THE ROLE OF COENZYME Q-10 IN AGING: A FOLLOW-UP STUDY ON LIFE-LONG ORAL SUPPLEMENTATION Q-10 IN RATS

K. Lönnrot [a], T. Metsä-Ketelä [b] and H. Alho [a]*

[a] Laboratory of Neurobiology, [b] Department of Pharmacology, University of Tampere, School of Medicine, POB 607, 33101 Tampere, Finland.

Key Words

Ubiquinone, coenzyme Q-10, supplementation, life-span, aging

Abstract

The essential role of coenzyme Q - ubiquinone - in biological energy transduction is well established. Reduced Q - ubiquinol- has also been shown to act as an antioxidant and to decrease the action of free radicals, which in turn could cause damage to structural lipids or proteins. The accumulation of lipopigments during aging in several peripheral organs and in the nervous system is considered to be related to the peroxidation of unsaturated fatty acids. An age-related decline of Q-10 has been suggested to occur in man and rats. In this study we followed the effects of life-long oral supplementation of coenzyme Q-10 on the development and life-span and pigment accumulation in peripheral tissues and the nervous system of laboratory rats. The Q-10 supplemented group showed a significant increase in Q-10 in plasma and liver, while it was unchanged in other tissues. There was no significant difference between the two groups in the development and mortality of the animals. No differences were observed in lipopigment accumulation. Our results indicate that in rats, life-long supplementation of Q-10 has no beneficial effects on life-span or pigment accumulation.

Introduction

Various hypotheses have been put forward in the attempt to understand the phenomena of aging and the accumulation of age pigment (see 1). Age-related

*Address for correspondence: H. Alho, Laboratory of Neurobiology, School of Medicine, University of Tampere, P.O. Box 607, 33101 Tampere, Finland

accumulation of pigment in the nervous system is considered to be a morphological marker for neuronal lipid peroxidation (1-4). Free radicals, the initiators of lipid peroxidation, are formed in all living cells, and are assumed to cause degenerative changes with aging (5,6). In addition to endogenous scavenger enzymes, tissues are defended against lipid peroxidation by exogenous antioxidants. Vitamin E deficiency is known to increase the accumulation of age pigments in some regions of the central nervous system, and to shorten the life span of some animals (7,8,9). Supplementation of certain other antioxidants such as (-)deprenyl (selegiline) has been shown to increase the life span of rodents (10,11).

Coenzyme Q-10 or ubiquinone in mammals is in origin both endo- and exogenous. Its function is fundamental for proton-based energy coupling. Failure of proper function, resulting in reduced energy supply, may be related to insufficient modification of lipid fluidity or lipid protein interactions and damage in receptors (12). The reduced form of coenzyme Q (ubiquinol) has also been shown to act as an antioxidant and to reduce the action of free radicals (12,13), which in turn could cause damage to structural lipids or proteins. An age-related decline of Q-10 has been suggested to occur in man and rats (14,15). One report indicates that administration of Q-10 increases the life span of mice (16). However, very little is known about the role of Q-10 in aging. Therefore we have studied the effects of life-long fair oral supplementation of Q-10 on the development and aging of Sprague-Dawley laboratory rats.

Material and methods

The experimental animals were supplemented with Q-10 from the early fetal life for the whole life-span. 16 pregnant female Sprague-Dawley rats were randomly divided into two groups and one group was fed with ubiquinone Q-10 added feedstuff (verum group) and the other with normal food (control group). From the born litters 75 male animals were included in each group (total 150).

Q-10 was mixed with soybean oil into normal animal foodstuff. All animal food required for this study was kindly provided by PharmaNord (Vejle, Denmark). According to the body weight of the animals, they were fed with foodstuff containing Q-10 either 50 mg/kg (animals up to100g bw), 125 mg/kg

(up to 150 g bw) or 250 mg/kg (more than 150 g bw). The control feedstuff contained 10± 0.5 mg/kg of Q-10. The concentration of Q-10 in feed was adjusted so that the daily Q-10 intake was 10 mg/kg bw/day in the experimental group (verum) and 0.25-0.5 mg/kg bw/ day in the control group. To control the daily energy intake so that it was at the same level in both groups, all animals were put on a restricted diet: for animals weighing less than 150 g the available food amount was 20 g / day and for animals weighing more than 150 g 25 g/day/ (approximately 15 % less than average *ad libitum* consumption).

Every six months 5-6 animals were sacrificed in both groups. The animals were anesthetized with chloralhydrate (250 mg/kg i.p.) and a blood sample was drained from the carotid artery. Several tissues were immediately collected either for histological analyses (immersion fixed with 4% paraformaldehyde solution for 2-6h at RT) or biochemical measurements (immediate freezing with liquid nitrogen). Some animals were perfused transcardially under deep anesthesia with physiological saline and with 4% paraformaldehyde solution. The histological analyses included normal pathological examination of the tissues, fluorescence histochemistry of adrenal gland and superior cervical ganglia for lipopigment estimation and electron microscopy of some tissues. The biochemical measurements included the measurement of plasma and tissue Q-10 and Q-9 concentrations, plasma vitamin E concentration and the measurement of total antioxidant capacity (TRAP) of plasma.

Ubiquinol and tocopherol were measured by the high performance liquid chromatography (HPLC) method as described by Lang et al. (24). TRAP was determined by the chemiluminescence method, which is described in full detail elsewhere (23,25). Briefly, thermal decomposition of ABAP produces peroxyl radicals at a constant rate. Peroxyl radical reactions are followed by luminol-enhanced chemiluminescence. The extinction time in chemiluminescence caused by an added sample of plasma or CSF was compared to that of a water soluble tocopherol *Trolox C*, which has a known capacity to quench two moles of peroxyl radicals per one mole of *Trolox C*. The time for which the added test sample extinguishes the reaction is directly proportional to the peroxyl radical-trapping antioxidant capacity of the sample, i.e., TRAP, which is expressed as micromoles of peroxyl radicals trapped by one litre of the sample. Lipopigment accumulation was determined by quantitative fluorescence microscopy as described in detail

elsewhere (4). Briefly, the fixed tissues were embedded in paraffin, sectioned serially and examined under a Nikon Mikrophot FXA fluorescence microscope. Quantitation of pigment autofluorescence was performed with an image analyzer (DPS-200 MTI image processor with Microscale software) as described previously (4). Autofluorescence intensity was measured at random from 80 sympathetic neuronal pericarya or cells of adrenal cortex at four different levels. The data are expressed as mean arbitrary units.

Differences between means were measured with Student's paired t-test. P-values < 0.05 were regarded as significant. The normality distributions was checked by comparing the means and the medians of the histograms. Linear regression analysis was used to show correlations between variables.

Results

The verum group showed a significant increase of Q-10 in plasma and liver, while all other tissues measured so far remained unchanged. The Q-9 levels were unchanged in all tissues (the Q-9 and Q-10levels will be published elsewhere).

Supplementation of Q-10 during ontogenesis had no teratogenic effects, the size of the litters was identical in both groups (10±4) and the puppies in both groups were healthy. The development of body weight and the growth of the animals throughout their life span was similar in both groups (Fig. 1.).

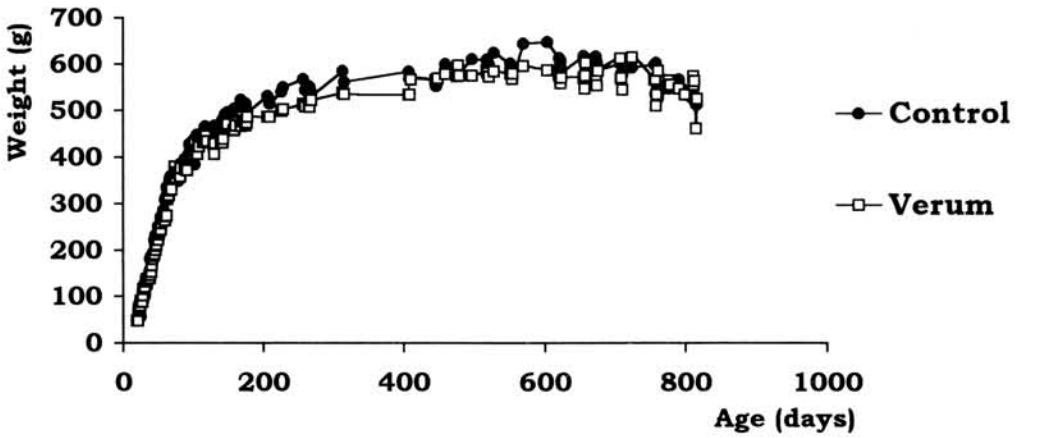

***Fig.1**. The monthly measured gain in body weight. Each point represents the mean of bw of all animals in each group, verum = Q-10 supplemented.*

The average life span was longer in the control group but the difference was not significant: in the control group it was 794±24, in the verum group 727.9±27.3 days in verum group (p = 0.076). The percentage cumulative mortality was also higher in the verum group (Fig.2.).

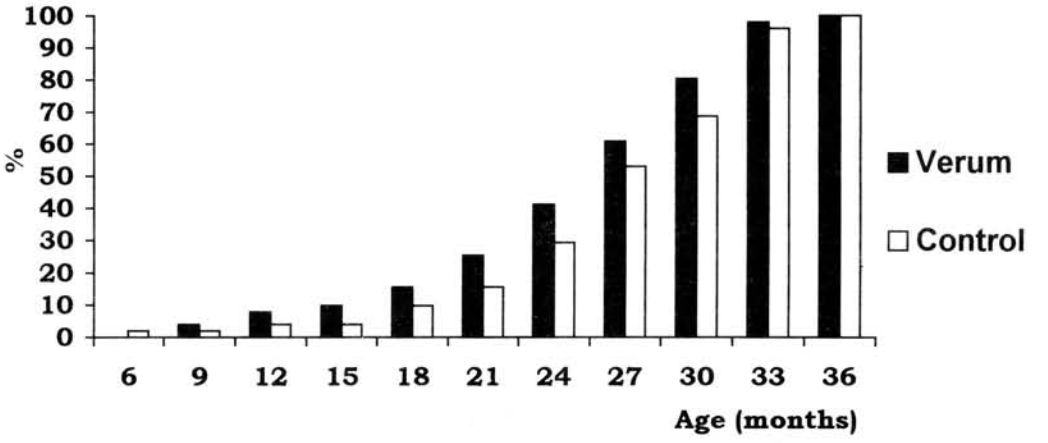

Fig. 2. *The percentage cumulative mortality at the end of the experiment*

Total antioxidant capacity (TRAP) was measured from the plasma of six month old animals and did not differ between the groups (TRAP: verum 356±18 and control 347 ± 11 μmol/l, n=6). Vitamin E level in plasma was also similar in both groups (012 ± 0.05 μmol/l, n= 6).

Lipopigment accumulation was examined at the age of 12 and 18 months in adrenal cortex (AC) and superior cervical ganglia (SCG) by calculating the number of pigment grains and by measuring the relative autofluorescence intensity by an image analyzer. A normal age-related accumulation of lipopigment in the SCG (Fig. 3.) and of pigment in the cortex of adrenal gland was observed in

both groups (Fig.4). Q-10 supplementation had no effect on the number or on the intensity of the pigment grains (Figs. 3-4).

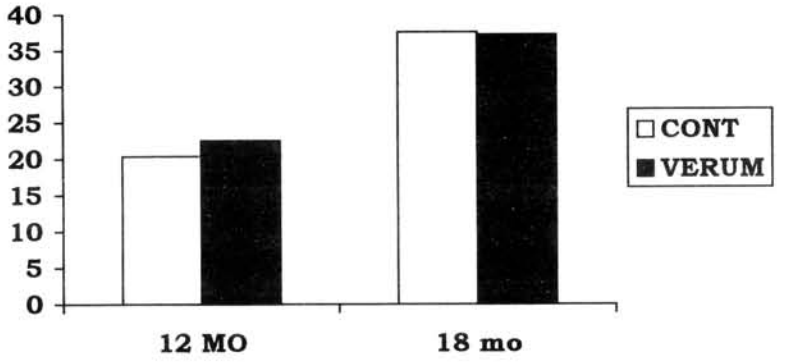

Fig.3. *The relative intensity (arbitrary units) of lipopigment autofluorescence in superior cervical ganglia at the age of 12 and 18 months, n=8 in each group.*

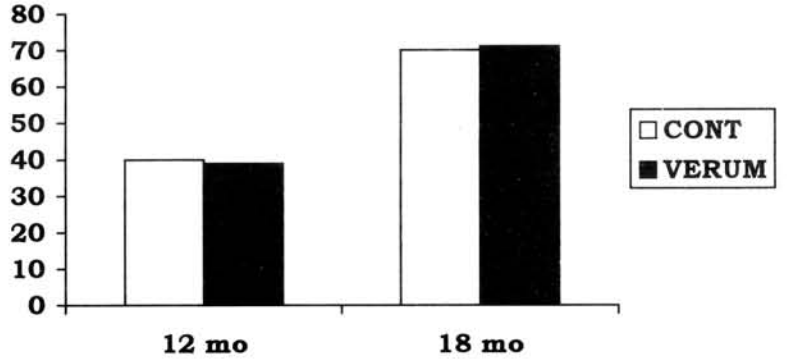

Fig.4. *The relative intensity (arbitrary units) of pigment autofluorescence in adrenal cortex at the age of 12 and 18 months, n=8 in each group.*

Discussion

Our results indicate that in the animal model we used, life-long supplementation of Q-10 had no effects on the longevity or mortality of the animals. This finding is at variance with the observation by Bliznakov (16) who reported that Q-10 supplementation resulted in a significant reduction of overall age-related mortality as compared with untreated mice of the same age. Furthermore, the mean life-span of the Q-10 treated group was significantly increased. The study design in Bliznakov's experiments differs from ours

considerably: the animals were old female mice and the dosage and administration was different. In Bliznakov's study an i.p. injection of 50 μg Q-10 /mouse was administered weekly for 81 weeks, commencing at age 10-18 months. The oral dosage in our study was 10 mg/kg bw, approximately 5 mg/ adult rat, for the whole life span. In our study good absorption of the orally administered Q-10 was confirmed by a highly significant increase of plasma and liver Q-10 during the whole life span (the concentration of Q-10 in plasma was three times higher than in the control). Bliznakov's study contains no reports on plasma or tissue levels of Q-10. The dosage in our study was considerably higher than in Bliznakov's study, but there were no signs of toxicity, teratogeneity abnormal growth or development. Also compared to the dosage used in humans (up to 400 mg/day), our dosage was not very high. One notable difference between these studies was the control of daily food consumption. In Bliznakov's study there were no restrictions on the daily intake of food, while our animals were placed on a restricted diet (restriction approximately 15 %). It has been shown in several studies that substantial restriction (daily restriction up to 40-50% of *ad libitum* calories or feeding every other day) is needed to increase the life span of rodents (17,18). It is therefore unlikely that the 15 % decrease in food in our study should have affected the longevity of the rats. This is also indicated by the fact that our control animal population lived up to the same age as rats generally in our animal house. While in Bliznakov's study the diet was not controlled, it is possible that the Q-10 supplemented group for some reason received less calories or had some other dietary changes that influenced the longevity of the animals. Other nutritional deficiencies could come onto play in these studies. In our study the animals received normal rat food, and among the nutritients effecting life span, vitamin E was controlled and was at the same level in both groups.

Although ubiquinone is used as a food additive and in human medicine (see 19), very little is known about the effects of life-long ubiquinone supplementation. In fact this is the first controlled study on life-long supplementation of Q-10 in mammals. There are some long-term human studies which indicate that no adverse effects or drug interactions, and Q-10 is described as non-toxic in the literature.(19,20). Our study confirms these observations, although the life span of the Q-10 supplemented animals was shorter (difference

not significant). The histopathological analyses and tumor incidences of the animals in our study will be published elsewhere in more detail.

Free radical induced lipid peroxidation has been suggested as the basic pathogenic mechanism behind the accumulation of lipofuscin in postmitotic cells (5,6). Pigment accumulation is thought to reflect the free radical induced irreversible damage to lipids and other macromolecules that takes places during aging and under oxidative stress (1-7). Both *in vitro* and *in vivo* studies have indicated that deficiency of dietary antioxidants and elevated tissue oxygen concentrations accelerate lipofuscin or lipofuscin like pigment material (ceroid) formation. Antioxidant supplementation (e.g. vitamin E, reduced glutathione) have been shown to retard the rate of pigment accumulation (3,7,8). Impaired poteolytic enzyme activities also induce the accumulation of lipofuscin-like material, as shown by thiol proteinase inhibitor treatments (21). A linear increase with age in the amount of lipofuscin in the peripheral sympathetic nervous system and especially in superior cervical ganglia has been reported (4), and autonomic neurons are considered a comparatively simple model for research on neuronal aging (22).

The reduced form of ubiquinone -ubiquinol - has antioxidant activity (12.13). Some reports indicate that in brain tissues there is a decline in Q-10 with age (14,15). Nervous tissue and adrenal glands contain relatively high concentrations, and thus provide an interesting model for studying the aging of the nervous system and the accumulation of pigments. However, our study shows that ubiquinone supplementation has no effects on pigment accumulation in adrenal gland or on lipofuscin accumulation in sympathetic ganglia.. This may indicate that exogenous ubiquinone does not pass into the nervous system, an assumption supported by our previous results in humans (23).

Based on the material analysed so far in this study, it can be concluded that life-long supplementation has no beneficial or harmful effects on the development or longevity of the laboratory rat. The extensive material collected will be further analysed and it may provide additional interesting data.

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Discussion after the talk of Dr. Alho

Brunk: Since Q-10 is generally not taken up by cells from the blood, could the enhanced liver values be due to plasma contamination?

Alho: In agreement with our studies there are several studies demonstrating that an oral supplementation of Q-10 increases plazma and liver Q-10 content, but not in other tissues. I do not know what is the mechanism behind the increased liver concentration, maybe liver cells can uptake Q-10 from plasma or it can be plasma contamination as you suggested.

Brunk: Since Q-10 is absorbed into the blood it might affect LDL oxidation. Did you find any difference between the granules with regard to degree of atherosclorosis?

Alho: As you know rat is not a good model for studing atherosclerosis because they have LDL content in plasma. However, we have collected from these animals also large arteries-like aorta, but we have not yet analyzed them.

Ivy: How does the dose in your rat study compare with the dose in the previous mouse study?

Alho: Our dose is relatively larger than that used in the previous mouse study, when the Q-10 dosage was 50 ug/animal. We can estimate that corresponds to approximately 0.5 mg/kg b.w. Our dosage was 10 mg/kg b.w.

Ivy: Do you think your higher dosage might explain your negative results?

Alho: No, I don't think so. Compared to the generally used human dosage, 100-400 mg/adult/day, our dose is high but not too high. No toxic effects of Q-10 have been shown so far.

Ivy: What was the sex of the mice in the previous study?

Alho: Females. This could have some impact on the results.

Ivy: Do you think that Q-9 administration may have yielded different results?

Alho: I can not say, very speculatively I should say no.

Zs.-Nagy: I cannot agree with the claim that Q-10 is a radical scavenger. It has been shown that most of the O_2^- (superoxide) radicals in situ derive from the redox-oscillation of ubiqinone in complex I of mitochondria. Your negative results derive from the fact that Q-10 is hardly

reaching the brain at all. If it is able to reach the brain cells, it will become toxic, as it is in the liver, at least above a certain level.

Alho: I cannot agree with you. Many in vivo and in vitro studies have shown that Q-10 can act as a radical scavenger. However, this study was launched more than three years ago, and now we have much more knowledge on the biogenesis of Q-10. Our data with humans supports this assumption that Q-10 from plasma does not enter the nervous system.