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Royal Jelly prolongs the life span of C3H/HeJ mice: correlation with reduced DNA damage

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Abstract

In this study, we investigate the effect of dietary Royal Jelly (RJ) on tissue DNA oxidative damage and on the life span of C3H/HeJ mice. In C3H/HeJ mice that were fed a dietary supplement of RJ for 16 weeks, the levels of 8-hydroxy-2-deoxyguanosine (8-OHdG), a marker of oxidative stress, were significantly reduced in kidney DNA and serum. Secondly, we determined the effect of dietary RJ on the life span in C3H/HeJ mice. The 50% mice survivals of intermediate- (about 6 mg/kg weight) and high-dose groups (about 60 mg/kg weight) were reached at significantly longer times than that of the control group according to the generalized Wilcoxon test ($p < 0.05$). The average survival times were 88 weeks for the control group vs. 79 weeks for the low-dose group (about 0.6 mg/kg weight), 112 weeks for the intermediate-dose group and 110 weeks for the high-dose group, respectively, showing that RJ extended the average survival time by about 25% compared to the control group. However, RJ did not extend the total life span. These results indicated that dietary RJ increased the average life span of C3H/HeJ mice, possibly through the mechanism of reduced oxidative damage.

Keywords: Royal Jelly; 8-Hydroxy-2′-deoxyguanosine; Life span; Mice; Dietary treatment

1. Introduction

Royal Jelly (RJ), a principal food of the honeybee queen, is produced by the hypo-pharyngeal and mandubular glands of worker honeybees. It has been reported that RJ has several pharmacological activities, including vasodilative and hypotensive activities (Shimoda et al., 1978), increase in growth rate of chick embryos (Kawamura, 1961), disinfectant action (Yatsunami and Echigo, 1985), anti-tumor activity (Tamura et al., 1987), antiinflammatory activity (Fujii et al., 1990), antihypertensive activity (Matsui et al., 2002), antifatigue activity (Kamakura et al., 2001) and antiallergy activity (Kataoka et al., 2001). Analysis of chemical composition shows that RJ is composed mainly of proteins, sugars, lipids, vitamins, and free amino acids, together with a large number of such bioactive substances as 10-hydroxy-2-decenoic acid (Blum et al., 1959), antibacterial protein (Fujiwara et al., 1990), a stimulating factor for the development of male mouse genital organs and a 350-kDa protein called apisin (Kato et al., 1988) that stimulates the proliferation of human monocytes (Watanabe et al., 1998). Therefore, RJ has been widely promoted as a commercially available medical, a health food and as a cosmetic in many countries. In addition, it has been said that RJ is a useful form of traditional medicine for longevity used in Europe and Asia.

Aging is usually defined as the progressive loss of function accompanied by decreasing fertility and increasing mortality with advancing age. Harman articulated a ‘free radical theory of aging’, which speculates that endogenous oxygen radicals are generated in cells, resulting in a pattern of cumulative damage (Harman, 1956). The theory originally implied that the targets of reactive oxygen species (ROS) are random and indiscriminate, and that the balance between ROS production and antioxidant defenses determines the degree of oxidative stress. Consequences of this stress include the modification of cellular proteins, lipids and DNA. Many studies have shown that aging cells and organisms accumulate increased levels of oxidant-damaged nuclear DNA. This endogenous DNA damage might be a major cause of aging and degenerative diseases associated

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with aging such as cancer and heart disease. In addition, mutations that increase oxidative damage could shorten life span.

Thus, we hypothesized that if the levels of over-produced free radicals in the body could be reduced the average lifespan might be extended in mammals due to suppression of oxidative damage to the body. Therefore, we first investigated the effect of RJ on levels of an oxidative DNA damage marker, 8-hydroxy-2-deoxyguanosine (8-OHdG). Next, we considered that if RJ could behave like an antioxidant and decrease DNA oxidative damage as reflected by decreased levels of 8-OHdG, we researched the effect of RJ on life span in C3H/HeJ mice should be addressed.

2. Materials and methods

2.1. Animals

Six week-old male C3H/HeJ mice were obtained from Japan CREA, Inc. (Tokyo, Japan) and Charles River Japan, Inc. (Tokyo, Japan). Mice were housed four per cage in an animal room controlled at 23 ± 2 °C, a humidity of 50 ± 10%, under a 12 hour-dark and 12 hour-light cycle. Bedding consisted of autoclaved wood chips. The diet, NMF powder (Oriental Yeast Co., Tokyo, Japan) and autoclaved water were provided ad libitum.

2.2. Royal Jelly and the experimental diet

Samples of RJ were collected from Paraibuna region in São Paulo, Brazil, and were kept frozen at −40 °C until used. Powdered RJ was prepared by adding Treharose powder® (Trehalose, Hayashibara Biochemical Laboratories, Inc., Okayama, Japan) at a ratio of 9:1 by volume to RJ. The experimental diets were prepared by adding powdered RJ at low (5 ppm), intermediate (50 ppm) and high (500 ppm) concentrations, thoroughly mixed with the commercial powdered diet, NMF, respectively.

2.3. In vivo experiments

Experiment 1. Firstly, we examined the effect of RJ on 8-OHdG levels in different tissues obtained from treated and control mice.

In experiment 1, Eight weeks-old male C3H/HeJ mice were given free access to the various diets and water. In this experiment, every group was composed of 10–12 mice. After 16 weeks of receiving RJ or control diet, mice were killed by diethyl ether, and the sera and various organs were collected to measure 8-OHdG levels.

Experiment 2. Eight weeks-old male C3H/HeJ mice were given free access to the various diets and water. The mice were observed daily during the period of their survival. In this experiment, each experimental group contained 19–20 mice. When mice died, the mice were autopsied to determine whether the cause of death were other than possibly age-related. The times at which mouse survival reached 75, 50 and 25% in the respective groups were recorded.

2.4. Measurement of 8-hydroxydeoxyguanosine

8-OHdG levels in the DNA present in the sera and various organs were determined using Highly Sensitive 8-OHdG Check (The Japan Institute for Control of Aging, Fukuroi, Shizuoka, Japan).

DNA was isolated from the various organs using the sodium iodide (NaI) method according to a previous report (Wang et al., 1994). Briefly, mouse tissues were homogenized in a dounce homogenizer in an ice-cold lysis solution, and then the homogenates were centrifuged at 10,000*g* for 20 s. The nuclear pellets were resuspended in enzyme reaction solution and after treating the pellets with 10 μg/ml proteinase K and 1 μg/ml RNAse, at 37 °C for 60 min, this solution was mixed with NaI solution. DNA was precipitated by ice-cold isopropanol and resuspended in water. The DNA concentrations were determined by measuring the absorbance of the samples at 260 nm.

The DNA samples were hydrolyzed as described (Kasai et al., 1986). Briefly, 40 μg DNA was dissolved in 20 mM acetate buffer and digested with DNAse I at 37 °C for 60 min. The digested DNA was then treated with alkaline phosphatase in 0.1 M Tris–HCl buffer at 37 °C for 30 min to hydrolyze the nucleic to free nucleotides. The 8-OHdG levels in the samples were determined by the highly sensitive 8-OHdG-check kit.

2.5. Statistical analysis

Statistical analyses were carried out using the generalized Wilcoxon test for the survival rates, and the Fischer's protected LSD test for differences in serum and organ DNA 8-OHdG levels to determine any significant differences ($p < 0.05$) between the means.

3. Results

3.1. Serum and various organ DNA 8-OHdG levels

The groups receiving dietary RJ did not show any changes in growth, food intake and in appearance when compared with control groups in any of our experiments (data not shown). The results of determinations of 8-OHdG levels in the serum and various organs are shown in Fig. 1. The 8-OHdG levels in kidney DNA of the intermediate- and high-dose RJ groups were significantly reduced when compared to the control group ($p < 0.05$). This effect was observed in a dose-dependent manner. In addition, serum 8-OHdG levels in the low-and high-dose RJ groups were

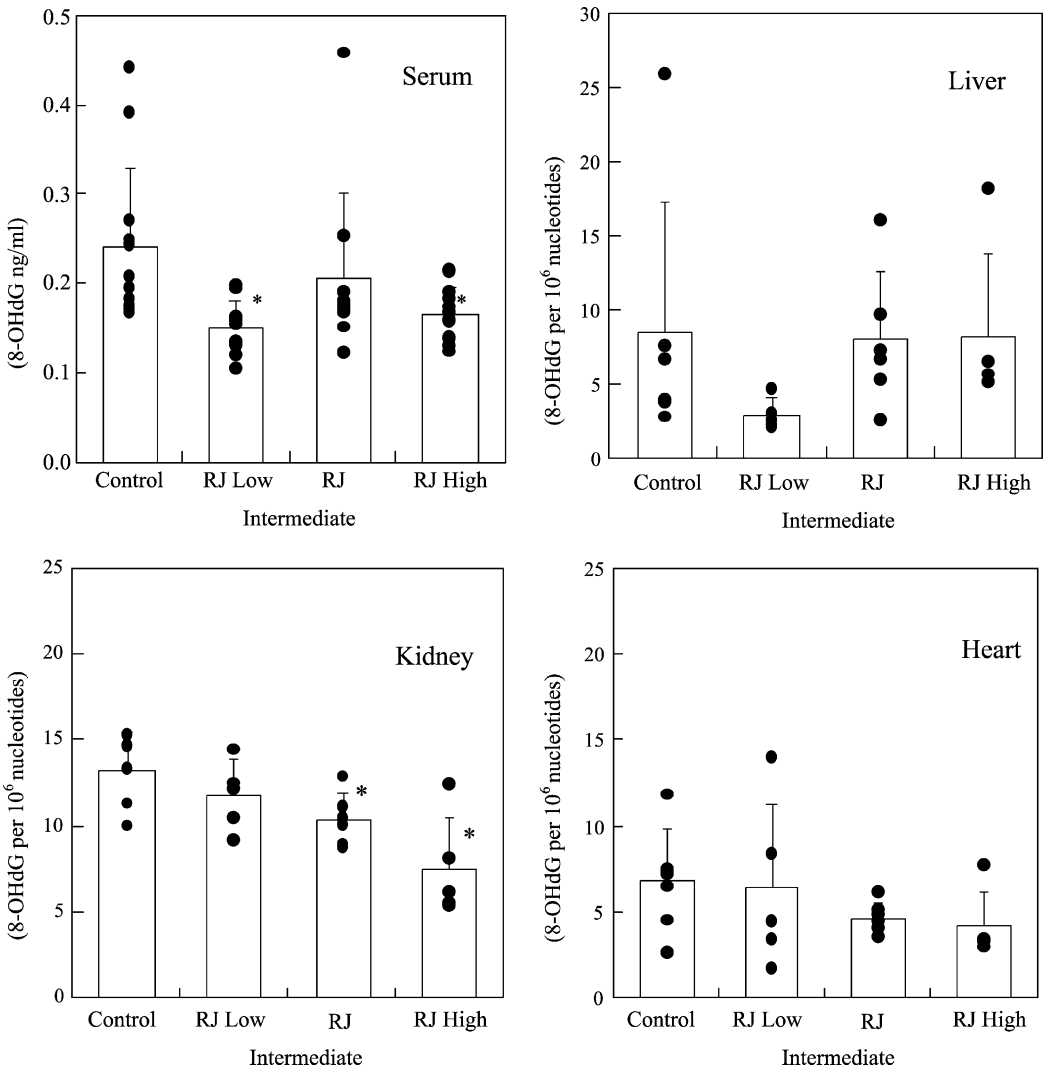

Fig. 1. Effects of Royal Jelly on DNA 8-OHdG levels in the serum and various organs. Data shown represents the means ± SD. Serum data, $n = 8–11$; organ DNA data, $n = 4–6$. Significantly different from control at $p < 0.05$.

significantly reduced compared with that of the control group ($p < 0.05$). DNA 8-OHdG levels in the liver and heart were similar among the different groups.

3.2. Effects of Royal Jelly on the survival rates and life span of mice

In this experiment, dietary RJ did not affect mouse growth, food intakes and appearances as compared with control groups (data not shown). The mortality data is represented as survival curves in Fig. 2. It was observed that the survival of the intermediate- and high-dose RJ groups were significantly longer than that of the control groups. The 50% mouse survivals of the intermediate- and high-dose RJ groups were reached at 112 and 110 weeks, respectively, vs. 89 weeks for the control group as shown in Table 1. In addition, the 75 and 25% mouse survivals for the intermediate- and high-dose groups were similar as shown in Table 1. These results suggested that RJ improved mouse

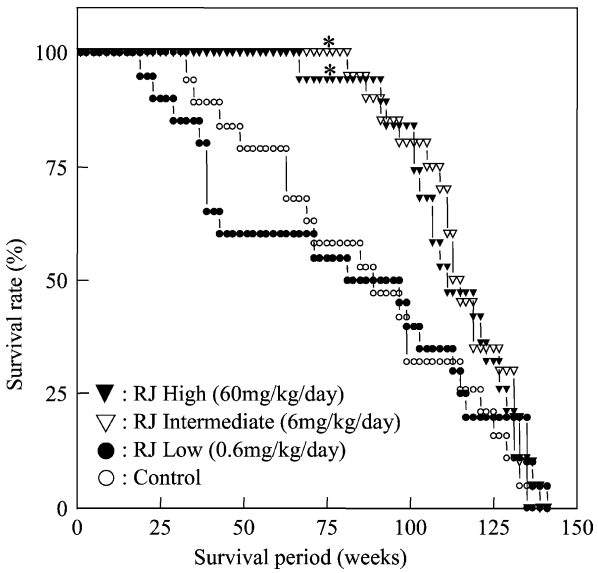

Fig. 2. Effect of Royal Jelly on survival rate in male C3H/He mice. Significantly different from control group at $p < 0.05$.

Table 1
75, 50 and 25% mouse survival periods

Mouse survival (%)	Group (No of weeks[a])			
	Control	Low	Intermediate	High
75	63	39	105	101
50	89	81	112	110
25	121	115	131	128

[a] Signifies the number of weeks elapsed from the start of the experiment before the indicated mouse survival was reached by the respective mouse groups.

survival by approximately 25% when compared with the control group, although no differences was observed between the control group and the RJ low-dose group. However, RJ was not able to extend the total life span of the mice as represented in Fig. 2.

4. Discussion

Aging or senescence is recognized as dysfunction of the body with the passage of time, and this dysfunction of various organs ultimately leads to death (Thomas and Steven, 2000). Some reports show that free radicals can induce oxidative damage to the body and that this damage might induce dysfunction of cells, organs and the whole body. Additionally, the activity of superoxide dismutase, that scavenges free radicals, has been correlated with life span (Tolmasoff et al., 1984). Mice which over-expressed enzymes that repair oxidative damage (Migllaccio et al., 1999), increased antioxidant intake (Massie et al., 1984; Heidrick et al., 1984; Blackett and Hall, 1981) and calorie restriction (Bartke et al., 2001; Yu, 1993) have been also reported to exhibition induce extended life spans. Accordingly, these are based on the control of free radical-induced damages to the body. Thiobarbituric acid reactants, phosphatidylcholine hydroperoxide and 8-OHdG are markers of oxidative damage in the tissues (Miyazawa et al., 1993; Nakae et al., 2000). Therefore, these markers may also be considered as markers for aging or senescence.

In this experiment, we have first examined the effect of dietary RJ on DNA 8-OHdG levels in the tissues of recipient mice. As shown in Fig. 1, RJ significantly reduced kidney DNA and serum 8-OHdG levels. It has been reported that the antioxidant effect of RJ was weaker than that of tocopherol in vitro (Kuwahara et al., 1996), indicating that RJ had very little potency at scavenging free radicals. Thus, we hypothesis that RJ does not show direct radical scavenger effects, nevertheless, there must be another mechanism for the suppression of DNA damaging oxidation. The mechanism for the protective effects against antioxidant action of RJ has not been clarified in detail, and the future experiments along these lines are to be planned.

It was reported that 8-OHdG accumulates in the tissues with aging (Kaneko et al., 1996). From the results of experiment 1, we speculated that RJ could retard senescence and improve survival due to a decrease in oxidation damage to the body. Therefore tried to examine the effects of dietary RJ on the survival of C3H/HeJ mice in experiment 2, since it has been reported that this strain frequently suffers from carcinogenesis of the liver or other organs with age. As shown in Fig. 2, RJ extended the 50% mice survival significantly. In other words, mice fed intermediate to high levels of RJ took a significantly longer time to reach 50% survival than the mice on low RJ diets or control mice. However, RJ could not extend the total life span of the mice. One possible mechanism for the improvement survival is the suppression of oxidative damage to the tissue such as the formation of 8-OHdG. We think that suppression of 8-OHdG by RJ might explain that RJ were alleviating a condition which was causing chronic inflammation in the organs such as kidney, possibly. Alternatively, RJ may contain some as yet unidentified substances with a direct on mouse survival. This will be one of the future subjects of investigation we will address in the new future.

In conclusion, RJ could improve the 50% survival rates of C3H/HeJ mice. This indicates that RJ allowed many more mice to reach their old age than those on control commercial chow and the substance warrants further studies on its multiple effects.

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