Sulforaphane Improves Oxidative Stress Response in *Caenorhabditis elegans* via SKN-1

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INTRODUCTION

The majority of previous research with SFN has concerned its anticarcinogenic effects: SFN has protective effects in normal human cell cultures, while diminishes their development and promotes apoptosis in cancer cells by increasing the production of reactive oxygen species (Briones-Herrera et al., 2018). SFN also reported to reduce lipid accumulation and insulin levels to mitigate some of the effects of diabetes (de Souza et al., 2016); improve glucose metabolism in rats (Axelsson et al., 2017); help prevent the rise of mean arterial blood pressure (Elbarbry et al., 2014); and regulate muscle metabolism (Whitman et al., 2013). Most significantly, SFN's cytoprotective effects are known to be associated with the upregulation of Nuclear factor E2-related factor 2 (Nrf2) (Briones-Herrera et al., 2018). However, limited research has been conducted on the effects of sulforaphane on aging, obesity, and stress resistance.

Caenorhabditis elegans is a common animal model for research involving aging, obesity and neurodegenerative diseases, and is a free-living nematode found in temperate soil environments. Wild-type worms have a lifespan of about 21 days and reach adulthood within 48 hours after hatching at 25℃. Their large brood size of 300 progeny per hermaphrodite allows for efficient experimentation of bioactive compounds in *C. elegans*. It is the first animal model to have its full genome sequenced, and over 65% of its genes are associated with human diseases. Since *C. elegans* have SKN-1, the ortholog of Nrf2, we used this model to determine the role of sulforaphane on aging, obesity and oxidative stress resistance.

The hypothesis of this experiment is if *C. elegans* are treated with SFN, then their lifespan will increase, fat accumulation will decrease and stress resistance will increase.

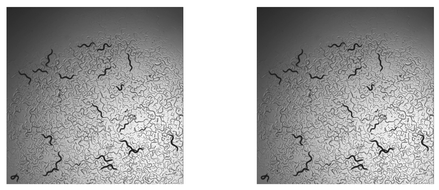


Image 1, 2: Images captured by the WormLab tracking system of N2 wild-type *C. elegans* on low-peptone NGM plate.

METHODS

Materials

- *C. elegans*: N2 wild type, TJ356 (zls356 IV) and LD1 [ldIs7] strains obtained from Caenorhabditis Genetics Center at the University of Minnesota
- Sulforaphane ($C_6H_{11}NOS_2$) obtained from Biopurify Phytochemicals LTD (CAS#: 4478-93-7)

Treatment

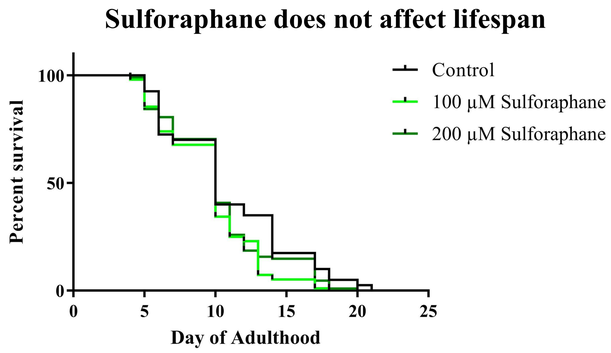
The *C. elegans* were treated with 100 and 200 µM sulforaphane in dimethyl sulfoxide (DMSO), and 0.2% DMSO was used as a control. *Escherichia coli* OP50 was used as food for the *C. elegans.*

Procedures

- Fluorescence microscope with SKN-1::GFP (translocation assay): The worms exhibited low, medium or high nuclear translocation in the intestine through subjective analysis.
- Oxidative stress and lifespan assay: 5mM paraquat was added to liquid media to induce oxidative stress, and the number of surviving worms in each well were counted daily; for aging, survival of worms was counted daily. Worms were treated with SFN at L4/young adult stage.
- Triglyceride and protein assay: Used Infinity Triglycerides Reagent to determine fat accumulation in worms; BCA protein reagent was used for protein assay to normalize data.
- Real-time PCR was quantified by the ΔCt and Fold Change: $2^{\wedge(-\Delta\,\Delta Ct)}$ (Schmittgen & Livak, 2008)
- Tracking assay: Locomotive behavior (speed and amplitude) and body size (length and width) were analyzed using WormLab. Worms were treated with SFN at L1 stage.

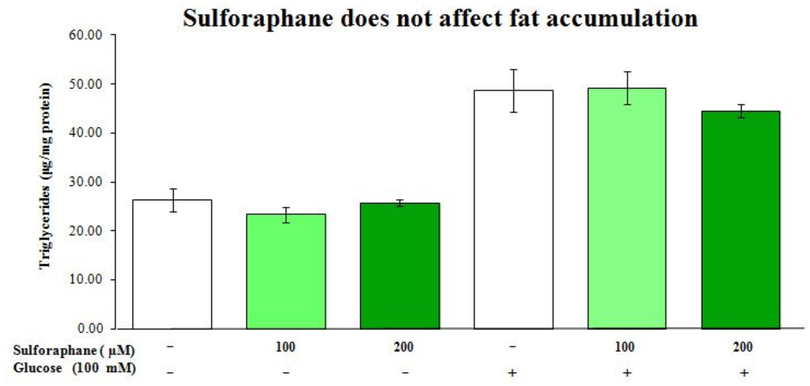
RESULTS

Sulforaphane has no effect on lifespan and fat accumulation



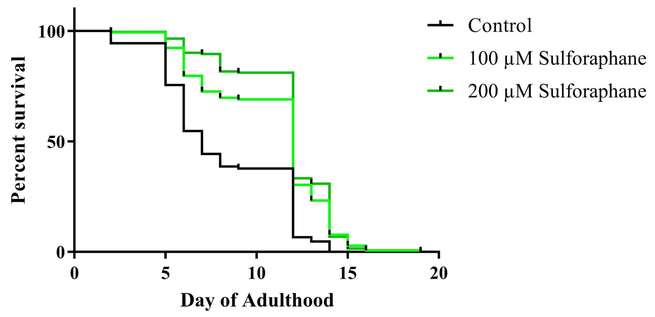
Graph 1: Using a logrank test, 100 and 200 µM SFN had no effect on *C. elegans* lifespan. n = 40-108; $P = 0.0614$

Sulforaphane	Control	100 µM	200 µM
Median survival	10	10	10
Maximum Survival	21	18	20



Graph 2: Glucose increased fat accumulation, but SFN did not change fat accumulation in either group. n = 4

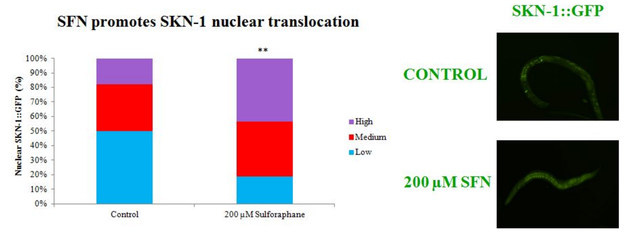
Sulforaphane provides oxidative stress resistance



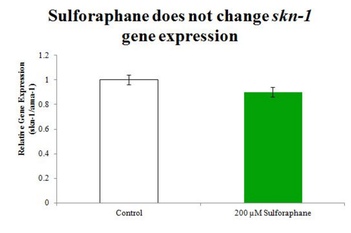
Graph 3: Using a logrank test, 100 and 200 µM SFN improved oxidative stress response in *C. elegans*. n = 106-201; $P < 0.0001$

Sulforaphane	Control	100 µM	200 µM
Median survival	7	12	12
Maximum Survival	14	19	19

Sulforaphane increases SKN-1 nuclear translocation

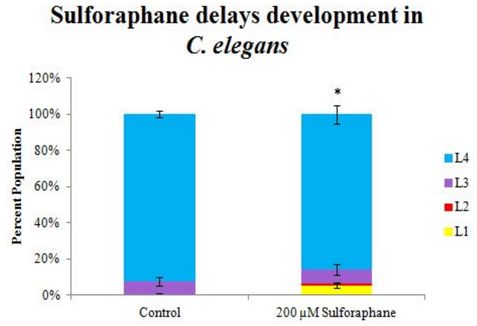


Graph 4: Sulforaphane promotes SKN-1 nuclear translocation in *C. elegans*. n = 67-88; ** $P \leq 0.01$

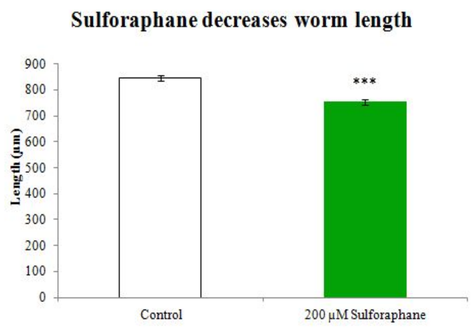


Graph 5: Sulforaphane does not change *skn-1* gene expression in *C. elegans*. n = 3

Sulforaphane may delay development and decrease worm length



Graph 6: Sulforaphane delayed development in *C. elegans*. n = 2 collected from 57-128 worms; *$P \leq 0.05$



Graph 7: Sulforaphane decreased mean *C. elegans* length. n = 62-66; ***$P \leq 0.001$

FUTURE WORK AND IMPLICATIONS

Because SFN has already been shown to have neuroprotective benefits, reduce inflammation and exhibit anticarcinogenic effects, it already has numerous possible benefits for humans. This study also supports previous research that SFN protects against oxidative stressors. The age-related accumulation of oxidative damage caused by free radicals is one of the major contributing factors to aging. This research could have an impact on the creation of preventative medication, supplements and diets for certain ailments. It can also be used within the field of food science, especially within the technology and nutrition subdivisions, to create avenues for more nutritional and sustainable food that can be distributed globally. With the global population exponentially increasing, the amount of food necessary to sustain it will exceed the capacity of current food production. Experiments with bioactive compounds can determine how to create nutrient-rich foods to supplement a large population at a low cost.

Nevertheless, future experimentation is necessary to determine SFN's effect on ROS production (and aging) and determine what possible genes and proteins are responsible for SFN's positive oxidative stress response in *C. elegans*. Lastly, in order for SFN's benefits to be realized, more trials will need to be run on all the experiments to ensure their accuracy, and especially in human subjects since this study used C. elegans.

SUMMARY

The main findings from the experiment are that SFN improves oxidative stress response and induces SKN-1 nuclear translocation. Additionally, the compound delays development of worms and reduces their mena length. Further observations are that SFN has no effect on lifespan and fat accumulation, does not impact movement or pumping rate and does not change *skn-1* gene expression.

CONCLUSION

Sulforaphane may improve oxidative stress response in *C. elegans* through post-translational regulation of SKN-1.

REFERENCES

All images and graphs are by Nitya Thakkar

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