Influence of L-Deprenyl Treatment on Mouse Survival Kinetics

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INTRODUCTION

The study of the mechanisms of aging received an important momentum from the use of animal models whose life span can be modified by definite controlled interventions. These models present, however, their limits when the interpretation of the results is attempted. At present, in fact, there is no agreement about how to measure the aging rate of a population or the biological age of an individual.[1] Even when the findings seem clear-cut as happens in caloric restricted rats, caution is necessary in extrapolating results to animals living in unrestricted conditions.

Despite the limitation that any model may have, manipulations able to prolong life span, and maximal life span in particular, remain among the most powerful tools to gain insights into basic aging processes. Thus, any new way of extending life is welcome, as from a multifaceted view a more complete and understandable picture can stem. In this framework, the observations of some authors on the effects of the monoamine oxidase inhibitor L-deprenyl on aged animals sound very interesting.[2-4]

It has been observed that this drug can prolong the life span of treated rats and improve their sexual activity at the same time.[5,6] Interestingly, the treatment was effective when started at the end of the second year of life.[5,7] It is also worth stressing that L-deprenyl can be considered a safe drug, thus very useful in long-term experiments.[4,6] In addition to the interest due to these characteristics, our attention was drawn by the possible relationships of its mechanism of action with that at the basis of our previous findings in old mice: age-related alterations of the adrenergic system in different tissues of aging animals.[8-11] These changes were demonstrated to be not definitive, being corrected by endocrine manipulation exerted just a little before two years of age.[12-14]

Since our previous experimental work on aging was done on mice, first of all a survival experiment was planned with the strain of mice of our own laboratory. The drug was administered to 22-month-old mice, as one of the most interesting characteristic to verify was the efficacy of a treatment starting late in life.

MATERIALS AND METHODS

Animals

Animals were taken from our own colony of Balb/c-nu mice. The term "nu" refers to the recessive nude mutation introduced into inbred Balb/c mice by crossing

them with nude mutants. Three animal groups of different ages (a two month difference from each other) were used, each one further subdivided into L-deprenyl-treated and control ones. The animals (D mice) of the three treated subgroups ($n = 62$) were injected subcutaneously with 0.25 mg/kg bw L-deprenyl three times a week until time of natural death. The animals (C mice) of the three control subgroups ($n = 46$) received saline using the same schedule. Treatments started when animals of each subgroup were 22 months old. Survivals were checked daily, while body weights were assayed individually once a week. At the end of the experiment, data from subgroups were collected together. L-Deprenyl was kindly donated by Chiesi Farmaceutici S.p.A., Parma, Italy.

Survival Analysis

Survival data were analyzed using both the nonparametric test of Kolmogorov-Smirnov (SPSS package) and a parametric mathematical model of survivorships proposed recently.[15] An outline of the parametric model is given as follows. It is based on two aspects of survival kinetics: the deterministic component of aging rate and the statistical distribution of vitality among the subjects of a population. Vitality is used with the meaning of an index of comprehensive biological efficiency which allows the organism to survive.[16] Let $Z(v,t)dv$ be the probability that an individual of age t has vitality in the range $v - v + dv$:

$$Z(v, t) = \mathrm{Fc}^{-1}[Z_1(v, t) - Z_2(v, t)]; \quad v < 1$$

where $Z_1(v,t)$ represents a normal distribution with mean $\mu(t)$ and standard deviation $S(t)$. $Z_2(v,t)$ only differs from $Z_1(v,t)$ in having its mean shifted of an amount equal to $2S(t)$; in this way, the resulting distribution of the positive vitality values has the upper boundary $v = 1$. Fc is a correcting factor which gives $l(0) = 1$:

$$\mathrm{Fc} = \int [Z_1(v, t) - Z_2(v, t)]dv \quad \text{for } t = 0$$

If we assume unity as an upper bound for v, that is, $Z(v,t) = 0$ for all $v > 1$, the probability $l(t)$ that an individual survives to age t is given by

$$l(t) = \int_0^1 Z(v, t)dv;$$

The function $\mu(t)$ is linked to $S(t)$ as

$$\mu(t) + S(t) = 1$$

and has the following structure:

$$\mu(t) = 1 - S_0 + S_0^{10} - S_0^{10} \exp \frac{\omega t}{S_0}$$

Sometimes it can be more useful to represent the survival function reporting the absolute number of subjects $L(t)$ alive at any age t instead of the survivorship probability $l(t)$. Thus

$$L(t) = N_0 l(t)$$

where N_0 is representative of the initial number of individuals present in the cohort at birth.

Thus, the model here outlined contains only two parameters, ω and S_0, whose

values can be determined fitting specific survivorship curves. The parameters are related to deterministic and stochastic factors: ω, a deterministic component describing the environmental and genetic influence on physiological functions, also used as an index of the aging rate of the population;[1] S_0, a stochastic component representing the fluctuating interaction of the living organism and its environment. Roughly, the deterministic parameter ω is related to the maximal life span of the population studied, while the stochastic parameter S_0 is linked to the shape of the curve.

The fitting of the model to experimental data was performed by Newton-Gauss nonlinear regression analysis, as described by Snedecor and Cochran.[17] Mean body weight (mbw) data were fitted by nonlinear regression analysis using Fig. P package, Biosoft.

RESULTS

Survival data are reported in FIGURE 1A. No dramatic changes took place in D mice with respect to the C ones, although in the middle period of the treatment, D mouse survival kinetics are slightly slower when compared with those from controls. The expected deprenyl-induced increase in maximum life span does not occur in our strain of Balb/c-nu mice. As a matter of fact, according to the nonparametric test of Kolmogorov-Smirnov, the two curves show no statistically significant differences. Since nonparametric tests are safe but often lack of power and do not give any information about the possible interpretation of the differences eventually observed,[1,18] the same data have been analyzed using the model outlined in the section Materials and Methods. The resulting fitting curves are shown in FIGURE 1B, while the goodness of the fit can be seen in FIGURES 1C and 1D. As expected, the two curves are very close to each other; the values of their estimated parameters together with their standard errors are given in the legend. While the difference between the two values of the parameter ω is extremely small, so that it hardly has any biological meaning, the difference found between S_0 values, although not dramatic, may deserve some attention.

Data on mbw of C and D mice are reported in FIGURE 2A. At first glance, the two patterns seem to present no differences. Both groups of mice show a slow progressive decrease in mbw with advancing age. However, a more thorough analysis shows that the two kinetics are different even from a qualitative point of view. While D mice present a roughly linear decrease of mbw, controls show a faster, nonlinear decreasing trend. When both sets of empirical data are fitted using a second-degree polynomial function, the two resulting curves are those shown in FIGURE 2B. The goodness of fit of C and D mouse data can be seen in FIGURE 2C and 2D, respectively. It is worth noting that D mice show a lesser degree of variability in mbw than C ones.

DISCUSSION

One of the most fruitful approaches to investigating the mechanisms of aging processes is the study of the animals with modified life span. Particularly important are the experimental models of extended maximal life span. At present, the caloric restricted rat is the best studied animal model, and many interesting data have recently been collected.[19] The efficacy of other treatments, such as antioxidant supplementation, is still questionable. In fact, some positive findings on survival

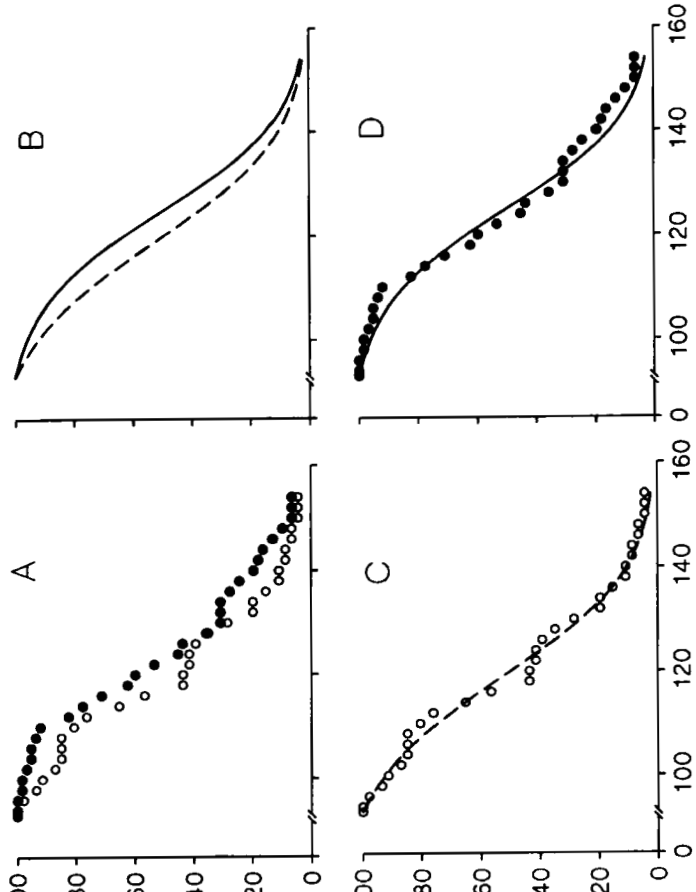

FIGURE 1. Survival data of C (open circles) and D (filled circles) mice. Data from both groups (A) are fitted using the mathematical model outlined in the section Materials and Methods. The two best fits are compared in B. They are also reported separately in C and D together with empirical data to better see the goodness of the fit. The values of the parameters ± SE for the best fits are given as follows: C mice, $\omega = 0.0275 \pm 0.0002$, $S_0 = 0.418 \pm 0.005$; D mice, $\omega = 0.268 \pm 0.0002$, $S_0 = 0.374 \pm 0.007$. D mice show a 10.5% decrease in S_0 value, which is statistically significant ($p < 0.01$).

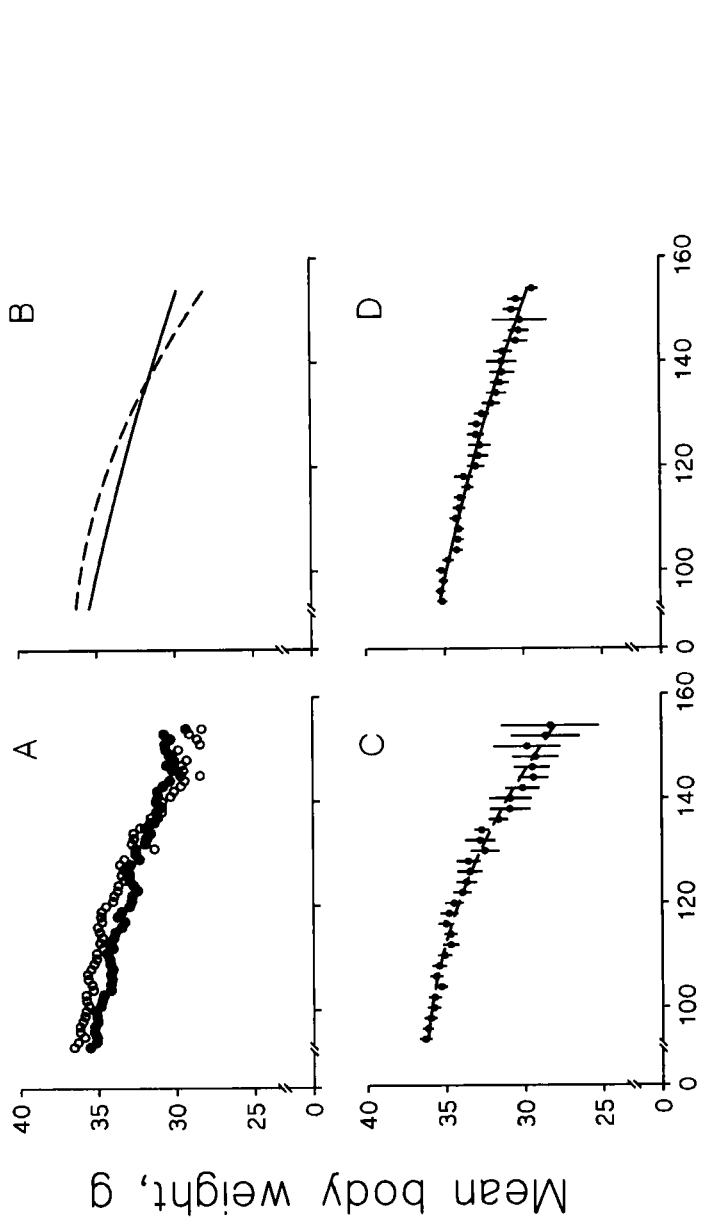

FIGURE 2. Data on mbw from C (open circles) and D (filled circles) mice are reported in A while their fitting curves are shown in B. Both C and D data and their own fittings are reported in the figures C and D, respectively. In order to avoid confusion, figures C and D report only every other point ± SEM, but all data were considered in the fitting procedure. Fits were first performed using linear functions. However, only D mouse data gave a good fit, thus the procedure was repeated with the equation $y = mx^2 + nx + p$. This function improves the goodness of the fit of C mouse data only, eliciting a qualitative difference in the kinetics of the two sets of data. The figures report the parabolic fits in order to make the comparison easier.

could be due to indirect underfeeding as a result of the bad taste of antioxidants given orally. As regards the caloric restriction itself, some basic questions cast some doubts on its use as a model of natural aging. On the one hand, we wonder whether restricted rats may live longer than ad libitum fed controls as a result of life shortening of controls due to their overeating. On the other hand, other important aspects to be taken into account are those concerning the survival capacity of restricted animals in nonprotected natural environments, or the interspecies differences in treatment efficacy.[20]

Interspecies differences have also to be taken into account in the analysis of present results, as L-deprenyl effects on survival obtained by other authors on rats differ from that found on our strain of mice. In fact, we did not observe any influence on maximal life span, while in other authors' experiments on rats, a life-prolonging effect was shown.[5–7] Since considerations about maximal life span are not reliable,[1,20] the aging rate of the population is often estimated. Even in this case, hardly any difference can be shown as the parameter ω entering our model is just an index of the aging rate. It seems safe to conclude that the schedule used in treating Balb/c-nu mice with L-deprenyl does not have any influence on the mechanisms that may control the pace of "physiological" aging.

Some feeble positive effects seem nevertheless to be present in treated mice when compared with their controls. The rate of mortality was slightly lower in the middle part of the survival curve, that is, a sort of mild rectangularization of survivorship was obtained. The very small difference between the two curves, which is not statistically significant according to the Kolmogorov-Smirnov test, would hardly have any interest per se. However, when a more sensitive tool such as a parametric model[15] is used, the slight changes induced in D mouse survival kinetics may reveal some interesting aspects. While the difference in ω values has not much biological meaning, that observed in S_0 deserves some attention, despite being as small as about 10%. Since S_0 is assumed to represent the stochastic component of the survival kinetics, its decrease can be representative of a narrower range of variability in the physiological functions of the D mouse population. Moreover, since maximal life span is not affected, the treatment is likely to influence most individuals with less physiological capacity. These considerations on individual function variability are supported by findings on mbw. D mice show lower standard error of the mean (SEM) of mbw than controls, a fact that is self-evident when the tails of the two curves are compared. Further support comes from the different kinetics of mbw: D mice show a decrease in mbw with age that is linear and slower than the parabolic one of their controls. The slowing down of the usual age-related decrease in D mouse mbw, already reported by other authors in rats,[7] also allows us to exclude an indirect undernourishment as the cause of the slightly different survival kinetics.

It is likely that this paper raises new questions instead of solving some. Certainly, many factors can be involved in and modulate any drug treatment. Species, duration of treatment, age at start, and breeding conditions are among the most important ones. The few data available until now on L-deprenyl survival effects might suggest that the same treatment induces a deep influence on basic processes of aging in rats prolonging their maximal life span, while it has no effect on maximal survival in mice, where it seems to have a beneficial influence on some pathological conditions, reflected in the mild rectangularization of their survival kinetics. It is not known, however, whether a more precocious beginning of treatment or different doses may be more effective even on mice. It may also be worth noting that from an applicative point of view, a strategy devoted to rectangularizing the survival curves may be more fruitful than one trying to prolong maximal life span, at its own turn more useful in studying basic aging processes.

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REFERENCES

1. PIANTANELLI, L., A. BASSO & G. ROSSOLINI. Ann. N.Y. Acad. Sci. (In press.)
2. KNOLL, J. 1982. *In* Strategy in Drug Research. G. A. B. Buisman, Ed.: 107–135. Elsevier. Amsterdam, the Netherlands.
3. KNOLL, J. 1985. Mech. Ageing Dev. **30:** 109–122.
4. BURCHINSKY, S. G. & S. M. KUZNETSOVA. 1992. Arch. Gerontol. Geriatr. **14:** 1–15.
5. KNOLL, J. 1988. Mech. Ageing Dev. **46:** 237–262.
6. KNOLL, J., J. DALLO & T. T. YEN. 1989. Life Sci. **45:** 525–531.
7. MILGRAM, N. W., R. J. RACINE, P. NELLIS, A. MENDONCA & G. O. IVY. 1990. Life Sci. **47:** 415–420.
8. PIANTANELLI, L., R. BROGLI, P. BEVILACOUA & N. FABRIS. 1978. Mech. Ageing Dev. **7**(3): 163–169.
9. PIANTANELLI, L., P. FATTORETTI & C. VITICCHI. 1980. Mech. Ageing Dev. **14:** 155–164.
10. VITICCHI, C., R. GRINTA & L. PIANTANELLI. 1990. Gerontology **36:** 286–292.
11. PIANTANELLI, L., S. GENTILE, P. FATTORETTI & C. VITICCHI. 1985. Arch. Gerontol. Geriatr. **4:** 179–185.
12. PIANTANELLI, L., A. BASSO, M. MUZZIOLI & N. FABRIS. 1978. Mech. Ageing Dev. **7**(3): 171–182.
13. ROSSOLINI, G., C. VITICCHI, A. BASSO & L. PIANTANELLI. 1991. Int. J. Neurosci. **59:** 143–150.
14. VITICCHI, C., S. GENTILE & L. PIANTANELLI. 1989. Arch. Gerontol. Geriatr. **8:** 13–20.
15. PIANTANELLI, L., G. ROSSOLINI & R. NISBET. 1992. Gerontology **38:** 30–40.
16. PIANTANELLI, L. 1986. Arch. Gerontol. Geriatr. **5:** 107–118.
17. SNEDECOR, G. W. & W. G. COCHRAN. 1989. Statistical Methods. Iowa State University Press. Ames, Iowa.
18. MODE, C. J., R. D. ASHLEIGH, A. ZAVODNIAK & G. T. BAKER. 1984. J. Gerontol. **39:** 36–42.
19. YU, B. P. 1990. *In* Review of Biological Research in Aging. M. Rothstein, Ed. **4:** 349–371. Wiley-Liss. New York, N.Y.
20. GAVRILOV, L. A. & N. S. GAVRILOVA. 1991. The Biology of Life Span: A Quantitative Approach. Harwood Academic Publisher. London, England.