(−)Deprenyl Increases the Life Span as Well as Activities of Superoxide Dismutase and Catalase but Not of Glutathione Peroxidase in Selective Brain Regions in Fischer Rats

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(−)Deprenyl was developed in Hungary about 30 years ago as a monoamine oxidase inhibitor[1] and was reported to be effective for Parkinson's disease.[2] Recently, the efficacy of the drug in the early stages of Parkinson's disease has been confirmed by several double-blind controlled studies.[3,4] Furthermore, Knoll reported that the drug, when first administered at the age of 24 months in male rats, increased the remaining life expectancy twofold compared with that of saline-treated control rats.[5] Since old rats do not develop a disease corresponding to human Parkinson's disease, the mechanism(s) for the life span extention, if real, may be different from those working in Parkinson's disease. Furthermore, if deprenyl really does extend life span, this fact should have a significant impact on experimental gerontology, since no other chemicals or pharmaceuticals have been reproducibly demonstrated to prolong the life span of animals. It is still the general consensus in experimental gerontology that the only means of prolonging the life span of animals (rodents in particular) is dietary restriction.[6–8]

More recently, Milgram *et al.* reported that the remaining life expectancy of male Fischer-344 (F-344) rats that began treatment at 24 months increased by only 16%,[9] while Knoll reported a more than 100% increase in 24 month-old rats of the Logan-Wistar strain.[5] This (16%) increase of life span found in the Canadian study was only marginally significant ($p = 0.048$, one-tailed t-test).[9] Thus, it appears that a

more extensive study is necessary to draw a definite conclusion with regard to the life-prolonging effect of the drug.

Knoll further reported that when animals were treated with deprenyl for 21 successive days, activities of superoxide dismutase (SOD) in striatum of brain were significantly increased in both male and female rats.[5] In contrast, increases in activities of catalase (CAT) and glutathione peroxidase (GSH Px) were not statistically significant.[5] Knoll suggested that the increase in SOD activity in striatum may be causally related to the life-prolonging effect of the drug.[5]

In the present report, we will summarize the results of our own life span study as well as of a series of recent studies on the effect of deprenyl on antioxidant enzyme activities, primarily in the rat brain.

MATERIALS AND METHODS

Animals

F-344 rats of both sexes raised in the aging farm of the Tokyo Metropolitan Institute of Gerontology were used throughout. Husbandry conditions, life spans, and pathologies that appear in the later periods of the animals' lives were reported elsewhere.[10]

Life Span Study

Male F-344 rats that had been raised in the aging farm of the Tokyo Metropolitan Institute of Gerontology began to be administered with deprenyl at the age of 18 months. Animals of experimental groups were injected subcutaneously (sc) with deprenyl 3 times a week at a dose of 0.5 mg/kg per day. Control groups were given isovolumetric saline solution injections. Each 35 animals of three different cohorts (15, 10, 10 animals) constituted control and experimental animal groups. During the drug treatment, they were raised with 3 animals in one cage in a clean conventional facility of the institute. Other husbandry conditions were identical to those in the SPF aging farm. Details of the study have been reported elsewhere.[11] Animals were observed until natural death. No intervention was made except for the measurement of body weight once a month.

Antioxidant Enzyme Activity Studies

Animals of both sexes and of different ages were treated with sc injection or infusion of deprenyl solution primarily for 21 successive days. Some animals were treated longer, up to 4 weeks. Animals were sacrificed by decapitation and several brain regions such as striatum, s. nigra, three different parts of cerebral cortex (frontal, parietotemporal, and occipital), hippocampus, and cerebellum were dissected on an ice-cold plate. The liver was also removed in some experiments. Tissue preparations for enzyme activity measurements are described in detail in our previous publications.[12–15]

Enzyme Activity Measurements

Superoxide Dismutase (SOD)

The activity of SOD was assayed by the method of Elster and Heupel[16] based on the inhibition of nitrite formation from hydroxylammonium in the presence of O_2^- generators. Differentiation of the two different types of SOD (Cu, Zn-SOD and Mn-SOD) was performed by the addition of potassium cyanide (5×10^{-4} M) to the incubation medium. Cu, Zn-SOD activities were defined as those inhibited by potassium cyanide. The difference between total and KCN inhibited enzyme activities was defined as Mn-SOD activity.

Catalase (CAT)

CAT activity was assayed by the method described by Beers and Sizer.[17]

Glutathione Peroxidase (GSH Px)

Activities of GSH Px were determined by the method described by Paglia and Valentine.[18] Two different types of GSH Px (selenium dependent and nonselenium dependent) were assayed using two different substrates, hydroperoxide and cumene-hydroperoxide. Details of procedures for these enzyme activities are described elsewhere.[12-15]

Statistical Analysis

Average life spans of three different cohorts in the same group were analyzed by means of one-way analysis of variance (ANOVA). Since no significant difference could be noted in either the control or the experimental group among three different cohorts, all data were pooled into one group (35 rats each) and the comparisons between control and deprenyl-treated animals were made by using the Student *t*-test for unpaired values (two-tailed test). Enzyme activities among different groups were primarily analyzed by means of one-way ANOVA. When values were judged to be significantly different with respect to deprenyl treatment, values of any set of two groups were analyzed by Scheffe's test. When only a control group and an experimental group were compared, the Student *t*-test for the unpaired values (two-tailed test) was applied. *p* values lower than 0.05 were judged to be significant for these analyses.

RESULTS

TABLE 1 summarizes the average life expectancies of both control and experimental groups calculated from day 0, 18 months (the start of treatment), and 24 months of age. For the calculation of the average survival after 24 months, 3 animals that died before 24 months were included by using minus days in age. Details of this study have been published elsewhere.[11] The increases in mean survival in deprenyl-treated animals compared to respective values in control animals were 5.6%, 15.0%, and

33.8% for survivals from 0 days, 18 months, and 24 months, respectively, and these increases were all statistically significant. Only the 10% longest survivals did not differ significantly between the two groups.

FIGURE 1 illustrates the results of our first experiments on the enzyme assays on the striatum of young male[12] F-344 rats as well as young and old female rats.[14] As is clearly shown, an sc injection of deprenyl at a dose of 2.0 mg/kg per day for 21 successive days yielded a nearly threefold increase in activities of both types of SOD enzymes as well as a 60% (significant) increase in CAT (but not of GSH Px) activities in the striatum of the young male rats. However, in young female rats, the same treatment caused a threefold decrease in SOD activities,[14] while in old female rats activities remained unchanged.[14] Subsequently, we found that the effect of deprenyl on antioxidant enzyme activities is not specific to the striatum, but is also selective to certain brain regions such as s. nigra and cerebral cortex, while there was no effect on enzyme activities in hippocampus or cerebellum, or the liver (FIGURE 2).[13]

The reason(s) why we observed three different results (i.e., an increase, a decrease, and no change) in three different rat groups (FIGURE 1) has been explained by our subsequent studies. FIGURE 3 summarizes some of our recent studies[14,19] using different doses of deprenyl. The duration of treatment was again 21

TABLE 1. Mean Survival Times (Days) of Two Different Rat Groups[a]

	Control Rats (n = 35)	Deprenyl-Treated Rats (n = 35)	Increase (%)	P
From 0 days	876.7 ± 108.7	926.3 ± 97.4	5.6	<0.05
From 18 months	328.7 ± 108.8	378.3 ± 97.4	15.0	<0.05
From 24 months[b]	146.7 ± 108.7	196.3 ± 97.4	33.8	<0.05
Ten percent longest survivals (each n = 4)	1057.5 ± 27.0	1074.8 ± 22.0	1.0	>0.05

[a]Reproduced from Reference 19 with the permission of the publisher.
[b]Survival of animals that died before 24 months were included as age in negative days.

successive days. All values in treated animals are expressed in FIGURE 3 as a percent of respective control values obtained from saline-treated animals.

The discrepant results obtained in our study (FIGURE 1) turned out to be due to differences in the optimal dose of the drug for increasing antioxidant enzyme activities among different animal groups. For example, in young male rats, an optimal dosage appears to be 2.0 mg/kg per day or even greater because higher doses were not tested, while in young female rats, an optimal dosage is around 0.2 mg/kg per day, which is at least 10 times lower than that of young male rats. The reason why the optimal dose for young male rats (2.0 mg/kg per day) decreased SOD activities in young female rats appears to be due to an actual overdose of the drug, since as the dose was increased above the optimal dose, it tended to lose its effect, and as the dose increased up to 2.0 mg/kg per day, it reversed the effect. Similarly, the reason why 2.0 mg/kg per day did not change SOD activities in old female rats is that this dose is just between an optimal dose (1.0 mg/kg per day) and an excessive dose to reduce activities. From FIGURE 3, it is clear that in female rats, aging increased an optimal dose fivefold, while aging reduced the optimal dose fourfold in male rats, the optimal dose in old male rats being 0.5 mg/kg per day (FIGURE 3). The reason for the variability of an optimal dose caused by sex and aging will be discussed later.

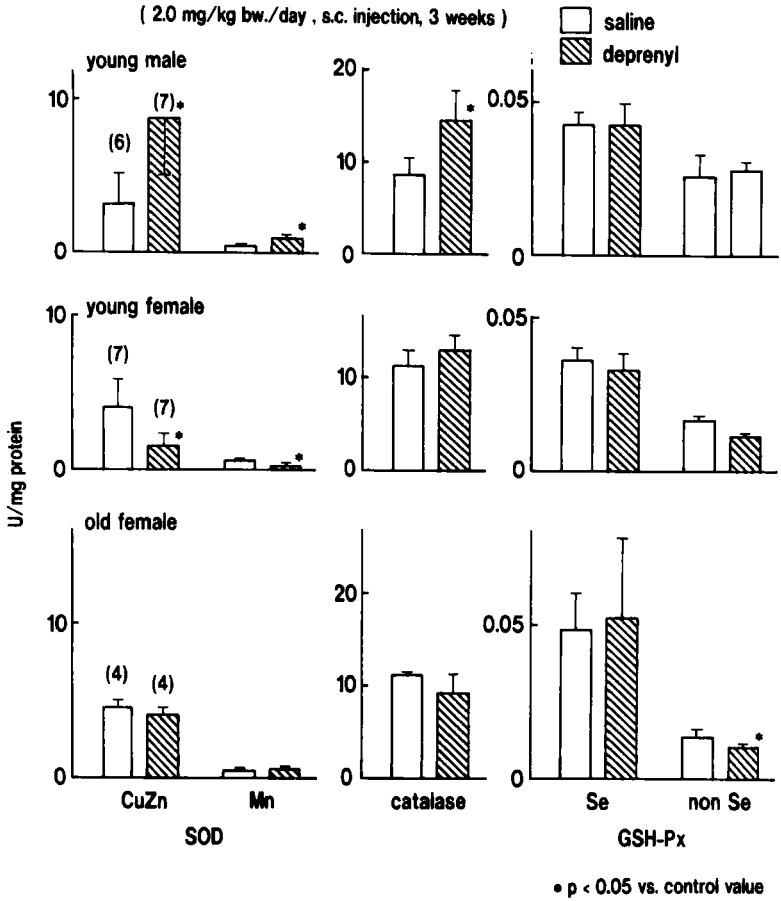

FIGURE 1. Enzyme activities of SOD, CAT, and GSH Px in the striatum of control rats (white column) and rats treated with sc injection of deprenyl for successive 21 days at a dose of 2.0 mg/kg per day (shadowed column). Redrawn from data previously reported by the authors for young male rats[12] and young and old female rats[14] with the permission of the publisher. Numbers in parentheses indicate the number of rats of each group. *Significantly different from corresponding control calues ($p < 0.05$, t-test).

FIGURE 4 summarizes the results of our recent study which examined sequential changes in SOD and CAT activities during the full 4 weeks of deprenyl infusion.[15] SOD activities in s. nigra and striatum started to increase 1 week after the start of infusion and continued to increase up to 4 weeks. In contrast, CAT activity remained unchanged after 1 week of deprenyl infusion and started to increase only after 2 weeks of infusion. Interestingly, unlike SOD activities, CAT activities peaked at 3 weeks and tended to decrease slightly at 4 weeks. This tendency is more clearly seen for CAT activities in frontal and parietotemporal cortices and for SOD activities in parietotemporal cortex, where a 4-week treatment lost its effect for increasing enzyme activities in this brain region.

DISCUSSION

Life Prolongation by Deprenyl

As previously discussed, the prolongation of the life span of animals by means of chemicals or pharmaceuticals has been claimed in a number of past studies. Thus far, however, none of these studies has been reproducible. The only reproducible way to prolong the life span of animals (especially rodents) is by dietary restriction.[6–8] However, with this paradigm, so many biochemical and physiological parameters are altered that we cannot discriminate which factors play a causal role in prolonging the life span of the animals. It is likely that most parameters that have been claimed to be altered by dietary restriction are all the result of the life prolongation, but not the cause. The twofold increase in the remaining life span of rats reported by Knoll[5] when deprenyl treatment was begun at 24 months is therefore an astonishing observation, if it is reproducible. Indeed, the first animal of the treated group died after all control rats died.[5] However, a subsequent study reported from Canada in this regard has cast doubt on the effect of the drug, although it was reported as a positive result.[9] A 16% increase of the remaining life span in this study after 24 months was only marginally significant with the p value of 0.048 by using one-tailed t-test. If an ordinary two-tailed test is applied, it easily loses its significance. Since the results of these studies differ so greatly, it is difficult to draw a simple conclusion about the effects of deprenyl. However, it should be noted that different rat strains were used in the two studies.

Our study design is somewhat different from the previous two studies.[5,9] Although we also used male F-344 rats as were used in the second (Canadian) study, we started to administer the drug at the age of 18 months, instead of 24 months as was

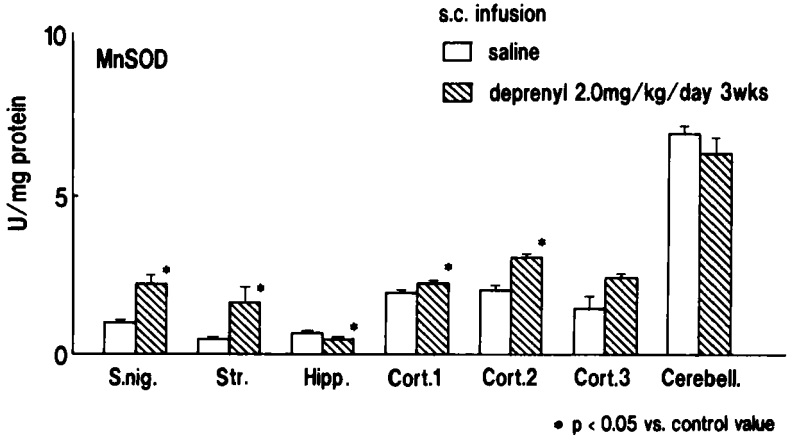

FIGURE 2. Mn-SOD activities in different brain regions in young male rats treated with saline solution (white column, $n = 6$) and rats treated with deprenyl infusion at a rate of 2.0 mg/kg per day for 21 days (shadowed column, $n = 4$). *Significantly different from corresponding control values ($p < 0.05$, t-test). (S. nig., substantia nigra; Str., striatum; Hipp., hippocampus; Cort 1, frontal cortex; Cort 2, parietotemporal cortex; Cort 3, occipital cortex; Cerebell., cerebellum). (Reproduced from Reference 13 with the permission of the publisher.)

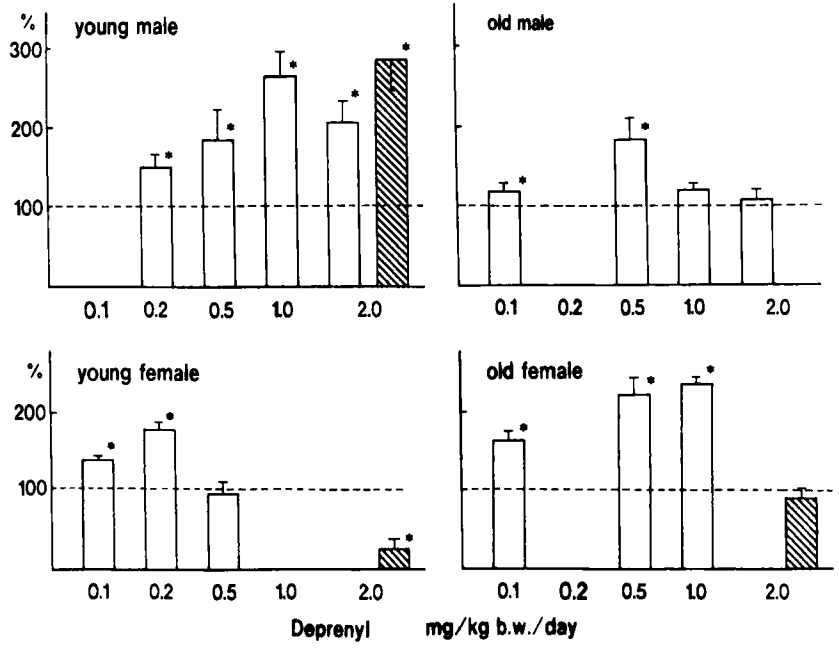

FIGURE 3. Relative enzyme activities of Cu Zn-SOD in striata from young and old rats of both sexes treated with different doses of deprenyl. All values are expressed as percentages of respective control values. White columns indicate values in rats given 21 day sc infusion, and shadowed columns represent values in rats given 21 day sc injection. *Significantly different from respective control values ($p < 0.05$). The number of rats studied in each group is 3 to 7 (mostly 4–5). (Reproduced from Reference 19 with the permission of the publisher.)

done in the previous two studies. Further, we used twice the dose (0.5 mg/kg per day) that was used previously (0.25 mg/kg per day).[5,9] Some quantitative differences between our study and that of the Canadian group may be explained by these differences in experimental design. The 34% increase obtained in our study was statistically significant ($p < 0.05$) as analyzed by means of two-tailed t test despite the fact that the number of animals in each group was almost one half (each 35) those used in the previous two studies (66).[5,9]

Our results, therefore, could be taken as clear evidence that the effect of deprenyl in prolonging the life span of rats is real. However, our results also suggest that such a robust effect as was originally reported by Knoll[5] is not always obtainable. It is possible, however, that if a more proper dosage is used, we can improve the results in terms of the percent increase of the remaining life span. If the real mechanism(s) underlying this phenomenon becomes clear, it will be a remarkable advance in our understanding of the mechanisms of aging. At the moment, however, nothing is known on the mechanism of deprenyl's action(s) except for an inhibition of MAO B and the alteration of antioxidant enzyme activities as will be discussed later.

The possibility that the life prolongation was obtained indirectly through the lower food intake of animals administered deprenyl could be ruled out, since the average body weights were almost identical for both groups throughout the observa-

tion period.[11] This also agrees with the observation by the Canadian group.[9] Interestingly, the standard deviation of body weight of the control group started to become greater after 24 months of age compared to the deprenyl-treated group. Although there was no specific comment on this observation, it was also true in the Canadian study.[9] This means that although the average body weight is the same at corresponding ages for both groups, some control animals lost body weights more quickly than did deprenyl-treated animals, while some others gained or lost body weight more slowly. The major reason why some control animals were heavier in their later lives was that many animals started to bear skin tumors after 24 months, some of them weighing more than 200 g in tumor weight alone.[11] Thus, it appears that deprenyl in some way retarded the growth of these skin tumors as well as the natural body weight loss that is known to occur in this animal strain in their later lives.

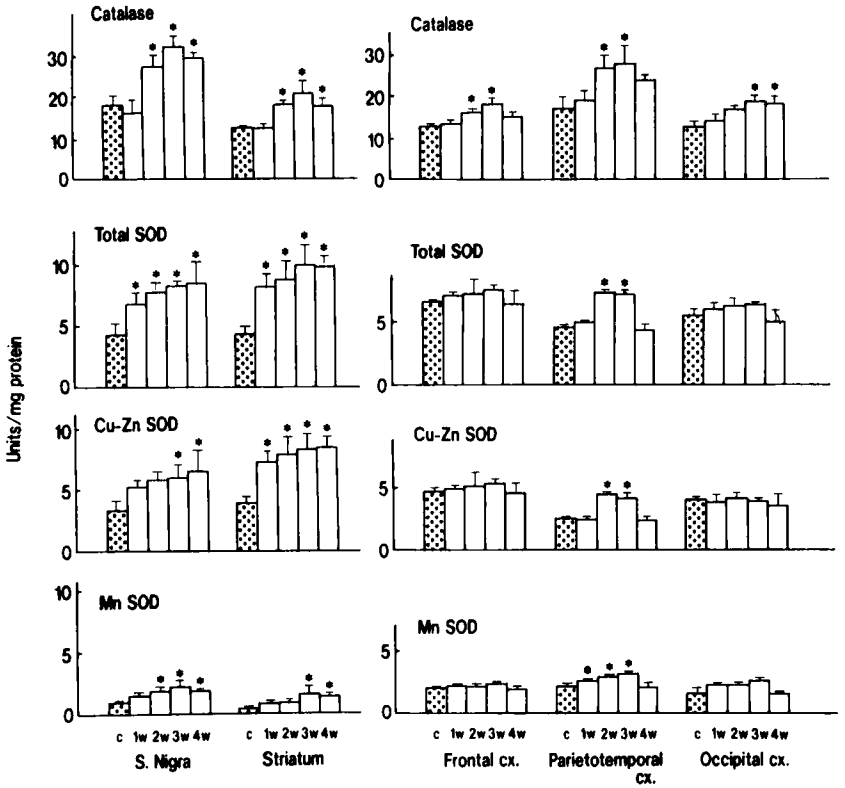

FIGURE 4. Sequential changes in activities of CAT and SOD in different brain regions from young male control rats (c) and rats treated with deprenyl for different time intervals. Deprenyl was continuously infused sc by osmotic minipumps. *Significantly different from corresponding control values (ANOVA + Scheffe's test, $p < 0.05$). Number of animals in each group was 4 except for the group treated with deprenyl for 2 wks ($n = 3$). (Reproduced from Reference 15 with the permission of the publisher.)

Increase in Antioxidant Enzyme Activities

At present, there is no direct proof that increases in antioxidant enzyme activities such as SOD and CAT in certain brain regions, especially the striatum and the s. nigra, are at least a partial cause for the life prolongation obtained by the administration of the (−)deprenyl. However, this phenomenon was demonstrated for all rat groups examined, if appropriate dosages were chosen.[14,19] Further, we have confirmed that it occurs in mice as well as in dogs (unpublished observations). Thus, this effect of the drug appeared to be a phenomenon not only in rats but also in a variety of animal species. Thus far, however, there are no life-prolongation studies in primate species, to the knowledge of the authors.

The question of why an optimal dose of deprenyl for increasing SOD and CAT activities varies so widely depending on sexes and ages has plausible explanations. First, the 10 times lower optimal dosage in young female than in male rats and the reduction of the optimal dose in male rats with aging can be at least partly explained by the possible differences in the metabolic rate for deprenyl among different animal groups. Deprenyl is known to be metabolized by *N*-demethylation or depropargylation, yielding nordeprenyl and depropargyl-deprenyl (methamphetamine), respectively, both eventually being further metabolized to (−)amphetamine.[20,21] These reactions are mediated mainly by the microsomal monooxygenase system in the liver.[20,21] In most rat strains, many of the reactions mediated by the monooxygenase system are known to be much faster in males than in females. Furthermore metabolic rates of these reactions are known to decline drastically during aging in male (but not in female) rat livers[22,23] (for review, see Reference 24). If we assume that young female rats have a 10 times lower metabolic rate than male rats, the 10 times lower optimal dose in the former can be accepted without any other explanation. In fact, Yoshida *et al.*[25] reported that the formation of amphetamine from deprenyl (the major metabolic pathway in rat liver), was 2-, 6-, and 11-fold lower in livers of female rats of Wistar, SD, and Donryu strains respectively than in males. With the known quantitative strain differences in regard to sex differences of drug metabolism in rat livers, it is not surprizing if we find a 10 times lower metabolic rate in females of the F-344 strain as was previously observed for Donryu rats.[25]

Since our group has repeatedly shown that, in general, drug metabolism in the livers of young male rats declines rapidly with age, approaching values in young (and old) female rats[22,23] (for review, see Reference 24), the reduction of an optimal dose in old male rats has a reasonable explanation as discussed above on the basis of difference in the metabolism of deprenyl. The increase in an optimal dose in female rats with aging, however, cannot be explained on the basis of metabolic rate differences, since hepatic drug metabolisms in female rats essentially stay unchanged during the whole aging process.[22,23] However, it has been well documented that not only in rats[26] but in humans[27] also, aging causes an increase in MAO B activity in many different tissues, such as brain and heart. Since deprenyl is irreversibly bound to the MAO B enzyme molecule and inactivates its enzyme activity, greater amounts of deprenyl may be required in old animals to block these enzymes. Consequently, the amounts of deprenyl that are used for increasing SOD (and CAT) activities may become more limited in old animals, if the same dose is used. This may be at least a partial cause for the increase in an optimal dose in old female rats compared with young ones. The same mechanism for increasing an optimal dose with age should also be operative in old males rats. However, the reduction of metabolic rate appears to have worked more strongly, actually decreasing an optimal dose in old male rats. Thus, we must recognize that sex and age are both strong determinants for an optimal dose in this animal strain.

As is shown in FIGURE 4, for some enzyme activities, a 4-wk treatment appears to be less effective than a 3-wk treatment. In this particular study,[15] however, we were not certain of this observation or of its interpretation. However, we have recently realized that the longer we treat animals, the smaller the optimal dose becomes. In old female rats treated for 6 months, 3 times a week, the results suggest that 0.25 (0.5 mg at largest) per mg/day is most appropriate.[28] An optimal dose of 1.0 mg/kg per day obtained in the study shown in FIGURE 3 for old female rats was the one obtained with 21 successive days of treatment. Therefore the dose of 1.0 mg/kg per day in this study is greater as a weekly dose than 2.0 mg/kg per day in a study where deprenyl was given only 3 times a week. It means that an optimal dose in a long-term study for increasing antioxidant enzyme activities should be at least 4 times lower (and probably less) than the one that was found in our initial short-term study. Our recent studies in progress in male rats, as well as in mice, have increasingly supported this contention. As is shown in FIGURE 1, an excessive dose of deprenyl not only becomes less effective in increasing activities of antioxidant enzymes but can eventually reduce these activities below physiological levels. From these observations, we suggest that a dose of deprenyl used for a long-term study, such as a life span study, must be planned very carefully, keeping in mind that an optimal dose varies widely depending on the sex, age, and, more importantly, the duration of drug treatment, at least in rats.

CONCLUSIONS

Although the underlying mechanism(s) remains unknown, it appears certain that deprenyl can prolong the life span of rats, when an appropriate dose is administered. The drug also increases SOD and CAT activities in selective brain regions, such as s. nigra, striatum, and cerebral cortex. However, an optimal dose for this effect varies widely depending on the sex and age and even the duration and mode of administration of the drug to the animal. Accordingly, the dosage of deprenyl used may considerably influence the results of long-term studies such as a life span study using this drug, if the effect on antioxidant enzyme activities is causally related to its effect on rat life span. Future studies, therefore, must be done with the caution in mind that the proper selection of an optimal dose of deprenyl is a critically important factor for studies evaluating the long-term effects of this drug.

SUMMARY

(−)Deprenyl, a MAO-B inhibitor that is also known to be effective for symptoms of Parkinson's disease, when injected subcutaneously (sc) in male Fischer-344 rats at a dose of 0.5 mg/kg per day (3 times a week) from 18 months of age, significantly increased the remaining life expectancy. The average life span after 24 months was 34% greater in treated rats than in saline-treated control animals. Furthermore, a short-term (3 wk) continuous sc infusion of deprenyl significantly increased activities of superoxide dismutase and catalase but not of glutathione peroxidase in selective brain regions such as s. nigra, striatum, and cerebral cortex, but not in hippocampus or cerebellum, or the liver. The optimal dose for increasing these activities, however, differed greatly depending on the sex and age of animals, with a 10-fold lower value for young female than male rats. Interestingly, aging caused an increase and a decrease in the optimal dose in female and male rats, respectively. In addition,

treatment for a longer term tended to reduce the optimal dosage in the same animal group. The results clearly demonstrate that deprenyl increases antioxidant enzyme activities in selective brain regions. If this effect of deprenyl is causally related to its life-prolonging effect, the dosage to be used for any life span study would be a critical factor, with the dosage differing widely depending on sex, age of animal, and mode and duration of drug administration.

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