Assessing the Effects of Deprenyl on Longevity and Antioxidant Defenses in Different Animal Models

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ABSTRACT: Among many pharmaceuticals that have been tested for their effects on longevities of different animal rodents, deprenyl is unique in that its effects on longevity has been tested in at least four different animal species by independent research groups and that the effect has been postulated to be due to its effect of raising such antioxidant enzyme activities as superoxide dismutase (SOD) and catalase (CAT) in selective brain regions. Thus far, in all four species of animals examined (rats, mice, hamsters, and dogs), a positive effect was demonstrated, although the extent of its effect is quite variable. Our group has examined the effect on longevities in rats and mice and on antioxidant enzymes in rats, mice, and dogs. Although in rats of both sexes, we have obtained positive effects on longevity, two studies with different doses in mice did not reveal a significantly positive effect. We have observed, however, significantly positive effects on SOD (in Cu, Zn-, and Mn-) as well as CAT (but not glutathione peroxidase) activities in the brain dopaminergic system such as in the S. nigra and striatum (but not in hippocampus) in all rats, mice, and dogs, although the effects were quite variable, depending on the doses used. In mice, however, a long-term administration (3x/w, 3 months) caused a remarkable decrease in the magnitude of activity as well as a narrowing of the effective dose range, which may explain a relatively weak effect of the drug on mouse longevity. Further, a recent study on aging beagle dogs by Ruehl *et al.* showed a remarkable effect on longevity, which agrees with our SOD study in dogs. Although deprenyl has been claimed to have several other effects, such as a radical scavenging effect and a neuroprotective effect, past reports on its effects on longevities and antioxidant defenses are compatible with the notion that the drug prolongs the life span of animals by reducing the oxidative damage to the brain dopaminergic system during aging. Further, our studies on F-344 rats as well as a dog study by Ruehl *et al.* suggest that the drug may at least partially prolong the life span of animals by enhancing immune system function and preventing tumor development in animals.

Attempts at prolonging the human life span with pharmaceuticals, nutrients, and many other means have been made throughout the long history of humans since ancient days. However, all of these attempts have been in vain, at least in modern scientific terms.

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Although the general consensus in experimental gerontology is that the only reliable means for prolonging the life span of rodents is by dietary restriction, recent attempts at pharmacological intervention in aging and age-associated pathologies in experimental animals are becoming more and more realistic. Nonetheless, these observations still lack solid scientific explanations.

Most of the efforts in the past as well as at present involve administration of so-called antioxidants; some of the past results are summarized elsewhere.[1] Although it has been suggested that (-)deprenyl works as a free radical scavenger,[2] as will be discussed in detail later, the drug is unique in that it also modifies endogenous antioxidant enzyme activities.[3–9] Since the initial report by Knoll,[3] that chronic administration of this drug caused a drastic increase in the life span of aging male rats, at least eight studies in four different animal species have been published. Except for mice, on which negative results were observed in several earlier studies, in all other studies in rats,[10,11] hamsters,[12] and dogs,[13] significantly positive results were reported for prolongation of life spans. It is not clear, however, how deprenyl worked to prolong the life span of these animals. Although other possibilities are not excluded, our own observations on the ability of deprenyl to increase antioxidant enzyme activities in selective brain regions, such as the substantia nigra (S. nigra) and striatum (but not the hippocampus or cerebellum), appear to include one likely possibility with regard to a mechanism underlying its effect on life span.

In this review, we summarize some of the past reports on the effects of deprenyl on the life span of animals as well as on antioxidant enzymes and will discuss the possible causal relationship between these effects. Furthermore in relation to these effects, we will also discuss several pharmacological effects of this interesting drug and its future potential.

EFFECTS OF DEPRENYL ON LIFE SPANS OF DIFFERENT ANIMAL SPECIES

Rats

The first study of deprenyl's effect on life span was reported by Knoll[3] in rats. In his study, he began administering deprenyl, sc 3 times a week, at a dose of 0.25 mg/kg to male Logan-Wistar rats at the age of 24 months.[3] In his initial study, he demonstrated that the remaining life expectancy of treated male rats after 24 months of age was more than two times longer than that of saline-treated control rats. The second study[10] on male Fischer rats given the same dose of deprenyl beginning at 24 months of age, as in Knoll's study, however, resulted in only a 16% increase in the remaining life span of treated animals.[10] In the third study, the drug administration was started in male Fischer rats at the age of 18 months at a dose 0.5 mg/kg (3 times a week, sc) and revealed an increase of 34% in the remaining life expectancy after 24 months of age[11] (FIG. 1). These studies are discussed in detail in our previous reviews.[14,15] To the knowledge of the authors, no further work has been thus far published on rats.

Mice

At least two studies have been previously published.[16,17] Interestingly, neither of these earlier studies obtained a significantly positive effect of deprenyl on longevity of the animals. The results of our own unpublished work on mice using two different doses of deprenyl (0.25 mg/kg, 0.5 mg/kg, 3 times a week) also failed to obtain significantly posi-

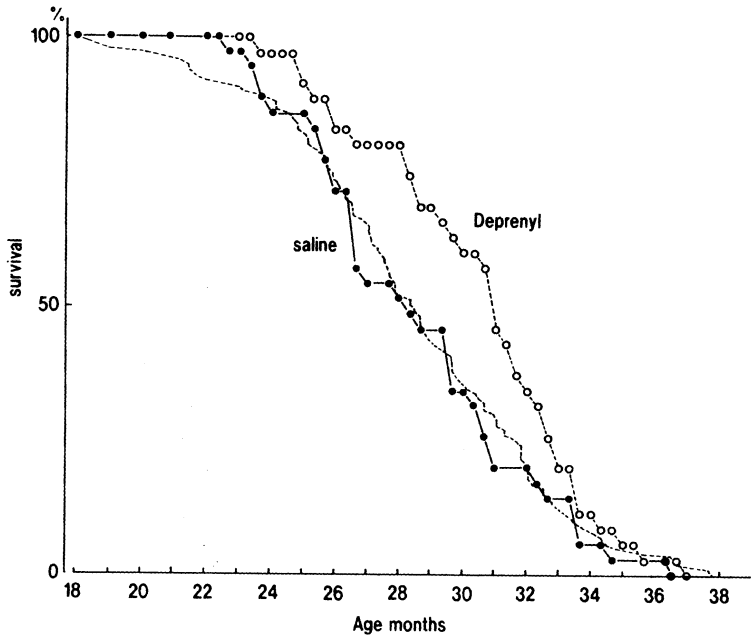

FIGURE 1. Survival curves of control (closed circles) and deprenyl-treated (open circles) rats as expressed from pooled data of three cohorts. Broken line without symbols indicates data from 100 animals raised in the specific pathogen-free farm of the institute as reported previously. (Kitani *et al.*[11] With permission from *Life Sciences*.)

tive results, although the 0.5 mg/kg dose could extend life span to some extent. Only one recent study has shown that deprenyl is also effective in significantly increasing the life span of this animal species.[18] However, mice appear to be the animal species in which a positive result is not easily obtained. For this reason our subsequent discussion will focus mainly on mice.

Hamsters

Stoll *et al.*[12] recently reported the results of their study on hamsters of both sexes (FIG. 2). Interestingly, they could demonstrate a significant effect of deprenyl on life span in female but not in male animals, although in males too, there were some positive modifications of the survival curve by the drug.

Dogs

Perhaps the most impressive result so far reported, except for the initial study by Knoll[3] on rats, is a recent study by Ruehl *et al.*[13] involving 82 beagle dogs. Although they did not see any significant difference between the life spans of young treated and control

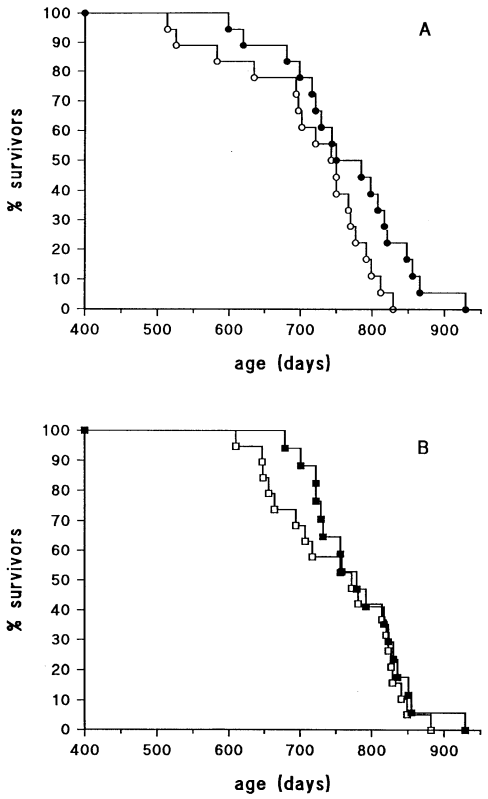

FIGURE 2. Survival curves of female (A, ○, controls; ●, selegiline) and male (B, □, controls; ■, selegiline) Syrian hamsters (female control: n = 18, female deprenyl: n = 18, male control: *n* = 19, male deprenyl: n = 17). Selegiline was provided at a dose of 0.05 mg selegiline per kg body weight per day via the diet beginning at the age of 13 months until natural death. The slopes of the curves of the female hamsters were significantly different ($p < 0.05$; chi-square = 3.85; 1, DF), analyzed by the log rank test. (Stoll *et al.*[12] With permission from the *Neurobiology of Aging.*)

dogs, when a subset of dogs older than 10 years of age was observed, there was a significantly positive effect of deprenyl on the survivorship of animals (FIG. 3). Within 800 days of observation, dogs treated with deprenyl at a dose of 1 mg/kg (oral administration, every day) could enjoy remarkably longer survivals, as shown in FIGURE 3, in comparison with placebo-treated control dogs. Twelve of 15 deprenyl-treated dogs (80%) survived to the conclusion of the study, whereas only 7 of 18 control animals (39%) survived. It is noteworthy that a remarkable difference in pathologies between the two groups was a decrease in the incidence of mammary tumors in treated animals, which is known to be prevalent in this beagle dog strain. Indeed, although no dog treated with deprenyl died of neoplasia during the observation period, 11 elderly dogs in the control group died of different kinds of neoplasia. Among seven female dogs that died of malignancies, four died of malig-

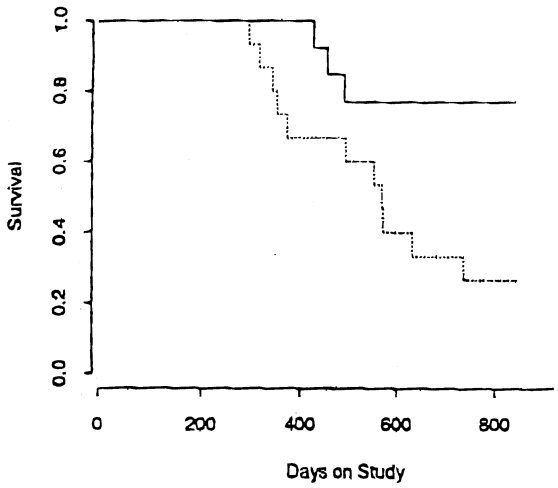

FIGURE 3. Survival of dogs between 10 and 15 years old at the start of the study and treated with deprenyl for at least 6 months. $p < 0.05$. ——, deprenyl; - - -, placebo. (Ruehl *et al.*[13] With permission from *Life Sciences.*)

nancy of mammary gland origin. We will come back to this point later, that is, the prevention of tumor occurrence by deprenyl.

EFFECTS OF DEPRENYL ON ANTIOXIDANT ENZYME ACTIVITIES

Again, Knoll was the first to point out that superoxide dismutase (SOD) activity was significantly elevated in the striatum of rats by administration of the drug for three weeks.[3] He reported, however, that catalase activities were not significantly raised. Further he showed that glutathione peroxidase (GSHPx) activities were also increased to some extent.[3] He later, however, found no significant effect of the drug on SOD activities in another rat strain and suggested that this effect of deprenyl may not always occur.[19]

SUMMARY OF OUR PAST OBSERVATIONS

Our group has worked quite extensively on the effect of deprenyl on antioxidant enzyme activities in various brain regions. Since these results have been reported elsewhere in detail,[4–9] our results will be only briefly summarized below. (1) Activities of any species of SOD (Cu, Zn-, and Mn-) can be increased by an appropriate dose of the drug, but the effect is generally greater for Mn-SOD.[4–9] (2) The effect is selective to such brain regions as S. nigra, striatum, and to some extent cerebral cortex; however, it is not selective in hippocampus, cerebellum, or the liver.[5,6] (3) To achieve an optimal increase in SOD activities, three weeks of treatment are needed, and the CAT increase is somewhat slower than that of SOD.[7] (4) Unlike the study of Knoll,[3] we found that CAT activities can be increased significantly in brain regions in which SOD activities are increased; however,

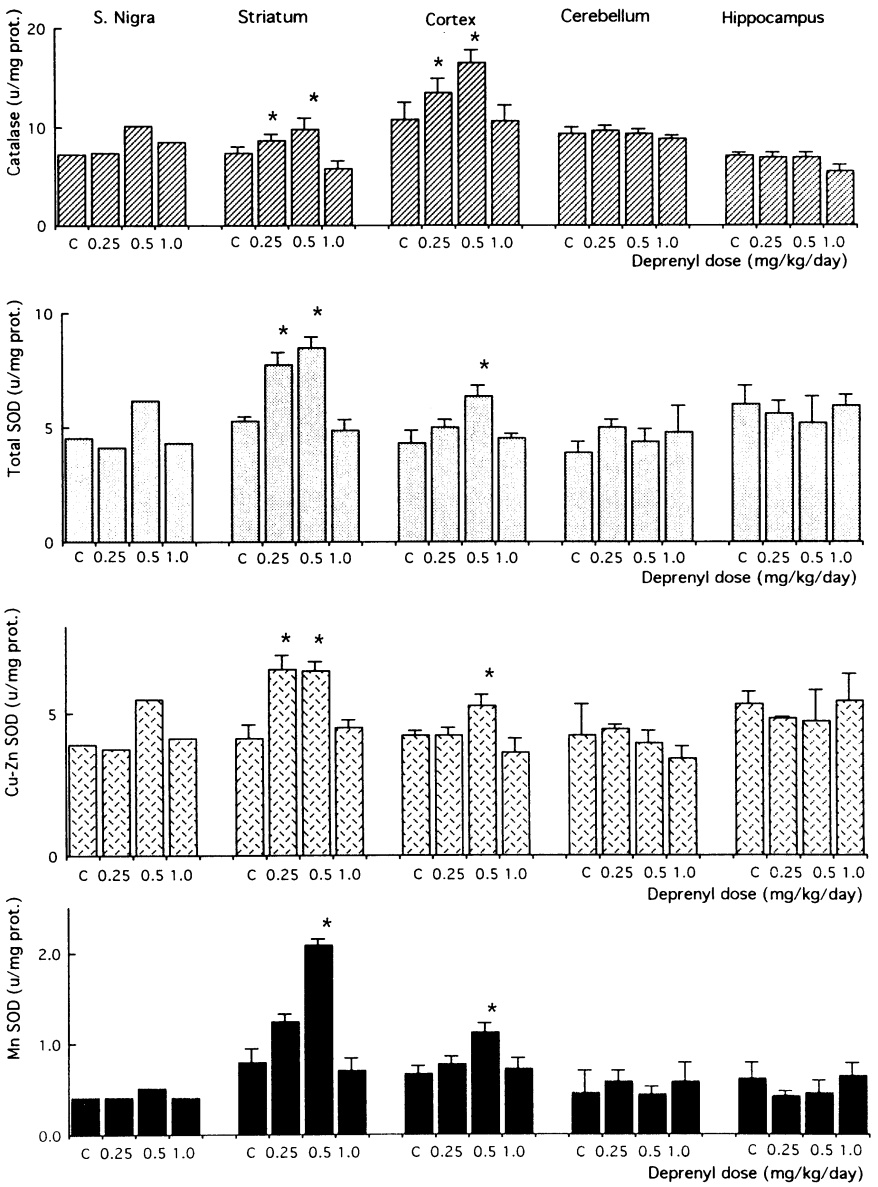

FIGURE 4. Catalase and superoxide dismutase activities in five different brain regions in old male mice treated with different doses of deprenyl for three months, starting at the age of 26 months. Doses indicated are those per injection, and sc injections were done 3 times a week for 3 months. *Significantly different from respective control values ($p < 0.05$, ANOVA + Scheffe's test). Number of animals in each group is 3 or 4.[22] C = control.

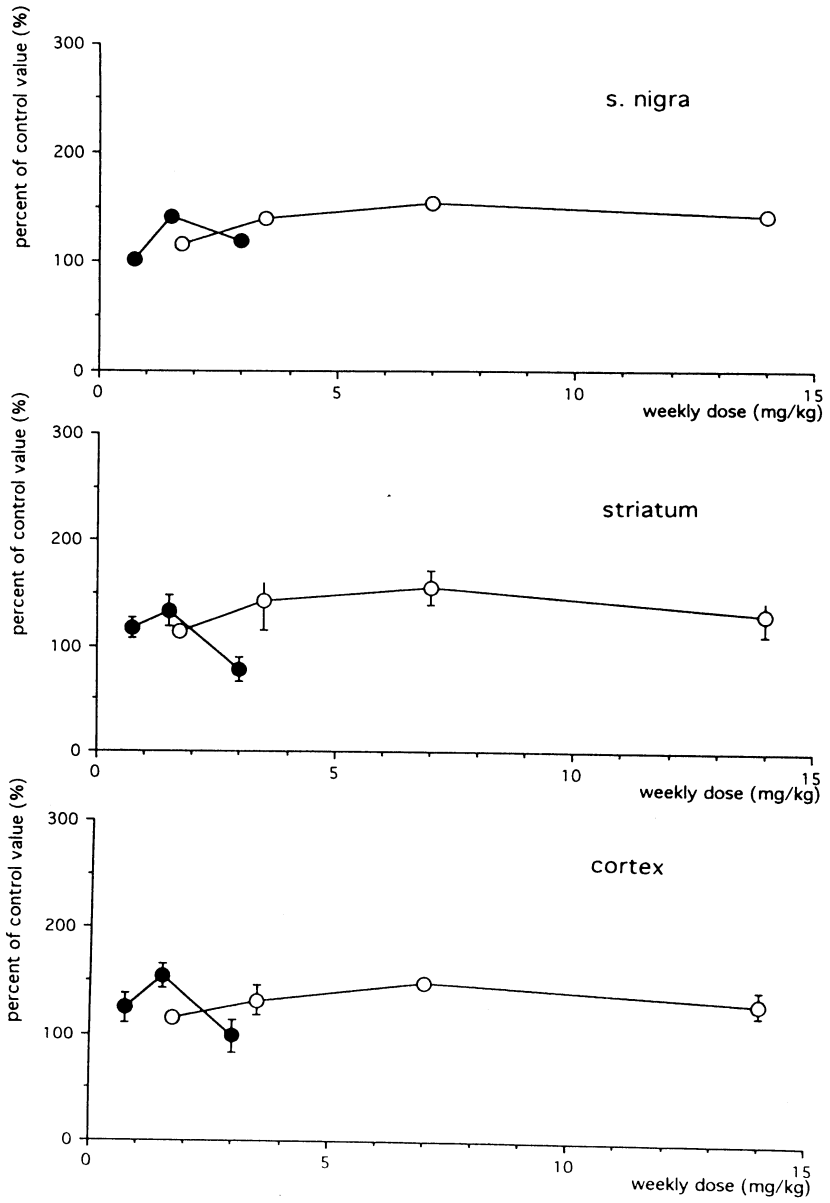

FIGURE 5. Relative catalase activities in three different brain regions in old mice treated with deprenyl for 3 weeks (open circles) or 3 months (closed circles). Enzyme activities in treated animal groups were expressed as percentages of respective values in control groups. In the short-term study,[20] deprenyl was continuously infused sc for 3 weeks (via osmotic minipump), whereas in the long-term study,[22] deprenyl was injected sc three times a week for 3 months. For the purpose of comparison, doses were recalculated as weekly doses for the two studies. (Carrillo *et al.*[22] With permission from *Life Sciences.*)

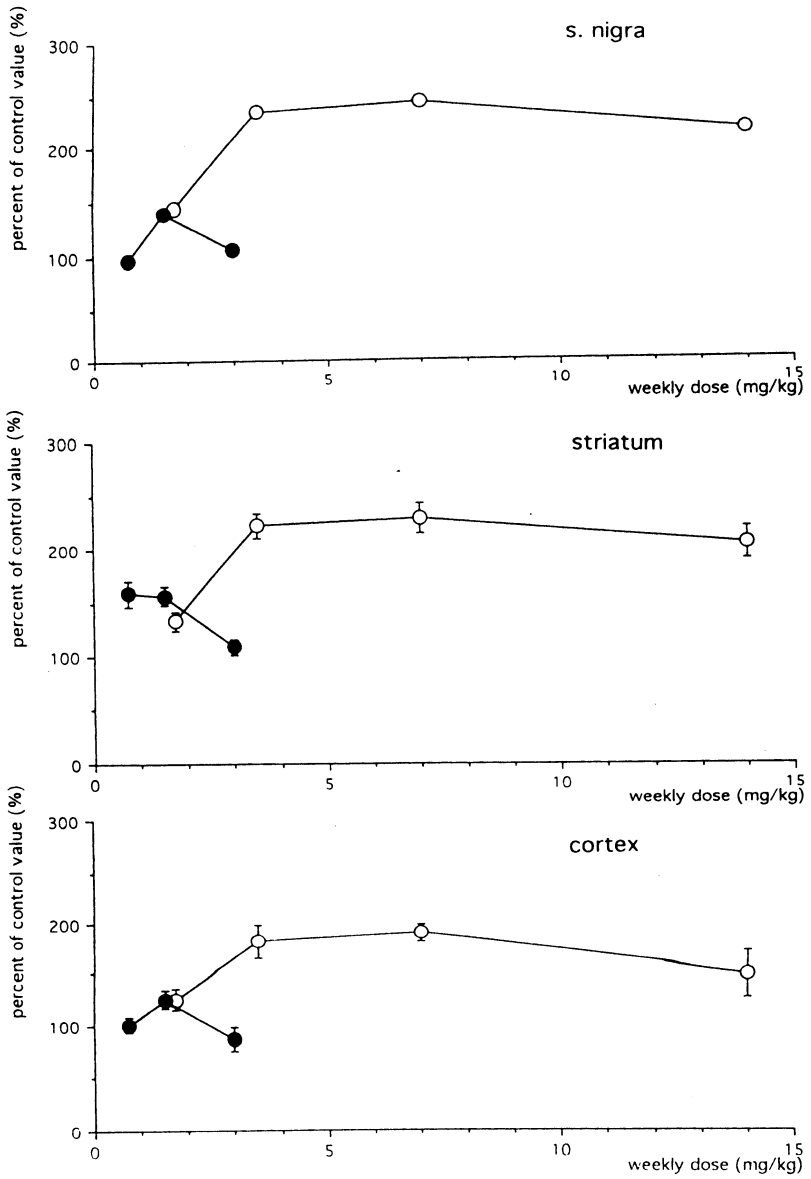

FIGURE 6. Relative Cu, Zn-SOD activities in three different brain regions in old male mice treated with deprenyl for 3 weeks (open circles) or 3 months (closed circles). Enzyme activities in treated animal groups are expressed as percentages of respective values in control groups. In the short-term study, deprenyl was continuously infused sc for 3 weeks,[20] whereas in the long-term study,[22] deprenyl was injected sc three times a week for 3 months. For the purpose of comparison, doses were recalculated as weekly doses for the two studies. (Carrillo *et al.*[22] With permission from *Life Sciences.*)

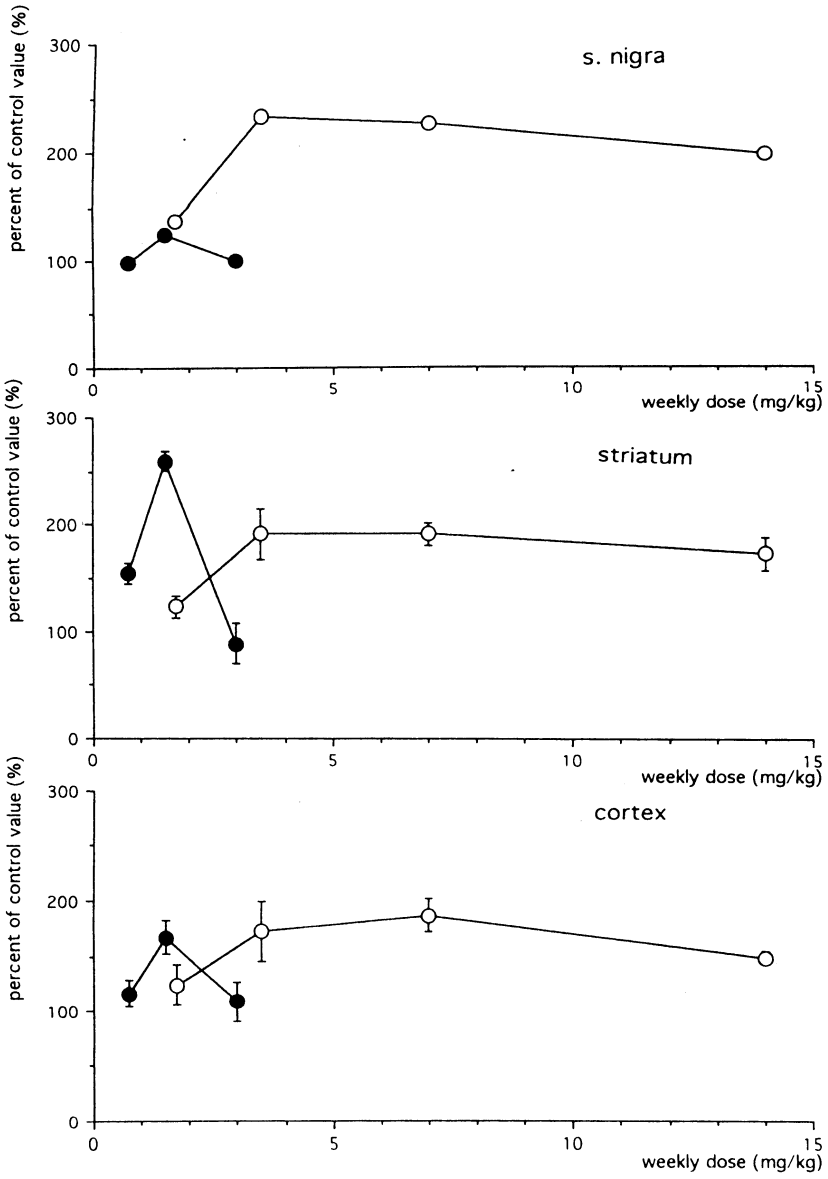

FIGURE 7. Relative Mn-SOD activities in three different brain regions in old mice treated with deprenyl for 3 weeks (open circles) or 3 months (closed circles). Enzyme activities in treated animal groups are expressed as percentages of respective values in control groups. In the short-term study,[20] deprenyl was continuously infused sc for 3 weeks, whereas in the long-term study,[22] deprenyl was injected sc three times a week for 3 months. For the purpose of comparison, doses were recalculated as weekly doses for the two studies. (Carrillo *et al.*[22] With permission from *Life Sciences*.)

there is no effect on GSHPx activities.[5,6] (5) These effects of deprenyl were demonstrated not only in rats but in mice[20] and dogs[21] as well. (6) The dose effect is remarkable. If the dose is too small, it does not cause a significant effect, and, as the dose is increased, it shows a dose-dependent increase in its effect; however, if a dose becomes excessive, the effect becomes less efficient and if it is further increased, it causes a decrease, rather than an increase in the activities, thus demonstrating a typical inverted U-shaped effect.[6,8] (7) To further complicate matters, the optimal dose is different depending on the animal species, strain, and especially the sex of rats, such that young male rats require a 10-fold higher dose (2.0 mg/kg) in comparison to young female rats (0.2 mg/kg) in order to demonstrate an optimal increase in antioxidant enzymes.[6,8] This effect may not hold for other species due to the specifics of the rat liver P450 system. (8) It should be emphasized that the age of animals is also a key factor in determining an optimal dose. In male rats, old rats require a lower dose to have an optimal effect than do young rats; however, in females, age has an opposite effect, resulting in an increase in the optimal dose with aging. Several possible causes for the variability of an optimal dose by these factors have been discussed previously.[6,8,14,15] (9) Our recent study on mice[22] has found that the duration of treatment is also an important factor, at least in this animal species. Thus, we will discuss this point in more detail.

Although a significant increase in SOD and CAT activities was previously demonstrated by us with a short-term treatment of three weeks in mice,[20] we wanted to know the effects of long-term treatment, as this would be crucial for performing a life span study.

FIGURE 4 summarizes the effects of a long-term (3 months) treatment with deprenyl in aging C57BL male mice, which began receiving injections of the drug at the age of 26 months (sc, 3 times a week). We have found that deprenyl significantly increases SOD and CAT activities; however, the effective dose range and the magnitude of increase in enzyme activities were much smaller than in a short-term treatment for three weeks.

FIGURES 5–7 summarize the comparison of the effects for a short-term (3 weeks) and a long-term (3 months) treatment with the drug. In the short-term treatment, we administered the drug continuously for 21 days, whereas in the long-term study we injected the drug three times a week. The comparison of the two studies was made on a weekly dose basis. It is apparent that long-term treatment caused (1) a narrowing of the effective dose range for both SOD and CAT activities; (2) a decrease in the magnitude of increase of various enzyme activities of SOD, with the exception of the Mn-SOD in striatum; and (3) a decrease in the optimal dose of deprenyl by a factor of 5 to 10. Although a similar tendency can be seen in rats too, especially for a decrease in the optimal dose, the narrowing of the effective dose range was not as remarkable in rats as it was in mice.[9]

A POSSIBLE CAUSAL RELATIONSHIP BETWEEN THE TWO EFFECTS OF DEPRENYL

Deprenyl is primarily a monoamine oxidase B (MAO B) inhibitor.[23] Further, numerous pharmacological effects have been reported for this drug. Some of these are summarized in TABLE 1. It should be emphasized that some of the effects itemized in this TABLE may be interrelated and may therefore not be independent. However, the prolongation of the average life span (in at least two studies, the maximum survivals were also extended[3,10]), which up to now has been demonstrated in at least four different animal species (rats,

TABLE 1. Pharmacology of Deprenyl

1. MAO B inhibition[19]
2. Antidepressant effect[23]
3. Increase in antioxidants (SOD, CAT, GSH)[3–9]
4. Catecholaminergic activity enhancer[19]
Increase of noradrenaline release
Inhibition of reuptake of dopamine
Recovery of sexual capability of old male rats
5. Life span extension[3,10–13,18]
6. Neuroprotective effect[34]
7. Radical scavenging effect[2]
8. Immunomodulation[25]

mice, hamsters, and dogs) may be the result of complex pharmacological effects of the drug and is not easily understood.

The physiological significance of increase in SOD and CAT enzyme activities in selective brain regions has remained totally unclear until recently. Some have suggested that it may have been the mere result of an oxygen crisis, possibly caused by the administration of the drug, and thus may not have had any beneficial effect on the organism, although we have insisted that such a possibility is very unlikely.[7,14,15] A recent study reported by Knollema *et al.*[24] has provided an answer to this question. After deprenyl administration for three weeks in male F-344 rats, animals were exposed to an acute hypoxia–ischemia episode followed by reperfusion. Twenty-four hours after the hypoxia treatment, animals were sacrificed, and a histological examination was done in several brain regions. They found that the previous deprenyl pretreatment had a definitive effect in preventing the neuronal damage caused by hypoxia and reperfusion in selective brain regions, such as the S. nigra and striatum, but not in the hippocampus. It is interesting that the deprenyl treatment also prevented neuronal damage in the thalamus. Although they did not examine activities of SOD and CAT, the selectivity of its effect on the dopaminergic system suggests that it is most likely that these preventive effects were due to the increase in SOD and CAT enzyme activities by deprenyl pretreatment. Although such an effect of deprenyl may have limited value in the treatment of stroke (because it requires an advance treatment with the drug prior to a stroke episode), regarding the longevity of animals, this work may have a significant impact, inasmuch as the brain dopaminergic system, especially S. nigra and striatum, is believed to be exposed to perpetual oxidative stress during aging. Thus deprenyl treatment may also be effective in preventing tissue damage due to chronic oxidative stress during aging.

HOW CAN THE PROTECTION OF THE DOPAMINERGIC SYSTEM AGAINST CHRONIC OXIDATIVE DAMAGE PROLONG THE LIFE SPAN OF ANIMALS?

The discussion herein is very speculative, with no direct proof. However, we want to raise the possibility of one mechanism of the action of deprenyl. The brain dopaminergic system is known to maintain such fundamental functions of the organism as locomotion

and reproduction. It has also been suggested that the dopaminergic system regulates many humoral factors, such as TNF and growth factor. Also, there are some suggestions that deprenyl is involved with the regulation of several interleukins.[25] Thus, once the dopaminergic system is better preserved during aging by deprenyl treatment, it may have a significant impact on the functions of the organism. For example, the dopaminergic system may work to prevent the development of tumors, possibly by releasing TNF and other antitumorigenic factors.

In our previous study on the life span of F-344 male rats, we observed one interesting phenomenon, that is, the smaller variability of body weight of deprenyl-treated rats during aging than in control rats, despite quite comparable average body weights throughout the observation period (FIG. 8).[11] Interestingly, a similar tendency can be seen in a previous Canadian study using the same strain of rat.[10] This strain of rat (Fischer 344) is notorious for developing many kinds of subcutaneous tumors late in life. Although they are mostly benign in nature, they grow so big that sometimes their weight becomes greater than the rest of the rat body, resulting in a tremendous increase in the apparent body weight. On the other hand, this strain is known to lose body weight after 24 months of age due to emaciation. These two opposing factors cause a continuous increase in the variation of body weight of animals as they become older (FIG. 8). Thus the smaller variation in body weight in deprenyl-treated rats with a comparable average body weight to control rats may mean a slower decrease of body weight with age due to emaciation and the slower development of

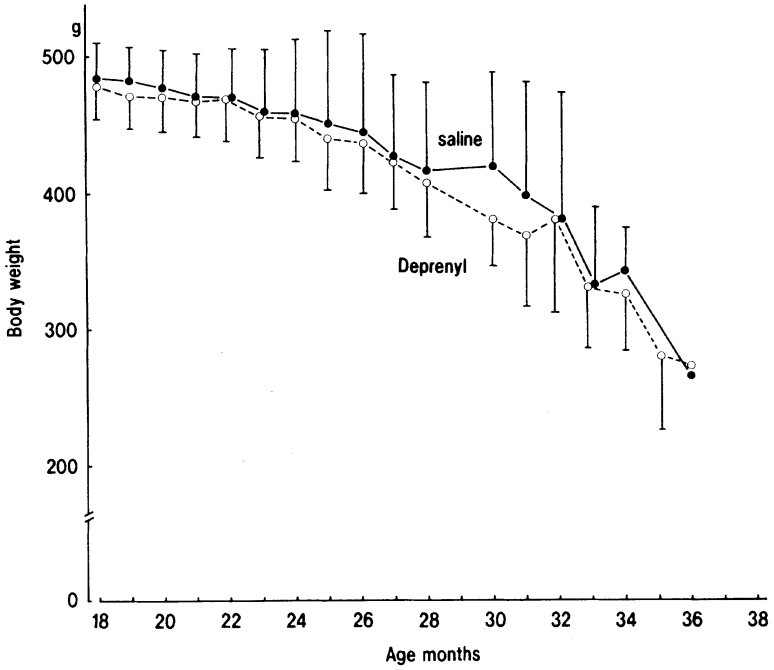

FIGURE 8. Sequential changes in body weights of control (closed circles) and deprenyl-treated (open circles) rats. Despite comparable average body weights for both groups, the variation in body weight in the deprenyl-treated group is constantly smaller than that in the control group. Vertical bars indicate 1 SD. (Kitani *et al.*[11] With permission from *Life Sciences.*)

tumors during aging in deprenyl-treated animals. We have previously suggested that one factor that caused a longer life span in deprenyl-treated F-344 rats would be the prevention or slowing of the occurrence (and development) of subcutaneous tumors, which can be an indirect cause of the death of animals.[7,11,15]

The data reported by Ruehl *et al.*[13] strongly support our previous contention that in some way deprenyl treatment can prevent tumor growth in the organism. It remains totally unknown whether this effect of deprenyl is the sole mechanism for the prolongation of the life span of animals. In the study of Stoll *et al.* on hamsters,[12] they demonstrated a significant prolongation of the averge life span despite comparable body weights. However, assuming that the increase in SOD and CAT activities is at least a partial cause for the prolongation of the life span of animals, we have a very reasonable explanation for the fact that in mice we have seen thus far the greatest difficulty in significantly prolonging their life span by the drug, inasmuch as we have shown that it is extremely difficult to maintain the higher SOD and CAT activities for a long period with deprenyl in this animal species. Although a 0.5 mg/kg (3 times a week) dose was shown to be effective in maintaining the SOD and CAT activities at levels higher than control mice,[22] it remains totally unknown how long deprenyl can maintain its effect when administration is continued for a longer time. Further, dosage could also explain discrepant results reported by Stoll *et al.*[12] between male and female hamsters, because it is quite possible that the dose used in their study was not equally effective regarding the elevation of SOD and CAT activities between the two sexes.

Other indirect evidence that the increase in SOD and CAT activities may be a causal factor for the life span prolongation of animals by deprenyl is that when the dose of 1.0 mg/kg (3 times a week) was administered in aging F-344 rats, after 13 months of treatment, the treated groups had shorter life spans than did control rats, and there was no effect of deprenyl on SOD and CAT activities in animals that survived, despite the fact that this dose was quite effective in increasing these activities, at least for one month of treatment.[26]

FUTURE PERSPECTIVES

The attempts to prolong the life span of animals by means of administration of pharmaceuticals and nutrients will very likely continue. For so-called antioxidant chemicals, which have a direct radical scavenging effect, the pharmacokinetics should be the key factor for the drug's success.[14] Deprenyl has also been claimed to be effective as a radical scavenger.[2] At present, there is no way to exclude the possibility that deprenyl's effect on the life span of animals is due to its direct effect as a radical scavenger. However, we believe that another possibility, as discussed here, is that deprenyl may be modifying the life span of animals by indirectly modulating endogenous antioxidant defense mechanisms. This, then, provides another approach to pharmacological intervention in aging and age-associated pathologies, that is, the modification of endogenous antioxidant defenses. In this context, deprenyl may be a prototype of drugs that possess such properties.

Finally, the clinical significance of deprenyl (and/or its analogues) may need some discussion. In terms of the life-prolonging effect of the drug in humans, we do not as yet have any solid optimistic evidence. Although the initial retrospecive study by Birkmayer *et al.*[27]

revealed a significantly prolonged remaining life expectancy in Parkinson's disease patients treated with deprenyl and levodopa in comparison with patients treated with levodopa only, to the knowledge of the authors, no well-conrolled study in patients has ever confirmed the significant effect of the drug regarding the life expectancy of subjects. Rather, one study from the United Kingdom[28] has reported a shorter remaining life span as well as a lesser effect of the drug in patients with Parkinson's disease. However, as far as the effect of the drug is concerned, this is the only study that reported a negative effect of the drug on the progression of Parkinson's disease, which is in contrast with a number of other well-controlled studies on Parkinson's disease[29–31] that all confirmed a beneficial effect of the drug on the course of this disease. The discrepancy between the study from the UK[28] and others[29–31] needs to be clarified in the future. However, it is the belief of the authors that the result of a single study from Britain should not be taken as evidence, for various reasons, against the further clinical trials of this most interesting drug.

The mechanisms(s) of how the drug counteracts the progression of Parkinson's disease must be carefully reconsidered. The primary idea that the drug inhibits MAO B oxidation, resulting in the decrease in the formation of such toxic dopamine metabolite(s) as 6-hydroxydopamine, so that the oxidative damage of striatal neurons is saved by deprenyl in Parkinson's disease, was originally a plausible one, inasmuch as MPTP induced parkinsonian-like disorders in experimental animals and this effect was efficiently prevented by deprenyl pretreatment.[32] However, the discovery that the drug can prevent disorders caused by MPP^+ (an oxidative metabolite of MPTP)[2,33] has blown up the initial idea. Several other suggestions are now being considered regarding the effect of the drug on Parkinson's disease. (1) There appears to be a radical scavenging effect. Wu *et al.*[2] have shown that the drug can effectively reduce the formation of salicylate adducts caused by infusion of MPP^+, suggesting that the drug may have a radical scavenging effect. This mechanism may result in the prevention of the loss of striatal neurons in Parkinson's disease. (2) Salo and Tatton have repeatedly shown that the drug has a peculiar neuroprotective effect,[34] which also may work to protect the deterioration of the striatal system in Parkinson's disease. (3) Modifications (upregulation) of SOD and CAT in the dopaminergic system, including the striatum, as discussed in this article may also have to do with the significant effect of deprenyl for Parkinson's disease.

Up to now, there is convinving evidence that the drug is not effective for amyotrophic lateral sclerosis (ALS).[35] By contrast, many arguments are now ongoing regarding its efficacy in Alzheimer's disease (AD). There have been many clinical trials on its efficacy in AD. Although some studies have reported a beneficial effect of the drug, at least on the symptomatology of AD, some other reports have argued against its effect. Only recently, one carefully controlled double-blind prospective study has reported a significant effect of the drug on the progression of AD, especially on its functional loss.[36] This study is also unique in its affirmative results on a significant effect of α-tocopherol on the progression of AD. It remains to be clarified whether these two drugs worked by means of the same mechanism(s), possibly as a direct radical scavenger, or by some other means.

Regardless of the underlying mechanism(s) of these drugs, it does not seem too optimistic to suggest that we appear to be having a clue for real pharmacological intervention in aging and age-associated disorders, which thus encourages our future endeavors in this kind of approach.

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