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MAINTENANCE ON L-DEPRENYL PROLONGS LIFE IN AGED MALE RATS

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Summary

The effect of l-deprenyl on longevity was examined in male Fischer rats Subcutaneous injections of either l-deprenyl (0 25 mg/kg) or saline were given every other day starting at 23 to 25 months of age The deprenyl-treated animals showed a significant increase in both mean and maximum survival The differences were largest in the longest surviving animals, suggesting that an earlier onset for treatment may be beneficial Analysis of body weights ruled out deprenyl-induced dietary restriction as an explanation for the group differences in survival To the contrary, after about four months of treatment, the animals on l-deprenyl showed a slower rate of decrease in body weight than the controls

L-deprenyl is a selective monoamine oxidase B (MAO-B) inhibitor which is widely used as an adjunct in the treatment of Parkinson's disease Until recently, the primary clinical benefit was thought to be an augmentation of the response to l-dopa (1) It is now known, however, that l-deprenyl also slows the development of severe motor symptoms (2) and prolongs the lifespan of patients being treated with l-dopa (3) It has been hypothesized that these effects are a result of slower degeneration of the nigrostriatal dopamine system, possibly due to the protective effects of l-deprenyl against toxic bi-products of MAO mediated metabolism (4,5) This hypothesis is consistent with evidence that l-deprenyl can counteract (4,6) the neurotoxic effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, a drug which can induce Parkinson-like symptoms and brain damage (7)

The suggestion that long-term administration of l-deprenyl delays degeneration of the nigrostriatal dopamine system has prompted speculation that l-deprenyl may also have applicability to normal aging Both dopamine levels and levels of dopamine type 2 receptor binding are known to decrease during aging (8) Since these processes reflect a deterioration of brain dopamine systems, it was hypothesized that l-deprenyl might also be able to delay brain aging (5) In support of this hypothesis, Knoll, Dallo & Yen (9) reported a significant increase in lifespan in rats injected with l-deprenyl three times weekly starting at 24 months of age Indeed, they found that the first death in the deprenyl group occurred almost two months after the last death of the control animals Further, the authors observed an increase in sexual activity in the deprenyl-treated rats These results were attributed to l-deprenyl providing

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protection of the nigrostriatal dopamine system Unfortunately, neither food intake nor body weights were reported, and it could not be established whether maintenance on l-deprenyl decreased caloric intake, which is known to prolong survival (10)

The present work was part of a larger investigation into the effects of l-deprenyl on brain aging Here, we report an analysis of the effects of deprenyl on survival in two experiments which used death of the subjects as the endpoint

Methods

Subjects were male Fischer 344 rats obtained from Harlan Sprague Dawley breeding farms in two batches at ages 21-22 months and 22-23 months In the first experiment, 62 animals from the first batch were randomly assigned to either a deprenyl (N=31) or control group (N=31) at 24 to 25 months of age, three months after the animals had been received from the breeding farm Blood samples were taken from these animals at the start of the experiment and again after 3 months, the animals were not subjected to any other experimental procedures The serum chemistries were blindly analyzed by a commercial laboratory (Vita Tech Canada) From each sample, blood concentrations were determined for A/G ratio, albumin, bilirubin, blood urea nitrogen (BUN), creatinine, glucose, total protein, SGOT and SGPT

Seventy animals (35 deprenyl and 35 controls randomly assigned from the second batch) were used in the second experiment, which started when the animals were between 23 and 24 months of age, 30 days after the animals had been received These animals were tested bimonthly for sexual behavior and monthly for locomotor activity and sensory-motor abilities The results of the behavioral testing will be reported elsewhere

L-deprenyl was dissolved in physiological saline and a 0 25% solution was prepared and injected subcutaneously using a dose of 0 25 mg/kg on every other day The controls were injected with an equivalent volume of physiological saline The drug dosage and route of administration were the same as used by Knoll, Dallo & Yen (9) and has previously been found to be effective in enhancing activity of the nigrostriatal system (11) The endpoint of both experiments was morbidity or death In some instances animals were sacrificed because of persistent stress or discomfort due to the growth of tumors These animals were assumed to have died naturally and were included in the analysis of the data At death, necropsies were performed The liver, spleen, heart, lungs, thymus, prostate, pituitary, adrenals, kidney, and testes were removed, weighed and fixed in formalin Determination of cause of death was based primarily on the results of the necropsies This information was supplemented by the results of the serum biochemistries in experiment 1

Results

In the analysis of longevity, animals were assigned a score equal to their survival in days following the start of treatment The results include data from 5 animals, 4 from the deprenyl and one from the saline group which were sacrificed because of tumors Exclusion of these animals from the study would not have changed the significance of the results The distributions of survival times for both experiments were found not to deviate significantly from normality using a Kolmogorov-Smirnov test for goodness of fit Furthermore, there was no censoring of

data, since all subjects completed the study We therefore used the student's t-test for group comparisons

We first compared the mean survival times between the two experiments and found no significant difference (p= 457) Consequently, the data from the two experiments were pooled for subsequent comparisons The results are summarized in Table I As a group, the deprenyl-treated animals survived significantly longer than did the controls This effect was largely due to differences in the longest surviving animals, as indicated by the magnitude of the difference between groups in maximum (90th percentile) survival

In the majority of cases, death could not be attributed to a single factor For example, many animals had tumors, and liver and kidney problems The data are summarized in Table II and do not reveal any significant differences between groups

TABLE I

Effect of L-Deprenyl on Three Measures of Survival in Male Fischer Rats

Group	Mean Survival	Maximum[3] (90th Percentile) Survival	Longest Surviving Animal
Control	(N=66) 114 7 ± 7 7[1]	(N=7) 212 1 ± 8 9[2]	251
Deprenyl	(N=66) 133 7 ± 8 3	(N=7) 248 4 ± 11 7	315

Scores represent means ± SEM in days following the start of the experiment
[1]Deprenyl treated rats were significantly different from controls (P= 048) using a one tailed test
[2]Difference between groups was significant (P= 015) using a one tailed test
[3]Maximum survival refers to the mean of the longest surviving 10% of the animals as defined by Weindruch and Walford (10)

TABLE II

Probable Causes of Death

	Multiple Factors	Tumor	Renal	Liver	Other*
Controls GR	35	15	16	1	0
Deprenyl Group	31	15	11	6	3

* GI tract, Cardiovascular

The serum biochemistry data are shown in Table III The only measure significantly affected by l-deprenyl was BUN which was higher in the control group at the 3 month test

Body weights were recorded on alternate days The analysis of the data was based on the mean body weights over successive 8 day periods Standard analysis of variance could not be used because of the decrease in sample size over repeated testing Therefore, the groups were compared using repeated student's t tests The results are summarized in Fig 1, which shows that there were no differences between the two groups during the first four months of the study In both groups, body weight decreased as the populations aged Fig 1 also illustrates that there was a divergence in body weights at about four months, and on five subsequent tests (representing a 40 day period), rats in the deprenyl group were significantly heavier than the controls

TABLE III

Effect of l-deprenyl on Measures of Serum Chemistry

	BASELINE			3 MONTHS		
Measure	Control N=24	Deprenyl N=29	Correlation with survival	Control N=22	Deprenyl N=23	Correlation with survival
A/G Ratio	97 ± 02	97 ± 03	10	75 ± 02	81 ± 04	14
Albumin g/L	29 92 ± 71	29 76 ± 89	13	24 0 ± 73	25 62 ± 74	51**
Bilirubin umol/L	3 05 ± 25	2 93 ± 24	-26	4 19 ± 41	4 43 ± 98	-35
BUN mmol/L	8 45 ± 29	8 18 ± 22	-12	14 02 ± 1 61	9 95 ± 45[1]	-51**
Creatinine umol/L	73 13 ± 1 97	70 93 ± 1 61	-01	85 28 ± 5 82	75 52 ± 2 68	-13
Glucose mmol/L	7 73 ± 1 03	7 19 ± 73	11	7 87 ± 76	8 50 ± 62	38*
Total Protein g/L	60 58 ± 4 93	60 31 ± 95	17	56 73 ± 1 47	57 69 ± 93	57**
SGOT U/L	108 96 ± 8 43	121 41 ± 1 10	07	135 32 ± 17 10	135 79 ± 32 00	47*
SGPT U/L	63 96 ± 4 72	64 52 ± 4 56	05	60 04 ± 6 03	67 04 ± 14 68	45*

Scores represent means ± SEM Correlations are with days survival from the start of the experiment
[1] significantly different from controls using a two-tailed test (P= 017)
* significant at 01 level
** significant at the 001 level

Discussion

These results demonstrate that the lifespan of aged rats can be extended by regular administration of l-deprenyl starting late in life Measurements of body weight provided no evidence that l-deprenyl suppressed food intake Thus, the effect of l-deprenyl on survival is not a secondary consequence of dietary restriction Indeed, we found a divergence in body weights after about four months, with the deprenyl group being heavier than the controls This divergence in body weights is consistent with the results of the survival data

As previously discussed, it was originally predicted that l-deprenyl would delay brain aging The analysis of the serum chemistries suggests that l-deprenyl also affects the aging of other organs At three months, the controls had a significantly higher level of BUN Since high levels of BUN are indicative of renal impairment, l-deprenyl appears to provide protection of renal function It is not clear whether this is a direct or indirect effect, but the result does provide one possible explanation for the increase in lifespan in rats treated with l-deprenyl In the present experiments the analysis of the necropsy material neither confirmed nor refuted this suggestion, since multiple factors contributed to the death of the majority of animals and since kidney

pathology was evident in the vast majority of both control and deprenyl-treated rats in this category

Although our results are consistent with previous work by Knoll et al , (9), we observed a much smaller effect The differences in magnitude probably reflect strain differences Knoll used a hybrid cross between Logan females and Wistar males, which had a mean lifespan of 35 months In contrast, the mean lifespan of our controls was 28 months, and we had no rats surviving to 35 months Our experiment started with rats at 23 to 25 months of age Previous studies starting at an earlier age have reported a mean lifespan of 22 to 24 months for the Fischer 344 rat (12,13) On the other hand, dietary restriction starting at 6-7 months can extend the mean lifespan of this strain to 35 months (12)

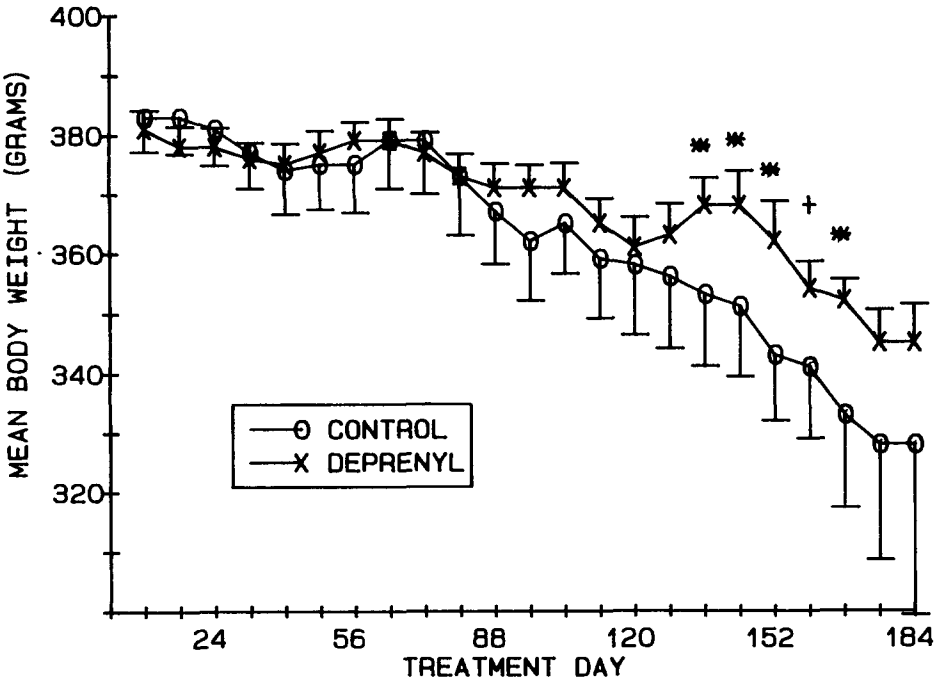

FIG 1

Changes in body weight in deprenyl-treated animals and controls Values show means and S E M Levels of significance are ** = $p < .05$, + = $p < .10$ (two tailed tests)

The strain differences could be important for two reasons One possibility is that many of our animals were already too old or sick for deprenyl to have had an effect It seems unlikely, for example, that much could have been done to prolong the life of most of the animals which died within the first two months At the start of the experiment the body weights of some of the animals were very low, which is generally indicative of poor health and impending death In other cases, tumorigenesis had already begun The second reason involves the duration of deprenyl treatment It is conceivable that the effect of deprenyl is cumulative, requiring several months of treatment before significant effects on mortality are established This

suggestion is consistent with evidence by Knoll et al (9) that deprenyl increased sexual behavior maximally between the 28th and 36th week of treatment

There are interesting parallels between our results and the results of clinical trials on patients with Parkinson's disease Deprenyl is less effective when treatment is started at an advanced stage of the disease (14) In a report by Birkmayer and Birkmayer (3), patients given both l-deprenyl and l-dopa survived 12% longer than patients given only l-dopa Similarly, our old rats on l-deprenyl survived approximately 16% longer than our controls In contrast, Tetrud and Langston (2) studied Parkinsonian patients who had had the disease for less than 5 years, and found that l-deprenyl delayed the development of the disease by almost 76% Also the rats treated with l-deprenyl in Knoll's experiment began treatment at a relatively earlier point in their lifespans than did rats in our study, and his rats survived 210% longer than the controls It seems very likely, therefore, that age and physical status at the start of treatment are critical covariates in predicting the response to long-term administration of l-deprenyl Further research on the role of both strain and age differences is clearly called for

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