INDEPENDENT CHEMICAL REGULATION OF HEALTH & SENESCENT SPANS

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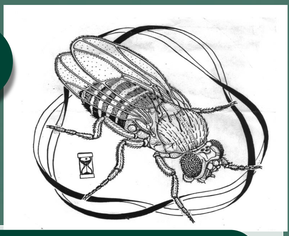


ABSTRACT

Standard protocol to identify pro-longevity drugs uses lifetime feeding. Testing two drugs with different epigenetic effects, we find that lifetime feeding yields no or harmful effects on longevity. Stage-specific feeding, however, yields significant positive effects on age-specific mortality & longevity. These data suggest that the search for DR mimetics may be enhanced by the use of stage-specific screening of candidate molecules.

INTRODUCTION

. The assumption underlying the strategy of lifetime feeding is that the animal can respond fully to the drug at any time in its ife cycle. However, temporal and tissue-specific alterations in gene expression patterns (GEP) have profound effects on aging of multicellular organisms. Most life spans may be demographically divided into Development (D), Health (H), Transition (T), and Senescent (S) phases or spans, with major GEP/aging differences between the H and S spans (Ref 1). There is no *a priori* reason to believe that the organism's response to any candidate drug will be uniform across its life cycle. There is a potential contradiction between our knowledge of stage/tissue-specific GEP and our standard protocols for testing drugs.

We tested that contradiction by determining whether a drug (curcumin), found to have no pro-longevity effect by two labs (Refs 2,3) but a mild effect by a third lab (Ref 4) under lifetime feeding protocols, would actually have pro-longevity effects if tested by stage-specific feeding of the drug as opposed to lifetime feeding. Our data show that lifetime feeding yields false negative data and does not uphold the assumptions underlying this standard testing protocol.

Stage-Specific Effects of Curcumin

Curcumin fed throughout the Adult Life span is Deleterious & Decreases Longevity.

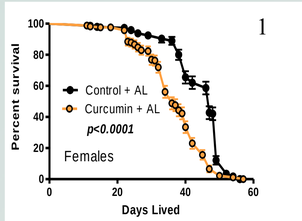


Fig 1. **Wildtype animals fed 100 mM curcumin during the entire adult lifespan (days 5-65) have a significantly earlier age of onset of senescence and a decreased median lifespan, relative to appropriate controls. This decrease is seen in both sexes. Curcumin has a negative effect if given in mid- or late-life.**

Curcumin Lowers the Mortality Rate & Extends the Adult Health Span Only if Fed Early in the Life Span.

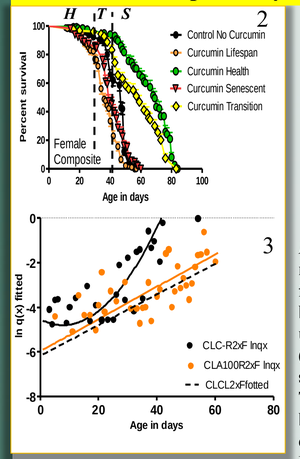


Fig. 2: **Wildtype animals fed 100 mM curcumin as adults but only from days 5 to 27, expressed a significant extension of the Health Span relative to the standard control (, p<0.0001). Wildtype animals fed curcumin only from days 28-40 (transition span) or days 38-89 (senescent span) expressed a significantly shorter life span. Dashed lines approximate these boundaries.**

Fig. 3. **The Ra female age-specific mortality rate (ln q_x) was calculated from the data of Figure 1, plotted, the best fit mortality curve fitted to the data using maximum likelihood analysis (Winmodest), and graphed. The data symbols represent the actual lnq_x values. The solid lines represent represent the best fitted curves; there is a significant difference between the Gompertz-Makeham plor for the Ra control and a Gompertz plot for the Ra experimental cohort. The dotted line represent the Gompertz curves of the long-lived La female controls**

Curcumin induces normal females to express mortality curves comparable to that of long lived control animals. (see Ref. 5 for details)

Stage-Specific Effects of HDAc1 Inhibitors

Feeding NaBu During The Adult Stage Decreases Longevity of Normal-lived Ra Adults

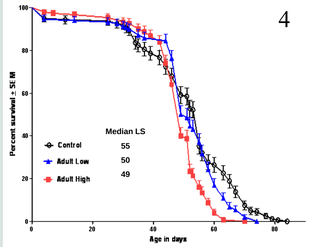
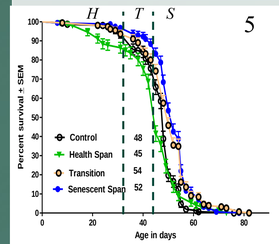
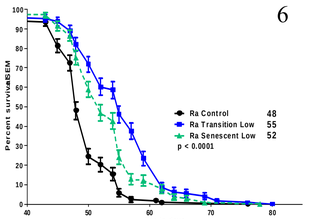


Fig. 4; **Lifetime feeding of NaBu at high dose (100 mM) yields significant decreases relative to controls (Ra p<0.0001, La p=0.0244 (not shown). Low dose (10 mM) has no significant effect relative to controls (p=0.0589 (Ra) or p=0.2869 (La). Median life spans are listed in Fig. 4.**

Stage-specific Feeding of NaBu to Ra Adults Has Stage-specific Effects on Late-Life Longevity





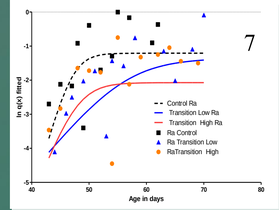


Fig 5: **Feeding 10 mM NaBu to Ra adults only during their transition (T) or senescent (S) spans leads to significant increases in median and late-life longevity (X^2 = 38.52, 1df, p<0.0001). In *Fig 6,* the Ra survival data from Figure 5 were rescaled to show the details of extended survival during the senescent span (days 43ff).**

Fig. 7 **Low dose NaBu treatment during the T span has a greater positive effect on survival and longevity in the S span than does intervention during the Sspan itself (p=0.0013). Treated flies have lower Gompertz-logistic mortality rate curves than controls.**

SAHA is also a HDAcI & also lowers the late life mortality rate & decreases the MRDT by 15% relative to controls (data not shown here; see Ref 6).

CONCLUSIONS

- **Three drugs, one (curcumin) with HAT and two (NaBu and SAHA) with HDAC1 inhibitor properties, yielded significant positive effects on age-specific mortality rates and stage-specific longevity only when fed to the animals during stage-specific periods.**
- **Full life feeding of either curcumin or NaBu yielded either no beneficial effect or a significant life-shortening effect.**
- **One of the tested drugs is reported to have no prolongevity effects, by labs using a. lifetime feeding protocol.**
- **Long-lived animals either do not benefit from these drugs, or are harmed by them, suggesting that long-lived animals may rely on the drug-induced processes.**
- **The data support a stage-specific paradigm for pro-longevity drug screens, particularly for drugs with epigenetic properties, so as to minimize the false negative reports.**

REFERENCES

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We thank Scott Pletcher (UM) for help with the mortality analysis