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THE EFFECTS OF LIFELONG UBIQUINONE Q10 SUPPLEMENTATION ON THE Q9 AND Q10 TISSUE CONCENTRATIONS AND LIFE SPAN OF MALE RATS AND MICE

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SUMMARY
The effect of lifelong oral supplementation with ubiquinone Q_{10} (10 mg/kg/day) was examined in Sprague-Dawley rats and C57/B17 mice. There were no significant differences in survival or life-span found in either rats or mice. Histopathologic examination of different rat tissues showed no differences between the groups. In Q_{10} supplemented rats, plasma and liver Q_{10} levels were 2.6 to 8.4 times higher at all age points than in control rats. Interestingly, in supplemented rats the Q_9 levels also were significantly higher ($p<0.05$) in plasma and liver at ages 18 and 24 months. Neither Q_9 nor Q_{10} levels were affected by supplementation in kidney, heart, or brain tissues. In spite of the significant changes in plasma and liver ubiquinone concentrations, lifelong Q_{10} supplementation did not prolong or shorten the lifespan of either rats or mice.

Key words: ubiquinone, aging, tissue concentration, rat, mice

INTRODUCTION

Ubiquinone, also known as coenzyme Q, is a versatile molecule with a number of distinct but related functions. It has numerous functions in energy metabolism which are well documented: it regulates succinate dehydrogenase (1), NADH dehydrogenase (2,3), and cytochrome b-c1 complex activities (4,5), and it also has an obligatory role in energy conservation in the protonmotive Q

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cycle (6). Among naturally occurring ubiquinone homologues, the number of isoprenoid units in the sidechain may vary from 1 to 12 (7). The most common ubiquinone homologue in rats is Q_9; in humans, Q_{10} (8).

The first observations of the antioxidative properties of ubiquinone were made by Lea and Kvietny (9) and Mellors and Tappel (10). They showed that quinones and quinols protect fatty acid emulsions or isolated mitochondria against lipid peroxidation induced by photo-oxidation. Since then it has been shown in numerous studies that ubiquinone, in its reduced form ubiquinol, has antioxidant properties by inhibiting lipid peroxidation both in model system and in biological membranes in vitro and in vivo (for review, see (11)).

During the last four decades there has been much debate on the role of free radicals in aging. According to the theory of free radicals in aging, first introduced by Harman (12) and Gerchman et. al.(13), all biological systems involve oxidative stress originating as a result of an imbalance between the generation of oxidizing species and cellular antioxidant defense. This can cause damage by peroxidation to all cellular macromolecules, including proteins (14), DNA (15) and lipids (16), thus leading to the cellular degeneration and damage related to aging. This theory has led to the suggestion that antioxidants such as ubiquinol may play a role in the prevention of the aging process.

Energy metabolism declines during aging in rats (for reviews, see (17)). It has been proposed that the tissue content of ubiquinone also decreases during aging (18,19), which may in part be responsible for the decline of energy metabolism. In the mitochondrial fraction, however, no such decrease was observed (20).

There are very few previous studies on long-term ubiquinone supplementation, and there are no reports about its effects on development and survival. The longevity of mice was highly increased after intraperitoneal ubiquinone injections in an uncontrolled study by Blitznakov (21). This finding has not, however, been confirmed. Therefore, we decided to study the effect of oral supplementation of ubiquinone Q_{10} on the survival of both rats and mice. We followed both populations from birth to death and monitored their growth (22)

and survival. In order to ensure that our observations were relevant and due to ubiquinone, we also measured ubiquinone Q_9 and Q_{10} concentrations in plasma and different tissues.

MATERIALS AND METHODS

Food

Our experiments consisted of two studies, i.e. a survival study with rats and mice, and a concentration study with rats. In both of these studies there was an experiment group fed with Q_{10} supplemented food and a control group. Q_{10} was used because most earlier studies have been performed with it. It was mixed into normal animal diet by using soybean oil as a vehicle. Soybean oil was also added into the control food. All the food was kindly provided by Pharma Nord (Vejle, Denmark). The feeding was adjusted so that the daily intake of Q_{10} was 10 mg/kg/day in the experimental group and less than 0.5 mg/kg/day in the control group. For rats weighing less than 150 g, the quantity of food made available was 20 g/rat/day; for rats weighing over 150 g, the amount was 25 g/rat/day. For the mice, the amount was 5g/mouse/day.

Animals

The mice used were male c57/B17 strain. At the age of two months, a total of 86 mice were randomly divided into two groups: receiving food with Q_{10} or receiving control food. The rats were of the Sprague-Dawley strain. We randomly divided 16 pregnant female Sprague-Dawley rats into two groups, receiving Q_{10} food and control food. We included a total of 150 male newborn rat pups (75 from both groups) in our studies.

Survival study

In order to identify differences in survival and longevity, we followed all 150 rats and 86 mice throughout their life-span. Animals were regularly weighed and inspected to follow their growth and general well-being. An autopsy was performed on all rats that died naturally, whenever possible within 24 hours of death. A total of 31 treated rats and 29 control rats were autopsied. An autopsy included a macroscopic evaluation of skin and internal tumors and pathology. Samples were also taken from heart, liver, kidney, lung, hypophysis, adrenals, and tumors for later microscopic examination. This included a normal pathological examination of tissues as carried out by a pathologist.

Concentration study

In order to examine the changes in ubiquinone plasma and tissue concentrations during aging and the effect of supplementation on these changes, we killed 4 to 10 rats from both groups by decapitation at the ages of 6, 12, 18, and 24 months. The rats were anesthetized with chloralhydrate (250 mg/kg i.p.). A blood sample was drained from subclavian artery into a heparin-containing Eppendorf tube. The tubes were kept on ice and the plasma was separated within 30 minutes. The samples were stored at -70 °C for later analyses. Several tissues were immediately collected for biochemical measurements. Tissues were frozen immediately with liquid nitrogen and stored at -70°C for later analyses.

Ubiquinone measurements

Biochemical measurements included determination of plasma and tissue total Q_9 and Q_{10} concentrations from the samples obtained from the concentration study. To remove excess blood from the heart samples, they were rinsed in physiological saline before freezing. Heart, liver, kidney, and brain tissues were dry homogenized with a microdismembrator (Mikro-Dismembrator, B. Braun Melsungen) before the measurements. An accurate amount of tissue or plasma (200 µl) was dissolved in 300 µl of 1:2 ethanol-water solution. We added 100 µl of β-carotene solution (0.5 g/l for tissue samples and 0.025 g/l for plasma samples, dissolved in ethanol) as an internal standard. The tissue samples were stirred with an ultrasonicator. Ubiquinone was reduced to ubiquinol with 10 mg of sodium dithionite to allow measurement of total ubiquinone content. The ubiquinol was extracted into 500 µl of hexane for tissue samples; 700 µl of 19:1 hexane-isopropanol solution was used for plasma samples. The hexane layer was dried under nitrogen and resuspended in 200 µl of chloroform-methanol (1:1). Electro-chemical detection (Antec EC-controller, potential 0.5 V) of total ubiquinol was performed by high performance liquid chromatography (HPLC, pump LKB 2150) according to a method described by Lang et al. (8). Each day samples containing a known amount of Q_9 and Q_{10} were analyzed and used as standards.

Statistics

In survival analysis the values are given as means ± SE. The differences between the means were tested according to the generalized Wilcoxon (Breslow) model. In concentration analysis all the values are means ± SD. The differences between means were measured with a 2-way ANOVA (Solo, BMDP Statistical Software, LA, CA). Values for $p < 0.05$ were regarded as significant.

RESULTS

Growth And Survival

Supplementation of Q_{10} during ontogenesis showed no teratogenic effects in rats. The number of the litters was identical in both the control and treatment group 10 (4) and followed the normal range of variation within our animal laboratory. The pups were healthy in both groups. There were no differences in weight gain or growth, Total (peroxyl) Radical-trapping Antioxidant Parameter (TRAP) and vitamin-E values, and lipopigment accumulation between the experimental and control groups as previously reported (22). For rats, the average survival in the control group was 26.5 (0.83) months and in the experimental group 24.3 (0.91) months. For mice the average survival times in the control and experimental groups were 28.1 (0.70) and 29.0 (0.74) months,

respectively. Survival analysis showed that in rats there was a slight tendency towards longer survival in the control animals (p=0.0727). In mice there were no statistical difference in survival between the control and experimental group (p=0.24) (see figure 1).

Tissue Histology

Alveolar histocytosis was observed in lungs in 31 % of the control group rats but in only 9.7 % of the experimental group rats. However, this difference was not statistically significant (incidence proportion ratio, IPR 3.21, 95 % confidence interval (CI) 0.96 to 10.7). In microscopic analysis these areas were found to contain cell debris and lipoid deposits within macrophages, but no inflammatory changes were seen.

In macroscopic examination we observed that 39 % of the experimental rats had renal stones compared to only 21 % in the control rats. The difference was not statistically significant (IPR 1.8, CI from 0.78 to 4.17). Microscopic analysis revealed no difference between the two groups in renal cystic changes (IPR 1.16, CI from 0.50 to 2.70) or interstitial nephritis (IPR 1.03, CI from 0.43 to 2.48). There were no differences between the experimental and control groups in the other tissues examined.

The Effect of Q_{10} Supplementation on Plasma And Tissue Q_9 and Q_{10} Concentrations

The Q_{10} concentrations were 2.6 to 8.4 times higher in the plasma (p value ranging from 0.0001 at 6 month to 0.0269 at 18 month) and 3.2 to 6.6 times higher in the liver (p value ranging from 0.0002 at 6 month to 0.0619 at 18 month) at all ages in the Q_{10} supplemented group than in control group (see figure 2). At 18 months in liver the Q_{10} concentration was not statistically different (p=0.0619) because of a wide standard deviation and a small sample number. Interestingly, the plasma and liver Q_9 concentrations were also higher in the Q_{10} supplemented group at ages 18 and 24 months than in control group. In plasma, Q_9 concentration was 1.9 times higher at 24 months in the treated group than in the control group (p=0.0013). In liver, the Q_9 concentration was in the treated group 1.7 times higher at 18 months (p=0.036)

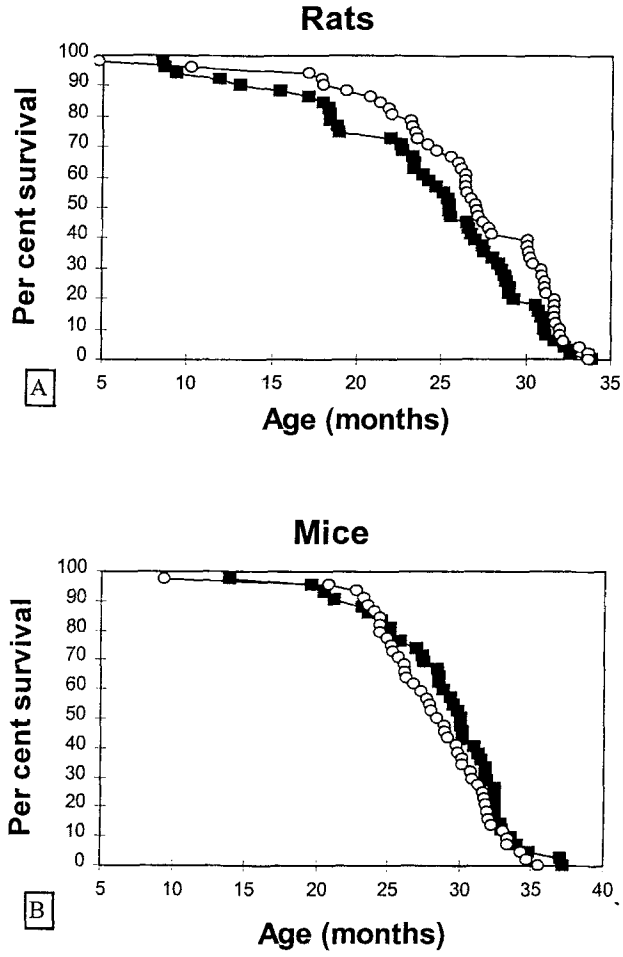

Figure 1. Percentage survival of Q_{10} supplemented and control rats (A) and mice (B). Mean for experimental rats: 24.3 SD ±0.91 months; and for control rats 26.5 ±0.83 months; p=0.073.Mean for experimental mice: 28.1 ±0.70; and control mice 29.0 ±0.74 months; p= 0.24

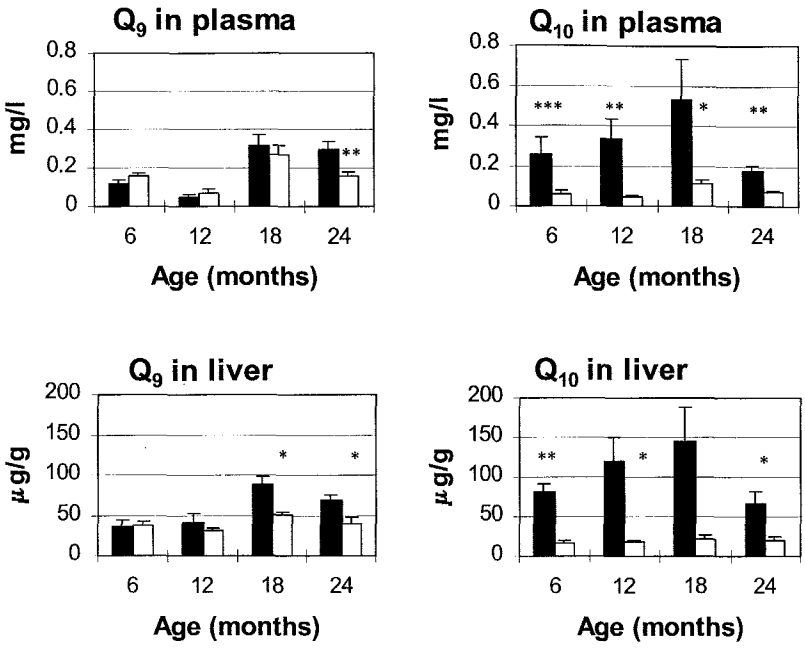

Figure 2. Total ubiquinone Q_9 and Q_{10} concentrations in rat plasma and liver at 6, 12, 18, and 24 months. In plasma, n = 10, 4, 4 and 4 animals per group, respectively. In liver, n = 4 animals per group. control, ▩ Q_{10} treated. Values are µg/g wt weight ± SD in liver and mg/l ± SD in plasma. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, compared to adjacent control group.

and 1.8 times higher at 24 months (p=0.037) than in the control group. In kidney, heart, and brain tissues, there were no differences between the control and the treatment groups in either Q_9 or Q_{10} concentrations, except that at 18 months the Q_{10} concentration in kidney was higher in the experimental group than in the control group (see table 1).

The Effect of Aging on Plasma And Tissue Q_9 and Q_{10} Concentrations

In both the experiment and the control group, the plasma Q_{10} concentration increased with age up to 18 months and then decreased (see fig 2.). The increase from 12 to 18 months was significant in the control group ($p = 0.005$). The decrease after 18 month was significant in both the experimental and the control group: the p-values were 0.0269 and 0.0417, respectively. Both Q_9 and

Table 1. Total ubiquinone Q_9 and Q_{10} concentrations in rat kidney, heart and brain tissues at 6, 12, 18, and 24 months. Values are μg/g wt weight ± SD. n= 4 to 6 animals per group.

	Q9 in kidney		Q10 in kidney	
age (months)	control	treated	control	treated
6	71.5 ± 11	79.3 ± 7.7	7.28 ± 1.0	8.50 ± 0.7
12	89.7 ± 3.8	78.2 ± 4.4	10.2 ± 0.6	8.12 ± 0.6
18	71.9 ± 9.3	86.5 ± 9.2	7.10 ± 0.7	9.81 ± 0.5
24	90.8 ± 13	83.4 ± 6.7	11.7 ± 0.8	9.70 ± 0.6
	Q9 in heart		Q10 in heart	
age (months)	control	treated	control	treated
6	217 ± 40	225 ± 20	19.1 ± 2.8	20.3 ± 1.5
12	294 ± 38	253 ± 20	36.1 ± 2.2	35.0 ± 4.7
18	275 ± 42	299 ± 46	35.1 ± 4.8	40.6 ± 7.5
24	289 ± 20	232 ± 12	38.0 ± 2.7	32.0 ± 3.5
	Q9 in brain		Q10 in brain	
age (months)	control	treated	control	treated
6	35.3 ± 3.8	45.0 ± 3.3	14.4 ± 1.8	18.6 ± 1.0
12	52.4 ± 3.4	44.4 ± 0.6	19.4 ± 2.2	16.8 ± 1.3
18	42.4 ± 1.5	45.8 ± 4.7	18.9 ± 1.3	19.9 ± 2.6
24	38.8 ± 9.4	52.7 ± 4.4	15.8 ± 3.4	21.0 ± 2.1

Q_{10} concentrations in the liver increased with age up to 18 months and then decreased. The Q_9 increase was significant (p=0.004) in the experiment group. In heart, kidney, and brain tissues, the only change observed with aging was the increase in the heart's Q_{10} concentration from 6 to 12 months in both the control and the experimental groups (p-values 0.0007 and 0.0021, respectively). Other than that, neither the Q_9 nor the Q_{10} concentration changed significantly with age in any of the tissues that were included in our study.

DISCUSSION

Our longevity study is the first attempt to measure the effect of ubiquinone supplementation on the whole life-span and survival of rats or mice. Blitznakov (21) reported that the i.p. administration of 50 μg/day of ubiquinone Q_{10} to aged mice increased their survival. However, plasma and tissue ubiquinone Q concentrations were not measured. In our longevity study, there were no differences between the groups in either rats or mice. We also carefully investigated rat tissue histopathology and observed no significant differences

between the groups. The incidence of alveolar histocytosis was lower in the Q_{10} treated animals. These are formations of foamy macrophages within subpleural alveoli. The lung surface area covered by these changes was not measured, but in macroscopic evaluations it was obvious that in all cases it was less than 50 per cent of the total surface area. The cause and significance of this is uncertain, but because of its common (23,24) and benign nature in rats, it is unlikely to influence survival.

In our study, the supplementation caused an increase in tissue concentrations of Q_{10} only in plasma and liver, but not in heart, kidney or brain tissue. After much shorter supplementation similar results have recently been obtained by Reahal and Wrigglesworth (25) and Zhang et al. (26), who both concluded that the dietary uptake of ubiquinone is limited. Lenaz et al. (20) and Zhang et al. (26) found in their studies that exogenous Q_{10} is mainly incorporated in liver into the lysosomal fraction. Interestingly, we observed that Q_{10} supplementation also caused an increase in liver Q_9 concentration. A similar result has been obtained by Lenaz et al. (20). The purpose of this increase in not known and awaits further investigation.

The effect of aging on ubiquinone concentration has previously been investigated in three studies (18-20). Beyer et al. (18) studied rats until 25 months of age and reported a decrease in ubiquinone after 18 months of age in heart and kidney, whereas liver, brain, and lung tissues concentrations remained fairly unchanged or even slightly increased. Kalen et al. (19) studied both rats and post mortem-human tissues. The rats in their study were followed until 300 days of age and showed controversially an evident decrease in ubiquinone Q_9 in lung, liver, spleen, and kidney tissues, while heart remained unchanged. Lenaz et al. (20) studied the mitochondrial fraction and found that there was no change in Q_9 concentration in rats aged between 2 and 26 months. In our ubiquinone concentration study, we followed rats until 24 months of age. The ubiquinone Q_9 concentration decreased in plasma and liver between 18 and 24 months while in heart, kidney, and brain the concentration remained unchanged. All these studies reported different results with regard to

changes in ubiquinone concentration during aging. The differences between the results may be explained by the use of different methods. In our study we measured total ubiquinone Q_9 and Q_{10} concentrations. Unfortunately, we were not able with our equipment to separate the oxidized and reduced form of ubiquinone.

In conclusion we demonstrated that lifelong supplementation with Q_{10} in rats caused an increase in plasma and liver Q_{10} concentration, and also in liver Q_9 concentration. However, lifelong Q_{10} supplementation had no significant effect, either positive or negative, on the lifespan of either rats or mice. In control rats aging caused a decrease in the plasma and liver Q_9 concentrations, while the levels in heart, kidney and brain tissues remained unchanged.

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