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RESEARCH ARTICLE

The effects of tetrahydrocurcumin and green tea polyphenol on the survival of male C57BL/6 mice

Kenichi Kitani · Toshihiko Osawa · Takako Yokozawa

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Abstract The effect of feeding of two different antioxidants, tetrahydrocurcumin (TC) and green tea polyphenols (PPs) on the survival of male C57BL/6 mice was examined. Mice that started to receive diets containing TC (0.2%) at the age of 13 months had significantly longer average life spans (days, mean ± SD) than control mice (797.6 ± 151.2 vs.882 ± 154.6, both $n = 50$, controls vs. TC treated, plus 11.7%, $P < 0.01$). The 10% longest survival was also significantly greater in TC-treated mice (plus 6.5%, $P < 0.01$). In contrast, in mice that started to receive TC in their 19th month of life, no significant difference from the control mice was found for either the average life span or the 10% longest survival. In mice that received water containing PPs (80 mg/l), the average life span was also significantly longer than in the control mice (801 ± 121.5 vs. 852.7 ± 88.2, plus 6.4%, $P < 0.05$), although the 10% longest survival was not significantly different from that in the control mice ($P > 0.05$). The body weights of the TC (but not PP) fed mice, were slightly (2–4%) but significantly ($P < 0.05$) lower than the values for the corresponding ages in the control mice in the first six months of treatment. Thereafter, the difference in average body weight between the control and the TC-fed animals was totally lost. Although an additional contribution of an unintended slight decrease in food intake due to TC feeding (suspected due to the difference in body weight) is not excluded, we suggest that the feeding of nutritional antioxidants such as TC and PPs may have the potential to beneficially modify the life spans of animals.

Keywords Aging · Green tea polyphenols · Life span · Male C57BL mice · Tetrahydrocurcumin

Abbreviations
PPs Polyphenols
TC Tetrahydrocurcumin

Introduction

Since the proposal of the free radical theory of aging by Harman half a century ago (Harman 1956), attempts to administer a number of antioxidant compounds to prolong the life spans of experimental animals have been performed (reviewed by Harman

K. Kitani (✉)
National Institute for Longevity Sciences, 36-3, Gengo, Moriokacho, Obu-shi, Aichi 474-8522, Japan
e-mail: kitani@nils.go.jp

T. Osawa
Graduate School of Bioagricultural Sciences, University of Nagoya, Furo-cho, Chigusa-ku, Nagoya 464-8600, Japan

T. Yokozawa
Institute of Natural Medicine, University of Toyama, 630 Sugitani, Toyama-shi 930-0194, Japan

1994). However, the results were not convincing enough for researchers to conclude that the feeding of antioxidants was a reproducible means to prolong the life spans of animals (Harman 1994). Lipman et al (1998) reported the effect of feeding five different preparations of antioxidants (vitamin E, glutathione and their combinations) on the life spans of male C57BL/6 mice. However, in none of the five groups fed with different antioxidant preparations could a positive effect on the survivals of the animals be observed (Lipman et al. 1998). Further, some antioxidants used in earlier studies turned out to be carcinogenic (Harman 1994). If we want to obtain some clinical implications from this type of study, it is desirable that the antioxidants to be used are the least toxic, and preferably, nutritional elements.

For this reason, we selected (a) curcumin, known to be a potent antioxidant and a component of turmeric in Indian curry (Osawa 2000, Osawa et al. 1995), and (b) green tea polyphenols (PPs). Turmeric, as well as green tea, has been used in oriental medicines for thousands of years for the treatment and prevention of many disorders (Kim et al. 1998; Crepsy and Williamson 2004; Ringman et al. 2005; Jagatia and Aggarwal 2007). Recently, purified curcumin and its more potent biometabolite, tetrahydrocurcumin (TC) and green tea (or PPs extracted from green tea) have been demonstrated to prevent experimentally induced pathologies such as cancers (Yoshizawa et al. 1987; Huang et al. 1994; Conney et al. 1997; Inano et al. 2000) renal injury (Okada et al. 2001; Nakagawa et al. 2004; Yokozawa et al. 2005) and arteriosclerosis (Yokozawa and Dong 1997; Osawa 2000; Yokozawa et al. 2002), and possibly Alzheimer's disease (Frautschy et al.2001; Lim et al. 2001; Ringman et al. 2005; Yang et al. 2005) etc. So far, however, no study has been reported concerning their effects on the life spans of animals, rodents in particular. We attempted to clarify whether a long-term feeding of TC or PPs can modify the survival of male mice.

Materials and methods

Male C57BL/6JHsd mice were obtained from Harlan-Spraque Dawley (Indianapolis, ID) 3 months prior to the study. Five animals each were placed in a cage in the clean conventional facility of the institute. Water and mouse pellets (MF, Oriental Ltd., protein 23.8%) sterilized by γ-ray radiation (30 kGy) were given ad lib. before the study. Daily light (6:00 to 18:00), room temperature (23°C ± 2°C) and humidity (50%) were controlled.

TC study

The TC was a generous donation of Nikken Fine Chemicals Co. Ltd., Aichi, Japan. The same batch of materials for MF pellets was divided into two. One was made into ordinary MF pellets and the other was mixed with TC (0.2%in concentration) then made into pellets. Both were sterilized by γ-ray radiation (30 kGy) and stored in a cold room in the animal facility of the institute.

Two series of studies were conducted: in the first, feeding with pellets containing TC pellets or standard pellets was initiated at the age of 19 months, in the second, this feeding was initiated at the age of 13 months

PPs study

The mixture of PPs used in this study was Sunphenon (Taiyo Kagaku Co., Yokkaichi, Japan), which was prepared from a hot-water extract of green tea with a recovery rate of 9.6% by weight of the original pulverized Japanese green tea, as reported previously (Sakanaka et al. 1989). It was composed mainly of (−)-epigallocatechin 3-*O*-gallate (18.0%), (−)-gallocatechin 3-*O*-gallate (11.6%), (−)-epicatechin 3-*O*-gallate (4.6%), (−)-epigallocatechin (15.0%), (+)-gallocatechin (14.8%), (−)-epicatechin (7.0%), and (+)-catechin (3.5%), (about 70% in weight). The preparation was dissolved in water (80 mg/l as PPs). The water containing PP was also sterilized by γ-ray radiation (30 kGy) and given to the experimental animals as drinking water. The study was begun when the animals were in their 13th month of age. Possible effects of γ-ray irradiation on TC and PPs will be discussed later.

All animals were maintained with no other intervention except for body weight measurements of once a month until they died. All values were expressed as the mean ± 1 SD Statistical analysis for survivals of animals as well as body weight at every corresponding month of age was made by means of one-way analysis of variance (ANOVA)

followed by a Student *t* test for unpaired values. The statistical analysis on survival rates of control and experimental groups at every month of age after treatments was made by Chi-square test. *P* values lower than 0.05 were judged to be significant. The study was evaluated from ethical points of view by the ethics committee for animal experiments inside the institute and was approved.

Results and Discussion

Figure 1 shows survival curves of TC feeding experiments 1 (Fig. 1a) and 2 (Fig.1b) which were begun at 19th (exp. 1) and 13th month (exp.2) respectively. In experiment 1, survival curves were not much different between the control and TC-fed animal groups, although the TC-fed group showed a slightly prolonged survival. In experiment 2, a clear tendency for the survival curve to shift towards the right could be observed in the TC-fed group. A significantly greater survival rate in TC fed mice than in control mice was observed at the age of 28th month ($P < 0.05$, Chi-square value 4.17). Figure 2 summarizes the sequential changes in average body weight measured every month in the TC studies. In both experiments 1 (Fi.2a) and 2 (Fig.2b), TC-fed animals showed slightly but significantly ($P < 0.05$, *t*-test) lower average body weights in the first 6-month period of TC feeding. However, the body

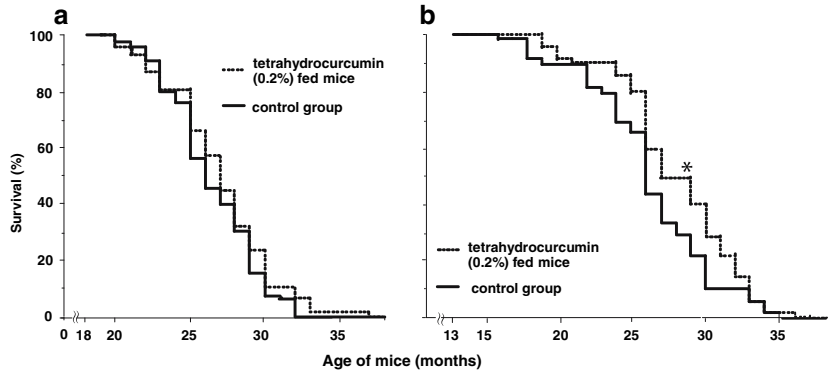

Fig. 1 Survival curves of control (solid line) and tetrahydrocurcumin (TC) (0.2%) fed (broken line) mouse groups. The left column (**a**): mouse groups which started to receive TC feeding at the age of the 19th month and the right column (**b**): those which started to receive TC feeding at the age of 13th month. Significantly different from the corresponding value in control mice (*$P < 0.05$, Chi-square test)

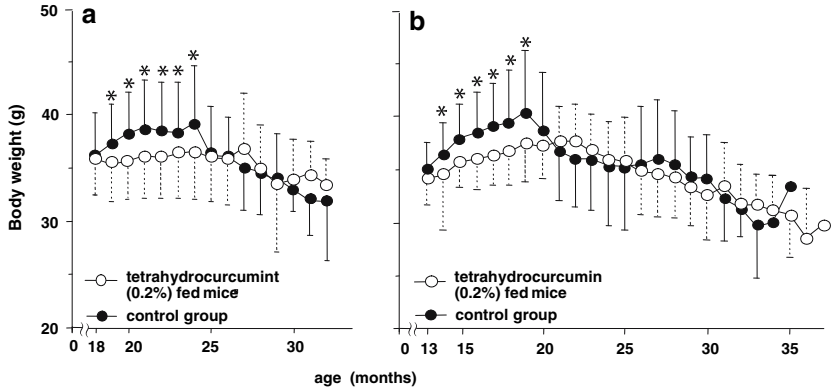

Fig. 2 Sequential changes in average body weights in control (closed circle with solid line) and TC fed (open circle with broken line) mouse groups. The left column (**a**): mouse groups which started to receive TC feeding at the age of the 19th month and the right column (**b**): mouse groups which started to receive TC feeding at the age of the 13th month. Significantly different from corresponding control values (*$P < 0.05$, *t*-test)

weight differences between the control and the TC-fed animals during these periods were very small (2–4%, maximally 4.7%) in both experiments. Thereafter, the average body weights became very close for control and TC-fed mice, with no significant differences between the two groups in either experiment 1 or 2.

Figure 3 shows survival curves (Fig. 3a) and sequential changes in average body weight (3b) of control and PP-fed mice. In PP-fed mice, the survival curve shifted toward the right in comparison with that of the control mice. At three successive age points (from 24th to 26th month), the differences in survival rate between control and PP fed mice were highly significant ($P < 0.01$, 0.01, 0.001, Chi-square values 6.53, 10.2, 25.5 for 24th, 25th, and 26th month respectively). Unlike in TC-fed mice, average body weights in PP-fed mice were not significantly different from the corresponding values in the control mice at most age points (Fig. 3b), showing almost identical values throughout the experimental period.

Table 1 summarizes survival days of the different mouse groups in two different experiments for TC. In TC experiment 1, both average life spans from day 0 and the 10% longest survival were slightly longer in the TC-fed mice than the corresponding values in the control mice group. However, the differences between the values in the control and TC-fed mice were not statistically significant ($P > 0.05$). In contrast, in experiment 2, both average life span and 10% survivals were significantly longer ($P < 0.01$) in the TC-fed mice than the corresponding values in the control mice. The average life span of PP-fed mice was significantly longer than that of the control mice ($P < 0.05$). However, the 10% longest survival was not significantly different between the control and the PP-fed animals ($P > 0.05$, Table 2).

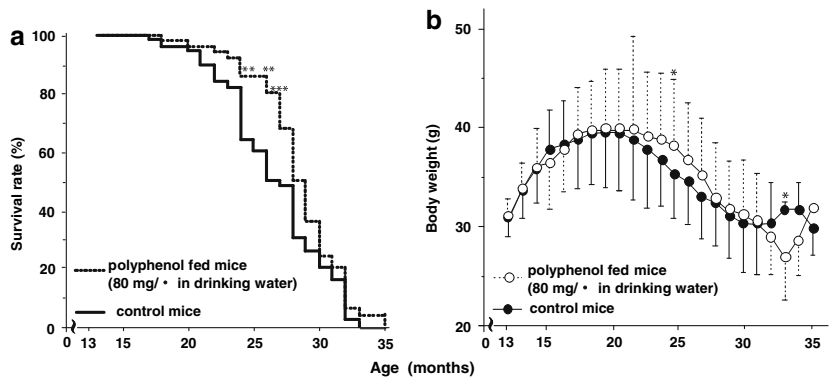

Fig. 3 Survival curves (**a**):(significantly different from corresponding control values, $^{**}P < 0.01$, $^{***}P < 0.001$, Chi-square test) and changes in average body weights (**b**):(significantly different from the respective control value, $^{*}P < 0.05$, *t*-test) in control and green tea polyphenols (PPs) fed mouse groups. A PPs feeding was begun at the age of the 13th month

Table 1 Mean survival times of control and tetrahydrocurcumin (TC) fed mice

Exp. 1			
(Feeding from 19th month)	Control mice	TC-fed mice	*P*
From day 0 (days)	806.1 ± 93.0 (46)[a]	828.7 ± 108.7 (47)	>0.05
10% longest survival (days)	963.0 ± 29.4 (5)	1,024.4 ± 67.3 (5)	>0.05
Exp. 2			
(Feeding from 13th month)	Control mice	TC-fed mice	*P*
From day 0 (days)	797.6 ± 151.2 (50)	882.7 ± 154.6 (50)	<0.01
10% longest survival (days)	1,040.8 ± 24.8 (5)	1,108.0 ± 33.5 (5)	<0.01

[a] The number in parentheses indicates the number of mice studied

The results of the present study have shown that TC feeding begun at the age of the 13th month significantly prolonged the average life span as well as the 10 % longest survival of mice. It could be argued, however, that the minor but significantly lower average body weights in the first 6 months of TC feeding compared with the corresponding values in control mice were the results of unintended dietary restrictions caused by the diet containing TC, which at least may have partially contributed to the prolongation of the life span of the animals. It could be probably true that the smaller food intake in both experiments 1 and 2 was caused by TC feeding. However, we have been unable to find any data in the past-published studies which have shown a significantly longer life span in dietary-restricted rodents with differences in body weight smaller than 5% in comparison with the control animals. Accordingly, we suggest that the significant prolongation of average life spans in TC fed mice in experiment 2 was at least partially caused by TC feeding, although an additional contribution of a very moderate dietary restriction may not be excluded. In the case of PP feeding, we observed a significant increase in the average life span but not in the 10% longest survival.

When Figs. 1b and 3a are compared, it appears that some differences exist in the effect of TC and PPs. TC at a 0.2% dose appeared to prolong the life span of animals rather continuously in the almost entire period of treatment up to the end of their lives resulting in a significant prolongation of even 10% longest survivals (Fig. 1b, Table 1), while PPs appeared to be most effective in the relatively early period of treatment resulting in highly significant differences in survival rate in the successive 3 months of life starting from 24th month (Fig. 3a) but with no significant prolongation in 10% longest survival. These differences may suggest that there might exist some difference(s) in the mode and/or site of action for these compounds. It would be interesting to examine the effect of the combined use of these compounds in the future.

The mechanism(s) for the prolonged survival of animals by TC or PP feeding, however, remains to be established. As discussed earlier both TC and PP feedings have been shown to prevent several disorders associated with aging in experimental animals. This preventative effect has been mostly attributed to their antioxidant properties. Further, for both TC and PPs, some biological properties other than antioxidants are also reported. In particular, anti-inflammatory (or immunomodulatory) properties are reported for both TC and PPs (Surh 2003; Yadav et al. 2005; Khan et al. 2006; Jagetia et al. 2007). Inflammation has been discussed as a partial mechanism of aging and age-associated disorders (Chung et al. 2001). Thus, these compounds may have affected the aging process itself by means of antioxidant and/or anti-inflammatory effects of these compounds. It could also well be that the apparent increase in the average life spans was caused by the prevention of some of age-associated disorders. In C57BL/6 mice, the disorder of the highest incidence in their later life is lymphoma. Accordingly lymphoma and cancers of different kinds may have been partially prevented by the immunomodulatory (and/or antioxidant) effects of these compounds. Further, since nephropathy is a common disorder in aging rodents in particular, it is possible that these antioxidants prevented and/or retarded the development of kidney disorders in aging mice. These possibilities need to be studied in the future.

Not only for these disorders which specifically occur in aging rodents, our results may also also agree with past experimental studies suggesting the possibility that these compounds are effective in preventing and/or retarding the progression of a number of disorders which occur exclusively in the elderly humans by means of antioxidant and/or anti-inflammatory (or immunomodulatory) effects. These

Table 2 Mean survival times of control and polyphenol (PP)-fed mice

	Control mice	PP fed mice	*P*
From day 0 (days)	801.1 ± 121.5 (50)[a]	52.7 ± 88.2 (50)	<0.05
10% longest survival (days)	976.4 ± 17.8 (5)[a]	989.1 ± 43.9 (5)	>0.05

PPs feeding (80 mg/l in drinking water) was begun at the age of the 13th month

[a] The number in parentheses indicates the number of mice studied

include Alzheimer's disease (Frautschy et al. 2001; Lim et al. 2001; Ringman et al. 2005; Yang et al. 2005) and disorders caused by arteriosclerosis (Zheng et al. 1996, Yokozawa and Dong 1997; Osawa 2000; Artas et al. 2001; Yokozawa et al. 2002) which are not observed in normal aging rodents.

Finally, either TC or PPs is not affected appreciably by a direct irradiation of the dose used (30 kGy) primarily because of a low molecular weight (TC) and a low concentration of PPs. However, we have had a suggestion by radiation chemists that PPs are likely to be indirectly affected by irradiated water in appreciable amounts. Most likely products by this effect are thought to be hydroxylated compounds of different kinds. It is possible that these presumably hydroxylated catechins still hold bioactivities as original molecules do. The observed effect of PPs containing water, therefore, may be interpreted to be due to the remaining PPs which are still sufficient to be effective and/or the bioactivity of hydroxylated catechins. In all possibilities, it would be important to pursue in the future the reproducibility of the results presented in this study as well as the underlying mechanism(s) of the present observation. Even if the basic mechanism(s) is not fully elucidated, the confirmation of the present study and clinical trials as currently being done for curcumin (Ringman et al) in the future may provide a rational approach for the nutritional interventions in aging and age-associated disorders in humans.

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