# Common properties for propargylamines of enhancing superoxide dismutase and catalase activities in the dopaminergic system in the rat: implications for the life prolonging effect of (−)deprenyl

**K. Kitani**[1], **C. Minami**[1], **W. Maruyama**[1], **S. Kanai**[2], **G. O. Ivy**[3], and **M.-C. Carrillo**[4]

[1] National Institute for Longevity Sciences, Aichi, Japan
[2] Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan
[3] Life Science Division, University of Toronto, Scarborough, On., Canada
[4] National University of Rosario, Rosario, Argentina

**Summary.** (−)Deprenyl has been reported to prolong the life span of different animal species. Further, the drug effectively increases antioxidant enzyme activities such as superoxide dismutase (SOD) and catalase (CAT) in brain dopaminergic regions. We have found that the effect of the drug on antioxidant enzyme activities is highly dose dependent, increasing with an increasing dose, however, a higher dose becomes less effective and an excessive dose becomes adversely effective. Most importantly, an optimal dose for the effect varies widely depending on animal species, strain, sex, age and duration of the treatment, which may at least partly explain discrepancies reported among different studies in the past. From the parallelism of the dose-effect relationship of the drug between life span extension and increasing endogenous antioxidant enzyme activity, we have suggested that the above two effects of (−)deprenyl may be causally related. This review summarizes our past series of studies and also reports our very recent observation that other propargylamines such as rasagiline and (R)-N-(2-heptyl)-N-methylpropagylamine (R-2HMP) also share the property of enhancing antioxidant enzyme activities. Further, our most recent study has found that these propargylamines increase antioxidant enzyme activities not only in brain dopaminergic regions but in extra-brain dopaminergic tissues such as the heart and kidneys. These observations are discussed in relation to the life prolonging effect of (−)deprenyl reported in the past.

## Introduction

(−)Deprenyl is a monoamine oxidase B (MAO B) inhibitor but possesses a variety of pharmacological effects such as neuroprotection, life span prolongation, anti-apoptotic effects, etc. (reviewed in Kitani et al., 1999). Knoll, who was initially involved in the development of the drug as an

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antidepressant, later reported that old rats which were treated chronically with (−)deprenyl lived for a significantly longer period than saline treated control animals (Knoll, 1988). He further reported that 3-week consecutive s.c. injections of the drug significantly increased activities of superoxide dismutase (SOD) but not of catalase (CAT) in the rat striatum (Knoll, 1988). Some years later, however, he cast some doubt on the latter observation, since the enhancing effect of (−)deprenyl on SOD activities was not reproduced in another strain of rats (Knoll, 1989).

Although a positive effect of (−)deprenyl in prolonging the life span of animals including rats (Knoll, 1988; Milgram et al., 1990; Kitani et al., 1993), mice (Archer and Harrison, 1996), hamsters (Stoll et al., 1997) and dogs (Ruehl et al., 1997) has been reported in recent years, other studies have failed to demonstrate a significant effect of the life prolongation of animals by this drug (Bickford et al., 1997; Gallagher et al., 1998; Ingram et al., 1993; Piantanelli et al., 1994). Our group has been involved in studies of these two effects of (−)deprenyl (i.e. life prolongation and antioxidant enzyme activities) and has obtained results which may at least partially explain the discrepancies between different studies in terms of the life prolonging effect as well as the effects on endogenous antioxidant enzyme activities of the drug (Carrillo et al., 1991; 1992a,b,c; 1993, 1994a,b; 1996; Kitani et al., 1993; for review, see Kitani et al., 1996, 1998a). Very recently we have examined other types of propargylamines such as rasagiline [N-propargyl-l(R)-aminoindan] (Finberg et al., 1998) and (R)-N-(2-heptyl)-N-methyl propargylamine (R-2-HMP) (Boulton, 1999; Boulton et al., 1997) and found that at least the effects on antioxidant enzyme activities are shared by all these propargylamines (Carrillo et al., 2000b). Further, we have found that antioxidant enzyme activities can be increased not only in dopaminergic brain regions as we have previously reported (Carrillo et al., 1991; 1992a,b,c; 1993; 1994; 1996) but also in extra-brain dopaminergic tissues such as the heart and kidneys (Carrillo et al., 2000b). In this chapter, we attempt to summarize briefly our past results of (−)deprenyl and other propargylamines with a special emphasis on the dose-efficacy relationship for these two effects which may explain discrepancies among past studies on the effects of the drug.

## Materials and methods

Some of the results presented here have been obtained at the Tokyo Metropolitan Institute of Gerontology (TMIG, Tokyo, Japan) using Fischer 344/Du (F-344/Du) rats originally purchased from Japan Charles River (Atsugi, Japan) and BDF1 and C57BL mice from SLC (Shizuoka, Japan).

Recent studies were performed at the National Institute for Longevity Sciences (NILS, Obu, Japan). Rats used at NILS were F-344/6JNia purchased from Harlan Sprague Dawley (Indianapolis, IN, USA) which were raised under a contract with the National Institute on Aging (NIA Bethesda, MD, USA). Husbandry conditions in the two institutes have been described elsewhere for TMIG (Nokubo, 1985) and NILS (Kitani et al., 1998b).

(−)Deprenyl is a generous gift from Fujimoto Pharmaceutical Company in (Matsubara-shi, Japan) and rasagiline was a generous gift from TEVA Pharmaceutical Company (Netanya, Isreal).

Procedures for tissue preparations and enzyme activity measurements are described in detail elsewhere (Carrillo et al., 1991; 1992a,b,c). In brief, SOD activities were determined by the method described by Elster and Heupel (1976). In some later studies, the original method of McCord and Friedovich (1969) was used. Cu,Zn-SOD activities were defined as the fraction which can be inhibited by the addition of KCN at a concentration of 0.5 mM. (KCN). Catalase (CAT) activities were determined immediately after the preparation of tissue samples by the method described by Beers and Sizer (1952). Glutathione peroxidase (GSHPx) activities were determined by the method described by Paglia and Valentine (1967). Protein concentration was determined by the method of Lowry et al. (1951).

## Results

### *The effect of (−)deprenyl on life span of rats*

Figure 1 shows the results of our initial study on male F-344/Du rats which began receiving s.c. injections of (−)deprenyl (0.5 mg/kg, 3 times per week) at the age of 18 months. The (−)deprenyl treatment modified the survival curve of animals leading to 50% survival times of 28.5 months in control rats and 31

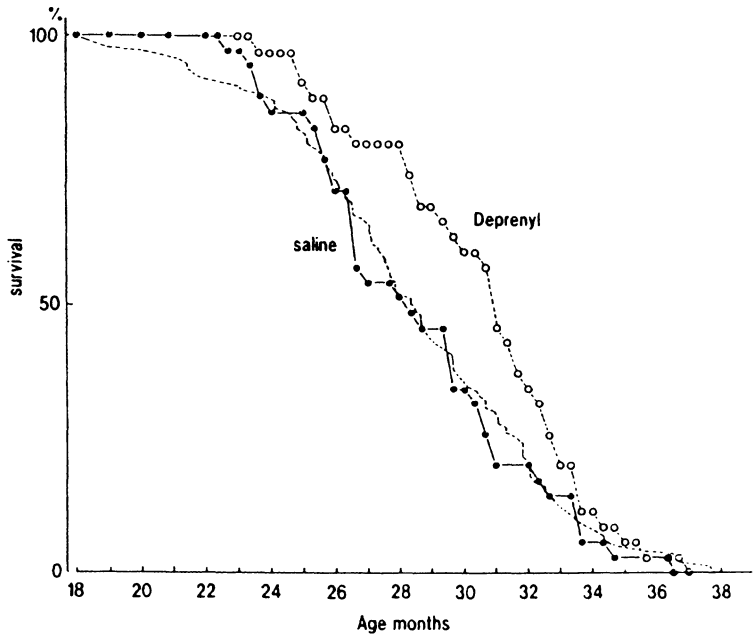


**Fig. 1.** Survival curves of control (closed circles) and deprenyl-treated (open circles) male F-344 rats as expressed from pooled data of three cohorts. Broken line without symbols indicates data from 100 animals raised in the specific pathogen-free farm of the institute (TMIG) as reported previously. Animals began deprenyl (0.5 mg/kg, 3 times a week) or saline s.c. injections at the age of 18 months. (Reproduced with permission from Kitani et al., 1993)

**Table 1.** Effect of deprenyl on life span of rats

| Strain (sex)<br>Effect | Dose<br>Authors |
|---|---|
| Logan-Wistar(M)<br>>+100%[a] | 0.25 mg/kg, s.c. (3x, a week)[b]<br>Knoll, 1988 |
| F-344(M)<br>+16%[a] | 0.25 mg/kg, s.c. (3x, a week)[b]<br>Milgram et al., 1990 |
| F-344(M)<br>+34%[a] | 0.5 mg/kg, s.c. (3x, a week)[c]<br>Kitani et al., 1993 |
| F-344(M)<br>no significant effect | 0.5 mg/kg, p.o. (daily)[d]<br>Bickford et al., 1997 |
| Wistar(M)<br>adverse effect<br>(shortening of life span) | 0.5 mg/kg, s.c. (3x, a week)[e]<br>Gallagher et al., 1998 |

[a] Average life expectancy after 24 months of age
[b] After 24 months of age
[c] After 18 months of age
[d] Between 54 wks–118 wks
[e] Between 3 months–23 months

months in treated animals. Accordingly, an average life expectancy calculated from 24 months of age was prolonged significantly by 34% in treated animals as compared with saline treated animals (Kitani et al., 1993). However, treated rats started to die more quickly after their 50% survival time than controls and the longest survival times did not differ significantly between the two groups (Kitani et al., 1993). Interestingly, when we increased the dosage of the drug two fold (1.0 mg/kg, 3 times a week), treated animals started to die more quickly resulting in the survival at the age of 31 months of 3 out of 12 animals in the treated groups, while 7 out of 12 controls were still surviving at this age (Carrillo et al., 2000a). SOD and CAT activities as examined at this age were almost identical between the two groups (Carrillo et al., 2000a) as will be discussed later.

Table 1 summarizes results of past studies on survivals of rats affected by (−)deprenyl treatment (Bickford et al., 1977; Gallagher et al., 1998; Kitani et al., 1993; Knoll, 1988; Milgram et al., 1991). Despite the three initial positive results (Kitani et al., 1993; Knoll, 1988; Milgram et al., 1991), some later studies have not been able to clearly demonstrate this effect (Bickford et al., 1993; Gallagher et al., 1998). A recent study from the UK has reported even an adverse effect (i.e. a shortening of the life span) of (−)deprenyl treated rats (Gallagher et al., 1998).

Table 2 summarizes results of past published studies on mice. The most recent study by Archer and Harrison (1996) clearly demonstrated a significantly positive result in life span extension in this animal species, while the other two published studies failed in demonstrating a significant effect (Ingram et al., 1993; Piantanelli et al., 1994). In our unpublished studies on

**Table 2.** Effect of deprenyl on life span of mice

| Strain (sex)<br>Effect | Dose<br>Authors |
|---|---|
| C57BL/6J (M)<br>No<br>(M)<br>No | 0.50 mg/kg/day[a] (daily)<br><br>1.00 mg/kg/day[a] (daily)<br>Ingram et al., 1993 |
| Balb/C (?)<br>No | 0.25 mg/kg, s.c. (3x, a week)<br>Piantanelli et al., 1994 |
| BDF1 (M)<br>+21% (n.s.)[b] | 0.50 mg/kg, s.c. (3x, a week)<br>Kitani et al. (unpublished) |
| BDF1 (M)<br>−1% (n.s.)[b] | 0.25 mg/kg, s.c. (3x, a week)<br>Kitani et al. (unpublished) |
| B6D2F1 + B6CBF1 (M + F)<br>positive | 0.25 mg/kg, s.c. (3x, a week)<br>Archer and Harrison, 1996 |
| NMRI(?)<br><br>>+100%[c]<br>(immunosuppressed) | 75 μg/kg, p.o. (daily, for 10 months)<br><br>Freisleben et al., 1997 |

*M* male, *F* female
[a] In drinking water, every day
[b] Average life expectancy after 24 months
[c] Average life expectancy from the start of study

BDF1 male mice, the difference in the average life expectancies after 24 months of age also did not attain a statistical significance between control and deprenyl-treated mice, although mice which received a dose of 0.5 mg/kg, 3 times a week lived for a longer period than control rats. A recent study from Germany reported a dramatic extension of survival of immunologically defficient mice (DD mice) by treatment with deprenyl (Freisleben et al., 1997). Although the initial report by Knoll also emphasized a dramatic recovery of decreased (or lost) sexual interest and capability of old male rats treated with the drug (Knoll et al., 1989), the study by Archer and Harrison (1996) has shown a clear decrease in fecundity of (−)deprenyl treated male mice. To our knowledge, there is no other report on the sexual capability in old animals affected by (−)deprenyl except for another study on old female rats which recovered estrous cycles after deprenyl treatment (ThyagaRajan et al., 1995) and this aspect of pharmacology of (−)deprenyl remains to be clarified in the future.

Ruehl et al. (1997) have successfully reported a significant extension of survivals of relatively old (10 to 15 years old) female Beagle dogs treated by the drug (Fig. 2). Stoll et al. (1997) have reported a significantly positive result in female but not in male hamsters in terms of life span prolongation. These rather discrepant data will be discussed later based on the possible dose-efficacy relationship as for the effect on antioxidant enzyme activities.

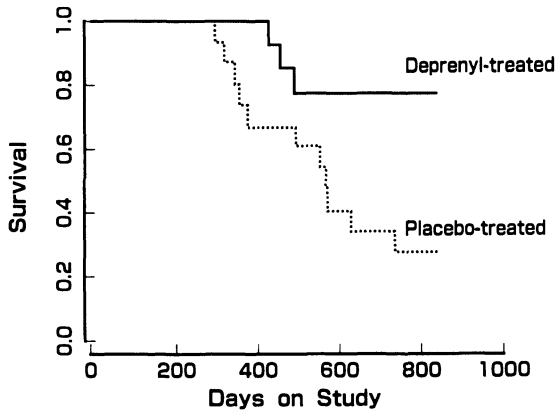


**Fig. 2.** Survival of dogs between 10 and 15 years old at the start of the study and treated with deprenyl for at least 6 months. $p < 0.05$ — deprenyl, ···· placebo (Reproduced with permission from Ruehl et al., 1997)

*Effects on antioxidant enzyme activities*

Knoll (1988) initially reported a significant increase in (total) SOD (but not CAT) enzyme activities in the striatum of male Logan-Wistar cross rats with consecutive s.c. injections of the drug for 21 days. However, he was unable to demonstrate a similar effect in another strain of rats with the same treatment (Knoll, 1989).

Figure 3 shows the results of our earlier work on male F-344/Du rats after the continuous infusion of the drug for 21 days at a dose of 2.0 mg/kg/day (Carrillo et al., 1992c). The results demonstrate two new observations which had not been documented by the previous study of Knoll (1988). One is a clear regional selectivity showing a clear effect on brain dopaminergic regions but not in others such as hippocampus and cellebellum (Carrillo et al., 1992a,b,c; 1993). Also, the drug did not affect enzyme activities in the liver (Carrillo et al., 1992a,b,c). The other is a clear and significant effect on CAT activities but not in activities of GSHPx (data not shown) (Carrillo et al., 1992a,b,c).

Figure 4 summarizes our observations in 4 different rat models, young and old F-344/Du rats of the two sexes (Carrillo et al., 1993). All values are expressed as percentage of corresponding control values obtained in rats treated with s.c. saline infusion (or injections). From these figures, we should recognize how important the dose selection of the drug is for obtaining an optimal effect to increase these enzyme activities. It is clear that the effect is dose-dependent with an increase in the efficacy with increasing doses, however, an excessive dose becomes less effective and very high doses become adversely effective, significantly decreasing enzyme activities as compared with control values (e.g. 2.0 mg/kg/day in young female rats in Fig. 4). Further, an optimal dose varies widely depending on the sex and age of rats. In young rats, there exists about a 10 fold difference in an optimal dose between the two sexes (2.0 mg/kg/day in males vs. 0.2 mg/kg/day in females, Fig. 4). Further aging caused an opposite effect in male and female rats,

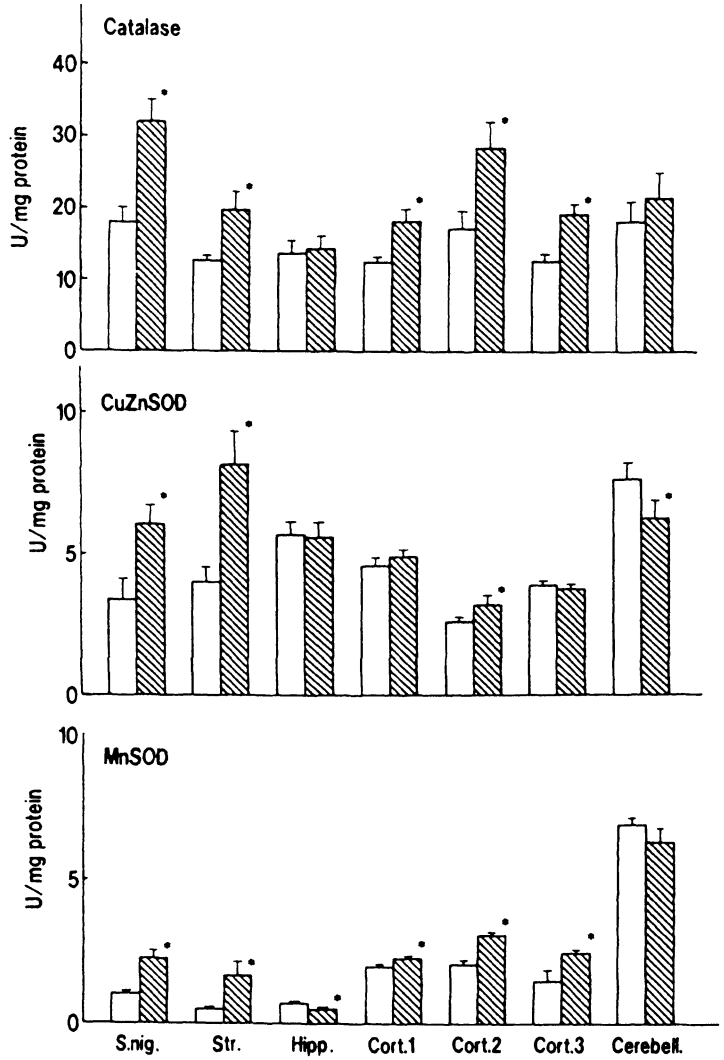


**Fig. 3.** Catalase (top panel) and superoxide dismutase (lower two panels) enzyme activities in young control (white columns) and deprenyl-treated (shaded columns) male F-344 rats. The dose of deprenyl is 2.0 mg/kg/day, s.c. continuous infusion for 3 weeks. *Significantly different from respective control values ($p < 0.05$) *S.nig.* substantia nigra; *Str.* striatum; *Hipp.* hippocampus; *Cort.1* frontal cortex; *Cort.2* parietotemporal cortex; *Cort.3* occipital cortex; *Cerebell.* cerebellum. (Reproduced with permission from Carrillo et al., 1992c)

decreasing the optimal dose in old males but increasing it in females (see Fig. 4, Carrillo et al., 1992b; 1993).

Possible mechanisms regulating variability of an optimal dose of the drug have been discussed in detail elsewhere (Kitani et al., 1996; 1998a; 1999) and only major points will be described here.

(−)Deprenyl is metabolized mainly by the liver microsomal P-450 enzymes by means of depropargylation, and N-demethylation leading to the formation of (−)amphetamine which is further hydroxylated by the same

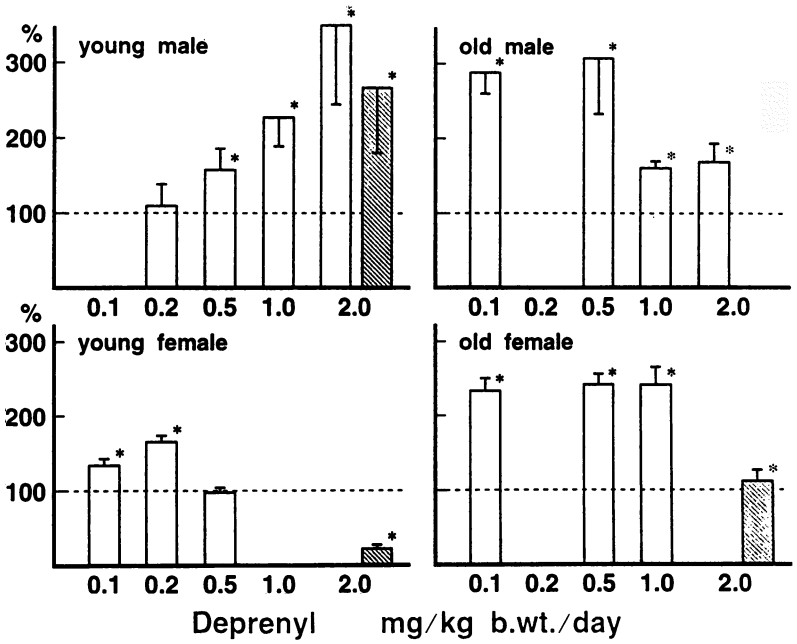


**Fig. 4.** Relative enzyme activities of Mn-SOD in striata from young and old rats of both sexes treated with different doses of deprenyl. All values are expressed as percentages relative to respective control values. White columns indicate values in rats given 21 day s.c. continuous infusion, and shaded columns represent values in rats given 21 day s.c. consecutive injections. *Significantly different from respective control values ($p < 0.05$). The number of rats studied in each group is 3–7 (mostly 4–5). (Reproduced with permission from Carrillo et al., 1993)

enzyme system (Yoshida et al., 1987). Although the rate of metabolism varies widely depending on rat strains, generally speaking P-450 enzyme activities are much greater (usually 5 to 10 times) in male than in female rat livers (Fujita et al., 1985; Kamataki et al., 1985; Kitani, 1988). This may largely explain a ten-fold difference in an optimal dose in increasing antioxidant enzyme activities between the two sexes, since in male rat livers, the drug is metabolized much more quickly than in females, leading to the need for a much greater optimal dose. This is an important point in interpreting much of the data on (−)deprenyl, since even in the same sex (e.g. females), inter-strain (quantitative) differences in P-450 enzyme activities are enormous (Yoshida et al., 1987; Kitani, 1988), which may result in the differences in an optimal dose in the same sex of the same species (Kitani et al., 1996, 1998a). With aging, P-450 functions decline drastically in male rats but stay essentially unaltered in females at least of F-344/Du rats (Fujita et al., 1985; Kamataki et al., 1985). This may explain a marked decline in the optimal dose in male rats with aging, since a drastic decrease in metabolism of the drug may lead to a greater availability of the drug in the body with aging. On the other hand, an increase in an optimal dose in old female rats can not be explained on the basis

of altered P-450 functions with age since unaltered P-450 functions with age have been clearly demonstrated in this sex of F-344 rats (Fujita et al., 1985; Kamataki et al., 1985; Kitani, 1988).

However, an alternative explanation for an increase in an optimal dose in aging female rats is possible. Several past studies have shown an increase in MAO B enzyme activities with age (Strolin and Keane, 1980). Since (−)deprenyl is a specific MAO B inhibitor which binds irreversibly with MAO B enzymes, an increase in MAO B enzyme molecules with age will increase the amount of administered (−)deprenyl that will be bound with MAO B, resulting in a decrease in bioavailability of this drug for modifying antioxidant enzyme activities. This may naturally increase the optimal dose of the drug for increasing enzyme activities with age in female rats. The same mechanism may also be operative in old males, however, the drastic decrease in P-450 enzyme activities in these animals may have offset the opposite effect caused by an increase in MAO B enzymes with age in males.

Figure 5 deliniates another important factor for regulating an optimal dose of (−)deprenyl in increasing antioxidant enzyme activities in mice (Carrillo et al., 1996). The dose which was quite effective in increasing activities when male mice were treated for only 3 weeks became totally ineffective when mice were treated for 3 months (Carrillo et al., 1996). A long term treatment caused not only a narrowing in the optimal dose range as compared with a 3-week treatment, but also decreased the magnitude of the increase as well as the optimal dose range. This tendency (i.e. the effect of a long term treatment of the drug) was not so marked when old female rats were examined after 3 weeks vs. 6 months of treatment (Fig. 6). However, when male rats were treated for 13 months from 18 to 31 months, the effect of the dose of 1.0 mg/kg/day (3 times a week) which was quite clear in animals treated for 1 month, was totally abolished showing almost identical enzyme activities for control and treated animal brains (Carrillo et al., 2000a).

From these results, we suggest that an optimal dose in increasing antioxidant enzyme activities is quite variable depending on animal sexes (especially in rats), strains and of course animal species. Since rat strain differences in P-450 enzyme activities are also well documented (Yoshida et al., 1987), the discrepancy between studies by Knoll himself using different rat strains but the same dosage of deprenyl is easily explained by this mechanism (Knoll, 1989). A recent study of Gallagher et al. (1999) which failed to find an upregulation of Cu,Zn-SOD activities in Wistar rats can also be reasonably explained by the long treatment period in the study and a possibly lower P-450 function for this particular rat line which may have lead to an over dosage of the drug. We have demonstrated, however, that this particular effect of (−)deprenyl (i.e. on antioxidant enzyme activities) can be reproduced in at least 3 different animal species [rats (Carrillo et al., 1991; 1992a,b,c; 1993), mice (Carrillo et al., 1996) and dogs (Carrillo et al., 1994)], suggesting that this property of the drug may be shared by a rather broad spectrum of animal

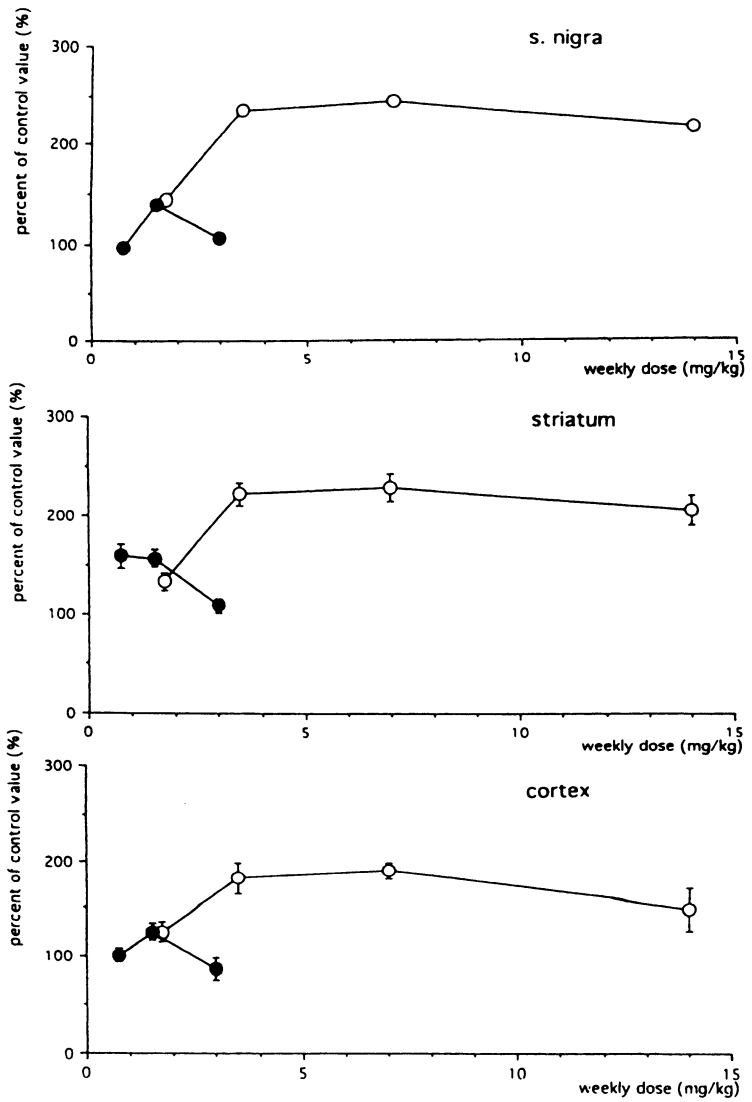


**Fig. 5.** Relative Cu,Zn-SOD activities in three different brain regions in old male mice treated with deprenyl for 3 weeks (open circles) or 3 months (closed circles). Enzyme activities in treated animal groups are expressed as percentages relative to respective values in control groups. In the short-term study, deprenyl was continuously infused s.c. for 3 weeks, whereas in the long-term study, deprenyl was injected s.c. three times a week for 3 months. For the purpose of comparison, doses were recalculated as weekly doses for the two studies. (Reproduced with permission from Carrillo et al., 1996)

species possibly including primates, although there exists a paucity of data in primates in this regard.

Figure 7 illustrates our very recent data on the effect of rasagiline (Carrillo et al., 2000b), another type of propargylamine which has been shown to possess not only an MAO B inhibitory effect but an anti-apoptotic potency as well (Finberg et al., 1998; Maruyama and Naoi, 1999). It is clear that this drug also has an enhancing effect on SOD and CAT activities in dopaminergic

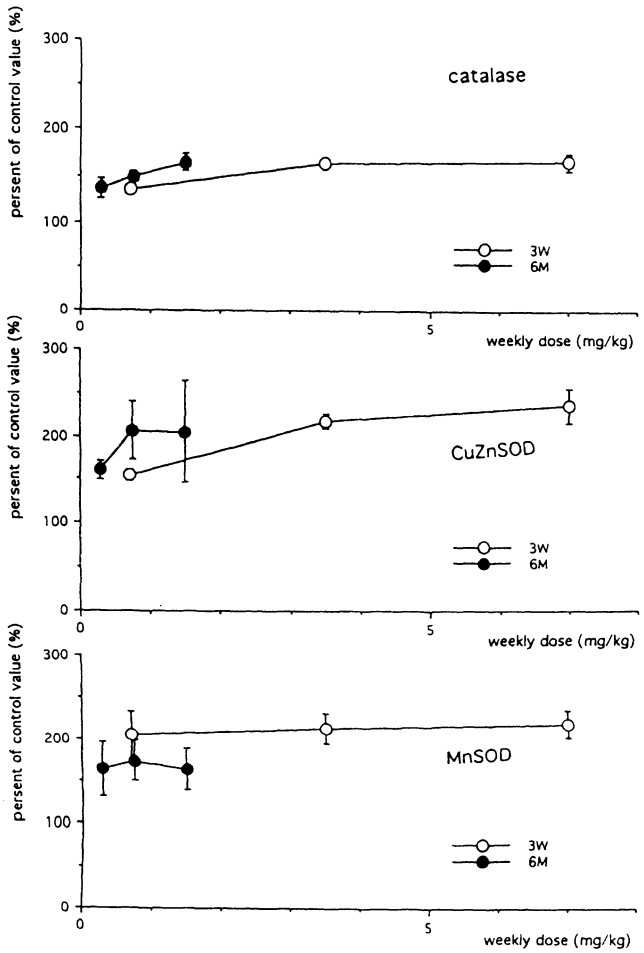


**Fig. 6.** Relative enzyme activities in striatum in old female rats treated with deprenyl for 3 weeks (open circle) or 6 months (closed circles). Enzyme activities in treated animal groups are expressed as percentages relative to respective control values. In the short-term study, deprenyl was continuously infused s.c. for 3 weeks (Carrillo et al., 1992a), whereas in the long-term study, deprenyl was injected s.c. three times a week for 6 months (Carrillo et al., 1994a). For the purpose of comparison, doses were recalculated as weekly doses for the two studies. The figure was drawn with permission from these two studies (Carrillo et al., 1992a; 1994a)

brain regions (Carrillo et al., 2000b) as was observed for (−)deprenyl (Carrillo et al., 1991; 1992a,b,c; 1993; 1996). Further, it is noteworthy that this drug also increases activities of CAT and especially SOD in dopaminergic tissues outside of the brain such as the heart and kidneys (Carrillo et al., 2000b). This property has been confirmed to exist for another propargylamine, (R)-N-(2-heptyl)-N-methylpropargylamine (R-2HMP) (Minami et al., unpublished observation). We have also confirmed that (−)deprenyl can also increase antioxidant enzyme activities (Minami et al., unpublished observation) in the heart and kidney, which has never been reported in the past.

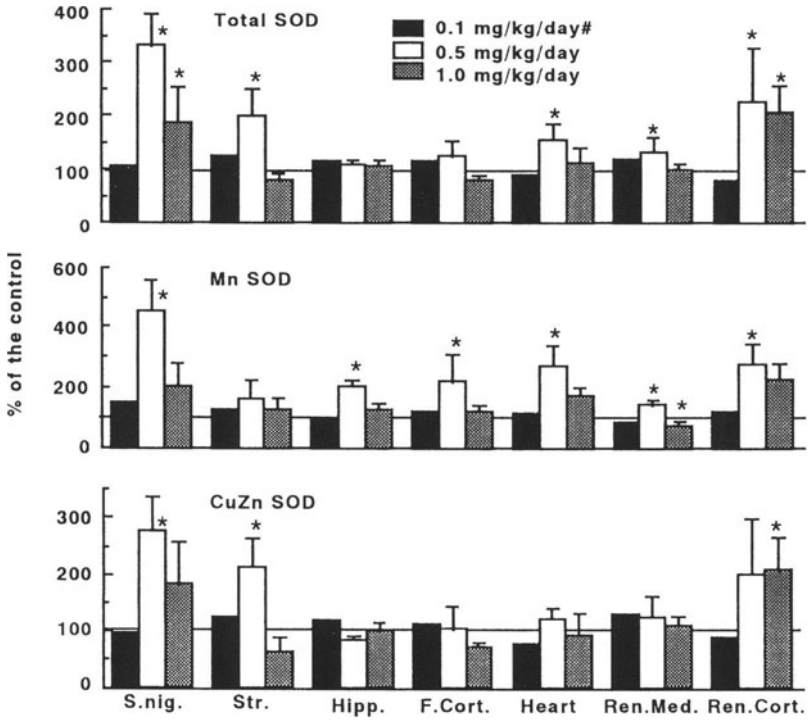


**Fig. 7.** Effect of rasagiline pretreatment on superoxide dismutase activities on different brain regions and tissues in 8-month-old male F-344 rats. All values are expressed as percentage relative to respective control values in rats given a saline solution infusion. *Significantly different from respective control values ($p < 0.05$). Black bars indicate values in a rat given a dose of 0.1 mg/kg/day for 3.5 weeks. White bars indicate values in rats given a dose of 0.5 mg/kg/day and hatched bars values in rats given a dose of 1.0 mg/kg/day for 3.5 weeks *S.nig.* substantia nigra; *Str.* striatum; *Hipp.* hippocampus; *F.Cort.* frontal cortex; *Ren.Med.* kidney medula; *Ren.Cort.* kidney cortex. (Reproduced with permission from Carrillo et al., 2000b)

## Discussion

### *The dose efficacy relationship of (−)deprenyl*

As has been illustrated in several figures in this chapter, there is no question that the dose efficacy of (−)deprenyl on antioxidant enzyme activities is quite variable depending on sexes, strains, species and age of animals studied. The effect of long term treatment is also clear, showing a narrowing of the optimal dose range as well as both a decrease in the magnitude of increase in enzyme activities and in an optimal dose itself, at least in mice, if the treatment is continued for a longer period. Interestingly, the effect of the longer treatment appears to be more pronounced in mice than in rats. It is more difficult to obtain convincing evidence that the variability of an optimal dose also exists with the effect of (−)deprenyl on life span of animals, although all emerging evidence is compatible with this notion (Gallagher et al., 1998; 1999; Kitani et al., 1996; 1998). Further, what is worthy of pointing out is a relative parallelism

of the dose-effect relationship between the two seemingly different aspects of (−)deprenyl's pharmacology (Kitani et al., 1996; 1998a; 1999). The MAO B inhibitory effect can be achieved with a much lower dosage of the drug than the effects on enzyme activities and survivals of animals. Similarly an anti-apoptotic effect can be achieved at a much lower dosage and tissue concentration and this effect is achieved very quickly, in one hour or two after administration of the drug (Maruyama and Naoi, 1999). In contrast, the optimal effect on antioxidant enzyme activities requires 3 weeks with repeated treatment (Carrillo et al., 1992b). The dose range for these antioxidant enzyme activities and survivals are very close to each other and a greater dose appears to become less effective for both effects. From this parallelism of the two effects of the drug, we still maintain our notion that these two effects may be causally related, although we are well aware that direct evidence to prove this thesis must be provided in the future.

### *Possible mechanisms for prolonging life span of animals by (−)deprenyl*

As has been repeatedly discussed, the pretreatment with (−)deprenyl was shown to protect brain dopaminergic systems against oxygen-induced tissue damage acutely caused by a hypoxia-reperfusion paradigm in rats (Knollema et al., 1995). Accordingly it is possible that chronic treatment with the drug also protects oxygen-induced chronic tissue damage in dopaminergic systems during aging. How can the protection of the dopaminergic system against chronic oxidative damage during aging prolong the lifespan of animals is a matter of pure speculation. We have speculated that reported modulation of release of many humoral factors including TNF and nerve growth factors by the drug may be involved (Kitani et al., 1996; 1998a; 1999). (−)Deprenyl has also been reported to be involved in the regulation of several interleukins (Müller et al., 1998; Ruehl et al., 1994; ThyagaRajan et al., 1998; 1999).

Thus, once the dopaminergic system is better preserved during aging by deprenyl treatment, it may have a significant impact on the functions of an organism by means of these humoral regulations. For example, the dopaminergic system may work to prevent the development of tumors, possibly by releasing TNF and other anti-tumorigenic factors (Kitani et al., 1996, 1998a). The surprisingly longer survival of aging female Beagle dogs given deprenyl appears to be causally related to a much lower incidence of breast cancer in the deprenyl treated group (Ruehl et al., 1997). The retardation of the development of subcutaneous (benign) tumors in F-344/Du rats has been pointed out to be at least a partial cause for the longer survival of this particular strain of rats by (−)deprenyl (Kitani et al., 1993; 1996; 1998a). More direct evidence that chronic treatment with (−)deprenyl in rats prevents carcinogen-induced (ThyagaRajan et al., 1998; 1999) as well as spontaneously developed breast cancer during aging (ThyagaRajan et al., 1995) has been recently provided. A recent report demonstrating a significantly longer survival of immunodeficient mice treated with deprenyl (Freisleben et al., 1997) also suggests that immunomodulation by the drug may be partially respon-

sible for this effect. Immunostimulant effects of the drug are also increasingly reported in the literature (Müller et al., 1998; ThyagaRajan et al., 1999). The causal relationship between effects of the drug on antioxidant enzyme activities and humoral factors including neurotrophic factors (Kontkanen and Castren, 1999; Li et al., 1998; Tang et al., 1998) remains to be established in the future.

Our recent findings that propargylamines, including deprenyl, elevate antioxidant enzyme activities in the heart and kidneys may provide other mechanisms for prolonging the life span of animals. For example, rodents (rats and mice) are known to develop nephropathies similar to nephrosis in humans losing huge amounts of proteins into the urine during aging (Burek, 1978). Although there is no direct proof that this lesion is a partial determinant for regulating the apparent survival of animals, it is possible that animals can live for a longer period if these lesions can be better prevented by an increase in antioxidant enzyme activities in the kidney.

In a previous study by Milgram et al. (1991), the only difference in biochemical parameters between control and deprenyl treated aging rats was a significantly lower blood urea nitrogen level in deprenyl treated rats. Although rodents do not usually bear cardiovascular lesions during aging, cardiovascular lesions as well as cancer are major killers of elderly humans. It remains to be studied how the increase of SOD and CAT activities affect cardiovascular functions and eventually age-related lesions in animal species including humans. Future work is definitely needed to clarify these issues.

### *Future problems to be resolved*

As has been discussed in this chapter, the two effects of (−)deprenyl on antioxidant enzyme activities and life span of animals are very unique and may help our future understanding of mechanisms of aging. However, many questions remain to be answered in order to further elucidate mechanisms of the complex pharmacology of (−)deprenyl and possiblly other propargylamines. First, the selectivity of the drug for dopaminergic brain regions and other tissues has to be explained. The mechanism(s) of enhancing antioxidant enzymes also requires an ample explanation. Further the complex nature of the pharmacology of the drug involving the whole neuro-immuno-endocrine system and interrelationships among seemingly different (but possibly interrelated) effects of the drug need to be elucidated. Finally, the mechanism(s) of prolongation of life span of animals has to be clarified. For this purpose, other propargylamines which have been newly introduced (Finberg 1998, Boulton 1999, Boulton et al., 1997) may be of considerable help.

## Conclusions

(−)Deprenyl and other propargylamines share the property of enhancing antioxidant enzyme activities in selective brain regions and other tissues

primarily of dopaminergic nature. Most importantly high doses become less effective and too excessive doses reduce activities. The optimal dose is very different among different animal models because of at least several different mechanisms. Emerging evidence suggests that the effect of (−)deprenyl to prolong the life span of animals can be reversed, resulting in a shortening of life span if too excessive a dose is used. Discrepancies observed in the past literature can be explained at least in part for these dose-efficacy relationships of the two different effects of (−)deprenyl. Further studies should be designed and interpreted with this important point in mind.

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Authors' address: Kenichi Kitani, National Institute for Longevity Sciences, 36-3, Gengo Moriokacho, Obu, Aichi, 4748522, Japan. E-mail: kitani@nils.go.jp