Life Sciences, Vol. 52, pp. 281-288
Printed in the USA

Pergamon Press

CHRONIC TREATMENT OF (-)DEPRENYL PROLONGS THE LIFE SPAN OF MALE FISCHER 344 RATS. FURTHER EVIDENCE.

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(Received in final form November 6, 1992)

Summary

Seventy male Fischer 344 (F-344) rats were treated with s.c. injection of (-)deprenyl (0.5 mg/kg, n=35) or physiological saline (n=35) 3 times a week from the age of 18 months until the time of their natural death. The fifty percent survival time was 28 months in control animals and 30 months in the deprenyl treated group. The mean survival time after the start of treatment (18 months) and after 24 months were 378.3 ± 97.4 days (mean ± SD) and 196.3 ± 97.4 days, respectively, in deprenyl treated rats and 328.7 ± 108.8 days and 146.7 ± 108.7 days in control rats. The increases in average life expectancies caused by deprenyl treatment (15% from 18 months and 34% from 24 months) were both statistically significant ($P < 0.05$, two-tailed t-test). The average body weights were comparable for both groups but the variation of body weight was greater in control groups, thus excluding the possibility that the life prolonging effect of deprenyl results from reduced dietary intake. The results confirm those of two previous studies (1,2) which reported a significant life prolonging effect of deprenyl in aged rats and lend added support to the results of a study on male F-344 rats where the effect was only marginally significant (16% increase after 24 months, P=0.048 by one-tailed t test) (2).

There have been many attempts to pharmacologically intervene in the life span of animals. However, no single pharmaceutical or chemical agent has been shown to be reproducibly effective in this regard. Thus far, the only means for significantly prolonging the life span of rodents is dietary restriction regime (for review, see 3). In 1988, however, Knoll reported a dramatic effect of (-)deprenyl on the life span of male rats of a Logan-Wistar strain. After 24 months of age when animals started to receive the drug, the remaining life span increased twofold in the deprenyl-treated group as compared to the saline treated controls. Indeed, the first deprenyl-treated animal died after all control animals had died (1). In a more recent study using male F-344 rats, the effect of deprenyl treatment was also reported to be significant, but the increase in life span by deprenyl was only 16% and the difference from the control value was only marginally significant (P=0.048) by a one tailed t-test (2).

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0024-3205/93 $6.00 + .00

Many variables may affect the results of these studies. The big difference between the two studies in terms of the life prolonging effect of deprenyl may be due primarily to a strain difference. It is, therefore desirable to repeat a study on the same strain and sex so that the results can be more directly compared. Our laboratory has worked on the effect of deprenyl on increasing antioxidant enzyme activities in brains of F-344 rats, an effect also initially reported by Knoll (1). We confirmed a significant increase in superoxide dismutase (SOD) activities in striatum (4-6) as previously reported by Knoll (1). In addition, we observed a significant increase in catalase (CAT) activity (4-6) which was not demonstrated in Knoll's study (1). Subsequent studies in our laboratory have revealed that the effect of this drug is selective for certain brain regions such as substantia nigra (s. nigra) and cerebral cortices in addition to striatum (5) but not in other regions such as hippocampus (5,6). Knoll initially suggested that the life prolonging effect of deprenyl is causally related to the increase in SOD activity (1).

In the present study, we wanted to determine if the significant effect of deprenyl on the life span of animals reported by Knoll (1) could be repeated. We used male F-344 rats for our study as was used by the Canadian group (2). We started to give (-)deprenyl earlier (at the age of 18 months) than the previous two studies in which the administration of the drug was started at the age of 24 months. Furthermore, we used a twofold higher dose (0.5 mg/kg/day) than the dose (0.25 mg/kg/day) used in the previous two studies (1,2). The results confirmed the life prolonging effect of this drug demonstrating a 30 % longer life expectancy in deprenyl-treated group than the control group after 24 months of age.

Materials and Methods

Male rats of the F-344 strain used in this study were originally purchased from Japan Charles River (Atsugi) at the age of 4 weeks in the specific pathogen-free (SPF) condition and were raised in the SPF aging farm of the institute. Husbandry conditions and survivals and pathological lesions found in the later lives of this strain have been were reported elsewhere (7). With 100 animals raised under SPF conditions in our aging farm, the 50% survival time was 28.5 months, which was comparable to corresponding values for this strain and sex of rat reported from other laboratories (2,8,9). Each of three different cohorts of rats (30, 20 and 20 animals) raised with different time intervals was randomly divided into two groups. Experimental groups were given s.c. injection of (-)deprenyl (a generous gift from Fujimoto Pharmaceutical Company, Osaka, Japan) dissolved in a physiological saline solution, three times a week at a dose of 0.5 mg/kg/day. Control groups were given an isovolumetric physiological saline solution injection. Three rats were housed in a same cage and maintained in clean conventional animal facilities after the start of the treatment. All cages were placed in racks supplied with a unilateral filtered air flow. Animals were fed CRF 1 rat pellets (Oriental, Atsugi, Japan) containing 23% protein. Pellets were pasteurized by means of autoclave-heating and added with vitamins. Drinking water was pasteurized by boiling and acidified (residual chlorine > 10 ppm, pH < 1.5). Animals were weighed every month. Except for the body weight measurements and s.c. injection of a deprenyl or saline solution, there was no intervention in the lives of these animals. Animals were observed until their deaths. After death, most animals were examined for gross pathology by autopsy. Histological examinations were also performed in tissues, if judged to be necessary especially to examine the nature of tumors found at autopsy.

All values were expressed as mean $\pm$ SD. Comparisons of the data among different cohorts given the same treatment were made by means of one-way analysis of variance (ANOVA). Since no significant difference was found among values of three cohorts (see results), all data were pooled into one group (35

rats each) and the comparisons between control and deprenyl-treated animals were made by using Student t test for unpaired values (two-tailed). P values lower than 0.05 were judged to be statistically significant.

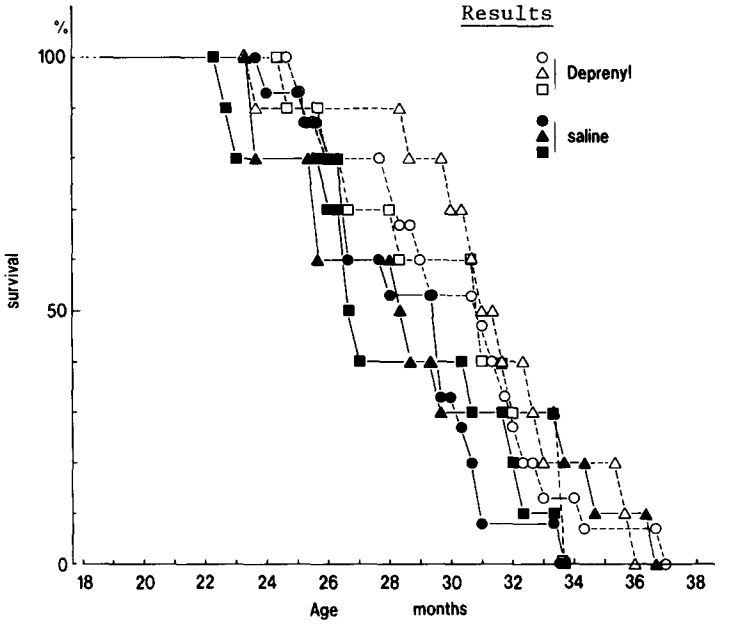

FIG. 1
Survival curves of three different cohorts given a saline (closed symbols) or deprenyl solution (open symbols).

FIG. 1 shows survival curves of three different cohorts of the two (control and deprenyl-treated) groups. In all three different cohorts, the deprenyl treated rats tended to survive longer. Mean survival times of three different cohorts are shown in TABLE I. Survival times among three different cohorts were not significantly different in either control or deprenyl-treated group (one-way ANOVA, $P>0.05$). Survival times in deprenyl-treated animals were generally longer than respective values in saline-treated control groups, however, the differences between control and deprenyl-treated rats of the same cohort were not statistically significant because of the small number of animals in each group (10 to 15).

TABLE I
Mean survival times of three different cohorts given differnt treatments

	Mean survival times (days)
Saline treatment	
Cohort 1 (15)*	872.3 ± 80.5
2 (10)	885.8 ± 134.7
3 (10)	874.3 ± 127.7
Deprenyl treatment	
Cohort 1 (15)	926.6 ± 100.4
2 (10)	932.4 ± 102.2
3 (10)	919.6 ± 98.0

*Number in parenthesis indicates the number of rats of each group.

FIG. 2 demonstrates the survival curves of control and deprenyl-treated groups in which three cohorts were pooled into one group. For comparison, the

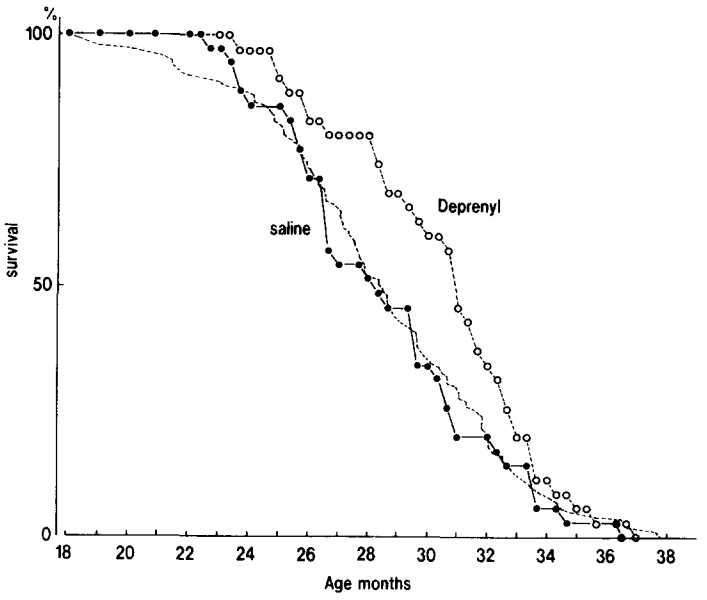

FIG.2
Survival curves of control (closed circles) and deprenyl-treated (open circles) rats as expressed from pooled data of three cohorts. Broken line without symbols indicate data from 100 animals raised in the SPF farm of the institute reported previously (7)

TABLE II
Mean survival times (days) of two different rat groups

	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*	146.7 ± 108.7	196.3 ± 97.4	33.8	<0.05
Ten % longest survivals (each n=4)	1057.5 ± 27.0	1074.8 ± 22.0	1.0	>0.05

*Survival of animals that died before 24 months were included as age in negative days.

survival curve previously obtained on 100 rats maintained in the SPF aging farm of our institute is also shown (7). Survival times of the two groups are summarized in TABLE II. It is seen that the survival curve of 35 control animals found in the present study is very close to the one previously found in the SPF aging farm of our institute. Although animals treated with deprenyl tended to live longer, the ratios of surviving animals in the deprenyl-treated groups were not significantly different from respective values in the control group at any time point during the study when evaluated by the X^2 test ($P>0.05$).

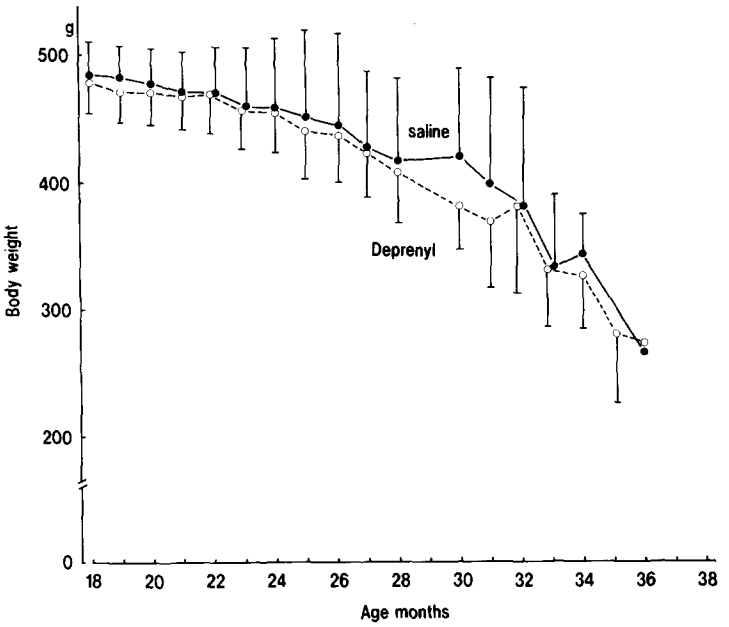

FIG. 3
Sequential changes in body weights of control (closed circles) and deprenyl-treated (open circles) rats.

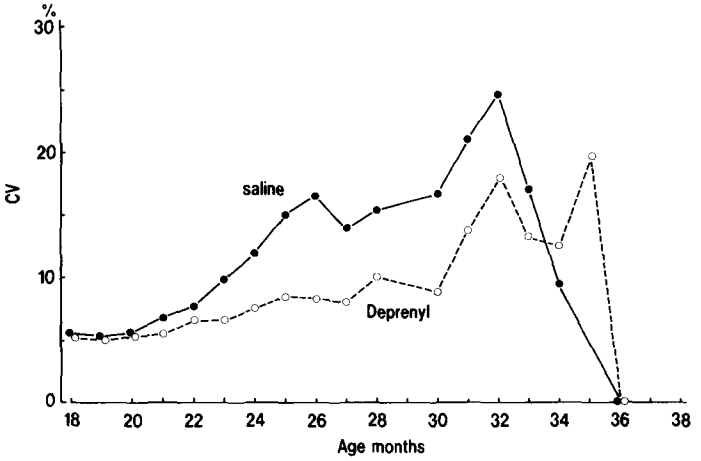

FIG. 4
Sequential changes in coefficients of variation of body weights in control (closed circles) and deprenyl-treated rats (open circles).

Mean survival times in deprenyl-treated animals were significantly longer as evaluated by Student t test (two-tailed). The mean survival time in deprenyl treated rats after 0 days, 18 months and 24 months of age were all statistically longer than corresponding values in control rats (TABLE II). In the calculation for the mean survival times after 24 months of age, three animals that died before 24 months of age were included by using minus days in age. The increase in mean survival times after 24 months in this calculation was about 34% in the deprenyl-treated group than the control group (TABLE II). Only when these three rats that died before 24 months of age were excluded from the calculation, the difference between the two groups marginally lost its statistical significance "control (n=33), 157.3 ± 102.7 days, deprenyl-treated (n=34), 202.6 ± 91.3, P=0.052, two-tailed test". However, the increase in mean life span was still 28.8% and it was statistically significant as evaluated by one-tailed t-test ($P<0.03$) as was done in a previous study (2).

FIG. 3 summarizes sequential changes in the body weight of the two groups. The mean body weight was not significantly different between the two groups at any time point studied. However, the standard deviation tended to become greater in control animals after 24 months. This is more clearly demonstrated in FIG. 4 as changes in coefficients of variation. The wider variation of body weight of control animals was due to the combined result of animals losing body weight due to a more rapid emaciation with age and animals with increasing body weight due to a growth of huge tumors in the control group.

The pathologies possibly related to the deaths of animals did not appear to be much different between the two groups. Details of pathological examinations will appear in a separate paper. Causes of death were not clearly determined in many cases due to multiple pathologies and occasional cannibalism which prevented a thorough pathological examination. Statistically, no clear difference was observed between the two groups. However, the impression that tumors of skin and muscles were generally bigger and grew earlier in control rats than deprenyl treated rats was supported by body weight changes (shown in FIG. 3).

Discussion

Although innumerable attempts have been performed in the past to prolong the life span of animals by means of administration of pharmaceuticals or chemicals, there has been no scientifically convincing and reproducible success in this attempt up to now. It is the general conclusion of experimental gerontology that the only means to make animals live longer is by dietary restriction (3,8,9). For this reason, the data reported by Knoll on deprenyl (1) was astonishing, since he reported a twofold increase in the remaining life expectancy after the start of the treatment at the age of 24 months. In fact, the first animal in the deprenyl-treated group died 2 months after the death of the last animal in the control group (1).

In comparison with the data reported by Knoll, a subsequent study by a different group on F-344 male rats was more modest (2). They found only a 16% increase in the life span after 24 months and the difference between control and deprenyl-treated groups was only marginally significant ($P=0.048$) using a one-tailed t test. In contrast, the increase of life span after 24 months in our study was more than 33% and the statictical significance was much higher. When evaluated by a one-tailed test as was done by the Canadian group, the P value was lower than 0.025 despite the fact that the number of animals in our study was about one half that of the Canadian study (each, n=60) (2). Only when three animals that died before 24 months of age were excluded from the calculation, the difference in the mean remaining life span after 24 months lost its statistical significance by a two-tailed test. However, the increase of life expectancy caused by deprenyl was still 28.8% as compared to 16% in the Canadian study, and the difference was significant when evaluated by one-tailed

test ($P<0.03$). Also it is quite conceivable that this comparison can also become statistically significant even by two-tailed test, if the numbers of animals are increased to corresponding values in the previous study (2).

Life spans of aging animal colonies differ greatly depending on husbandry conditions even for animals of the same strain and the sex (for review see Ref. 10). It is also known that life spans can differ among different cohorts even in the same husbandry conditions in the same facility (10). For this reason, the comparison of life spans for differently treated groups must be done with the utmost caution. In this regard, life spans of three different cohorts of control animals in our study were very close to each other. Furthermore, it is noteworthy that the average life spans of animals of the control group given physiological saline is close to that for our aging colony, as previously determined in our SPF facility (7), as well as for values reported in past studies (8,9) for animals presumably maintained in standard husbandry conditions. The 50% survival time for control animals is also very close to the value reported for the control male F-344 rats previously studied (2,8,9). Thus, despite possible differences in husbandry conditions, including diets, the data in past studies including ours are very comparable in regard to average life span. Accordingly the life span of control animals in our study can be regarded as that of an animal group which has been kept in standard husbandry conditions comparable with past studies (2,8,9). Thus, our conclusion that long term treatment with deprenyl can prolong the natural life span of rodents strongly supports the conclusion drawn in the previous two studies (1,2).

Although all these three studies agree in that (-)deprenyl has a significant effect in prolonging the life span of aging male rats, large quantitative differences were observed. A much greater effect of deprenyl in Knoll's study (1) than the other two studies may largely be explained as due to the strain differences. It is quite conceivable that major factors limiting the apparent life span of animals are pathologies (diseases) related to aging which differ widely among different strains of animals, as well as of animals raised in different husbandry conditions (10).

Knoll used a Wistar derived strain (1), while two other studies were made on F-344 rats. The effect of deprenyl in preventing or delaying the occurrence of certain pathologies may differ greatly among different strains. In addition, we started treatment at the age of 18 months, instead of 24 months as was adopted in the previous two studies (1,2). Furthermore, we used a 0.5 mg/kg dose instead of the 0.25 mg/kg used in the other two studies. These two factors may partly explain the difference between the result of a previous study (2) and that of ours using the same F-344 rats. If these two factors are at least partial causes for the difference in the results of the two studies, it is possible that the effect of deprenyl can be greater than that found in our study, if we find the truly optimal dosage and timing for treatments.

Another difference between our study and the other two (1,2) was found on the longest survival. Although the number of animal groups in our study was very small (each $n=4$), mean 10 percent longest survival times were almost identical for the two groups, while in the other two studies, a much clearer effect of deprenyl was found on this parameter. Even in the study from Canada, where the mean survival was only marginally significant, the mean longest survival was far greater in deprenyl treated animals and thus statistical significance was much higher ($P<0.01$). The reason for this difference is also not clear. One possibility is that our dose was not optimal in very old animals presumably becoming less effective or even adversely effective. Comparison of survival curves (FIG.2) in our study indicated that the greatest difference in the ratio of surviving animals between the two groups can be seen only in the middle of the observation period and that, as they got older, the

difference between the two groups became smaller. In fact, the longest surviving animals in the control group (1095 days) and in deprenyl-treated group (1107 days) were very close to each other. If the effect of deprenyl on the life span of animals is actually related to its effect in increasing antioxidant enzyme activities as was advocated by Knoll (1), it is possible that an over dosage leads to a deleterious effect on the life span of animals, since we have already shown that an overdosage of deprenyl decreases (rather than increases) the activities of these enzymes (6). For this reason, further trials on the effect of this drug must be done and the results must be evaluated with great caution regarding optimal dosage of the drug, which may well differ among strains, sexes and species of animals tested.

The mechanism(s) whereby deprenyl prolongs the life span of rats remains unresolved. The original contention by Knoll (1) that the increase of antioxidant enzyme activities such as SOD caused by deprenyl may prevent tissue damage caused by oxygen radials in specific brain regions is an interesting hypothesis. In this regard, we confirmed that the deprenyl treatment increases activities of not only SOD but of CAT (though not of glutathione peroxidase) in striatum of rats (4-6). Furthermore, we found that this effect is selective for certain brain regions such as striatum, s. nigra and cerebral cortex but not hippocampus, cerebellum or liver (5). Thus, it is possible that the activities of antioxidant enzymes in certain brain regions such as the nigrostriatal axis are more important in regulating the life span of animals. This however needs a more direct validation in the future. Furthermore, in view of large quantitative differences in the effect of deprenyl in prolonging the life span of animals observed among three studies, a more extensive study should be peformed using animals of different strains, species and sexes to draw a more general conclusion in regard to this very interesting effect of deprenyl.

Acknowledgements

This study was in part supported by grants in aid from Japan Foundation for Aging and Health and from Tokyo Metropolitan Institute of Gerontology. The skillful secretarial work of Ms. T. Ohara is gratefully acknowledged.

References

1. J. KNOLL, Mech. Ageing Dev. 46 237-262 (1988).
2. N.W. MILGRAM, R.J. RACINE, P. NELLIS, A. MENDONCA and G.O. IVY, Life Sci. 47 415-420 (1990).
3. R. WEINDRUCH and R.L. WALFORD, The retardation of aging and disease by dietary restriction. Charles C. Thomas, Springfield (1988).
4. M.-C. CARRILLO, S. KANAI, M. NOKUBO and K. KITANI, Life Sci. 48 517-521 (1991).
5. M.-C. CARRILLO, K. KITANI, S. KANAI, Y. SATO and G.O. IVY, Life Sci. 50 1985-1992 (1992).
6. M.-C. CARRILLO, S. KANAI, M. NOKUBO, G.O. IVY, Y. SATO and K. KITANI, Exp. Neurol. 116 286-294 (1992).
7. M. NOKUBO, J. Gerontol. 40 409-414 (1985).
8. B.Y. YU, E.J. MASORO and E. McMAHON, J. Gerontol. 40 655 (1985).
9. B.Y. YU, E.J. MASORO, I. MURATA, H.A. BERTRAND and F.T. LYND, J. Gerontol. 37 130-141 (1982).
10. J.D. BUREK, Pathology of Aging Rats, CRC Press, Florida (1978).