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Pharmacology of delayed aging and extended lifespan of *Caenorhabditis elegans*

James J. Collins, Kimberley Evason, Kerry Kornfeld *

Department of Molecular Biology and Pharmacology, Washington University School of Medicine, St. Louis, MO 63110, USA

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Abstract

The identification and analysis of compounds that delay aging and extend lifespan is an important aspect of gerontology research; these studies can test theories of aging, lead to the discovery of endogenous systems that influence aging, and establish the foundation for treatments that might delay normal human aging. Here we review studies using the nematode *Caenorhabditis elegans* to identify and characterize compounds that delay aging and extend lifespan. These studies are considered in four groups: (1) Studies that address the free-radical theory of aging by analyzing candidate compounds with antioxidant activities including vitamin E, tocotrienols, coenzyme Q, and Eukarion-8/134. (2) Studies that analyze plant extracts (blueberry and *Ginko biloba*) that contain a mixture of compounds. (3) Studies of resveratrol, which was identified in a screen for compounds that affect the activity of the Sir2 protein that influences lifespan. (4) Studies based on screening compound libraries using *C. elegans* aging as a bioassay, which led to the identification of the anticonvulsant medicines ethosuximide and trimethadione. There has been exciting progress in the analysis of compounds that influence *C. elegans* aging, and important challenges and opportunities remain in determining the mechanisms of action of these compounds and the relevance of these observations to aging of other animals.

Keywords: Aging; *C. elegans*; Pharmacology; Lifespan

1. Introduction

In 1513, the Spanish explorer Ponce de Leon set sail seeking gold and the mythical fountain of youth, whose magical waters could restore youthful vitality. He discovered Florida, but gold and the fountain of youth eluded him. The quest continues today, and the modern explorers are biologists seeking factors that modulate aging. The model organism *Caenorhabditis elegans* is a free-living soil nematode that plays a prominent role in aging research. Here we review studies of compounds that extend lifespan and influence aging in *C. elegans*. The identification and characterization of such compounds is an important aspect of aging research. First, this approach can lead to the discovery of endogenous pathways that influence aging. Second, a well-characterized drug is a valuable reagent that can be used to test theories of aging and investigate how different endogenous systems influence aging. Drugs are versatile reagents because dosage and time of administration can be controlled precisely. Third, these studies may lead to therapeutics for normal aging or age-related diseases, so that the fountain sought by Ponce de Leon may materialize one day at the pharmacy.

2. Measuring *C. elegans* aging

Measurements of aging are critical for determining whether a compound affects aging and then defining how it affects aging. However, measuring aging is a significant challenge. Aging is a complex process that involves progressive, degenerative changes in multiple organ systems. The different organ systems may begin degenerating

* Corresponding author. Tel.: +1 314 747 1480.
E-mail address: kornfeld@wustl.edu (K. Kornfeld).

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at different times and may degenerate at different rates. Therefore, any single measurement provides very limited information, and multiple measurements are necessary to provide a more comprehensive and detailed account of the aging process. In addition, age-related changes are variable between individuals, suggesting that stochastic events influence aging (Herndon et al., 2002). Because of this variability, aging must be measured in large populations of animals, and differences between populations must be validated using statistical analysis.

Lifespan is the most common measurement of the aging process, and it is always included in *C. elegans* drug studies. *C. elegans* lifespan measurements typically begin with the first larval stage (L1) or the fourth larval stage (L4). The major advantages of the lifespan measurement are that it is straightforward (alive vs. dead) and it is an overall measure of the function of the life-support systems. The major limitation is that it includes periods of pre-adult development and adult maturation as well as the period of functional decline. Therefore, a drug that extends lifespan might extend the period of development and maturation only, the period of functional decline only, or both periods. It is also possible to measure the decline of specific physiological functions, such as reproduction, body movement, or pharyngeal pumping (Chow et al., 2006; Croll et al., 1977; Garigan et al., 2002; Hosono et al., 1980; Huang et al., 2004; Johnson, 1987). This approach has the advantage that it permits quantitative measures of vitality. Age-related changes of molecular or biochemical features, such as increases in the levels lipofuscin and protein carbonylation, can also be analyzed (Klass, 1977; Adachi and Ishii, 2000). Similar to lifespan, these measurements have the limitation that they are not specific for aging since they may include maturational periods. Because of the limitations with each assay, a combination of assays provides the most comprehensive and specific information about the aging process.

3. Determining the mechanism of drug action

For a drug that is demonstrated to extend lifespan or delay aging the next critical question is what is the mechanism of drug action? Determining the mechanism can identify endogenous systems that influence aging and increase the usefulness of the drug as a reagent to investigate aging. For some drugs, one or more direct targets have been identified, whereas other drugs are less well-characterized. Several approaches are useful for determining the mechanism of action whether or not the direct target of the drug is established. First, drug treatment can be combined with genetic or environmental manipulations that influence aging. These experiments can be interpreted like genetic epistasis experiments, but it is noteworthy that important caveats apply to these interpretations. If lifespan extension caused by drug treatment is additive with lifespan extension caused by a mutation, it suggests the drug and the mutation act by independent mechanisms. However, a caveat is that each manipulation may have a partial effect on the same system. If drug treatment causes a lifespan extension that is not additive with a lifespan extension caused by a mutation, it suggests that the drug and the mutation may have a similar mechanism. However, a caveat is that combining the treatments may result in toxicity that obscures the lifespan effects. Second, if drug treatment causes phenotypes in addition to delaying aging, these phenotypes may elucidate the mechanism of drug action. For example, if drug treatment increases heat resistance of young animals and extends lifespan, it suggests the hypothesis that heat resistance causes the lifespan extension. Third, structure–activity studies of related compounds can elucidate mechanisms. For example, structurally related compounds might not increase heat resistance and yet still extend lifespan, and these data would not support the hypothesis that heat resistance causes the lifespan extension.

If a drug has a putative direct target, then specific experimental approaches can be used to investigate whether the putative target mediates the effects on aging. First, does the drug bind the target in extracts of treated worms? Second, is a similar concentration of drug necessary to bind the target in a purified system and affect aging in worms? Third, do mutations in the gene encoding the target affect sensitivity to the drug? Fourth, do mutations in the gene encoding the target affect aging? These experimental approaches can rigorously test the hypothesis that the drug affects aging by acting on the candidate target protein.

Hormesis refers to the phenomenon that low-level exposure to stress induces a protective stress response. Low-level exposure to stress can extend the lifespan of worms and some other animals, suggesting that hormesis can delay aging (Rattan, 2004). Thus, hormesis is a potential mechanism of action for drugs that extend lifespan. In particular, the finding that a drug is toxic at high doses and extends lifespan at low doses might suggest that the drug is mildly toxic at low doses and functions by hormesis. However, many and perhaps all compounds are toxic at sufficiently high doses, indicating that high dose toxicity is not specific evidence for a hormesis mechanism of action.

Caenorhabditis elegans is typically cultured with live *Escherichia coli* as a food source, raising the possibility that the drug directly affects the *E. coli* and indirectly affects the worms. Because nutrition affects lifespan and aging (Klass, 1977), a drug that decreases the nutrient value of *E. coli* might indirectly affect worm aging by causing caloric restriction. In addition, *E. coli* is pathogenic to older worms, and a drug that decreases the pathogenicity of *E. coli* might extend worm lifespan. For example, antibiotics that reduce bacterial pathogenicity extend worm lifespan, and worms cultured on non-pathogenic bacterial strains display an extended lifespan (Garigan et al., 2002; Garsin et al., 2003). To address these issues, experiments can be conducted with non-pathogenic bacteria, such as *Bacillus subtilis*, with *E. coli* that has been killed by UV irradiation or antibiotic treatment, or with worms grown in defined liquid media that lacks bacteria. Drugs that affect worm

aging in one or more of these alternative culture conditions are unlikely to act directly on the *E. coli*.

Drug delivery, dosage, and time of administration are important variables (reviewed by Rand and Johnson, 1995). *C. elegans* are typically cultured in Petri dishes containing nutrient agar or in liquid culture, and the compounds are typically added to media. Hydrophobic compounds can be delivered with solvents such as DMSO or ethanol. In these culture conditions worms soak in a drug solution. It is likely that uptake is primarily intestinal but other routes, such as absorption through the cuticle, are possible. The drug concentration external to the worms can be calculated, but the internal drug concentration must be measured. Studies indicate that worms do not equilibrate with the external drug concentrations. Instead worms can effectively exclude or metabolize drugs. For example, worms treated with an external concentration of 400 mM ethanol have an internal concentration of 22 mM ethanol (Davies et al., 2003); worms treated with an external concentration of 2 mg/ml ethosuximide have an internal concentration of 30 μg/ml ethosuximide (Evason et al., 2005). Determining the effective internal concentration is important because the value can be compared to other assays of drug activity such as target binding. Time of administration experiments are also useful in determining whether drugs function during development or during adulthood to influence aging.

We divided compounds that have been reported to extend lifespan in *C. elegans* into four groups: (1) compounds that were analyzed to test the free-radical theory of aging, (2) plant extracts containing mixture of chemicals, (3) a compound identified in a screen based on measuring the biochemical activity of a purified protein shown to affect lifespan, and (4) compounds identified in a screen based on measuring aging in intact animals. We summarize the evidence that these compounds affect aging and the results that address the mechanism of action of these compounds.

4. Pharmacological tests of the free-radical theory of aging

Reactive oxygen species (ROS) can damage a variety of macromolecules, leading to the theory that ROS are one cause of age-related degeneration. This theory predicts that compounds that reduce ROS-mediated damage will delay aging and extend lifespan, and several investigations have used *C. elegans* to test this prediction.

4.1. Vitamin E

Vitamin E, also known as α-tocopherol, is an endogenous molecule in *C. elegans*. Purified vitamin E is an antioxidant (Kamal-Eldin and Appelqvist, 1996), suggesting it might reduce ROS-mediated damage *in vivo*, and vitamin E extends the lifespan of other animals (Porta et al., 1980; Driver and Georgeou, 2003).

Harrington and Harley (1988) showed that treating wild-type *C. elegans* with 200 μg/ml vitamin E increased mean lifespan by 17–23% (Table 1). The mechanism of action was investigated by analyzing reproduction of self-fertile hermaphrodites. Vitamin E treatment reduced the total number of progeny and delayed the time to peak reproduction. These authors conclude, "These data do not support the free-radical scavenging mechanism for vitamin E-induced lifespan extension in *C. elegans*. Instead they support a general mechanism in which, within a narrow range of treatment, otherwise deleterious agents can increase longevity by slowing growth and development."

4.2. Tocotrienols

Tocotrienols are endogenous molecules that differ from vitamin E (α-tocopherol) by having an isoprenoid instead of a phytyl side chain (Table 1). Tocotrienols are potent antioxidants (Kamal-Eldin and Appelqvist, 1996).

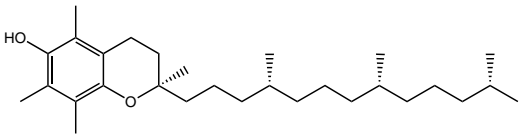
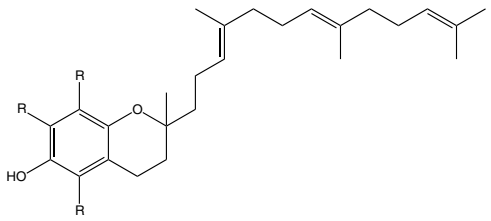
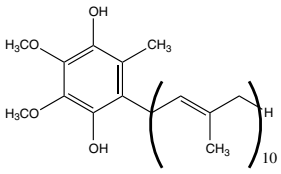
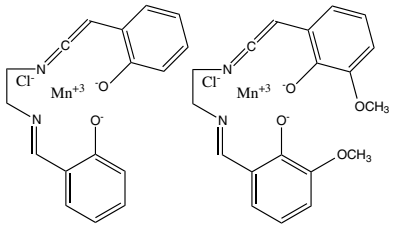
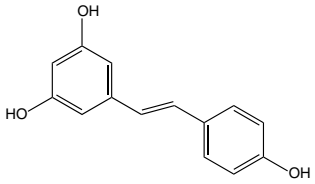
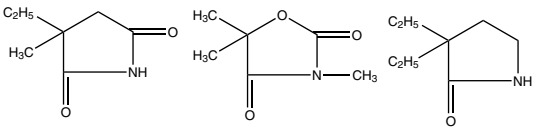
Adachi and Ishii (2000) analyzed the tocotrienol rich fraction (TRF) from palm oil which has the following composition: α-tocopherol, 22%; α-tocotrienol, 24%; γ-tocotrienol, 37%; δ-tocotrienol, 12%. Wild-type *C. elegans* displayed a 20% increase in mean lifespan when grown on agar plates containing TRF (Table 1). The mechanism of action was addressed by analyzing protein carbonyl content, a specific indicator of oxidative damage to protein. Protein carbonyl content displayed an age-related increase, and treatment with TRF reduced protein carbonyl content in 15-day-old animals. Treatment with TRF also blunted the effects of lifespan shortening caused by UV B irradiation. This study did not examine the timing or levels of reproduction. Adachi and Ishii (2000) conclude that these results support the model that tocotrienols extend lifespan by reducing damage caused by reactive oxygen species.

4.3. Coenzyme Q

Coenzyme Q (CoQ) (isoprenylated benzoquinone), also known as ubiquinone, is an endogenous *C. elegans* molecule that is necessary for the mitochondrial electron transport chain. Purified Coenzyme Q displays antioxidant activity (Ernster and Dallner, 1995). Furthermore, coenzyme Q has been linked to lifespan control in *C. elegans* by the *clk-1* gene which encodes a demethoxyubiquinone mono-oxygenase necessary for the synthesis of CoQ (reviewed by Rodriguez-Aguilera et al., 2005). Loss-of-function *clk-1* mutants have an extended lifespan (Ewbank et al., 1997).

Ishii et al. (2004) investigated the effects of treating wild-type *C. elegans* cultured on agar plates with CoQ_{10}; 150 ug/ml CoQ_{10} increased the average lifespan by 18% (Table 1). To address the mechanism of action, Ishii et al. (2004) showed that treatment with CoQ_{10} increased the lifespan of *mev-1* mutants that are hypersensitive to oxidative stress and reduced apoptosis in *mev-1* mutants. In addition,

Table 1
Compounds that influence lifespan and aging in *C. elegans*

Name	Structure	Aging phenotypes	Non-aging phenotypes	Reference(s)
Vitamin E		17–23% increase in mean lifespan	Reduced level of reproduction and delayed time to peak reproduction	Harrington and Harley (1988)
Tocotrienols		20% increase in mean lifespan; delays age-related increase in protein carbonyl content		Adachi and Ishii (2000)
Coenzyme Q_{10}		18% increase in mean lifespan	In *mev-1* mutants, decreased apoptosis and mitochondrial SOD production	Ishii et al. (2004)
EUK-8/ EUK-134		54% increase in mean lifespan or No significant increase in mean lifespan and dose-dependent decrease in mean lifespan	Increased SOD activity in worm extracts; Increased resistance to oxidative and thermal stress	Melov et al. (2000), Keaney and Gems (2003), Keaney et al. (2004), Sampayo et al. (2003)
Blueberry extract	Polyphenol mixture	28% increase in mean lifespan; delays age-related declines of pharyngeal pumping and increases in lipofuscin accumulation and heat shock protein inducibility	Increased thermotolerence	Wilson et al. (2006)
Ginko biloba extract (EGb761)	Complex Mixture	8% increase in mean lifespan	Increased resistance to oxidative and thermal stress	Wu et al. (2002), Strayer et al. (2003)
Resveratrol		10–18% increase in mean lifespan	Increased expression of stress response genes	Wood et al. (2004), Viswanathan et al. (2005)
Ethosuximide/ Trimethadione/ DEABL		17–47% increase in mean lifespan; delays age-related declines of pharyngeal pumping and body movement	Increased rates of egg-laying, body movement, and aldicarb sensitivity	Evason et al. (2005)

treatment with CoQ_{10} decreases levels of superoxide anion production from the mitochondria of *mev-1* mutants but not of wild-type animals. Ishii et al. (2004) conclude that the lifespan extension caused by CoQ_{10} is a consequence of reduced oxidative stress in the mitochondria.

Other studies indicate that reduced levels of CoQ can extend *C. elegans* lifespan (Ewbank et al., 1997; Larsen and Clarke, 2002). The long-lived *clk-1* mutant is defective in CoQ biosynthesis and lacks the endogenous form of CoQ. These mutants require dietary CoQ from the bacterial food source for survival (Jonassen et al., 2001) and presumably have a lower level and an altered form of CoQ compared to wild-type. Consistent with this model, Larsen and Clarke (2002) demonstrated that a CoQ deficient diet can extend lifespan. The results that both dietary supplementation with CoQ and restriction of dietary CoQ can extend lifespan may indicate that metabolism can be perturbed by an excess or deficiency of CoQ.

4.4. EUK-8 and EUK-134

Eukaryion-8 (EUK-8) and EUK-134 are salen-manganese compounds that display enzymatic activities of superoxide dismutase and catalase in purified systems (Doctrow et al., 2002). Studies of mice showed that these compounds can extend the lifespan of short-lived mice that have a defect in reactive oxygen metabolism (Melov et al., 2001).

Two groups of investigators have analyzed *C. elegans* exposed to EUK-8 and EUK-134, and these studies have resulted in substantially different conclusions. Melov et al. (2000) analyzed wild-type worms maintained in liquid culture and measured mean and maximum lifespan; drug treatment increased mean lifespan by as much as 54%. Gems and colleagues analyzed the lifespan of wild-type worms maintained in a variety of culture conditions; drug treatment did not increase lifespan but rather caused a dose-dependent decrease in lifespan and fertility (Keaney and Gems, 2003; Keaney et al., 2004) (Table 1).

To address the mechanism of action of the increased lifespan, Melov et al. (2000) performed dosage experiments and observed no overall dose response. The drugs extended the lifespan of *mev-1* mutants that are defective in reactive oxygen metabolism. The drugs did not significantly affect growth or fertility. Melov et al. (2000) conclude that the drugs likely act by affecting reactive oxygen metabolism. Consistent with this interpretation, Sampayo et al. (2003) showed that EUK-8 and EUK-134 cause a dose-dependent increase in resistance to oxidative stress caused by paraquat, indicating that these compounds act as antioxidants *in vivo*. Gems and colleagues addressed the mechanism of action by determining if the drugs were active in animals (Keaney and Gems, 2003; Keaney et al., 2004). Extracts of drug treated animals had elevated superoxide dismutase activity. Furthermore, drug treatment prevented the lifespan shortening caused by treatment with reactive oxygen species generators. Gems and colleagues conclude that EUK-8 and EUK-134 act as superoxide dismutase mimetics in worms, and this activity can rescue the lifespan of animals exposed to reactive oxygen species generators, but it does not extend the lifespan of animals grown in standard conditions.

Both groups of investigators conclude that EUK-8/134 are functional as SOD mimetics in the worm and can counteract the effects of damage caused by reactive oxygen species. The finding of Gems and collegues that these compounds do not extend lifespan indicates that in the culture conditions used by these investigators, ROS-mediated damage does not limit *C. elegans* lifespan. The culture conditions used by Melov et al. (2000) may have involved a higher level of oxidative stress that did limit *C. elegans* lifespan. In two other invertebrates, the house fly *Musca domestica* and the fruit fly *Drosophila melanogaster*, administration of EUK-8/134 did not extend lifespan (Magwere et al., 2006; Bayne and Sohal, 2002).

Pharmacological studies of *C. elegans* designed to test the free-radical theory of aging have produced several important results. The antioxidants vitamin E, tocotrienols, and coenzyme Q can extend lifespan. These studies have the potential to provide strong support for the free-radical theory of aging, but the critical issue is what is the mechanism of action of the compounds. Additional studies are required to demonstrate that the mechanism of action is a reduction of ROS-mediated damage and not an alternative mechanism such as delaying maturation as suggested for vitamin E.

5. Analysis of plant extracts that contain a mixture of chemicals

5.1. Blueberry extract

Extracts of blueberries have been shown to have beneficial effects in aged rats (Joseph et al., 1999). These extracts contain a complex mixture of polyphenols that can have antioxidant and anti-inflammatory effects. Based on these studies, Wilson et al. (2006) analyzed the effects of blueberry extracts on *C. elegans*.

Blueberry extracts increased mean lifespan of *fem-1* mutants by 28% (Table 1). This treatment also delayed age-related functional declines in pharyngeal pumping, age-related increases in lipofuscin levels, and age-related increases in the inducibility of transcripts encoding heat shock proteins. Thus, treatment with blueberry extracts affects multiple age-related changes.

To address the mechanism of action, Wilson et al. (2006) tested fractions of the blueberry extract and showed that the proanthocyanids (PAC) enriched fraction contains the lifespan extending activity. Treatment with the PAC fraction increased thermotolerence but did not increase resistance to oxidative stress. The extract did not affect bacterial growth and appears unlikely to primarily to act on the bacteria. To determine how the mechanism of action of the extract relates to genetically defined pathways, Wilson et al. (2006) showed that blueberry extract extended the lifespan of

loss-of-function mutants of *daf-16* and *skn-1* and a strain that overexpresses *sir-2.1*, indicating that it acts by a different mechanism than these genes. Blueberry extract did not extend the lifespan of *osr-1*, *sek-1*, and *unc-43* mutants. These genes are required for resistance to specific stresses, and these results implicate the OSR-1/UNC-43/SEK-1 pathway as a target for blueberry polyphenols.

5.2. Ginko biloba extract (EGb 761)

Ginko biloba is an extremely long-lived tree, and extracts of the leaves are a part of traditional and modern medicine. The standard extract of *Ginko biloba* leaves, EGb 761, includes ginko-flavone glycosides (24%) and terpenoids (6%). In the United States ginko extracts are a popular herbal medicine, and in Europe it has been used to treat age-related deterioration of mental function. Based on these observations, Luo and colleagues investigated how *Ginko biloba* extracts affect *C. elegans* aging.

Wu et al. (2002) showed that 100 μg/ml EGb761 extended the median lifespan and maximum lifespan by 1 day (less than 10%) of wild-type worms on agar plates (Table 1). One component of the extract, the flavenoid tararixetin, extended median lifespan by 28% but did not affect maximum lifespan.

To address the mechanism of action, Wu et al. (2002) showed that treatment with EGb761 increased resistance to oxidative stress caused by the chemical jugulone and to thermal stress. Strayer et al. (2003) showed that EGb761 blunted the transcriptional induction of the heat shock protein *hsp-16.2* caused by a heat shock or jugulone. EGb761 reduced H_2O_2-related reactive oxygen species. The authors suggest that the EGb761 extract decreases cellular stress caused by exogenous stressors, and this protection against stress may be responsible for the lifespan extension.

An important issue with the studies of plant extracts that contain a mixture of chemicals is whether the effect requires multiple chemicals or is due to a single chemical. If a single chemical causes the effect, then an important goal is to identify that chemical. Determining the mechanism of action of plant extracts that contain a mixture of chemicals will be difficult, since some chemicals may cause effects that are unrelated to delayed aging.

6. Compounds identified by screening chemical libraries for effects on the biochemical activity of proteins that influence aging

6.1. Resveratrol

Overexpression of the Sir2 protein extends the lifespan of *C. elegans* and other organisms such as flies and yeast (Guarente, 2005). Sir2 is a NAD^+-dependent protein deacetylase, and Howitz et al. (2003) reasoned that a compound that activates Sir2 might cause a lifespan extension similar to Sir2 overexpression. These investigators screened for small molecules that stimulate the NAD^+-dependent protein deacetylase activity of purified Sir2 using a fluorescently tagged substrate. They identified resveratrol (3,4′,5-trihydroxystilbene), a plant-derived polyphenolic compound (Table 1). Two subsequent reports indicated that while resveratrol stimulates Sir2 deacetylation of the fluorescently tagged substrate used in the initial screen, resveratrol does not effectively stimulate Sir2 deacetylation of several physiologic substrates (Borra et al., 2005; Kaeberlein et al., 2005).

Two studies have analyzed the effects of resveratrol on *C. elegans* lifespan. Wood et al. (2004) showed that 100 μM resveratrol extends the mean adult lifespan of wild-type worms cultured on agar plates by 10%. Viswanathan et al. (2005) showed a dose-dependent increase of mean adult lifespan of wild-type worms cultured on agar plates with resveratrol; the largest effect of 18% was observed with 1 mM resveratrol. To address the mechanism of action, Wood et al. (2004) showed that the deacetylase activity of purified *C. elegans* Sir2.1 is stimulated by resveratrol. Resveratrol did not decrease fecundity or the pharyngeal pumping rate, indicating that it does not cause defective feeding. Resveratrol was effective in worms fed live or heat-killed *E. coli*, suggesting that the drug does not effect bacterial pathogenicity. Resveratrol did not extend the lifespan of a *sir-2.1* null mutant, indicating that *sir-2.1* may be necessary for the effect of resveratrol and consistent with the model that resveratrol acts by stimulating Sir2 activity. Wood et al. (2004) conclude that resveratrol acts by stimulating the activity of *sir-2.1*. Viswanathan et al. (2005) also reported that resveratrol did not extend the lifespan of *sir-2.1* mutants. Resveratrol did extend the lifespan of loss-of-function *daf-16* mutants and strains that overexpress *sir-2.1*. Gene expression analysis of resveratrol treated worms suggests that resveratrol induces expression of a family of stress response genes, including *abu-11,* which is necessary for the lifespan extension effect of resveratrol. Viswanathan et al. (2005) conclude that resveratrol may bind Sir2 and have affects on Sir2 activity that are substrate-specific, potentially reducing Sir2 activity depending on the substrate.

The analysis of resveratrol is an exciting advance, because it is the first drug that affects *C. elegans* lifespan that was identified by screening with a protein implicated in longevity control. In addition to the effects observed in *C. elegans*, resveratrol extends the replicative lifespan of yeast and the chronological lifespan of *Drosophila* and the short-lived fish *Nothobranchius furzeri* (Howitz et al., 2003; Wood et al., 2004; Valenzano et al., 2006). Further experiments are necessary to establish the mechanism of action of resveratrol and the significance of the effects of resveratrol on Sir2 activity.

7. Compounds identified by screening chemical libraries for effects on *C. elegans* aging

7.1. Ethosuximide/Trimethadione/DEABL

The approach used to identify resveratrol requires the identification of proteins that influence aging. Evason

et al. (2005) reasoned that many processes that influence aging have not yet been identified, and drugs that delay aging could be identified with minimal assumptions by screening collections of drugs using *C. elegans* aging as a bioassay. In a screen of 19 drugs that are FDA-approved for human use, Evason et al. (2005) identified ethosuximide as a lifespan extending drug. Ethosuximide is a small, heterocyclic ring compound of the succinimde class that is approved for human use as an anticonvulsant (Katzung, 1998). Ethosuximide extended the mean adult lifespan of wild-type animals grown on agar dishes by 17% (Evason et al., 2005 and Table 1). The effect is dose-dependent, and at high doses ethosuximide causes toxicity.

To conduct a structure–activity study, Evason et al. (2005) analyzed the structurally related anticonvulsants trimethadione, an oxazolidinedione, and DEABL, a lactam. Trimethadione increased mean lifespan by 47%, a very robust effect (Table 1). DEABL extended mean lifespan by 31% (Table 1). In addition, ethosuximide and trimethadione extend the span of time that animals display fast body movement and pharyngeal pumping. Therefore these drugs delay age-related functional declines in addition to extending lifespan. Time of administration experiments demonstrate that trimethadione functions in adults to extend lifespan.

Ethosuximide and trimethadione have been shown to affect the activity of multiple ion channels, including T-type calcium channels (Katzung, 1998). The relationship between these activities in cultured cells and the anticonvulsant activity is whole animals has yet to be defined fully. To address the mechanism of action during aging, Evason et al. (2005) conducted several experiments that indicate that the lifespan extending activity of the drugs is related to their anticonvulsant activity. Structure–activity studies analyzing the three anticonvulsants and succinimide, the parent compound of ethosuximide that does not possess anticonvulsant activity, demonstrated a correlation between anticonvulsant activity and lifespan extending activity. The concentration of ethosuximide in worm extracts is similar to the therapeutic dose of ethosuximide in humans, suggesting that there may be a similar target in worms and humans.

Ethosuximide and trimethadione cause several behavioral effects in young adults, including increasing the rate of egg-laying, so that eggs are deposited at earlier stages of development, increasing movement activity, and causing hypersensitivity to the acetylcholinesterase inhibitor aldicarb. These phenotypes indicate that the anticonvulsants stimulate synaptic transmission in the worms consistent with their activity on the nervous system in vertebrates. The relationship between these behavioral phenotypes and delayed aging remains to be determined. The lifespan extension effects of ethosuximide and trimethadione are additive with mutations that extend lifespan by affecting the insulin/IGF-1 signaling pathway (*daf-2*, *age-1*, etc.), mitochondrial function (*clk-1*), caloric intake (*eat-2*), and neuronal activity (*unc-64*, *unc-31*, *tax-4*, *aex-3*). These findings suggest that ethosuximide may act by mechanisms that are different from these genetic pathways.

The studies of anticonvulsant medicines suggest that these drugs act on the *C. elegans* nervous system and thereby affect aging. The mechanism appears to be different from the mechanism of genetic mutations that affect neural function. While further experiments are necessary to define the direct targets of these drugs and elucidate how neural systems influence aging, these studies demonstrate how pharmacology can identify endogenous systems that influence aging.

These studies also demonstrate the feasibility of using *C. elegans* aging as a bioassay for drug screening. Evason et al. (2005) screened a relatively small number of compounds using standard assay methods. Lithgow and colleagues have developed high-throughput assays for *C. elegans* survival, and these methods have the potential to accelerate the process of discovering drugs that influence aging using *C. elegans* (Gill et al., 2003). A recent report by Kwok et al. (2006) describes the use of *C. elegans* to screen about 14,000 small molecules for non-aging phenotypes, further demonstrating the potential of the system for high-throughput analysis. These exciting advances can result in the discovery of new drugs and endogenous pathways that influence aging.

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