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Optimizing Pharmacological Lifespan Extension: Testing Chemical Compounds for Additive Effects on Longevity

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**Optimizing Pharmacological Lifespan Extension: Testing Chemical
Compounds for Additive Effects on Longevity**

A culminating thesis submitted to the faculty of Dominican University of California
and the Buck Institute for Research on Aging in partial fulfillment of the requirements for
the degree

Master of Science in Biology

By
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San Rafael, California
May, 2016

CERTIFICATION OF APPROVAL

This thesis, written under the direction of the candidate's thesis advisor and approved by the thesis committee and the MS Biology program director, has been presented and accepted by the Department of Natural Sciences and Mathematics in partial fulfillment of the requirements for the degree Master of Science in Biology at Dominican University of California. The written content presented in this work represent the work of the candidate alone.

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Abbreviations

Alzheimer's disease: AD

Parkinson's disease: PD

amyotrophic lateral sclerosis: ALS

familial amyotrophic lateral sclerosis: fALS

Huntington's disease: HD

dietary restriction: DR

berberine chloride: brcl

curcumin: curc

myricetin: myr

gossypolone: gosy

diallyl trisulfide: dial tris

lithium chloride: LiCl

quercetin: quer

salicylic acid: sal acid

nitrophenyl piperazine-1: NP1

thioflavin t: ThT

zinc: Zn

copper: Cu

zinc chloride: ZnCl

copper chloride: CuCl

superoxide dismutase-1: SOD1

ethylenediaminetetraacetic acid: EDTA

Federal Drug Administration: FDA

Caenorhabditis Genetics Center: CGC

wild-type SOD1 (WTSOD1)

wild-type N2 (WTN2)

Abstract

Aging occurs in almost all living organisms. As we grow old, we are at risk for many age-associated diseases like cardiovascular disease, diabetes, Huntington's disease, and amyotrophic lateral sclerosis, to name a few. The underlying causes of these diseases are being heavily studied in the scientific community, in hopes that the diseases could be prevented or delayed. Studying the mechanisms and causes of chronic and neurodegenerative diseases could further result in various therapeutics to treat age-related diseases.

One approach to studying aging is the use of pharmacological agents and how they can extend lifespan and/or promote healthy aging. There are several reports that chemical compounds extend lifespan in various model organisms including *Caenorhabditis elegans*, *Drosophila melanogaster*, and *Mus musculus*. Pharmacological agents likely extend lifespan through activation of pro-longevity pathways and/or inhibition of pro-longevity pathways. Researchers have demonstrated that many mechanisms controlling aging are conserved, even across these diverse organisms. Therefore, pharmacological agents that target these pathways may eventually be able to improve human health.

To study aging using a pharmacological approach, I utilized the nematode *Caenorhabditis elegans* (*C. elegans*) as a model organism. I investigated a number of synthetic compounds and natural products in combination that are known to have a positive effect on the lifespan of *C. elegans*. Next, I examined the chemical compounds effects for improvements on the general health of the organism during the aging period (healthspan). Finally, I examined these compounds for any positive effect in a *C. elegans*

model of a human neurodegenerative disease. Overall, my results demonstrate that combinations of compounds extend lifespan and promote health aging in *C. elegans*.

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Chapter 1

Testing Chemical Compounds for Additive Effects on Longevity

1. Introduction

Aging occurs in most life forms over time, and ultimately leads to the death of an organism. With aging, complex biological functions and pathways tend to decline and become imbalanced, leading to many chronic diseases. Aging is known to be associated with numerous neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), and Huntington's disease (HD) (Nussbaum-Krammer and Morimoto, 2014). The process and causes of aging are not fully understood. By understanding the mechanisms of such causes, scientists may be able to create interventions to delay the onset of these diseases. The impact of these findings could be the promotion of healthy aging and longevity.

C. elegans as a model organism

The transparent nematode *Caenorhabditis elegans* (*C. elegans*) is a very useful model for aging studies. *C. elegans* share many biological structures and pathways with more complex organisms including humans, thus can be utilized as a model organism to study age-related disorders. Although we are distantly related, biological structures and processes such as cell signaling, metabolism, gene regulation, and specialized cells are shared between *C. elegans* and humans (Kaletta and Hengartner, 2006). *C. elegans* also has a short lifespan, an average of 21 days, and can be used in a variety of experimental approaches such as chemical compound treatment, environmental manipulations, and disease modeling (Olsen et al., 2006). The microscopic worm can be easily manipulated to produce mutant and transgenic strains by various methods, which has allowed scientists to study many biological processes and diseases. It is simple to study large numbers of age-synchronized populations as they are easily cultured and produce over

300 progeny via self-fertilization in five to seven days after hatching. These characteristics allow scientists to utilize *C. elegans* with far greater ‘through-put’ than other model organisms such as the lab mouse.

Pharmacological studies of aging

Studying various classes of compounds allows scientists to gain more knowledge about causes of aging by understanding a given compound’s mechanism of action. These classes include antioxidants, vitamins, laboratory made compounds, and anticonvulsants. Multiple chemical compounds have been shown to have an effect on aging in *C. elegans*. Previously reported studies show that antioxidants such as resveratrol (Wood et al., 2004) and disaccharides like trehalose (Honda et al., 2010) increase *C. elegans* lifespan. These group of compounds are known as natural products and have been shown to modulate lifespan in *C. elegans* and other organisms such as *Drosophila melanogaster* and mice (Spindler et al., 2013)

Natural products are found in our environment and are produce by living organism such as plants. An example of a natural product is diallyl trisulfide. It has been shown to increase worm lifespan by enhancing the function of the pro-longevity transcription factor SKN-1 (Powolny et al., 2011). The transcription factor SKN-1 in *C. elegans* is orthologous to mammalian nuclear respiratory factor (Nrf) family of transcription factors. The Nrf family of transcription factors plays a critical role in mediating adaptive responses to cellular stress and defends against neurodegeneration and aging (Staab et al., 2013).

Additionally, natural products such as gossypolone, quercetin, and salicylic acid also have been shown to be beneficial. Gossypolone, the major metabolite of the

naturally-occurring compound gossypol, possesses anti-steroidogenic and anti-reproductive activity (Gilbert et al., 1995). Gossypol has demonstrated anti-cancer activity and has been shown to have synergistic anti-tumor effects with valproic acid (Chen et al., 2016; Dong et al., 2016; Gong et al., 2015). Further investigation of both gossypol and gossypolone has been evaluated as a potential therapeutic for human breast cancer. Quercetin, a flavinoid found in many fruits and vegetables, is being increasingly proposed as a nutraceutical for its healthful activities (Dueñas et al., 2013). Salicylic acid, a naturally-occurring compound found in plants, displays antioxidant activity (De La Cruz et al., 2004; Guerrero et al., 2004). In addition to those natural products, the salt ionic compound, Lithium chloride, is also known to be beneficial. It is currently used in the treatment for bipolar disorder (Vosahlikova and Svoboda, 2016) and has been shown to increase lifespan of *C. elegans* (McColl et al., 2008). Lithium chloride has even been correlated with longevity in humans (Zarse et al., 2011).

Synthetic compounds, including FDA approved drugs, have also been tested for their effects on aging in *C. elegans*. Anticonvulsants have been observed to increase the lifespan of *C. elegans* (Collins et al., 2008; Evason et al., 2005; Kornfeld and Evason, 2006). Anticonvulsants are a diverse group of pharmacological agents that modulate neural activity in vertebrates (Villalba et al., 2016). There is an extensive study of various synthetic compounds that has been shown in the Lithgow Laboratory to increase the lifespan of *C. elegans*. Alavez and colleagues found that the chemical compound thioflavin T (ThT), an amyloid-binding dye, extended the lifespan of *C. elegans* by improving protein homeostasis (Alavez et al., 2011). Another pro-longevity chemical compound identified in the Lithgow Laboratory is nitrophenyl piperazine-1 (NP1). NP1

was discovered by screening diverse drug like compounds for effectiveness at prolonging the lifespan of *C. elegans*. Lucanic and colleagues have shown that NP1 works through a dietary restriction (DR) mechanism (Lucanic et.al, in press at *Aging Cell*).

Overview

With *C. elegans* as a model, I studied the additive effects of natural products and synthetic compounds on lifespan extension and healthspan promotion. I tested mixtures of natural products and a specific combination of synthetic compounds. I examined whether or not combinations of compounds could result in additive or synergistic effects on *C. elegans* lifespan extensions and whether these combinations of compounds could promote healthy aging. By understanding the effects of these compounds on lifespan and healthspan, we can further understand the role of aging and age-related diseases, with the goal of preventing age-related pathologies such as AD, HD, ALS, and PD. Additionally, identifying a vital mix of compounds can lead to novel therapeutics to treat age-associated diseases.

2. Materials and Methods

2.1 C. elegans strains

The wild-type (N2), TJ1060:*spe-9(hc88);fer-15(b26)* strains were obtained from the *Caenorhabditis* Genetics Center (CGC) (St. Paul, Minnesota).

2.2 Synchronization of Worm Culture

2.2.1 Standard Synchronization

Wild-type (N2) worms were synchronized via standard synchronization. The process began with 50 gravid adults placed in fresh nematode grown media (NGM) plates and were allowed to lay eggs for 1-2 hours. After the period of egg laying, the 50 gravid

adults were removed from the agar plate with a flattened platinum wire. The eggs left on the plates were placed back in the 20°C incubator and allowed to hatch. Three days later, the resulting synchronized population of day 1 adults was then used for aging experiments.

2.2.2 Hypochlorite Synchronization

TJ1060 animals were synchronized via hypochlorite synchronization. The animals were floated off the media of a plate with S-basal solution (a bacterial and nematode buffer). The liquid was then pulled off using a pipette and transferred to a 15mL conical tube. The tube was centrifuged at 1700 RPM for 1 min. and 30 sec., and the supernatant was aspirated. A hypochlorite solution (5mL KOH, 5mL NaOCl, and 40mL water) was added to the tube, and it was shaken and vortexed for approximately 5-7 minutes, or until the bodies of the adult worms were no longer visible. The tube was centrifuged again, and the hypochlorite solution was removed. The resulting eggs were washed with three washes of S-basal solution and centrifuged and aspirated each time. The eggs were placed in 50mL conical tubes containing fresh S-basal on a shaker for 48 hours yielding synchronous starved L1 larvae. After 48 hours, L1 larvae were spotted onto NGM plates and incubated at 25°C until experimental use at day 1 adulthood.

2.3 Standard Media Preparation

Unless otherwise stated, worms were cultured at 20°C on NGM plates. Standard NGM plates were made with Bacto™ agar, an algae-derived polysaccharide, Bacto™ peptone, a protein digest, and sodium chloride. NGM plate ingredients are as follows: 17g of agar, 2.5g of peptone, 3g of NaCl, 975mL of water totaling a volume of 1L. In addition to those, 25ml of phosphate buffer (5M KPO₄), 1mL of 1M MgSO₄, 1mL of 1M

CaCl₂, 1mL Cholesterol (5mg/mL) were added. Worm cultures were fed a strain of *Escherichia coli* called OP-50.

2.4 FUdR Media

For all lifespan assay plates, media with a chemical that inhibits DNA synthesis were prepared to prevent larval contamination. Media is prepared according to the standard preparation as mentioned above. In addition to the ingredients listed, 5-Fluoro-1-[4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl]-1H-pyrimidine-2,4-dione (FUdR) is added, resulting in a final concentration of 10µg/mL FUdR.

2.5. Natural Product Screen

For the high-throughput screen, 96-well plates with all possible pair-wise combinations were tested in triplicate. One plate consisted of a master key of the 25 natural products used and the other plates consisted of one single natural product. Using a multi-channel pipette, 0.3 µL was obtained from the master key plate and was pipetted in 0.3 µL of the single natural product plate producing combination pairs. This was repeated until all natural products were mixed with one another. Approximately 10 gravid TJ1060 animals were then placed in each well exposed to the pair-wise combinations. Plates were placed in 25°C until day 13. On day 13, plates were scored manually with a microscope. A percentage of live animals over total number of worms were obtained.

2.6 Compound Treatments

Compounds tested were added to the NGM plates after addition of food (final concentration listed in parenthesis): diallyl trisulfide (5µM), gossypolone (22µM), lithium chloride (10mM), quercetin (100µM), salicylic acid (100µM), berberine chloride (100µM), vitamin A (50µM), myricetin (50, 100µM), curcumin (50, 100µM), NP1 (10,

50, 100, 200, 500 μ M), ThT (1, 10, 50, 100, 200 μ M). Compounds were dissolved into concentrated stock solutions and further diluted to working solutions prior to addition to assay plates. Compounds were always added at least one-day prior to use to allow for distribution of the chemicals throughout the cultured plates. Control vehicle was DMSO at a concentration of 0.25% unless otherwise stated.

2.7 Lifespan Analysis

Synchronized worm populations (from 1 hour egg lays) were added to 3cm NGM plates supplemented with 10 μ g/mL FUdR at day one of adulthood. All lifespan experiments were performed at 20°C unless otherwise stated. Worms were scored and transferred every other day, with weekly transfers to fresh NGM plates.

Scoring/measuring was achieved by counting the worms on each plate and marking what was dead, alive, or lost/bag. Worms that failed to display touch-provoked movement were scored as dead. Worms that died from causes other than aging, such as sticking to the walls of the plates, internal hatching of eggs ('bagging'), or gonadal extrusion were censored as lost/bag worms.

Data from the lifespan experiments were entered in a binary fashion into Prism™ 6.0 for Mac OS X (GraphPad Software, San Diego, California). Various tests were used within the Prism™ software to calculate statistical significance including log-rank (Mantel Cox) test and unpaired t-test.

2.8 Healthspan Analysis

2.8.1 Body Movement Measurement

Age-related decrease in worm movement was analyzed as part of a healthspan measurement. Worms were recorded inside the bacterial lawn of either a 3cm NGM agar

plate or a 24-well, 2cm NGM plate. For experiments using the 3cm NGM plates, the Leica microscope (DFC 400, San Bernardino, CA) was used to capture 15-second digital video of a population of worms at 1.6x magnification and converted into audio video interleaved (AVI) file, a sound and motion picture format, prior to analysis using WormLab software (MBF Bioscience, Williston, Vermont). Each movie was analyzed using the average peristaltic speed of the worms. Peristaltic speed is defined as the forward movement of the worm (peristaltic track length) divided by seconds moving.

For experiments using the 24-well NGM plates, the Zeiss microscope (Stemi 508, Pleasanton, CA) with a Canon Rebel (EOS Rebel T5i) attachment was used to capture 15-second digital videos. The videos were at 1.6x magnification and converted into AVI file, prior to analysis using WormLab software. Each movie was analyzed using the average peristaltic speed metric.

3. Results

Natural product screen for pair-wise combinations effects on aging phenotype and longevity

Single compounds have been shown to extend *C. elegans* lifespan but historically lack potency compared to genetic mutations. Genetic mutations in *C. elegans* are able to produce large lifespan effects, reports vary dramatically and examples include a robust doubling as well as an unusual 10-fold increase (Kenyon et al., 1993; Shmookler Reis et al., 2009). In addition, double mutations in *C. elegans* synergistically prolonged longevity by over 400% extension compared to control (Chen et al., 2013). However, single chemical compounds have exhibited relatively moderate effects on the lifespan of *C.*

elegans with between 20-30% considered strong and 40-60% relatively exceptional (Lucanic et al., 2013; Ye et al., 2014).

To determine if combining chemicals in a pair-wise manner could mimic the lifespan effects of genetic mutations, a high-throughput screen was conducted using a list of natural products suspected or previously reported to extend lifespan. Compounds were tested in pairs with the intent of systematically finding additive and synergistic effects. Results from the screen identified a set of compounds that appeared to have positive lifespan effects on *C.elegans*. Noting the chemical structures, the products were not structurally related, with the exception of myricetin and quercetin (Fig. 1). These compounds were then used in standard lifespan assays for further evaluation, which would allow for the rational discovery of more complex mixtures.

Figure 1. Chemical structure of natural products.

Natural products effects on lifespan

Many compounds have been shown to produce an effect on the lifespan of model organisms including *C. elegans*. In the research community, attention has been focused on the protective role of naturally occurring antioxidants, generally in biological systems (Dkhil et al., 2015). Natural products are of interests because the Federal Drug Administration (FDA) generally recognizes many natural products such as vitamins, antioxidants, metabolites, and micronutrients as safe. With many beneficial effects of natural products, I chose to examine specific combinations to see if there may be additive benefits on the lifespan extension in *C. elegans*.

To determine whether or not combinations of compounds could cause positive effects on *C. elegans* lifespan, I generated a master mix of natural products and performed a lifespan experiment. These compounds were the top candidates from a high-throughput screening of natural products for lifespan effects on *C. elegans* (Fig.1). Specifically, I studied the following cocktail mixture of natural products: diallyl trisulfide (5 μ M) +gossypolone (22 μ M) +lithium chloride (10mM) +quercetin (100 μ M) +salicylic acid (100 μ M). The mixture contained all five compounds at the concentration as mentioned. I performed the lifespan experiments at 25°C using TJ1060 animals, which are sterile when grown at this temperature. Assay was repeated at 20°C using wild-type (wt) N2 animals. Results from the lifespan at both 25°C and 20°C were similar in that the mixture of the five natural products did not produce any significant positive effects on the lifespan (Fig. 2 A and B). With the exception of lithium chloride, none of compounds individually increased lifespan.

Figure 2. *C. elegans* Survival Analysis with Various Treatments. **A.** Lifespan curve of synchronous population of genetic strain TJ1060 animals of *C. elegans* exposed to different compounds at 25°C. Control (n=45), dia tris (n=41), gossy (n=48), LiCl (n=36), quer (n=39), sal acid (n=42), mix (n=57) **B.** Lifespan curve of synchronous population of wild-type (N2) animals of *C. elegans* exposed to different compounds at 20°C. Control (n=109), dia tris (n=54), gossy (n=59), LiCl (n=55), quer (n=59), sal acid (n=58), mix (n=58). Statistical tests based on log-rank (Mantel-Cox) test. For more statistical analysis see table 1 and 2.

Table 1. Statistical Analysis of TJ1060 Survival

Intervention	Median	n [censored]	p-value (Mantel Cox) vs. control	Ratio of median intervention vs. control	95% CI of ratio
Control	9	45 [14]			
diallyl trisulfide 5μM	9	41 [16]	0.4256	1	0.6550 to 1.527
gossypolone 22μM	9	48 [12]	0.7514	1	0.6658 to 1.502
lithium chloride 10mM	12	36 [35]	0.0059/ **	1.333	0.8602 to 2.067
quercetin 100μM	10	39 [21]	0.1156	1.111	0.7237 to 1.706
salicylic acid 100μM	8	42 [19]	0.8539	0.8889	0.5837 to 1.354
Mix: dial tris +gossy +LiCl +quer +sal acid	10	57 [6]	0.763	1.111	0.7516 to 1.642

Table 2. Statistical Analysis of N2 Survival

Intervention	Median	n [censored]	p-value (Mantel Cox) vs. control	Ratio of median intervention vs. control	95% CI of ratio
Control	11	109 [10]			
diallyl trisulfide 5μM	11	54 [6]	0.0896	1	0.7217 to 1.386
gossypolone 22μM	11	59 [1]	0.9917	1	0.7285 to 1.373
lithium chloride 10mM	12	55 [6]	0.0061/ **	1.091	0.7889 to 1.509
quercetin 100μM	11	59 [1]	0.4236	1	0.7285 to 1.373
salicylic acid 100μM	11	58 [3]	0.547	1	0.7272 to 1.375
Mix: dial tris +gossy +LiCl +quer +sal acid	11	58 [2]	0.0546	1	0.7272 to 1.375

Berberine chloride pair-wise combination affects lifespan

The fact that my initial approach of combining multiple compounds at once did not appear to extend lifespan, I began to find pair-wise combinations for testing. Using the results from the previously mentioned natural compound screen, I chose pair-wise combinations that were considered to produce additive lifespan extension in *C. elegans*. In the screen, berberine chloride, a naturally occurring plant alkaloid was among the top candidates with a positive effect on lifespan.

Berberine chloride is well studied and known to have many biological effects such anti-inflammatory and anti-oxidative activities (Battu et al., 2010; Küpeli et al., 2002; Misík et al., 1995). Berberine chloride combined with vitamin A, myricetin, gossypolone, or curcumin showed positive effects in the results from the screen, so I therefore performed thorough lifespan experiments with pair-wise combinations. Results for one experiment are shown in Fig. 3. In all of the combinations shown in Fig. 3 A-D, berberine chloride significantly increased lifespan compared to the control and the combinations acted similarly as berberine chloride itself. This suggests that there were no added benefit of the other natural products, since berberine chloride on its own could produce just as much of an extension. In all cases, the combination of compounds had the longest maximum lifespan compared to the individual compounds on their own and the control.

Figure 3. *C. elegans* Survival Analysis with Berberine Chloride Pair-wise Treatments. Lifespan curve of synchronous population of TJ1060 strain of *C. elegans* exposed to natural products at 25°C. **A.** brcl+vitA combination ($p<0.0004$, $n=19$). **B.** brcl+myr combination ($p<0.0003$, $n=38$). **C.** brcl+gossy combination ($p<0.0073$, $n=40$). **D.** brcl+curc combination ($p<0.024$, $n=30$). Statistical tests based on log-rank (Mantel-Cox) test. For more statistical analysis see table 3.

Table 3. Statistical Analysis of Berberine-Chloride Survival

Intervention	Median	n [censored]	p-value (Mantel Cox) vs. control	Ratio of median intervention vs. control	95% CI of ratio
Control	7	47 [3]			
berberine chloride 100μM	12	23 [4]	0.0028/ **	1.714	1.041 to 2.823
gossypolone 22μM	9	42 [12]	0.1259	1.286	0.8480 to 1.949
vitamin A 50μM	9	20	0.2078	1.286	0.7619 to 2.170
myricetin 50μM	7	45 [1]	0.1188	1	0.6645 to 1.505
curcumin 50μM	9	41 [3]	0.0842	1.286	0.8457 to 1.955
berberine chloride + gossypolone	12	40 [1]	0.0073/ **	1.714	1.125 to 2.613
berberine chloride + vitamin A	14	19 [7]	0.0004/ ***	2	1.174 to 3.408
berberine chloride + myricetin	12	38 [3]	0.0003/ ***	1.714	1.118 to 2.629
berberine chloride + curcumin	12	30	0.024/ *	1.714	1.084 to 2.710

Combinatorial Mixture Increases Lifespan

From previous results, I found that berberine chloride on its own could produce a lifespan extension. In addition, a combination of berberine chloride and another natural compound could also produce a lifespan extension compared with control (Fig. 3). I then decided to revert back to my initial approach of combining more than two compounds to find a working cocktail mixture. I combined berberine chloride and three other compounds, vitamin A, curcumin, and myricetin, that have been shown to increase lifespan, in my own observation, data from the Lithgow Lab, and results from the screen, to see if I could find additive benefits.

Vitamin A, a nutritional organic compound, has been proven to be beneficial for the maintenance of the immune system and vision (Guo et al., 2012; Indrevær et al., 2013; Maeda et al., 2009). Both curcumin, a natural phenol, and myricetin, a flavonoid compound, are known to have anti-inflammatory and antioxidant properties (Franceschi et al., 2016; Gokce et al., 2016; Latief et al., 2015). All of the compounds have also been shown to mediate lifespan extension in *C. elegans*. To determine whether this cocktail mixture could produce any positive lifespan effects, I performed a lifespan assay of the four compounds in combination and saw the following results shown in Fig. 4. The combinations of four compounds were able to produce significant extension compared to control. Two different concentrations of curcumin and myricetin were used because of the solubility issues that occurred. Nonetheless, both concentrations were able to produce significant lifespan extension when combined with the other two compounds. Berberine chloride alone also significantly increased the lifespan. These results were similar to the results shown in Fig. 3 A-D.

Figure 4. *C. elegans* Survival Analysis with Various Treatments. Lifespan curve of synchronous population of wild-type (N2) strain of *C. elegans* exposed to natural products at 20°C. **A.** Mix 1 containing brcl (100μM), vitA (50μM), curc (100μM), and myr (100μM) significantly increased lifespan compared to control ($p < 0.0001$, $n = 62$). **B.** Mix 2 containing brcl (100μM), vitA (50μM), curc (50μM), and myr (50μM) significantly increased lifespan compared to control ($p < 0.0001$, $n = 59$). Statistical tests based on log-rank (Mantel-Cox) test. For more statistical analysis see table 4 and 5.

Table 4. Statistical Analysis of Mix 1 Survival

Intervention	Median	n [censored]	p-value (Mantel Cox) vs. control	Ratio of median intervention vs. control	95% CI of ratio
Control	15	93 [11]			
Mix 1: brcl +vitA +cur (100µM) +myr (100µM)	19	62 [6]	< 0.0001/ ****	1.267	0.9185 to 1.747
berberine chloride	22	54 [8]	< 0.0001/ ****	1.467	1.049 to 2.051
vitamin A	15	64 [6]	0.7613	1	0.7274 to 1.375
curcumin 100 µM	19	62 [4]	0.0031/ **	1.267	0.9185 to 1.747
myricetin 100 µM	15	64 [5]	0.8221	1	0.7274 to 1.375

Table 5. Statistical Analysis of Mix 2 Survival

Intervention	Median	n [censored]	p-value (Mantel Cox) vs. control	Ratio of median intervention vs. control	95% CI of ratio
Control	15	93 [11]			
Mix 2: brcl+vitA+cur(50µM)+m yr(50µM)	22	59 [11]	< 0.0001/ ****	1.467	1.057 to 2.036
berberine chloride 100µM	22	54 [8]	< 0.0001/ ****	1.467	1.049 to 2.051
vitamin A 50µM	15	64 [6]	0.7613	1	0.7274 to 1.375
curcumin 50µM	19	69 [4]	< 0.0001/ ****	1.267	0.9277 to 1.729
myricetin 50µM	15	63 [4]	0.2498	1	0.7263 to 1.377

Nitrophenyl Piperazine-1 (NP1) and thioflavin T (ThT) in Combination Increases Lifespan

In testing for more robust additive effects, I took another approach by looking at lifespan extension mediated by synthetic compounds. Prior to my arrival, there were two well studied chemical compounds in the Lithgow Laboratory that have been shown to produce significantly large lifespan extension. NP1 data has been reported to modulate the lifespan of *C. elegans* by Mark Lucanic. NP1 modulates sensory pathways that can elicit physiological responses associated with dietary restriction in *C. elegans* to extend their lifespan (Lucanic et.al., in press at *Aging Cell*). Sylvestre Alavez, a former post-doctoral researcher in the Lithgow Lab, discovered that thioflavin T (ThT) had effects on lifespan in *C. elegans* (Alavez et al., 2011). ThT, an amyloid-binding dye, extended lifespan by improving protein homeostasis.

To determine whether these two compounds could produce additive or synergistic effects on lifespan, I performed a lifespan experiment with both treatments administered simultaneously. I found that NP1 50 μ M and ThT 50 μ M additively increased lifespan compared to the control (Fig. 5). The median lifespan of the combination treated populations were greatly increased by 93% compared to the control animals. Critically, the combination also performed better than the individual compounds alone in terms of increasing lifespan.

Figure 5. NP1 and ThT Combined Produced Additive Lifespan Effects. **A.** NP1 chemical structure. **B.** ThT chemical structure. **C.** Lifespan curve of synchronous population of wild-type (N2) animals of *C. elegans* exposed to NP1 50µM, ThT 50µM, and NP1 50µM+ThT 50µM at 20°C from four technicians in four parallel experiments. All compounds extended lifespan compared to control. NP1 and ThT together produced a significant effect in extension ($p < 0.0001$, $n = 176$). Statistical test based on log-rank (Mantel-Cox) test. For more statistical analysis see table 6.

Table 6. Statistical Analysis NP1+ThT Survival

Intervention	Median	n [censored]	p-value (Mantel Cox) vs. control	Ratio of median intervention vs. control	95% CI of ratio
Control	15	245 [24]			
NP1 (50µM)	19	221 [24]	< 0.0001/ ****	1.267	1.056 to 1.519
ThT (50µM)	19	140 [133]	< 0.0001/ ****	1.267	1.029 to 1.559
NP1 (50µM) + ThT (50µM)	29	176 [67]	< 0.0001/ ****	1.933	1.593 to 2.346

Optimizing NP1 and ThT Lifespan Effects on C. elegans

NP1 and ThT repeatedly showed robust effects in producing a lifespan extension (Fig. 6). To determine the optimal dosage of NP1, I conducted a dose response assay using different concentrations of NP1. NP1 increased lifespan at various concentrations (Fig. 7). NP1 at 50 μ M demonstrated a 47% increase in median lifespan while NP1 at 100 μ M indicated a 73% increase and 200 μ M indicated a 60% increase median lifespan compared to control. On the other hand, NP1 exhibited toxicity at a high concentration of 500 μ M and significantly decreased lifespan of *C. elegans*.

To determine the optimal dose of ThT, I also performed a ThT dose-dependent response assay similar to the NP1 dose-dependent assay. Results presented in Fig. 8 show that ThT increased lifespan at 10 μ M, 50 μ M, and 100 μ M. Results indicate that 10 μ M of ThT increased median lifespan by 47% compared to control (Fig. 8). ThT at 50 μ M and 100 μ M both demonstrated a 13% increase in median lifespan compared to control. Similar to NP1 at 500 μ M, ThT at 200 μ M displayed extreme toxicity and significantly decreased lifespan.

Figure 6. **Median Lifespan of Multiple Experiments Exposed to NP1, ThT, NP1+ThT Treatments.** Median lifespan of individual lifespan experiments. Median lifespan was taken from survival analysis of synchronous population of wild-type (N2) exposed to NP1 (50µM), ThT (50µM), and NP1 (50µM)+ThT (50µM). Each dot represents an individual experiment consisting of an average of 65 worms. Standard error bars are shown.

Figure 7. *C. elegans* Survival Analysis with NP1 Treatment. Lifespan curve of synchronous population of wild-type (N2) animals of *C. elegans* exposed to 10µM of NP1 up to 500µM of NP1 at 20°C. NP1 significantly extended lifespan at 10µM ($p < 0.0001$, $n = 181$), 50µM ($p < 0.0001$, $n = 210$), 100µM ($p < 0.0001$, $n = 228$) and 200µM ($p < 0.0001$, $n = 169$). NP1 decreased lifespan at 500µM ($p < 0.0994$, $n = 180$). Statistical test based on log-rank (Mantel Cox) test. Graph represents three parallel experiments from three technicians. For more statistical analysis see table 7.

Table 7. Statistical Analysis of NP1 Dose Response

Intervention	Median	n [censored]	p-value (Mantel Cox) vs. control	Ratio of median intervention vs. control	95% CI of ratio
Control	15	216 [12]			
NP1 10µM	15	181 [15]	< 0.0001/ ****	1	0.8208 to 1.218
NP1 50µM	22	210 [13]	< 0.0001/ ****	1.467	1.213 to 1.773
NP1 100µM	26	228 [10]	< 0.0001/ ****	1.733	1.439 to 2.088
NP1 200µM	24	169 [26]	< 0.0001/ ****	1.6	1.308 to 1.957
NP1 500µM	8	180 [43]	0.0994	0.5333	0.4376 to 0.6500

Figure 8. *C. elegans* Survival Analysis with ThT Treatment. Lifespan curve of synchronous population of wild-type (N2) animals of *C. elegans* exposed to 1µM of ThT up to 200µM of ThT at 20°C. ThT extended lifespan at 10µM ($p < 0.0001$, $n = 162$), 50µM ($p < 0.0043$, $n = 171$), and 100µM ($p < 0.362$, $n = 163$). ThT significantly decreased lifespan at 200µM ($p < 0.0001$, $n = 177$). Statistical test based on log-rank (Mantel-Cox) test. Graph represents three parallel experiments from three technicians. For more statistical analysis see table 8.

Table 8. Statistical Analysis of ThT Dose Response

Intervention	Median	n [censored]	p-value (Mantel Cox) vs. control	Ratio of median intervention vs. control	95% CI of ratio
Control	15	190 [18]			
ThT 1 µM	16	163 [47]	0.1391	1.067	0.8653 to 1.315
ThT 10 µM	22	162 [50]	< 0.0001/ ****	1.467	1.189 to 1.809
ThT 50 µM	17	171 [38]	0.0043/ **	1.133	0.9218 to 1.393
ThT 100 µM	17	163 [32]	0.0362/ *	1.133	0.9193 to 1.397
ThT 200 µM	3	177 [24]	< 0.0001/ ****	0.2	0.1630 to 0.2454

NP1, ThT, and NP1+ThT Improves Movement

There is no general definition of healthspan, in that it could mean a healthy period of time in a person's life or a term used to predict age-related mortality (Crimmins, 2015). In laboratory settings, particularly using *C. elegans*, we can broadly define healthspan by measuring physiological parameters over the lifespan of the worm (Bansal et al., 2015). One parameter that can be measured to study healthspan is the body movement of the worms. It has been published that age-related decline in movement of the worms is correlated with lifespan (Huang et al., 2004).

Based on these findings, I examined the effects of NP1, ThT, and the combination, to see if these compounds would affect age-dependent decline in body movement of *C. elegans*. Results suggest that as worms age, their movement declines (Fig. 9). For the control animals, decline in movement plateaus from day 8 and beyond. With compound treatment, there is observable preservation of movement from day 9 to day 15, compared to controls (Fig. 9B). Animals with compound treatment performed better than control animals that were not on compound. The combination of NP1 and ThT did not exhibit additive or synergistic effects compared to the compounds alone. These results suggest that chemical compounds could delay the detrimental effects of movement in late-stage animals.

Figure 9. *C. elegans* Movement Analysis with NP1, ThT, and NP1+ThT Treatment. Peristaltic speed (forward movement/time) of wild-type (N2) animals was measured over the course of 19-23 days. **A.** Average peristaltic speed of N2 animals on various compounds treatments. **B.** Quantitative analysis of day 9, day 10, day 12, and day 15. Error bars represent standard deviation. Statistical test based on unpaired t-test generated using Prism. For more statistical analysis see table 9.

Table 9. Statistical Analysis of *C. elegans* Movement

	Day 9		Day 10		Day 12		Day 15	
Intervention	n	unpaired t test vs. control	n	unpaired t test vs. control	n	unpaired t test vs. control	n	unpaired t test vs. control
Control	24		18		15		8	
NP1 50 μ M	15	0.0277/ *	13	0.0042/ **	14	ns	15	0.003/ **
ThT 50 μ M	15	0.0172/ *	15]	0.005/ **	12	0.0195/ *	15	0.0355/ *
NP1 50 μ M+ ThT 50 μ M	15	0.0002/ **	14	ns	14	0.0194/ *	13	0.0031/ **

4. Discussion

Various chronic diseases increase in frequency with advancing age. These include an array of neurodegenerative diseases such as Parkinson's disease (PD), Huntington's disease (HD), and amyotrophic lateral sclerosis (ALS), and chronic diseases like cardiovascular disease, osteoporosis, and diabetes (López-Otín et al., 2013; Lucanic et al., 2013). In almost all cases, diseases accumulate from many factors such as disruption of protein balance, improper nutrition, genetic and environmental factors. The mechanism that links old age and diseases have yet to be fully determined but scientists have proposed many models. One method that holds promise for protecting against the harmful effects of aging is the use of novel compounds as potential treatments for age related diseases. My goal was to find novel combinations of compounds that could have potential effects in extending lifespan and further promote healthy aging, using a *C. elegans* model.

Additive effects of chemical combinations

Chemical effects on aging are widely studied in various model organisms including *C. elegans*. In this thesis, I demonstrated that lifespan extension could be

achieved by combining chemical compounds. Whether they were synthetic or natural products, this procedure could generate an increase in lifespan. However, there were instances where a combination of the compounds, in a cocktail mixture or pair-wise manner, did not cause additive effects (Fig. 2 and 3). There could have been a variety of reasons why these mixtures did not increase lifespan or have any additive benefits. One reason is the solubility of the compounds when combined. Once combined some of the compounds appeared to come out of solution. Another reason may be due to the fact the natural products were not at its optimal dosages. A solution to the dosage problem is to perform dose-dependent assays with each natural product similar to the dose-dependent assays described here for NP1 and ThT (Fig. 7 and 8).

Additionally, these compounds could be targeting the same pathway to increase lifespan, thereby perhaps precluding additive effects. On the other hand, the compounds could be activating pathways that have negative effects towards one another. For instance in Fig. 4, berberine chloride and curcumin alone, produced just as much of an extension as the mixture. Berberine chloride even produced a greater maximum lifespan compared to the mixture. The other compounds in Fig. 4, vitamin A and myricetin, had no effect on lifespan, therefore adding them to berberine chloride and curcumin in a mixture provided no further increase. More research needs to be conducted to further analyze the mechanism of actions.

Optimizing compound dosage for robust effects on longevity

In an attempt to optimize the correct dosage for the purpose of ensuring robustness of chemical compounds in *C. elegans* lifespan, NP1 and ThT dose-dependent assays were conducted (Fig. 7 and 8). From my observations of the various

concentrations of NP1, I found that 50 μ M of NP1 was the optimal dosage. This is mainly due to solubility issues with NP1 at 100 μ M and 200 μ M. At these doses, NP1 came out of solution when seeded on the NGM plates and precipitation from the chemical was visible, which was a confounding factor for the remainder of the experiment. However at 50 μ M of NP1, there was no observable precipitation and therefore was chosen as the optimal dosage for further experiments. This concentration is also consistent with previous data obtained from the Lithgow Laboratory.

In the case of ThT, 10 μ M showed the most robust lifespan extension, in contrast to the previously published dose response where 50 μ M and 100 μ M had the most robust effect. This effect may be due to the fact that the authors' started their lifespan at the fourth larval stage (L4) of the worms while I started my lifespans at day 1 of adulthood. In addition, I observed ThT increased the maximal lifespan by 21% at 50 μ M and 100 μ M compared with control. Although toxicity at the early ages of the worms is observable at 50 μ M, I chose to use 50 μ M of ThT as my optimal dosage, which is consistent with the published paper. The cause of toxicity is yet to be determined.

Healthy Aging

How long we live, and what proportion of that life is spent in healthy years, has important implication for individuals. These implications could range from potential burden of caregiving on surviving family members, valuing life-insurance, and adequacy of retirement benefits and savings (Beltrán-Sánchez et al., 2015). Improving healthspan and delaying or preventing age-related diseases would have huge benefits for society and on the individual level. The idea that chemical compounds that produced lifespan extension could promote healthy aging, steered me to test healthspan metrics.

Here I have demonstrated that chemical compounds could improve healthspan in late-aged worms. Interventions that make animals live longer have the potential to make them healthier. These compounds' combined mechanisms of action are not fully understood or have not been determined. One hypothesis for how lifespan extending compounds additively improve healthspan would be through accumulation of beneficial pathways that work jointly to produce such positive effects. In the case of NP1 and ThT, both compounds are known to be potent in different ways. NP1 is a compound, which elicits DR and thereby promotes lifespan extension. ThT's mechanism of action is still unclear, but is believed to act through improved protein homeostasis for lifespan extension. Additional study and analysis needs to be conducted in order to define the molecular pathways of these compounds and to determine whether stimulation of these distinct pathways results in some emergent pathway effects not observed in either single treatment as suggest by the apparent synergistic effects we sometimes observed.

Another idea for determining the mechanism of action is to look at structure activity of the compounds (Fig. 1 and 5A and B). By examining the structures, we can identify more stable forms with better drug-like properties, perhaps improving efficacy and delivery. We can manipulate the structures such as removing or adding chemical groups and find the importance or differences between one structure and another and ask what happens to the activity. One example of chemical structures being closely related but produced opposite effects is the chemical compound BTA-1, a derivative of ThT. BTA-1 differs from ThT in that BTA-1 has three less methyl groups. In a preliminary experiment, I have determined that BTA-1 is very toxic in *C. elegans* even in the nM

range (data not shown), perhaps suggesting that methyl groups are of importance in stabilizing the structure for positive effects.

5. Conclusion

Aging is a time-dependent functional decline that affects most organisms. By understanding how aging occurs and why various diseases are associated with aging, we can find ways to live a healthier life. Using *C. elegans* as model, I have demonstrated that chemical compounds in combination could extend its lifespan and further promote healthy aging. From such a small organism, research work in *C. elegans* can be translated into mice models furthering the potential for human clinical trials. The combinatorial approach will be key to finding the cocktail of compounds that might benefit mammalian healthspan.

Chapter 2

Probing Effects of Compounds on A Neurodegenerative *C. elegans* Model

1. Introduction

Amyotrophic lateral sclerosis (ALS) is the most prominent adult-onset motor neuron disease (Witan et al., 2008). There are two types of ALS: sporadic and familial. Sporadic ALS is the most common form of the disease in the U.S. accounting for 90-95% of all cases while familial ALS (fALS) is an inherited disease and accounts for 5-10% of all cases (Bocchitto et al., 2012). ALS is a complex disorder with a spectrum of phenotypes that could comprise a single disease or represent several closely related diseases. The clinical progression is generally rapid, beginning with muscle loss followed by muscle degradation, paralysis, and respiratory problems (Clerc et al., 2016). By understanding the mechanism behind this progression, we are better able to find therapies to treat ALS.

Among some fALS cases, mutations in the copper (Cu)/ zinc (Zn)- superoxide dismutase-1 (SOD1) gene have been identified, but the general mechanism behind the genetic mutation of SOD1 causing such disease is debatable (Ogawa et al., 2015). A hallmark in SOD1-related disease is abnormal accumulation of misfolded proteins, further producing toxic protein aggregation build-up. SOD1 is a thermally stable enzyme that removes superoxide at the bound Cu ion and can be stabilized by binding of the Zn ion (Furukawa and O'Halloran, 2005).

There is only one approved FDA drug, riluzole, which slows the progression ALS, but there are many symptomatic therapies (Salameh et al., 2015; Clerc et al., 2016). More research will have to be conducted to gain greater insight into human genes involved in ALS and understanding the disease phenotype at a molecular level. In chapter 1 of this thesis, I surveyed many compounds that could increase lifespan and promote

health aging in *C. elegans*. I next examined if these compounds could also be protective in an ALS phenotype. Specifically, I asked whether or not these compounds could interfere with the proteostasis network in relation to aging to increase lifespan and improve movement.

C. elegans, whose genome contains homologs of approximately two-thirds of all human disease genes, are popular model organisms for studying human diseases (Olsen et al., 2006). To study ALS in *C. elegans*, I examined the transgenic mutant human SOD1 overexpression model, G85R. *C. elegans* expressing human G85R SOD1 accumulate SOD1 aggregates and demonstrate reduced mobility (Bocchitto et al., 2012). I studied the effects of compounds, which were known to extend lifespan, on the mobility of the ALS animals. Because Cu/Zn is important for stability of the SOD1 protein, I also examined the effects of Cu and Zn on improving the ALS phenotype. The significance of these findings will lead to future studies, translated to different model organisms such as the lab mice, for better understanding of ALS.

2. Materials and Methods

2.1 *C. elegans* strain

The wild-type (N2) strain was obtained from the *Caenorhabditis* Genetics Center (CGC) (St. Paul, Minnesota). The transgenic *C. elegans* lines G85R (line 10) and wild-type human SOD1 (line 7) were provided from the Wang lab, John Hopkins University (Baltimore, Maryland) (Wang et al., 2009).

2.2 Synchronization of worm culture

See Chapter 1: Materials and Methods, 2.2

2.3 Standard media prep

See Chapter 1: Materials and Methods, 2.3

2.4 Compound treatment

Compounds tested were added to the NGM plates after addition of food (final concentration listed in parenthesis): NP1 (50 μ M), ThT (50 μ M), trehalose (5mM), mianserin (50 μ M), ethylenediaminetetraacetic acid (EDTA) (2.8mM), ZnCl (1mM), CuCl (0.27mM). For further details see Chapter 1: Materials and Methods, 2.6.

2.5 Healthspan Analysis: Thrashing/Body Bends Measurement

Movement via body bends of N2, G85R, and SOD1 strains were measured as an alternative healthspan metric. Synchronous populations were transferred to chemical plates at day 1 of adulthood. After 24 hours, adult animals were transferred to 10 μ L S-Basal buffer. After 30 seconds of adaptation to S-Basal, the number of body bends was then counted for 30 seconds. Movement of the worm from pharynx to tail defines a body bend. An average body bends of the worms from each compound and compound mixtures were plotted using Prism 6 software. Statistical tests such as an unpaired t-test was acquired via Prism 6 software.

3. Results

Temperature and FUDR effects on ALS phenotype

Before we began examining the effect of compounds on the ALS phenotype, I tested whether temperature had any effects on adult body bends. In addition to temperature, I also examined the worms on FUDR and non-FUDR. It has been shown that FUDR improved protein homeostasis, stress resistance, and healthspan in wild-type animals (Angeli et al., 2013). I examined the worms at 15°C, 20°C, and 25°C on non-FUDR and FUDR-treated plates. Results in Fig. 1 show that at 15°C, worms on FUDR and

non-FUdR had no significant changes between the wild-type N2 (WTN2) and wild-type SOD1 (WTSOD1) compared with the mutant G85R. At 20°C, I observed that the mutant G85R moved a lot less than the two wild-type models and that there were no differences between FUdR and non-FUdR treatments. At 25°C, the mutant G85R had little movement which could mean that the worms development was severely affected at 25°C. Taken together these results, I moved forward in my experiments using FUdR conditions at 20°C since these conditions allowed me to cultivate worms for a longer period of time and there were no difference in movement between FUdR and non-FUdR treatments. Using FUdR-treated plates also allowed me to conduct experiments using synchronized populations without interference of hatchlings.

Figure 1. **Temperature Effects on ALS Phenotype.** Synchronous population of WTN2, WTSOD1, and G85R animals exposed to FUdR and non-FUdR conditions at day 1 of adulthood at temperatures of 15°C, 20°C, and 25°C. n=8 for each condition at all temperatures.

Compound Treatments Improve Healthspan

In chapter 1, I identified compounds that had positive effects on lifespan and promoted healthy aging. Given these, I next wanted to examine whether compounds that have beneficial effects on lifespan could also improve movement (Fig. 2). G85R animals exhibited abnormal movement and in some cases paralysis. Using G85R mutants, I

examined whether the compounds had any effects on improving a movement phenotype. The compounds used are as follows: trehalose (5mM), ThT (50 μ M), NP1 (50 μ M), mianserin (50 μ M), and EDTA (2.8mM). Trehalose and ThT are both involved in having beneficial effects for protein misfolding disorders (Alavez et al., 2011; Mardones et al., 2016). NP1 and mianserin, an anti-depressant, both have been reported to extend lifespan in *C. elegans* (Lucanic et al., 2013; Rangaraju et al., 2015). EDTA is a chelating agent that binds to metal ions such as iron and copper, forming stable complexes (Ibad et al., 2016). The worms were treated with these compounds at day 1 and scored at day 2 and day 8.

Results in Fig. 3 show that the mutant G85R phenotype could be rescued at a mid-stage in life. At day 2, the G85R animals had poor performance in movement and we observed minimal to no rescue with the compounds. At day 8, the compounds ThT, mianserin, and EDTA rescued the effects in the G85R animals but it occurs at similar levels as WTN2 and WTSOD1. Collectively, this outcome potentially infer that there is an improvement in healthspan but it is probably is not specific to the ALS phenotype.

Figure 2. **Chemical structures of compounds used for thrashing assay.** Chemical compounds shown above refer to compounds used in thrashing assay except for NP1 and ThT. For chemical structures of NP1 and ThT, see chapter 1 Fig. 5.

Figure 3. **Body Bend Analysis of G85R Animals with Various Compound Treatments.** Synchronous population of WTN2, WTSOD1, and G85R animals exposed to different compounds. n=8 for all conditions at day 2 and day 8. Error bars represent standard error. *= $p \leq 0.05$, **= $p \leq 0.01$. Statistical test based on unpaired t-test generated using Prism.

Additional Metal-binding Ions Improve Movement

G85R animals are impaired metal-binding mutants which have largely reduced activity (Witan et al., 2008). Mutations in SOD1 can change the binding affinity for Cu and Zn. The fact that Cu/Zn is important for stabilizing SOD1, I examined whether or not changing the stoichiometry of zinc and copper ions could improve movement by preventing the misfolding of SOD1.

By introducing zinc chloride (ZnCl) and copper chloride (CuCl), results show that the compounds could rescue the effects of movement. In Fig. 4, at day 2, CuCl elicited a slight rescue in movement on G85R animals but occurred below wild-type levels. On the other hand, ZnCl on day 2 did not significantly improve movement of G85R animals. On day 7, ZnCl and CuCl displayed significant increase in movement compared to the G85R control animals. These rescue also occurred below the levels of WTSOD1 animals.

Figure 4. Body Bend Analysis of G85R Animals with ZnCl and CuCl Treatments.

Synchronous population of WTN2, WTSOD1, and G85R animals exposed to ZnCl and CuCl. Thrashing was measured on day 2 and day7. n=8 for all conditions at day 2 and day 8. Error bars represent standard error. * = $p \leq 0.05$, *** = $p \leq 0.001$, **** = $p \leq 0.0001$. Statistical test based on unpaired t-test generated using Prism.

4. Discussion

ALS is a progressive and fatal neurological disease that has no cure (Browne and Abbott, 2016). Pathogenic mechanisms that occur during this progression include but are not limited to mitochondrial dysfunction, inflammation, oxidative stress, and glutaminergic excitotoxicity (Cluskey and Ramsden, 2001; Zhu et al., 2015). While there is a variety molecular pathway associated with neuronal death in ALS, there is no clear primary driving force for the onset of ALS. Considering the high incidence of ALS and lack of viable therapies, more research should be conducted.

In order to gain a better insight of ALS, I surveyed a variety of compounds in an attempt to decrease the effects of the observable movement phenotype. In this thesis, I have demonstrated that compound treatments could improve movement in animals expressing the ALS phenotype at a mid-late stage. However, this improvement may not be directly associated with ALS in general. One hypothesis to differentiate between observing the improvement to ALS-specific phenotype is to expose the wild-type strains to the compounds. This will allow us to study effects of the wild-type SOD1 mutants and N2 animals to G85R mutants. Likewise, perhaps the compounds that were chosen in this chapter were not at the correct dosage. A solution to this is to conduct dose-response assays similar to chapter 1 (Fig. 7 and 8).

Mutations in TARDBP, encoding TAR DNA-binding protein-43 (TDP-43), are also associated in ALS (Guo et al., 2011). ALS patients display presence of aggregated TDP-43 proteins. Mutant TDP-43 proteins expressed in *C. elegans* motor neurons are susceptible to misfolding, leading to protein insolubility and aggregation (Aggad et al., 2014). The *C. elegans* model, TDP-43 proteinopathy, is an interesting model for further

research including the use of compounds that promote proteostasis. By using another model of ALS, we will be able to compare mutations associated with ALS.

5. Conclusion

Neurodegenerative diseases are incurable and result from progressive deterioration and/or death of nerve cells. The underlying causes of neurodegenerative diseases remain a mystery due to the fact that there are many mechanisms involved in causing detrimental effects. Using a *C. elegans* ALS model, I have demonstrated that chemical compounds could promote improve movement in aged animals. This is a small step towards mechanistically finding how the compounds are able to interact with biological pathways to induce movement. Perhaps the approach of combinatorial compounds will be a strategic method that could provide a benefit in neurodegenerative disease models not only in *C. elegans* but also in *Drosophila melanogaster* and mice with hopes that the benefits will be translated to humans.

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