Longer Life Foundation Final Research Report
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Grant Title: Anticonvulsant effects on lifespan
Grant dates: 10/1/05 – 10/31/07

Abstract

There are currently no well-documented pharmacological treatments that delay normal human aging and extend human lifespan. To identify drugs that can delay aging, we chose to screen drugs used to treat a variety of human diseases, reasoning that these compounds might have effects on aging that had not been previously identified. After screening about 20 compounds, we identified two drugs that significantly extended *C. elegans* life span, the anticonvulsant medication ethosuximide and the neuroactive medication valproic acid. Ethosuximide and valproic acid are approved for human use. These drugs increase mean and maximum life span of *C. elegans* and delay age-related declines of physiological processes, indicating that these compounds retard the aging process. Our major accomplishments during the grant period included characterizing the mechanism of action of these drugs in extending lifespan. We demonstrated that valproic acid acts differently from ethosuximide, and these two drugs can be combined to produce additive lifespan extensions. Valproic acid may influence the insulin/IGF signaling pathway. We demonstrated that ethosuximide extends lifespan by inhibiting the activity of specific ciliated neurons in the head of the animal. These studies contribute to understanding how the nervous system influences lifespan, and demonstrate the possibility of using drugs that affect the nervous system to affect longevity.

Summary and Description in lay language

We are interested in identifying drugs that can delay human aging and extend human lifespan. As a first step, we tested whether drugs that are approved for human use can delay aging and extend the lifespan of a simple animal that is easy to study: a small soil worm. We found that two drugs that are used to treat seizures in humans can delay aging in the worms. With the support of the Longer Life Foundation, we characterized how these drugs function to extend lifespan. We demonstrated that one of these drugs inhibits the activity of specific cell in the nervous system, demonstrating that the nervous system can control lifespan. Our results suggest that drugs that affect neural activity have promise for lifespan extension.

Introduction/ Brief literature review

The quest for methods to delay aging is a longstanding human endeavor. While modern society has produced a wealth of improvements that have increased longevity and improved the quality of life in later years, such as improvements in nutrition and treatments for specific diseases, there are still no pharmacological agents that have been demonstrated to delay human aging. Major challenges to understanding and treating human aging are the fact that humans live so long and age so slowly. Thus, a critical need in aging research is animal models that are short lived and amenable to experimentation. A logical progression for developing anti-aging therapies would be to conduct the first phase of screening in a short lived animal model, such as nematode worm. With the support of a Longer Life Foundation Research Grant (10/03 – 10/04), I conducted a screen for medications that can delay aging in nematode worms. This experiment was a major success, since we discovered that a class of anticonvulsant medications that are used to treat seizures, including ethosuximide and trimethadione, delayed age-related degenerative changes and significantly extended nematode lifespan.

To characterize age-related degenerative changes and life span, we used the nematode worm *Caenorhabditis elegans*. *C. elegans* is a complex animal that displays extensive conservation of

fundamental biological processes with other animals. For example, many key regulators of neural function are highly conserved in worms and mammals. It is ideal for studies of aging because the adults display the progressive, degenerative changes that are typical of aging in larger animals but the adult lifespan is only about 15 days. Genetic analysis of *C. elegans* has resulted in the discovery of several pathways that regulate aging, including an insulin/insulin-like growth factor pathway that appears to play a conserved role in regulating vertebrate aging. These findings validate the relevance of the *C. elegans* system for studies of vertebrate aging.

Methods, Results and Discussion

We made significant progress during the two years of my Longer Life Foundation grant in two areas of aging research: First, the analysis of drugs that can delay aging in *C. elegans*, and second, the analysis of aging in the reproductive system. In this report I will describe our accomplishments in these two areas. In addition, I have listed our aging-related publications during the grant period at the end of the narrative. Before describing our recent findings, I want to say that it was an honor to be supported by the Longer Life Foundation, and the financial support has been vital to allow our explorations of aging.

We made significant progress analyzing a new drug, valproic acid, that we discovered by testing FDA-approved compounds for the ability to extend lifespan of *C. elegans*. Valproic acid is an important human pharmaceutical used to treat seizure disorders and migraine headaches. It has not previously been reported to influence lifespan or aging. We have now done extensive dose response studies with valproic acid. Treatment with 0.5 mg/mL valproic acid extended the mean adult lifespan was 20.9 ± 7.0 days, a 29% increase compared to no drug treatment ($P<0.0001$). The maximum adult lifespan was 33.3 ± 2.1 days, a 43% increase compared to no drug treatment ($P<0.0001$). Treatment with 1 mg/mL valproic acid extended the mean adult lifespan 21.9 ± 7.0 days, a 35% increase compared to no drug treatment ($P<0.0001$). The maximum adult lifespan was 33.1 ± 2.1 days, a 42% increase compared to no drug treatment ($P<0.0001$). At low doses below 0.1 mg/mL in the media, valproic acid has no significant affect on adult lifespan; at doses higher that 3 mg/mL in the media, valproic acid causes toxicity and shortens lifespan. In addition to extending lifespan, valproic acid delays the age-related decline of body movement that is characteristic of worm aging.

Valproic acid is a small, carboxylic acid that was first licensed for use in humans as an anticonvulsant in 1978. More recently, valproic acid has been used to treat psychiatric disorders in humans, including bipolar disorder. Valproic acid is also a teratogen in humans and rodents. Valproic acid is likely to have multiple therapeutic targets, which may explain its diverse clinical effects. Several mechanisms have been proposed for valproic acid, including effects on gamma–amino butyric acid (GABA) levels, sodium channels, histone deacetylase (HDAC), and the endoplasmic reticulum stress response pathway. A group of compounds that are structurally related to valproic acid have been used to conduct elegant structure-activity studies. We have discovered that one of these related compounds, valproimide, can also extend the lifespan of *C. elegans*.

To determine the developmental stage when valproic acid functions to extend lifespan, we administered the drug from conception until the fourth larval (L4) stage or from the L4 stage until death. Exposure to valproic acid only during embryonic and larval development had no significant effect on mean lifespan. In contrast, exposure to valproic acid only during adulthood extended mean lifespan to 20.8 ± 7.5 days, a 28% increase ($P<0.0001$). Thus, valproic acid functions in adults to delay age-related degenerative changes.

If valproic acid has a different mechanism of action than the heterocyclic ring anticonvulsants, then valproic acid might further extend the lifespan of worms cultured with ethosuximide or

trimethadione. To investigate this possibility, we measured the lifespan of worms cultured with two of these drugs. We tested several different combinations of drug concentrations. Worms cultured with 4 mg/mL trimethadione and 1 mg/mL valproic acid displayed the largest lifespan extension. This combination of drugs resulted in a mean lifespan of 26.1 ± 7.8 days, a 61% increase compared to control worms cultured without drugs. Importantly, the mean lifespan of worms treated with both drugs was significantly greater than the mean lifespan of worms treated only with 4 mg/mL trimethadione ($P<0.005$) or only with 1 mg/mL valproic acid ($P<0.0001$). These findings support the hypothesis that valproic acid and trimethadione have different mechanisms of action. These studies also demonstrate the potential of combining drugs to produce a longer extension of lifespan.

To investigate the relationship between the activity of valproic acid and the insulin/IGF signaling pathway, we analyzed how valproic acid affects dauer larvae formation and the localization of the DAF-16 transcription factor that is the critical target of the signaling pathway. Valproic acid promoted dauer larvae formation, consistent with the possibility that the drug influences the insulin/IGF signaling pathway. Furthermore, valproic acid promoted nuclear location of DAF-16. Both findings are suggestive that valproic acid may extend lifespan by influencing the insulin/IGF signaling pathway. A manuscript describing these studies of valproic acid was recently accepted for publication by *Aging Cell*, pending minor revisions.

The other focus of our work on drugs has been the analysis of the heterocyclic anticonvulsant ethosuximide. Our main goal has been to elucidate the mechanism of action of the drug in extending lifespan. To identify genes that are necessary for the activity of ethosuximide, we conducted a genetic screen for mutations that cause resistance to the lethal effects of high doses of ethosuximide. We identified a large number of mutations that cause resistance to ethosuximide, and we mapped many of these mutations to positions in the *C. elegans* genome. We have now molecularly identified two genes that can be mutated to cause resistance, the genes *osm-3* and *che-3*. Both genes are necessary for the function of a small number of ciliated neurons in the head of the animal. We have now shown that mutations in several other genes that affect these neurons cause resistance to ethosuximide, indicating that these ciliated neurons mediate the effects of the drug. Consistent with this conclusion, we have shown that ethosuximide treatment causes many phenotypes associated with diminished function of these neurons, including defects in chemotaxis, dauer larvae formation, and L1 arrest. These findings indicate that ethosuximide extends lifespan by decreasing the activity of ciliated neurons in the head of the worm. These neurons are important for sensing chemical cues in the environment, including food, suggesting that ethosuximide may extend lifespan by decreasing the perception of food. It is interesting that ethosuximide treatment does not cause caloric restriction, since treated animals have normal numbers of progeny. Therefore, animals that have impaired food perception and normal food intake may experience a lifespan extension. A manuscript describing these studies is in an advanced state of preparation.

Our second major focus was measuring the development and aging of the reproductive system. We have carefully monitored daily progeny production of self-fertile and mated *C. elegans* hermaphrodites. We have conducted these studies in a variety of mutant backgrounds to understand the influence of genes on these processes. We have also examined the effects of environmental factors such as temperature and drugs that extend longevity. We have used sophisticated statistical approaches to analyze these data. Our main conclusions are that we identified four factors that can delay reproductive aging of mated hermaphrodites that have abundant sperm: cold temperature, caloric restriction, reducing the activity of the insulin/insulin-like growth factor pathway, and the anticonvulsant drug ethosuximide. Surprisingly, using the reproductive tract to generate progeny early in life neither accelerates nor delays reproductive aging. This observation suggests that reproductive aging is not controlled by use-dependent factors. A manuscript describing these studies was recently published in *PLoS Genetics*.

Publications during grant period

Evason, K., and **K. Kornfeld.** 2006. Effects of anticonvulsant drugs on lifespan. *Archives of Neurology*, **63**: 491-496.

Collins, J.J., K. Evason and **K. Kornfeld**. 2006. Pharmacology of delayed aging and extended lifespan of *Caenorhabditis elegans*. *Experimental Gerontology* **41**: 1032-1039.

Hughes, S., K. Evason, C. Xiong and **K. Kornfeld**. 2007. Genetic and pharmacological factors that influence reproductive aging in nematodes. *PloS Genetics* **3**: e25.

Collins, J.J., C. Huang, S. Hughes and **K. Kornfeld**. The measurement and analysis of age-related changes in *Caenorhabditis elegans*. (December 7, 2007), *WormBook*, ed. The *C. elegans* Research Community, WormBook, doi/10.1895/wormbook.1.137.1, http://www.wormbook.org.

Evason, K., J.J. Collins, C. Huang, S. Hughes and **K. Kornfeld**. 2008. Valproic acid extends *C. elegans* lifespan. *Aging Cell*, accepted pending minor revisions.

Collins, J.J., Evason, K., Schneider, D.L. and **K. Kornfeld**. The anticonvulsant ethosuximide acts through neurons in the head to extend lifespan. Manuscript in preparation.