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Research article

Lack of an effect of vitamin E on lifespan of mice

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Abstract

It has been speculated that ageing results from accumulation of damage to macromolecules, particularly DNA, owing to the action of oxidising free radicals. This possibility would predict that administration of anti-oxidants might prolong lifespan, but previous data on this prediction are conflicting. Three groups of mice were exposed throughout life, from the time of conception until death, to 20, 40 and 400 mg/kg of vitamin E in the diet. No effect on lifespan was observed and the median lifespans in the three groups were 804, 830 and 801 days, respectively. The design of the study also enabled an effect of parental age on lifespan of female progeny to be sought, but no effect was detected.

Introduction

The most popular stochastic theory of ageing is the somatic mutation theory, which suggests that ageing is predominantly a result of progressive accumulation of damage to nuclear and/or mitochondrial DNA. Supporting this theory are the observations that mutations in both nuclear (Morley et al. 1982; Trainor et al. 1984; Jensen et al. 1988; McCarron et al. 1989; van-Leeuwen et al. 1989) and mitochondrial (Cortopassi and Arnheim 1990; Linnane et al. 1990; Hattori et al. 1991; Munscher et al. 1993; Zhang et al. 1993) DNA accumulate with age and that the number of nuclear mutations is appropriately increased or decreased by inherited or acquired manipulations which lead to alteration in the rate of ageing (Dempsey et al. 1993; Odagiri et al. 1998). The DNA damage which leads to mutations could result from a variety of chemical mechanisms but undoubtedly the mechanism that has aroused the greatest experimental interest is damage to DNA as a result of the action of oxidising free radicals. Support for the aetiological role of free radicals in ageing came from the observation of Orr and Sohal that life span could be substantially increased in Drosophila which had been transgenically engineered to express increased levels of catalase and superoxide dismutase (Orr and Sohal 1994).

If oxidising free radicals are important in ageing, it would be predicted that treatment of animals with agents which scavenge free radicals might result in anti-ageing effects, either on the phenotypic features associated with ageing or, more definitively, on lifespan itself. However, evidence on these points is conflicting. The pigment lipofuscin, oxidised lipid and oxidised proteins all accumulate with age and vitamin E has been observed to decrease the rate of accumulation (Reddy et al. 1973; Blackett and Hall 1981; Poulin et al. 1996). However, in a study of a number of tissues in the transgenic Big Blue mouse, Moore et al. (1999) found that administration of vitamin E produced only an equivocal decrease in mutations and then only in adipose tissue. Using lifespan as a more definitive measure of ageing, extension following vitamin E administration has been observed in nematodes (Kahn and Enesco 1981), Drosophila (Miquel et al. 1973), rotifers (Enesco and Verdnoe-Smith 1980)

and Paramecium (Thomas and Nyberg 1988) but it is not clear whether this effect represents a true anti-ageing or an effect on maturation or metabolism. By contrast, anti-ageing effects of anti-oxidants in mammals have been difficult to detect and observations have been conflicting. On the one hand, Porta et al. (1980) observed that addition of vitamin E to the diet prolonged median lifespan in rats and Heidrich et al. (1984) observed that addition of 2 mercaptoethanol prolonged median lifespan in mice. On the other hand, a number of observers have failed to detect any effect of administration of anti-oxidants to rodents (Berg 1959; Kohn 1971; Tappel et al. 1973; Ledvina 1980; Blackett and Hall 1981; Lipman et al. 1998).

In all of the previous experiments involving administration of free radical scavengers, these agents had been commenced after weaning or in mid or late life. We speculated that the most important DNA damage might actually occur early in life and that this might set in train positive feedback loops such that exponentially increasing DNA damage became evident later in life. We therefore investigated whether increased levels of the free radical scavenger vitamin E from the moment of conception might lead to prolongation of life span in mice.

Methods in animals

Balb/c mice were used in the study. They were fed standard laboratory chow containing either 20, 400 or 4000 mg/kg of vitamin E. The content of vitamin E in standard chow used in this laboratory is 40 mg/kg. The breeding females and the progeny used for lifespan measurement were maintained on a constant vitamin E diet throughout the study whereas the breeding males were rotated to a different set of females and thus a different vitamin E level for each round of breeding. There were three sequential rounds of breeding. Each mating cage contained 1 male and 5 females, and in total each round involved 4 breeding males and 20 breeding females for each vitamin E level. The progeny were weaned at three weeks of age and males were discarded. The females were held at 4–10/cage until death.

Results

Between 42 and 45 mice in each vitamin E group were retained from each round of mating and the final numbers of mice were 135, 132 and 134 in the 20, 400 and 4000 mg/kg vitamin E groups respectively. Vitamin E levels in plasma were determined in 6 mice from each group when they were 21 months of age and they were (mean $\pm$ SE, μg/ml) 2.83 $\pm$ 0.34, 2.96 $\pm$ 0.36 and 4.63 $\pm$ 0.20 in the three groups respectively. The median lifespans in the three groups were 804, 830 and 801 days and the overall survival curves are shown in Figure 1. The data indicate that vitamin E administration had no effect on life span.

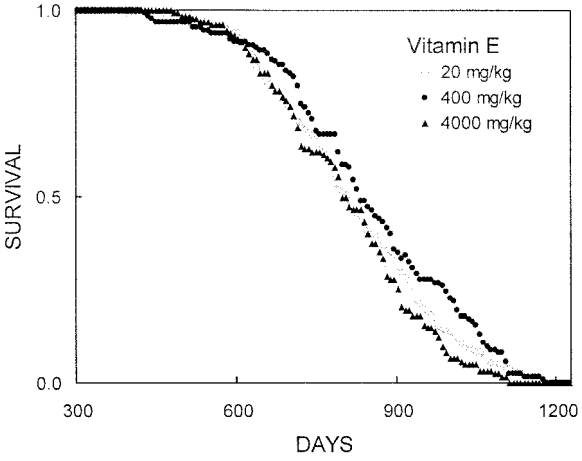

Figure 1. Relationship between lifespan and the level of vitamin E in the diet in female Balb/c mice.

The design of the study also made it possible to study the effect of parental age on life span of the female progeny. The cohorts of animals which were born from the three rounds of matings had parents which were 14–22, 32–39 and 43–47 weeks of age. These ages are equivalent to approximately 15, 31 and 39% of the median life span of these mice. The survival data are shown in Figure 2 and again indicate that there was no influence of the age of the parents on the life span of the progeny. It should, however, be emphasized that lifespan was only studied in female progeny.

Discussion

The results of this study are in accord with the previous data showing that administration of free radical scavenging agents does not affect life span in experimental animals. Although the results provide some evidence against the free radical hypothesis of ageing, it is still possible that the negative finding may have been due to the fact that vitamin E is not a suffi-

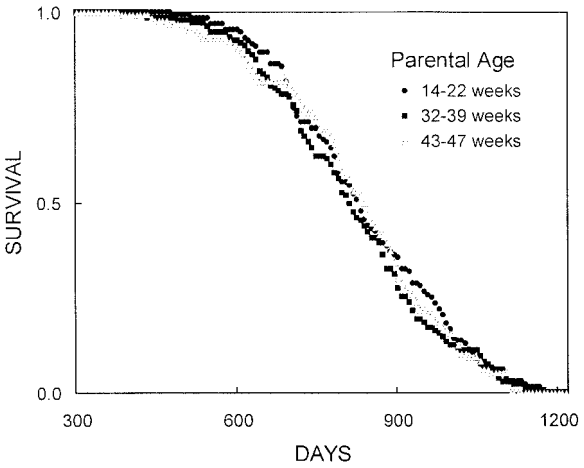

Figure 2. Relationship between lifespan of female offspring and parental age in Balb/c mice.

ciently potent scavenging agent, that sufficiently high levels of vitamin E were not achieved, that vitamin E levels at the key sites of free radical damage were too low, or that multiple free radical scavengers are required to produce detectable life-prolongation.

Although free radicals may yet prove to be the most important DNA damaging agents, there is some evidence that the majority of nuclear mutations may not be due to free radical damage. We have recently observed that exposure of human lymphocytes to hydrogen peroxide, a potent source of oxidising free radicals, results predominantly in mutations which are due to mitotic recombination (Turner et al. 2001). Hydrogen peroxide also produces some whole gene deletions but it produces very few point mutations and intragenic deletions. By contrast, the in vivo spectrum of nuclear mutations in humans shows that approximately two-thirds of mutations are due to intragenic mutations, approximately one-third are due to mitotic recombination and a very small proportion are due to whole gene deletion (Morley et al. 1990). Thus the spectrum of *in vivo* mutations differs from the spectrum of mutations produced by hydrogen peroxide and this suggests that agents other than oxidising free radicals may be important in the generation of the majority of *in vivo* mutations.

New mutations responsible for several autosomally or sex-linked recessively inherited disorders show increased incidence with increasing paternal age. It might therefore be predicted that increasing parental age might be associated with transmission of an increased number of recessive mutations to progeny and this might result in an inverse relationship between parental age and life span of the off-spring. Lansing (1947) observed this in experimental animals and Gavrilov et al. (1997) have recently reported an inverse association in humans between paternal age and life span of daughters. However, in this study we were unable to observe any relationship between parental age and lifespan of female offspring.

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