJ constituents involved are not heat-stable (i.e., they may not be vitamins or 10-HDA).

Conclusions

Examination of the effect of RJ on hepatic gene expression in mice by means of a DNA microarray technique revealed decreased gene expression of SQLE, which is a key enzyme in cholesterol biosynthesis, and increased gene expression of LDLR, which is involved in cholesterol incorporation into the liver. RJ also reduced gene expression of sterol regulatory element-binding protein (SREB)-1, which may be a transcriptional factor of SQLE (Figure 1). These results indicate that the hypocholesterolaemic effect of RJ may be due to a decrease in SQLE and an increase in LDLR. Further studies will be required to identify the active constituent(s) in RJ.

References

Armstrong, M. L. (1976) Regression of atherosclerosis. *Atheroscl. Rev*. **1**: 137–182

Belong, R. P., Masek, J. A. (1971) The effect of royal jelly of bees for treatment of cerebral atherosclerosis. *Vrach. Deso*. **8**: 26–28

Blum, M. S., Novak, A. F., Taber, S. (1959) 10-Hydroxy-δ^2-decenoic acid, an antibiotic found in royal jelly. *Science* **130**: 452–453

Brown, G., Albers, J. J., Fisher, L. D., Schaefer, S. M., Lin, J. T., Kaplan, C., Zhao, X. Q., Bisson, B. D., Fitzpatrick, V. F., Dodge, H. T. (1990) Regression of coronary artery disease as a result of intensive lipid-lowering therapy in men with high levels of apolipoprotein B. *N. Engl. J. Med*. **323**: 1289–1298

Butcher, R. W., Baird, C. E. (1968) Effects of prostaglandins on adenosine 3′,5′-monophosphate levels in fat and other tissues. *J. Biol. Chem*. **243**: 1713–1717

Davis, B. J. (1964) Electrophoresis of proteins. *Ann. NY Acad. Sci*. **121**: 404–427

Echigo, T., Takenaka, T., Yatsunami, K. (1986) Comparative studies on chemical composition of honey, royal jelly and pollen loads. *Bull. Fac. Agriculture, Tamagawa Univ*. **26**: 1–8

Fujii, A., Kobayashi, S., Kuboyama, N., Furukawa, Y., Kaneko, Y., Ishihama, S., Yamamoto, H., Tamura, T. (1990) Augmentation of wound healing by royal jelly (RJ) in streptozotocin-diabetic rats. *Japan. J. Pharmacol*. **53**: 331–337

Fujiwara, S., Imai, J., Fujiwara, M., Yaeshima, T., Kawashima, T., Kobayashi, K. (1990) A potent antibacterial protein in royal jelly. *J. Biol. Chem*. **265**: 11333–11337

Haydak, M. H. (1970) Honey bee nutrition. *Ann. Rev. Entomol*. **15**: 143–156

Horton, J. D., Shimomura, I., Brown, M. S., Hammer, R. E., Goldstein, J. L., Shimano, H. (1998) Activation of cholesterol synthesis in preference to fatty acid synthesis in liver and adipose tissue of transgenic mice overproducing sterol regulatory element-binding protein-2. *J. Clin. Invest*. **101**: 2331–2339

Horton, J. D., Goldstein, J. L., Brown, M. S. (2002) SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. *J. Clin. Invest*. **109**: 1125–1131

Howe, S. R., Dimick, P. S., Benton, A. W. (1985) Composition of freshly harvested and commercial royal jelly. *J. Apic. Res*. **24**: 52–61

Kamakura, M., Fukuda, T., Fukushima, M., Yonekura, M. (2001a) Storage-dependent degradation of 57-kDa protein in royal jelly: a possible marker for freshness. *Biosci. Biotechnol. Biochem*. **65**: 277–284

Kamakura, M., Mitani, N., Fukuda, T., Fukushima, M. (2001b) Antifatigue effect of fresh royal jelly in mice. *J. Nutr. Sci. Vitaminol*. **47**: 394–401

Kamakura, M., Suenobu, N., Fukushima, M. (2001c) 57-kDa protein in royal jelly enhances proliferation of primary cultured rat hepatocytes and increases albumin production in the absence of serum. *Biochem. Biophys. Res. Commun*. **282**: 865–874

Kamakura, M., Maebuchi, M., Ozasa, S., Komori, M., Ogawa, T., Sakaki, T., Moriyama, T. (2005) Influence of royal jelly on mouse hepatic gene expression and safety assessment with a DNA microarray. *J. Nutr. Sci. Vitaminol*. **51**: 148–155

Kato, A., Onodera, M., Ishijima, Y. (1988) Effect of royal jelly on development of genital organs in male mice. *J. Tokyo Vet. Anim. Sci*. **35**: 1–4.

Kawamura, J. (1961) Influence of gelee royale on embryos. *J. Showa Med. Assoc*. (in Japanese) **20**: 1465–1471

Laemmli, U. K. (1970) Cleavage of structural proteins during the assembly of the head bacteriophage T4. *Nature* **227**: 680–685

Loscalzo, J. (1990) Regression of coronary atherosclerosis. *Science* **323**: 1337–1339

Mach, W. J., Hodis, H. N., Blankenhorn, D. H. (1993) Regression of atherosclerosis: a report from two clinical trials. *Atheroscl. Rev*. **25**: 301–309

Makarova, V. G. (1969) The effect of course-wise administration of different doses of Apilak upon some lipid metabolism indices. *Farmak. Toks*. **32**: 63–65

Masson, D., Staels, B., Gautier, T., Desrumaux, C., Athias, A., Le Guern, N., Schneider, M., Zak, Z., Dumont, L., Deckert, V., Tall, A., Jiang, X. C., Lagrost, L. (2004) Cholesteryl ester transfer protein modulates the effect of liver X receptor agonists on cholesterol transport and excretion in the mouse. *J. Lipid Res*. **45**: 543–550

Matuszewski, J., Kaczor, M. (1961) Effect of royal jelly on mammals. *Acta Physiol. Pol*. **5**: 745

Nakajin, S., Okiyama, K., Yamashita, S., Akiyama, Y., Shinoda, M. (1982) Effect of royal jelly on experimental hypercholesterolemia in rabbits. *Yakugaku Zasshi* (in Japanese) **36**: 65–69

O'Brien, K. D., Xue-Qiao, Z., Albers, J. J., Brown, B. G. (1993) A review of the familial atherosclerosis treatment study: implications for the regression of coronary atherosclerosis and prevention of clinical cardiovascular events. *Atheroscl. Rev*. **253**: 311–319

Patel, N. G., Haydak, M. H., Gochnauer, T. A. (1960) Electrophoretic components of the proteins in honeybee larval food. *Nature* **186**: 633–634

Ross, R. (1976) Hyperlipidemia and atherosclerosis. *Science* **193**: 1094–1100

Ross, R. (1993) Cellular mechanism of atherosclerosis. *Atheroscl. Rev*. **25**: 195–200

Sakakura, Y., Shimano, H., Sone, H., Takahashi, A., Inoue, N., Toyoshima, H., Suzuki, S., Yamada, N. (2001) Sterol regulatory element-binding proteins induce an entire pathway of cholesterol synthesis. *Biochem. Biophys. Res. Commun*. **287**: 311

Seidel, D. (1993) Significance of risk factors. *Atheroscl. Rev*. **25**: 293–299

Shinoda, M., Nakajin, S., Oikawa, T., Sato, K., Kamogawa, A., Akiyama, Y. (1978) Biochemical studies on vasodilative factor in royal jelly. *Yakugaku Zasshi* (in Japanese) **98**: 139–145

Sitar, J., Cernochova, Z. (1967) Treatment of angina pectoris with Vita-Apinol Spofa. Some metabolic effects of the drug. *Vnitr. Lek*. **14**: 1798–1805

Stahl, A. (2004) A current review of fatty acid transport protein (SLC27). *Pflugers Arch*. **44**: 722–727

Takenaka, T. (1982) Chemical composition of royal jelly. *Honeybee Sci*. **3**: 69–74

Takenaka, T., Yatsunami, K., Echigo, T. (1986) Changes in quality of royal jelly during storage. *Nippon Shokuhin Kogyo Gakkaishi* (in Japanese) **33**: 1–7

Tamura, T., Fujii, A., Kuboyama, N. (1987) Antitumor effect of royal jelly. *Folia Pharmacol. Japon* (in Japanese) **89**: 73–80

Townsend, G. F., Morgan, J. F., Hazlett, B. (1959) Activity of 10-hydroxydecenoic acid from royal jelly against experimental leukemia and ascitic tumors. *Nature* **183**: 1270–1271

Vitteck, J. (1995) Effect of royal jelly on serum lipids in experimental animals and humans with atherosclerosis. *Experientia* **51**: 927–935

Yatsunami, K., Echigo, T. (1985) Antibacterial action of royal jelly. *Bull. Fac. Agriculture, Tamagawa Univ*. **25**: 13–22

Zaicev, G., Poriadin, V. (1962) The use of royal jelly in the treatment of endarteritis and atherosclerosis of peripheral arteries of extremities. *Inf. Bjul O Mat. Mol. III, Rjazan* **3**: 62