Biogerontology (2005) 6:297–302
DOI 10.1007/s10522-005-4804-4

Research article

Dose-dependency of life span prolongation of F344/DuCrj rats injected with (−)deprenyl

K. Kitani[1,*], S. Kanai[2], K. Miyasaka[2], M.-C. Carrillo[3] & G.O. Ivy[4]
[1]*National Institute for Longevity Sciences, 36-3, Gengo, Moriokacho, , Obu-shi, Tokyo, Japan;* [2]*Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan;* [3]*Institute for Experimental Physiology, University of Rosario, Rosario, Argentina;* [4]*University of Toronto, Scarborough, Canada; *Author for correspondence (e-mail: kitani@nils.go.jp; fax: +81-562-48-6668)*

Received 29 March 2005; accepted in revised form 20 August 2005

Key words: antioxidant enzymes, (−)deprenyl, F344 male and female rats, optimal dose range, specific pathogen free rats, survival prolongation

Abstract

The effect of (−)deprenyl (D) on prolonging survival has previously been reported in different species of animals. In rats, three studies reported a positive effect, while one study reported a shortening of life spans. In the present study, we attempted to clarify past discrepancies in the results based on the speculation that there exists a certain effective dose range for this effect of the drug. F344/DuCrj rats of both sexes began to receive subcutaneous (s.c.) injections of D at the age of 18 months at a dose of 0.25 mg/kg/injection (inj.), 3 times a week. Control animals were given a vehicle (a saline solution). Average life spans of animals (days) were significantly increased in both male (895 ± 109.7, $n = 30$; 967.8 ± 88.6, $n = 30$, control vs. D treated, $P < 0.01$, *t*-test) and female (924.7 ± 132.2, $n = 38$; 987.1 ± 133.4, $n = 39$, $P < 0.05$) rats by 8.1% and 6.7%, respectively. We have previously reported that a dose of 0.5mg/kg/inj. (s.c.) significantly increased the life span of male F344 rats, while a dose of 1.0 mg/kg/inj. somewhat shortened the life span, although the difference was not statistically significant. The results of the present study coupled with our previous reports clearly indicate that a proper dose of D within a certain dose range can significantly increase the life span of animals of both sexes, but that a greater dose becomes less effective and may actually adversely affect the life span of rats. The presence of this effective dose range of D may explain discrepancies in the effect of D on life spans of animals previously reported.

Abbreviations: CAT – catalase; D – (-)depreny; inj – injection; s.c. – subcutaneous; SOD – superoxide dismutase; TMIG – Tokyo Metropolitan Institute of Gerontology

Introduction

(−)Deprenyl (D) is an MAO-B inhibitor which was initially developed as an antidepressant. Knoll (1988) in Hungary first reported a more than 2-fold prolongation of the average life expectancy of rats, when rats began receiving the drug at a dose of 0.25 mg/kg/inj., 3 times a week, at the rather old age of 24 months. Since then, at least two other studies including our own (Milgram et al. 1990; Kitani et al. 1993) reported a significant effect of the drug on the life span of rats, although these studies have never been able to reproduce such a robust increase of life expectancy as was originally reported by Knoll (1988). On the other hand, at least one study reported no significant effect (Bickford et al. 1997) and another (Gallagher et al. 1998) reported an adverse effect, that is a shortening of the life span of rats. We speculated from our series of studies that higher

doses of D become less effective and may even shorten the life span of animals (Kitani et al. 1998, 2002a, b; Carrillo et al. 2000), because we clearly demonstrated an exact inverse U shape effect of the drug dose efficacy relationship, known as a hormetic effect as defined by Calabrese and Baldwin (2002) for increasing antioxidant enzyme activities in selective brain dopaminergic regions (Kitani et al. 1998, 2002a, b; Carrillo et al. 2000). While in our previous study, a dose of 0.5 mg/kg/inj., 3 times a week, s.c. significantly prolonged the average life span of male F344 rats (Kitani et al. 1993), a 2-fold higher dose (1.0 mg/kg/inj.) shortened the life span of the same rat strain of the same sex (Carrillo et al. 2000). We speculated that there may exist a certain effective dose range for D to increase the life span of animals (Kitani et al. 1998, 2000a, b; Carrillo et al. 2000). If this speculation is correct, a dose lower than 0.5 mg may also be effective in increasing the life span of animals, since a 0.5 mg dose appears to be rather close to an upper limit of the effective dose range.

We thus decided to cut the dose successfully used previously (Kitani et al. 1993) in half and to now use 0.25 mg/kg/inj. Further, in the present study we also examined the effect of the drug in female rats, since in the past studies on life spans, including ours, only male rats have been used. The reason for the use of the dose of 0.25 mg/kg/inj. in female rats in this study (as opposed to 0.5 mg) will be discussed later.

Materials and methods

We used F344/DuCrj rats of both sexes which were originally obtained from Charles River Japan (Atsugi). They were bred and maintained in the specific pathogen free (SPF) aging farm of the Tokyo Metropolitan Institute of Gerontology (TMIG). Husbandry conditions for animals used in the present study are the same as were reported previously (Nokubo 1985). In brief, distilled water and rat pellets (CRF1, Oriental, Tokyo, protein content, 18%) were given ad lib. Three male rats, or four female rats were housed per cage. Room temperature (23 ± 2 °C) and humidity (about 50%) were controlled. Lighting was controlled with 12 h light on (8:00–20:00) and 12 h light off (20:00–8:00). Experimental animals began receiving D (a generous gift of Fujimoto Pharmaceutical Company, Osaka) at the age of 18 months in the clean conventional facility of the same institute (TMIG) as was described previously (Kitani et al. 1993). D was dissolved in saline solution and given subcutaneously (s.c.). The dose was 0.25 mg/kg/inj., 3 times a week in both male and female rats. Control animals received isovolumetric vehicle (saline solution) injection. Animals were observed until their natural deaths without any intervention except for the measurements of body weight once every month. All values were expressed as mean ± S.D. When two values were compared, one way analysis of variance (ANOVA) was applied. The student *t*-test was used for a statistical comparison. *P* values lower than 0.05 were considered to be significant.

Results

Figure 1 shows the survival curves of D and saline treated male (Figure 1a) and female (Figure 1b) rats. The survival curve of D treated rats of both sexes shifted towards the right, showing a tendency of prolongation of the survival. Table 1 summarizes the average life expectancies calculated from day 0 and 2 years of animal age. The calculation of the latter was made by including animals which died earlier than 2 years using minus days for their life span. The average life spans of male rats from day 0 were 895 and 968 days for saline and D treated male rats, respectively. Females had longer life spans than respective male groups. The differences between saline and D treated rats were statistically significant in both sexes. Increases in life spans (from day 0) were by 8.1% in males and 6.7% in females. When the average life expectancy was calculated from 2 years of age, the increase was 44% and 32% for male and female rats, respecitively (Table 1). However, ages of the 10% longest survivals were not significantly different between the two groups (data not shown). Figure 2 summarizes the sequential changes in body weight in rats of both sexes. Average body weights were slightly higher in D treated male rats than saline treated rats in the later period of

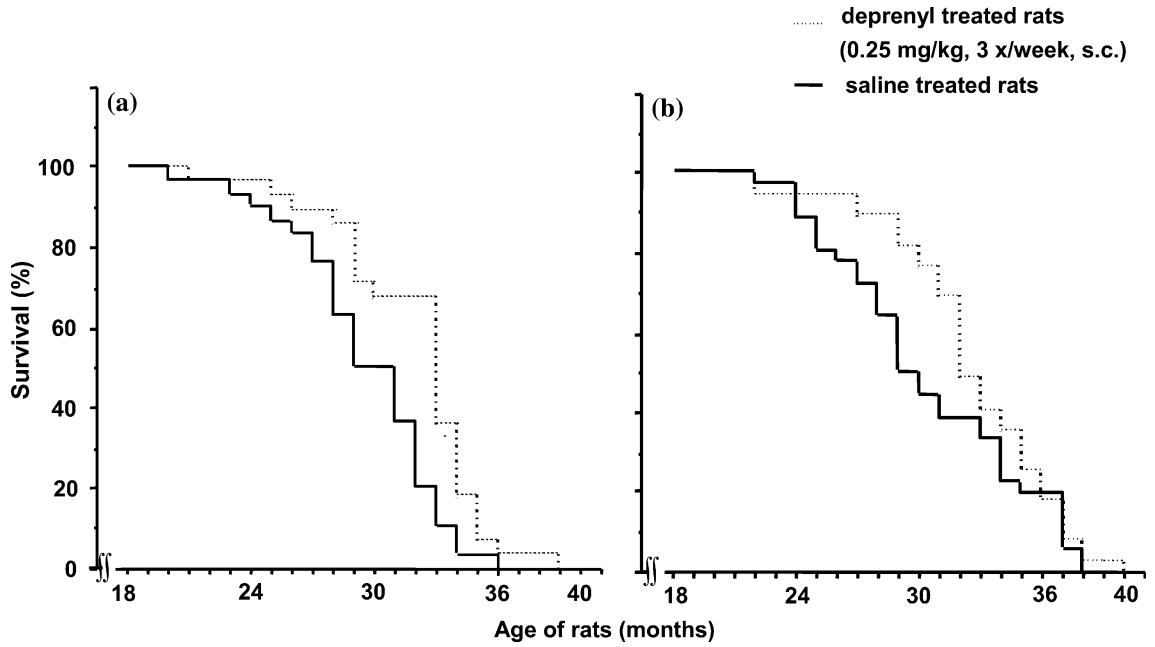

Figure 1. Survival curves of deprenyl treated (broken line) and saline treated (solid line) male (a) and female (b) F344/DuCrj rats.

their lives but were not significantly different between control and D treated rat groups (Figure 2a). In female rats, body weights were even closer than in male rats between the control and experimental group, showing almost identical average values at every corresponding rat age in months (Figure 2b).

Discussion

The results of the present study coupled with our previous experiences using different doses (0.5 and 1.0 mg/kg/inj., 3 times a week, (Kitani et al. 1993; Carrillo et al. 2000) indicate that the dose effect relationship of D on life spans of animals is a hormetic response rather than a sigmoid shaped one leading to an adverse effect with a dose above a certain dose level. This is quite similar to what we observed for the effect of the drug on antioxidant enzyme activities in dopaminergic brain regions (Carrillo et al. 2000) which was demonstrated to be an exact inverse U shape, a typical hormetic response. In that study, the dose of 1.0 mg/kg/inj., 3 times a week for 1 month was in the middle of the optimal dose range in 27-month-old male rats. However, the same dose, when continued for 13 months (from 18 to 31 months) did not increase SOD and CAT activities and the survivals were shortened (Carrillo et al. 2000). Apparently, a longer treatment period reduces this positive effect of the drug,

Table 1. Mean survival times (days) of saline and deprenyl treated rat groups.

	Saline treated rats	Deprenyl treated rats	Increase (%)	*P*
Male rats				
From 0 days	895.20 ± 109.67 (30)[a]	967.77 ± 88.62 (30)	8.1	< 0.01
From 24 months[b]	165.20 ± 109.67 (30)	237.78 ± 88.62 (30)	43.9	< 0.01
Female rats				
From 0 days	924.73 ± 132.15 (38)[a]	987.04 ± 133.37 (39)	6.7	< 0.05
From 24 months[b]	194.74 ± 132.14 (38)	257.05 ± 133.37 (39)	32.0	< 0.05

[a]Numbers in parenthesis indicate the number of rats studied.
[b]Survivals of animals that died before 24 months were included as age in negative days.

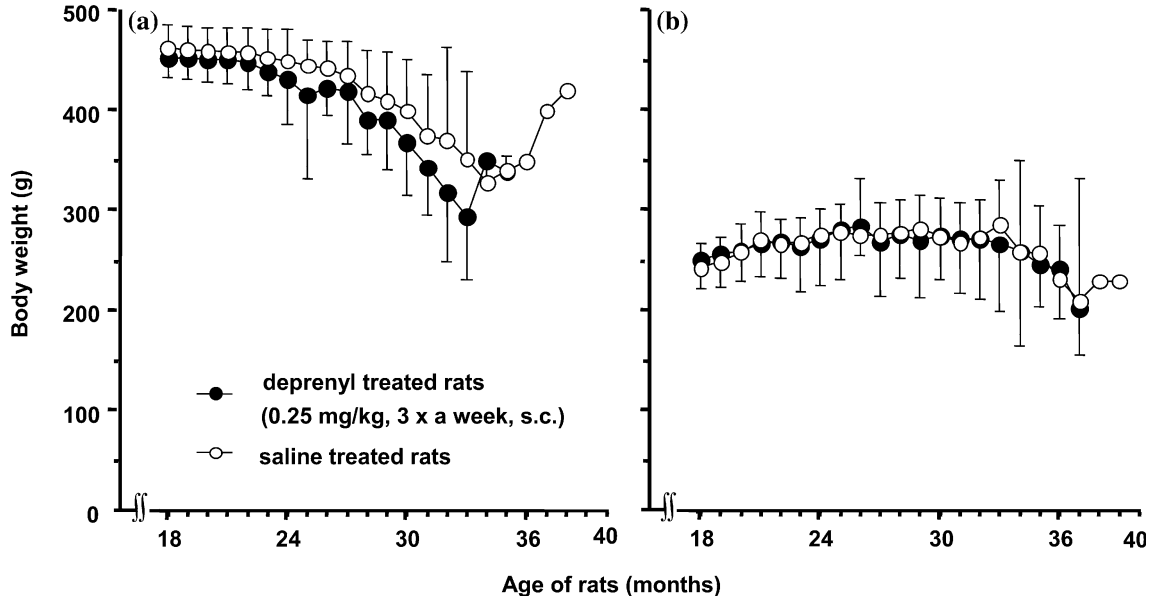

Figure 2. Sequential changes in average body weights of deprenyl treated (open circles with broken line) and saline treated (closed circles with solid line) male (a) and female (b) F344/DuCrj rats.

which may point to a reduction of an optimal dose for long-term treatment. From these observations we speculated about the possibility that the effect of D on the life span of animals may be the same. That is, a larger (or longer) dose of D exceeding an optimal dose range may become less effective and can even shorten the life span of animals, (a hormetic response), as we clearly observed for antioxidant enzyme activities (Carrillo et al. 1992, 1993, 2000; Kitani et al. 2002a, b).

The result of the present study agrees with our above speculation that there exists an effective dose range for the effect of D on life span in male F344 rats, which is certainly below a 1.0 mg/kg dose. In two previous studies (Knoll 1988; Milgram et al. 1990), both groups succeeded in prolonging the life span of male rats using the dose of 0.25 mg/kg/inj.

We also previously reported that an optimal dose for the antioxidant enzyme activities in young male rats is 10-fold greater than in female rats (Carrillo et al. 1992), however, as rats get older, the optimal dose became closer for different sexes, since in males it decreases with age, while in females the optimal dose increases with age (Carrillo et al. 1992, 1993).

A possible mechanism for the opposite trend of age-related changes in an optimal dose to increase antioxidant enzyme activities has been fully discussed previously (Carrillo et al. 1992, 1993; Kitani et al. 2002a, b). From these observations, we speculated that the effective dose for increasing the life span of aging female rats may be close to that of aging male rats. Furthermore, we have previously confirmed that the dose of 0.25 mg/kg/inj. 3 times a week maintained significantly higher activities of antioxidant enzymes for at least a 6-month-period (from 18 to 24 months) in F344/DuCrj female rats (Carrillo et al. 1994). If increased activities of antioxidant enzymes are at least a partial cause for the prolongation of life span, as we discussed previously (Kitani et al. 1998, 2002a, b), this dose (0.25 mg/kg/inj.) should be a reasonable choice for our life span study in females rats. The 0.25 mg/kg/inj. dose presently used for male and female rats was successful for rats of both sexes, which is compatible with our earlier speculation. To our knowledge, this is the first study which reported a significantly positive effect of D on life spans of female rats. Since an optimal dose of D to increase antioxidant enzyme activities can vary depending on different strains of rats as previously discussed by the authors (Kitani et al. 1998, 2002a, b), it is possible that the effective doses for the life spans we found in F344/DuCrj

rats of both sexes may not be optimal in other strains of rats. Indeed, the failure of obtaining a positive effect by using a 0.5 mg/kg dose in Wistar derived male rats (actually they found a shortening of life span) (Gallagher et al. 1998) can be best explained by the possibility that this animal model had much lower hepatic microsomal enzyme activities than F344/DuCrj male rats, which caused a much lower dose to be optimal for prolonging the life span and possibly for increasing antioxidant enzyme activities, and led to a possible over dosage of the drug with the same of 0.5 mg dose. In fact, the authors were unable to find significantly higher SOD activities in brain dopaminergic regions in their long treated rats, although CAT activities remained significantly higher than in control rats (Gallagher et al. 1999). Conversely, the study by Bickford et al. (1997) which did not find a positive effect on life spans of male F344 rats can be explained as this dose was too small, since they administered D in drinking water. Although they estimated that the amount taken to be about 0.5 mg/kg per day, the first pass effect of D by the liver is more than 90% (Mahmood et al. 19994) and the majority of D orally administered does not enter the systemic circulation. When calculated as a s.c. dose, it should be lower than 0.05 mg/kg per day, too small dose to be effective. From the forgoing, we conclude that the hormetic response for the effect of D on life spans of animals demonstrated in our studies can explain the discrepancies found in results reported by different groups in the past, since it is quite conceivable that the optimal dose also differs even in rats of the same sex and age but of different strains (Kitani et al. 2002a, b).

The precise mechanism(s) for the effect of D in prolonging the life span of animals, however, remains unelucidated. Almost comparable body weight changes in saline treated and D treated rats of both sexes agree with our previous study in the same F344/DuCrj rats (Kitani et al. 1993) as well as with another study in F344 (possibly F344/NHsd) rats (Milgram et al. 1990) both of which also showed almost identical body weight changes for control and experimental groups. These observations are enough to exclude the possibility that the life prolongation was due to an unintended calorie restriction by D treatment, since there is no study reported in the past which showed a significant life span prolongation by calorie restriction with no significant body weight reduction. Although we suggested that an up-regulation of antioxidant enzyme activities may be at least a partial cause for this effect, D has been shown to possess a variety of pharmacological effects such as an antitumor effect (ThyagaRajan et al. 1995, 1998, 1999), mobilization of various cytokines and other humoral factors involving immunomodulation (Kitani et al. 2002a, b), and many of these effects appear to be causally interrelated (Kitani et al. 2002a, b).

(−)Deprenyl is the only clinically available drug which possesses the property of reproducibly prolonging the life span of animals. We suggest that the elucidation of the mechanism(s) of the effect of the drug in increasing the life span of animals may help further our understanding of the mechanism(s) of biological organismic aging processes.

Acknowledgement

The skilful secretarial work of Ms T. Ohara is gratefully acknowledged.

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