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Study on life span extension efficacy by Korean Red Ginseng

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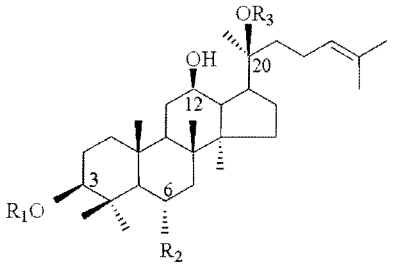
Abstracts : The backbone structure of ginsenosides, active ingredients of *Panax* ginseng, is similar with that of sterol, especially cholesterol. *Caenorhabditis elegans* (*C. elegans*) is one of free living nematodes and is well-established animal model for biochemical and genetic studies. *C. elegans* cannot synthesize *de novo* cholesterol, although cholesterol is essential requirement for its growth and development. In the present study, we investigated the effects of Korean red ginseng total extract (KRGE), ginseng total saponins (GTS) on life span of *C. elegans* in cholesterol-deprived and -fed medium. Cholesterol deprivation caused damages on life span of worms throughout F1 to F3 generations. KRGE or GTS supplement to cholesterol-deprived medium restored the life span of worms as much as cholesterol alone-fed medium. In study to identify which ginsenosides are responsible for life span restoring effects of KRGE, we found that ginsenoside Rc supplement not only restored life span of worms grown in cholesterol-deprived medium but also prolonged life span of worms grown in cholesterol-fed medium. These results show a possibility that ginsenosides could be utilized by *C. elegans* as a sterol substitute and further indicate that ginsenoside Rc is the effective component of Korean red ginseng that prolongs the life span of *C. elegans*.

Key words : Korean red ginseng; sterol; *C. elegans*; life span; ginsenoside Rc

INTRODUCTION

Ginseng, the root of *Panax ginseng* C. A. Meyer, has been used as a representative tonic for two thousand years in the Far East countries like Korea, China, and Japan. Now, ginseng is one of the most famous and precious herbal medicines consumed around the world.[1] Although ginseng exhibits multiple pharmacological actions *in vitro* or *in vivo* studies, its mechanisms on various efficacies are still elusive. Recent accumulating evidences show that ginseng saponins (or ginsenosides) are the main active ingredients of ginseng (Fig. 1). Ginseng root contains 3-4% of ginseng saponins. Ginseng saponins are especially abundant in fine roots rather than main body of ginseng root. Ginseng saponins are one of glycoside saponins and one of the derivatives of triterpenoid dammarane consisting of thirty carbon atoms. Each ginsenoside has a common hydrophobic four ring cholesterol-like backbone structure with sugar moieties attached. About 30 different types of ginseng saponins have been isolated and identified from the root of *Panax ginseng* (Fig. 1).



Ginsenosides	R1	R2	R3
Rb_1	-Glc_2-Glc	-H	-Glc_6-Glc
Rb_2	-Glc_2-Glc	-H	-Glc_6-Ara(pyr)
Rc	-Glc_2-Glc	-H	-Glc_6-Ara(fur)
Rb_1	-H	-O-Glc	-H
Rb_2	-Glc	-H	-H
Rg_1	-H	-O-Glc	-Glc
Rg_3	-Glc_2-Glc	-H	-H

Fig. 1. Structures of the representative ginsenosides. They differ at three side chains attached the common steroid ring. Abbreviations for carbohydrates are as follows: Glc, glucopyranoside; Ara (pyr), arabinopyranoside; Rha, rhamnopyranoside. Superscripts indicate the carbon in the glucose ring that links the two carbohydrates.

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Caenorhabditis elegans (*C. elegans*) is one of nematode species and is one of free living animals under the earth rather than is parasitic in host animals or plants. *C. elegans* takes its food from dead animals or plants. Since *C. elegans* has short life cycle and large amounts of worms can be easily grown for biochemical assays or genetic studies in the laboratory, the organism is one of the well-established genetic models for life span. Interestingly, *C. elegans* cannot synthesize sterols unlike other animals and requires dietary sterol, which is usually supplied as cholesterol in the laboratory. [2,3] Although the functions of sterols in *C. elegans* have not been fully characterized, *in vitro* sterol deprivation results in short life span throughout the first (F1), second (F2), and third (F3) generations.[3,4]

On the other hand, some species of parasitic nematodes are found in wild and cultivated ginseng root. For example, *Pratylenchus subpenetran* is one of main parasitic animals and causes damage to ginseng roots.[5] It penetrates into fine roots or outer spaces of ginseng root from soil and makes a lot of small humps in fine roots, because they die if they are exposed to air. Its parasitic actions in fine roots of ginseng interfere to absorb nutrients from soil. It is known that if it is not treated with anti-nematode agents, it causes to severe damages on normal development of ginseng during young period, resulting in delay of the growth and further make ginseng fragile to other microbial infections. Although these observations suggest a possibility that ginseng roots might provide suitable environment(s) for survival of the parasitic nematodes, little is known on effects of ginseng extract, ginseng saponins or individual ginsenosides as main ingredients of ginseng on the life span of nematodes.

We performed the present study with two-fold purposes: One was to examine whether supplements of Korean red ginseng total extract (KRGE) or ginseng total saponins (GTS) show any effects on the life span of *C. elegans* grown in cholesterol-deprived and fed-medium. If KRGE or GTS exhibits physiological effects on life span prolongation on *C. elegans* grown in cholesterol-deprived and fed- medium, the second aim was to identify which ginsenoside(s) are responsible for life span prolongation on *C. elegans* grown in cholesterol-deprived or -fed medium. We found that cholesterol deprivation induced significant damages on the life span of *C. elegans*. However, KRGE or GTS supplement to cholesterol-deprived medium restored the life span of worms as much as cholesterol-fed medium. We found that ginsenoside Rc is responsible for restoration of life span of worms grown in cholesterol-deprived medium and further prolonged the life span of worms grown in cholesterol-fed medium. These results indicate that ginsenoside Rc supplement meets the sterol requirements of worms grown in sterol-deprived medium. Finally, the effects of KRGE, GTS or ginsenosides to worms grown in cholesterol-deprived condition might be due to worms' ability to utilize the structural similarity of ginsenosides with sterol.

MATERIALS AND METHODS

Materials

Electrophoresis grade agarose was obtained from Becton, Dickinson and Company (Sparks, MD, USA) and peptone was obtained from Amresco (Solon, OH, USA). Cholesterol and all other analytical agents were obtained from Sigma (St. Louis, MO, USA). Cholesterol stock solution for cholesterol treatment group was prepared at 5 mg/ml of ethanol. The final ethanol concentration was 0.01%. KRGE, GTS and individual ginsenosides, isolated according to the method of Tanaka *et al.*(1966) and Shibata *et al.*(1966), respectively, was kindly provided by the Korea Ginseng Cooperation (Taejon, Korea).[6,7] Briefly, to remove phytosterols such as sitosterol or campasterol, the concentrated water extract was partitioned between water and n-hexane. The aqueous layer was further partitioned with butanol (BuOH) to obtain BuOH fraction, ginseng total saponin fraction. The BuOH fraction, after concentration, was further processed to remove non-saponin components remaining in BuOH fraction using Diaion HP-20 column.[8,9] After chromatography, we confirmed no existence of phytosterols by HPLC. GTS contained ginsenoside Rb_1 (17.1 %), Rb_2 (9.07 %), Rc (9.65 %), Rd (8.26 %), Re (9 %), Rf (3 %), Rg_1 (6.4 %), Rg_2 (4.2 %), Rg_3 (3.8 %), Ro (3.8 %), Ra (2.91 %) and other minor ginsenosides. GTS was diluted with NGM medium before use. Fig. 1 shows the structures of the eight representative ginsenosides.

Media and *C. elegans* growth

The nematodes were grown and maintained on NGM agarose plates (3 g/l NaCl, 2.5 g/l peptone, 5 mg/l cholesterol, 1 mM $CaCl_2$, 1 mM $MgSO_4$, 25 mM KH_2PO_4, pH 6.0, and 17g/l agar) with the *Escherichia coli* (*E. coli*) OP50 strain in an incubator at 20°C.[10] To obtain cholesterol-free conditions, agar was replaced by agarose, which was extracted three times with chloroform.[11] We also extracted peptone with ether in a large beaker in a fume hood.[12] For this, the peptone powder was mixed with an

excess volume of ether, allowed to settle, decanted, and the process was repeated twice more. The extracted peptone was allowed to dry overnight in the hood to remove the remaining ether.[12] *E. coli* strain OP50 was also grown directly in this sterol-free medium. Wild-type N2 *C. elegans* (Bristol type) was provided by the *Caenorhabditis* Genetics Center and was maintained according to the methods of Brenner (1974).[10] For definition of various generations of worms, we first cultured worms (Po, n =10-20) in each treatment group such as cholesterol-deprived, cholesterol (5 μg/ml)-fed group, various concentrations of GTS or KRGE + cholesterol-deprived, or GTS + cholesterol (5 μg/ml)-fed group. When eggs from Po animals grown in each treatment were again placed on various treatment group plates as mentioned above, the resulting animals are referred to as F1 generation. When eggs from F1 animals were grown in various treatment group plates, the resulting animals are referred to as F2 generation. Again when eggs from F2 animals were grown in various treatment group plates, the resulting animals are referred to as F3 generation.

Measurement of life span

For synchronous culture, L1 larvae were allowed to hatch by overnight at 20°C incubation in M9 buffer and transferred to different media plates to develop to L4 larvae. L4 larvae were transferred to NGM plates supplemented with 40 μM 5-fluoro-2'-deoxyuridine (FuDR) to suppress the production of their progenies. NGM agarose-FuDR plates were also divided into cholesterol-deprived, cholesterol-fed, KRGE, GTS or ginsenoside + cholesterol-deprived, or KRGE, GTS or ginsenoside + cholesterol-fed group. The worms were examined daily and dead worms, which did not move after touching their heads with a platinum wire, were removed to count. The dead worms due to scrawling up the walls of plates were excluded from this analysis. The worms on the plates contaminated by other microorganisms were also excluded from analysis, since lifespan of worms were affected by the concentration of dietary bacteria throughout F1 to F3 generations. The life span was determined from the percentage of worms alive on a given day.

RESULTS

Effects of KRGE or GTS on worm life span in cholesterol-deprived or -fed medium

Since worms grown in cholesterol deprived-medium show shorter life span than those grown in cholesterol-fed medium,[4,13] we also examined whether KRGE or GTS supplement to cholesterol-deprived medium affects worm life span. For this, L1 larvae were allowed to hatch by overnight at 20°C incubation in M9 buffer and transferred to different kinds of plates to develop to L4 larvae. L4 larvae were transferred to NGM plates supplemented with FuDR to suppress the production of their progenies. The synchronized L4 worms were again divided into cholesterol-deprived (n = 100), cholesterol (5 or 50 μg/ml)-fed

Table 1. Effects of Korean Red ginseng extract (KGRE) on life span of *C. elegans* grown in cholesterol-deprived and fed-medium

F1	Life span	
	Mean days	Maximum days
- Chol	12.7	15
- Chol + KRGE 1 (mg/ml)	17.2*	20
+ KRGE 2	17.5*	21
+ KRGE 4	17.1*	20
+ KRGE 6	17.5*	21
+ Chol	17.0*	20

*$p<0.01$, significantly different from cholesterol-deprived group.

Table 2. Effects of GTS on life span of *C. elegans* grown in cholesterol-deprived and fed-medium

F1	Life span	
	Mean days	Maximum days
- Chol	12.8 ± 0.2##	15
- Chol + GTS 5 (μg/ml)	16.5 ± 0.1**	20
+ GTS 100	17.2 ± 0.4**	22
+ GTS 300	17.4 ± 0.3**	24
+ Chol 5	16.5 ± 0.2**	20
+ Chol + GTS 300	15.7 ± 0.6*	21
+ Chol 50	15.0 ± 0.1*	19
F2		
- Chol	13.7 ± 0.4#	17
- Chol + GTS 5	16.2 ± 0.0*	20
+ GTS 100	17.5 ± 0.3*	23
+ GTS 300	16.4 ± 0.1*	22
+ Chol 5	16.2 ± 0.2*	20
+ Chol + GTS 300	16.7 ± 0.1*	22
+ Chol 50	16.5 ± 0.1*	21
F3		
- Chol	13.4 ± 0.3##	17
- Chol + GTS 5	17.7 ± 0.2**	22
+ GTS 100	17.7 ± 0.4*	23
+ GTS 300	16.0 ± 0.2*	22
+ Chol 5	17.2 ± 0.2*	22
+ Chol + GTS 300	15.8 ± 0.3*,#	24
+ Chol 50	16.5 ± 0.5*	22

*$p<0.01$, **$p<0.001$, significantly different from cholesterol-deprived group.
#$p<0.01$, ##$p<0.001$, significantly different from cholesterol-fed group.

(n = 118), KRGE (1, 2, 4, or 6 mg/ml) or GTS (5, 100, or 300 μg/ml) + cholesterol deprived (n=95-100 for each concentration), and cholesterol-fed + GTS (300 μg/ml) (n = 98) in the presence of FuDR. The average life span and maximum survival days of worms grown in various treatments were summarized in Table 1 and 2. As shown in Table 1 and 2, worms grown in cholesterol-deprived medium showed a significantly shorter life span than those grown in cholesterol-fed medium, consistent with previous report.[4,13] Through life span study, we could observe that although worms grown in KRGE or GTS supplement to cholesterol-deprived medium did not show a longer life span than those of cholesterol-fed group in F1, F2 and F3 generations, KRGE or GTS supplement to cholesterol-deprived group produced significantly a longer life span of worms by about 4-5 days than those of cholesterol-deprived group throughout F1 to F3 generations. KRGE or GTS supplement in cholesterol-deprived medium restored normal life span as much as cholesterol-fed condition. However, supplement of GTS (300 μg/ml) to cholesterol-fed medium or supplement of ten-fold higher concentrations (50 μg/ml) of cholesterol to normal cholesterol (5 μg/ml)-fed medium did not prolong the life span compared with cholesterol-fed group (Table 2 and Fig. 2). We could also observe that co-supplement of GTS to cholesterol-fed medium did not affect the life span of worms compared with worms grown in cholesterol-fed medium. Again, these results demonstrated that cholesterol deprivation in culture medium caused a short life span as shown in previous reports [4,13] but KRGE or GTS supplement to cholesterol-deprived medium restored the normal life span.

Ginsenoside Rb_1 and Rc are main ingredients for the restoration of life span of worms grown in cholesterol-deprived mediums and ginsenoside Rc prolonged the life span of worms grown in normal cholesterol-fed mediums

Above results suggest that there might be active ginsenoside(s) responsible for life span restoration of worms grown in cholesterol-deprived medium in GTS. To identify active ingredient(s) for life span prolongation of worms, we tested the effects of several individual ginsenosides such as ginsenoside Rb_1, Rc, Rg_1, Rg_3, Rh_1, and Rh_2 (300 μM each) on life span of worms in cholesterol-deprived or fed-medium. Among them ginsenoside Rg_3, Rh_1, and Rh_2, which are minor ginsenosides and are present as a trace of amount compared with ginsenoside Rb_1 and Rc, did not affect on life span of worms grown in

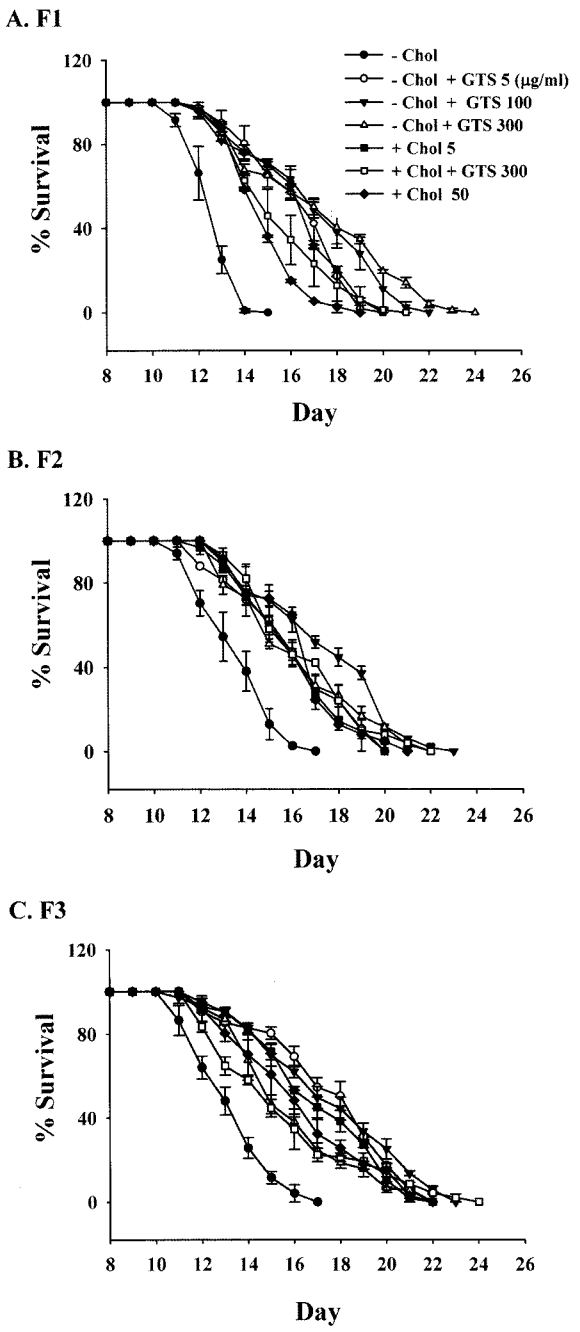

Fig. 2. Effects of GTS on life span of worms grown in cholesterol-deprived or -fed medium from F1 (A), F2 (B), and F3 (C) generations at 20°C. Life span of worms grown in indicated conditions was measured as described in Materials and methods. Eggs laid by P0, F1, or F2 animals were placed on plates containing the following treatments: – Chol; – Chol (cholesterol-fed, 50 μg/ml) + GTS (5 μg/ml); – Chol + GTS (100 μg/ml); – Chol + GTS (300 μg/ml); + Chol; + Chol + GTS (300 μg/ml); + Chol (cholesterol-fed, 50 μg/ml). The average values were obtained from 94-118 worms.

Table 3. Effects of individual ginsenosides on life span of *C. elegans* grown in cholesterol-deprived and fed-medium

F1	Life span Mean days	Maximum days
- Chol	12.6 ± 0.1##	16
- Chol + Rg_1	14.4 ± 0.2*, ##	19
+ Rb_1	16.8 ± 0.6**	23
+ Rc	17.0 ± 0.1**	22
+ Chol	17.2 ± 0.1**	21
+ Chol + Rg_1	17.2 ± 0.3**	19
+ Chol + Rb_1	17.1 ± 0.6*	22
+ Chol + Rc	18.3 ± 0.4**	23
F2		
- Chol	13.0 ± 0.4#	16
- Chol + Rg_1	13.2 ± 0.1##	17
+ Rb_1	17.4 ± 0.5*	21
+ Rc	17.4 ± 0.4*	20
+ Chol	17.8 ± 0.4*	21
+ Chol + Rg_1	17.4 ± 0.1**	19
+ Chol + Rb_1	17.1 ± 0.6*	24
+ Chol + Rc	19.4 ± 0.1**	24
F3		
- Chol	13.2 ± 0.3#	16
- Chol + Rg_1	15.8 ± 0.1*	19
+ Rb_1	17.7 ± 0.3**	23
+ Rc	16.9 ± 0.4*	23
+ Chol	16.4 ± 0.3*	23
+ Chol + Rg_1	15.9 ± 0.3*	20
+ Chol + Rb_1	18.1 ± 0.4**	25
+ Chol + Rc	19.5 ± 0.7*, #	26

*p<0.01, **p<0.001, significantly different from cholesterol-deprived group.
#p<0.01, ##p<0.001, significantly different from cholesterol-fed group.

both cholesterol-deprived and cholesterol-fed medium rather inhibited the development and growth of worms (data not shown), whereas supplement of ginsenosides such as Rb_1 or Rc but not Rg_1 to cholesterol-deprived medium restored the life span as much as cholesterol-fed group. The average life span and maximum days in F1, F2, and F3 generations of worms grown in cholesterol-deprived, cholesterol-fed, ginsenoside Rb_1, Rc, or Rg_1 + cholesterol-deprived or -fed medium was summarized in Table 3. Interestingly, co-supplement of ginsenoside Rc but not Rb_1 and Rg_1 to cholesterol-fed medium not only prolonged the average life span but also extended the maximum days of life span by 2, 3 and 3 days compared with cholesterol-fed group in F1, F2, and F3 generations, respectively (Fig. 3 and Table 3). Since low concentrations of GTS supplement (5 μg/ml) to cholesterol-deprived medium can substitute cholesterol for life span elongation (Table 2), we also tested the effects of ginsenoside Rb_1, Rc, or Rg_1 at low concentration (5 μM each) on worm life

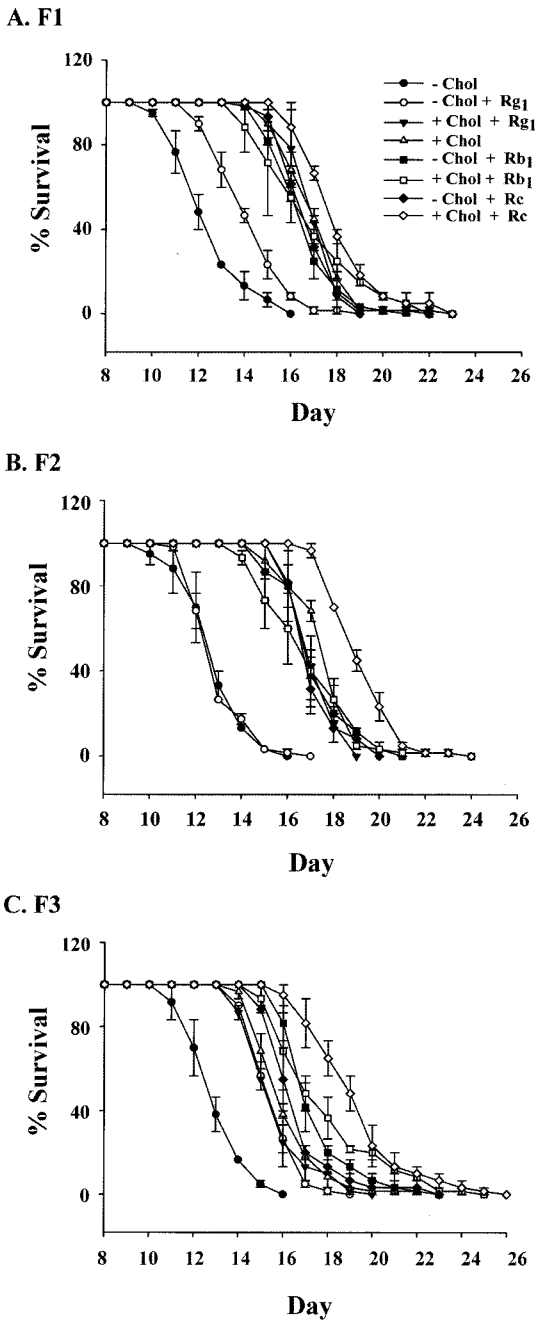

Fig. 3. Effects of ginsenoside Rb_1, Rc, or Rg_1 on life span of worms grown in cholesterol-deprived or -fed medium from F1 (A), F2 (B), and F3 (C) generations at 20. Life span of worms grown in indicated conditions was measured as described in Materials and methods and Fig. 3. We used ginsenoside Rb_1, Rc, or Rg_1 (300 μM each). The average values were obtained from sixty worms. The average values were obtained from 60-66 worms.

span in cholesterol-deprived medium. As shown in Table 3, we found that supplement of ginsenoside Rb_1, Rc, or

Rg_1 to cholesterol-deprived medium slightly but not significantly extended the life span of worm. Thus, we found among several individual ginsenosides tested that ginsenoside Rb_1 and Rc at 300 μM are main ingredients for the restoration of life span of *C. elegans* grown in cholesterol-deprived mediums. We further found that ginsenoside Rc is the main component of *Panax* ginseng that prolongs the life span of *C. elegans*.

DISCUSSION

Nematodes, including free-living *C. elegans*, require sterol for its normal development, growths, and life span as a nutritional source,[14] since *C. elegans* is unable to biosynthesize sterol *de novo*.[14] However, *C. elegans* is usually able to obtain cholesterol or cholesterol-like sterols for their growth by metabolizing natural sterols such as phytosterols present in many plants or sterols from the animal body in the soil.[15] Although some species of nematodes are known to be parasitic in wild and cultivated ginseng roots,[5] little is known on the physiological roles of ginseng saponins or ginsenosides to life span of *C. elegans*.

In the present study, we investigated the effect of GTS on life span of *C. elegans* in cholesterol-deprived- or -fed medium. Life span of worms grown in cholesterol deprivation medium was significantly shorter than those of cholesterol-fed group. However, even low amount of GTS supplement (5 μg/ml) or KRGE to cholesterol-deprived group extended life span as much as cholesterol-fed group (Table 1 and 2). We also found that ginsenoside Rb_1 and Rc were main ingredients that are responsible for GTS beneficial effects to worms grown in cholesterol-deprived medium. Interestingly, co-supplement of ginsenoside Rc further extended life span and maximal survival days of worms grown in cholesterol-fed medium. However, we could not observe the same life span extending effects in worms grown in medium supplemented with ten-fold higher concentration (50 μg/ml) of cholesterol than normal medium (Fig. 2 and Table 2). These results indicate that *C. elegans* could use GTS and its individual ginsenosides such as ginsenoside Rb_1 and Rc as a sterol substitute. Further ginsenoside Rc has life span extending effect in *C. elegans*.

Although there are many publications on the presence of saponins from various kinds of plants, the physiological roles of saponins in plants are not yet fully understood.[16] Recent studies showed that most of saponins are usually known to be antimicrobial, to inhibit mould, and to protect plants from insect attack. For example, it is known that saponins such as avenacosides in oat are activated by the plant's enzymes in response to tissue damages or pathogen attacks.[17] Thus, most of saponins may be considered a part of plants' defense systems and have been included in a large group of protective molecules found in plants named 'phytoanticipins' or 'phytoprotectants'.[18]

However, we found in the present study that KRGE or GTS helps *C. elegans* survive normally rather than does harm to them, since KRGE or GTS supplement extended life span in spite of cholesterol deprivation. One speculation on the beneficial effect of KRGE might be due to GTS in KRGE, since KRGE contains the diverse ginsenosides than other ginseng preparation. Recent accumulating evidences showed that ginseng saponins in worms mimic the actions of steroid hormones such as estrogen and progesterone as in mammalian cells.[19] Thus, these steroidal actions of ginseng saponins might directly meet sterol requirement as sterol source. The other speculation is that *C. elegans* might modify ginseng saponins present in medium in sterol-deprivation environments and produces sterols from ginseng saponins for its utilization. This speculation might be supported from filipin staining. We used sterol-specific filipin staining, which shows fluorescence upon forming a complex with 3-hydroxysterols,[12] to detect sterol accumulation in *C. elegans*.[20] Worms grown in ginsenoside Rb_1 or Rc supplement were bigger than those grown in cholesterol-deprived medium and exhibited stronger fluorescence in whole body with discrete internal organs than those grown in cholesterol-deprived medium, although ginsenoside Rb_1 and Rc have no 3-hydroxy group at carbon-3 instead have two carbohydrates (data not shown). Moreover, treatment of azacoprostane-HCl, which is known as -dehydrocholesterol reductase inhibitor,[21] blocked the beneficial effects (i.e. normal fertility, development, growth, and life span) of GTS to cholesterol-deprived group (data not shown). These results suggest that *C. elegans* might have metabolic pathways for modification of GTS, ginsenoside Rb_1, or Rc in order to produce its required sterol. However, we could not exclude other possibilities that GTS or individual ginsenosides might participate in other physiological roles besides sterol substitute, since co-supplement of GTS to cholesterol-fed medium produces much larger brood size and bigger worms than those observed in cholesterol-fed medium (data not shown). Moreover, low concentration of GTS supplement to cholesterol-deprived group significantly extended life span compared with cho-

lesterol-deprived group (Fig. 2 and Table 2), although low concentration of individual ginsenosides such as Rb_1, Rc or Rg_1 had no significant effects (data not shown). However, co-supplement of ginsenoside Rc (300 μM) to cholesterol-fed medium also induced prolongation of life span (Fig. 3), which was not observed in much higher concentration of cholesterol, indicating that ginsenoside Rc has life span extending effect in *C. elegans* (Table 3). Future studies will be further required to elucidate the physiological roles and metabolic pathway of GTS or ginsenosides in *C. elegans*.

Among individual ginsenoside Rb_1, Rc and Rg_1, we could observe that life span extending effect of ginsenoside Rg_1 on worms grown in cholesterol-deprived or fed-medium was less effective than those of ginsenoside Rb_1 and Rc (Fig. 3). As shown in Fig. 1, the differences of chemical structure between ginsenoside Rg_1 and Rb_1 or Rc are that ginsenoside Rg_1 has one carbohydrate at carbon-6 and has hydroxyl group at carbon-3, whereas ginsenoside Rb_1 and Rc has only two carbohydrates at carbon-3. Thus, the carbohydrate attached at carbon-6 of ginsenoside Rg_1 might play a role in exhibiting less physiologically beneficial effects in prolonging the life span in cholesterol-deprived or fed-medium.

In summary, using *C. elegans* as model system we herein used KRGE, GTS and individual ginsenosides to know its role in cholesterol-deprived or fed-medium. We have obtained novel evidences for the first time that KRGE and GTS could substitute cholesterol for normal life span of *C. elegans* and that supplement of KRGE, GTS, ginsenoside Rb_1 or Rc to cholesterol-deprived medium extended life span as much as worms grown in cholesterol-fed medium. We also found that supplement of ginsenoside Rc to cholesterol-fed group further extended life span in cholesterol-fed group. These novel findings provide new insights that *C. elegans* utilizes subtypes of ginsenosides as sterol source.

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