HFPO-DA Causes for Impactful Regulation of Aging and Damage Control Genes

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Summary

- Damage control genes such as *gcs-1*, *lmd-3*, and *prx-11* have been found to be up-regulated after exposure to the HFPO-DA chemical.
- Genes that have been found to be down-regulated to any extent due to the effects of HFPO-DA are shown to have involvement in regulation processes such as regulation of heat shock protein, lipid desaturase, and peroxisomal membrane proteins.
- *pqm-1* and *nhr-49* which are part of determining the adult lifespan are greatly down-regulated in the experimental group.

Abstract

HFPO-DA (Gen X) is a chemical that is already commonly attributed to several negative symptoms such as: cancer and neurodevelopmental effects. (Chase, 2006) (Shabalina, Panaretakis, Bergstrand, & Depierre, 1999). However, HFPO-DA has also shown that it can affect cellular processes such as apoptosis (*Carcinogenesis*, Volume 20, Issue 12)(Conley et al., 2020). This experiment has investigated the relation between *C. elegans* exposure to HFPO-DA and the genes *gcs-1*, *lmd-3*, *nhr-49*, *gst-4*, *daf-16*, *hsp-70*, *pxmp-4*, *sir-2.1*, and *pqm-1* in *C.elegans*. First, an experimental group of *C.elegans* were exposed to 280 ng/L of HFPO-DA, at which point the RNA of these *C.elegans* were extracted. This product is then analyzed for fold change values and then compared to view the overall change in regulation for the genes. In the experiment, there was an upregulation of the damage control genes *gcs-1*, *lmd-3*, and *prx-11*, and there was a down regulation in the genes *pqm-1* and *nhr-49*, which affect *C. elegans* lifespan. There is also a down regulation of *Sir-2.1*, *daf-16*, and *gst-4* which all affect other miscellaneous processes. This pattern shows that exposure of HFPO-DA will affect *C. elegans* lifespan and cause a strong anti-toxicant reaction. This shows that the HFPO-DA gene is dangerous as it elicits a response similar to that of a toxicant and can affect the lifespan of organisms which indicates the chemical is more dangerous than initially thought.

Introduction

Hypothesis

When *C. elegans* are exposed to 280 ng/L GenX for 2 days then a repression of *hsp-70*, *pxmp-4*, *sir-2.1*, and *pqm-1*, and induction of *gcs-1*, *lmd-3*, *nhr-49*, *gst-4*, and *daf-16* will occur within the cells of the *C. elegans* because *gcs-1*, *lmd-3*, *nhr-49*, gst-4, and *daf-16* take part harm response towards dangers such as arsenic or oxidative stress while *hsp-70*, *pxmp-4*, *sir-2.1*, and *pqm-1* take part in regulation of protein levels that will be down regulation to improve function of damage control genes.

Overview

• HFPO-DA (GenX) was chosen as a stressor on specific protein and gene types within *C. elegans* N2 Wild type to determine the effects of stress on aging and protein aggregation through the living environment.

Genes and Proteins

- **hsp-70**: A heat shock protein that exists in virtually all living organisms. (Common in Parkinsons disease)
- **nhr-49:** Regulates expression of lipid desaturase, fat-7. (Common in diabetes)
- **pxmp-4:** A gene that encodes a peroxisomal membrane proteins.
- **pqm-1:** controls oxygen consumption rates, suppresses hypoxic glycogen levels.
- **gst-4:** balances oxidative stress placed on brain cells**.** (Associated with neurodegenerative diseases)
- **daf-16:** It is responsible for activating genes involved in longevity, lipogenesis, heat shock survival and oxidative stress responses. This is a major piece of transcription within C. Elegans.
- **sir-2.1**: Included in chromosomal organization, lifespan, and intrinsic apoptotic signaling.
- **prx-11:** This protein is responsible for defense response against Gram-negative bacterium.
- **lmd-3:** catalyzes the rate-limiting step in the de novo synthesis of GTP
- **gcs-1:** A gene that is included in glutathione biosynthetic process, arsenic response, and superoxide response. (Associated with stress induced detoxification)

Significance and Reasoning

•The effects of stressors such as PFOA and PFOS have caused disease such as cancers, metabolism disruption, and neurodevelopment effects as seen in many lawsuits in 2006(Chase, 2006).
• Birth defects have also been found in studies related to the *Sprague-Dawley rat* from HFPO-DA (GenX) (U.S. Environmental Protection Agency/ Office of Research & Development)(Shabalina, Panaretakis, Bergstrand, & Depierre, 1999).
•These chemicals also cause a much greater impact on a fundamental level of our genes and proteins by inducing apoptosis and disrupting the cell cycle (*Carcinogenesis*, Volume 20, Issue 12)(Conley et al., 2020).
• By studying how stressors such as HFPO-DA (GenX) can affect the function and process of protein aggregation in *C. elegans* on the transcriptional level this may show that HFPO-DA could be more harmful than previously thought.
• This study aims to find the effects of HFPO-DA on *C. elegans* at the molecular level to discover correlation between dangerous chemicals used by companies and the harm that they may bring to us with modern issues of pollution and chemical exposure

Benefits of using *C. Elegans*

•*C. elegans* have neurons, skin, gut, muscles, and other tissue that have similar form and function to that of humans.
•Because of their short 4-day generation time and ability to be easily cultured in a lab it allows for experiments to be conducted in short periods of time with similar results to that of one that may take years with other specimens.
•They have extremely short lifespans and are inexpensive to raise.
•Experimental group is easily manipulated due to the availability to induce chemicals on the *C. elegans* source of food.

Methodology

Exposure Group and Control Group
Incubated with 280ng/L hexafluoropropylene oxide dimer acid (HFPO-DA) for 2 days. Create controlled group that are raised at 23 degrees Celsius, fed E. coli OP50, and Grown on the NGM (Nematode Growth Medium)

Sample Collection
Wash the plate with 5 mL of M9 Buffer and collect 1,000 adult worms in a tube. Store pelleted worms at –80 degrees Celsius for 4 hours.

RNA Extraction
Remove supernatant from the thawed pellet and add 1 mL of cold Guanidinium thiocyanate-phenol-chloroform (GTCp) reagent. Add 200 micro-Liters of cold chloroform to the solution of worms and GTCp. Precipitate the RNA with isopropanol. (Fig. 1) Remove and discard the supernatant. Wash pellet with 75% ethanol.

RNA Quantitation
Quantitate the RNA using the Nanodrop

qRT-PCR
Use one Step qRT-PCR (Fig. 2) to transition the RNA to cDNA and then to PCR product as well as iTaq™ Universal SYBR® Green One-Step Kit.

Primers: gcs-1 : Forward: GCAGGTGAATGCGATGCTTG
Backward: GCAAGCGATGAGACCTCCGT
hsp-70: Forward: GCCGGTTGAAAAGGCACTTC
Backward: GCAGTTGAGGTCCTTCCCATT
nhr-49: Forward: TTTTGTTAGTCAACTCATTCCGTT
Backward: GCGGAAGAATCCCTTACACC
pxmp-4: Forward: TGCTGCTCTTGTCATGGGAT
Backward: TGGGAAGACTGGGAAGTTTGG
pqm-1: Forward: TTGCAGGCATAGCTCTCAGC
Backward: CGGCTGCATTAGGTTTACTGTG
gst-4: Forward: TGCTGAGCCAATCCGTATCA
Backward: AATGGGAAGCTGGCCAAATG
daf-16: Forward: GCACAAGTTTACGAATGGATGGT
Backward: GTACGCCGTGGATTCCTTCC
sir-2.1: Forward: GTGTGTCGCGATTTCTGCTC
Backward: GCAAGACGAACCACACGAAC
prx-11: Forward: TTCTGGGCCTAGCGAAACAG
Backward: GTCACTAAGCCAGGCGAGAA
imd-3: Forward: GGGATTGAAAATGGGGCGAAG
Backward: ATGCAAATACGCCCGCATGA

Data Analysis
Using data from the Control Group, Exposure Group, and the endogenous control, begin to calculate the fold change through the 2^-(ΔΔCt) system of equations. Use the P-test with a level of significance of 0.05 to determine if the data is valuable.

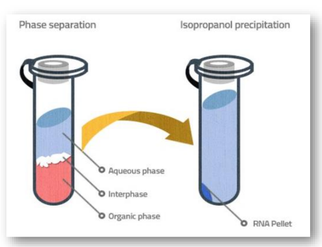


Figure 1: Isolation of RNA using the phenol-chloroform method for isolation.

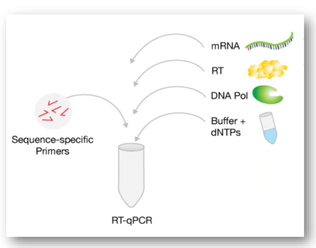


Figure 2: A depiction of the contents that are placed within the tube for One step qRT-PCR to take place.

Results

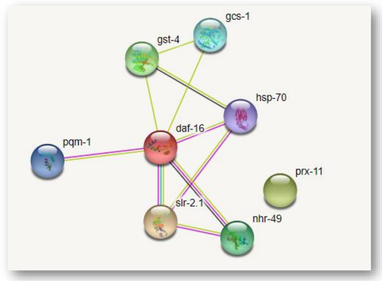
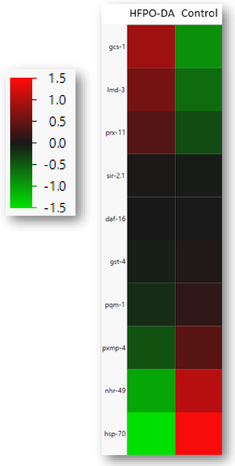


Figure 3: STRING Map of target genes. The STRING chart gives insight on the connections between the target genes by creating a map. The colored lines that connect the targets represent gene fusions (red), gene co-occurrence (blue), gene neighbors (green), text mining (yellow), co-expression (black), and protein homology (blue).

Figure 4: Heat Map of HFPO-DA exposed worms. The Heat Map here is a visual representation of the fold change for all the target genes. When exposed to HFPO-DA there is an overall increase in the fold change between experimental and control for *gcs-1 (*fold change:5.032 P-value: 0.0.213*)*, *lmd-3 (*fold change:2.094 P-value: 0.269*)*and *prx-11 (*fold change:2.26 P-value: 0.527*)*. There is hardly any visible change between the experimental and control heatmaps for *sir-2.1 (*fold change:0.556 P-value: 0.9876*)* and *daf-16 (*fold change:1.063 P-test: 0.9876*)* . There is an overall decrease in the fold change between experimental and control heatmaps for *gst-4 (*fold change:1.06 P-value: 0.0.893*)*, *pqm-1 (*fold change:0.89 P-value: 0.0.773*)*, *pxmp-4 (*fold change:0.56 P-value: 0.2766*)*, *nhr-49 (*fold change: 0.278 P-value: 0.0.0729), and *hsp-70 (*fold change: 0.1655 P-test: 0.056*)*.



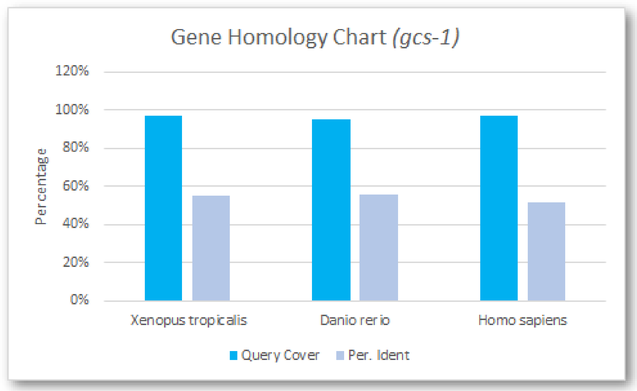


Figure 5: Gene Homology Chart comparing gcs-1 within multiple species. The graph shown above shows the Query Cover and Per. Ident of the gene, *gcs-1*, among multiple species. The Query Cover percentage shows the percentage of the search sequence that overlaps with the aligned sections. The Per. Identity is the percentage of characters within the covered part of the query that are identical.

Discussion/ Conclusion

Conclusion/ Discussion

By using the control group fold change values as a baseline, it allows for comparison within the experimental group and find the changes caused by the HFPO-DA chemical that is displayed in the heat map (Fig. 4). The primary genes that were up regulated because of the effects of HFPO-DA were *gcs-1*, *lmd-3*, and *prx-11*. These genes all deal with stress or damage response such as oxidative stress *(lmd-3)*, arsenic response *(gcs-1), and* gram-negative bacterium response *(prx-11),* which is consistent with the harmful nature of HFPO-DA presented, and their use in toxic chemical and harmful foreign element response. *Sir-2.1*, *daf-16*, *and gst-4* were slightly down regulated. *gst-4* exhibits glutathione transferase activity, *daf-16* mostly deals with defense against other living organisms and binding of DNA protein, and enzyme binding, and *sir-2.1* exhibits deacetylase activity. These are consistent with the findings that HFPO-DA causes large change in defense genes against foreign elements and because these genes deal with binding and internal activity, they are only slightly down regulated. *pqm-1*, *pxmp-4*, *nhr-49*, and *hsp-70* were all down regulated. *hsp-70* is a heat shock protein, *pqm-1* and nhr-49 takes part in determining the adult lifespan, and pxmp-4 encodes for peroxisomal membrane protein 4. The STRING map (Fig. 3) shows that most of the genes have some sort of connection except prx-11. This information tells us that the genes that combat the effects of HFPO-DA are being upregulated. Due to this, genes that take part in important processes and determine adult lifespan of *C. elegans* are being down regulated, which shows how HFPO-DA can cause a shorter lifespan in *C. Elegans*. The homology chart (Fig. 5) shows the significance and application of this data in a variety of life forms.

Study Limitations

- Because some genes may serve multiple functions, it is difficult to pinpoint the exact cause of up regulation or down regulation in the gene. This is seen in the gene *daf-16* , which participates in the binding of proteins, enzymes, and DNA.
- Because all the cDNA produced is used up in the following qPCR step, there is no way to repeat the experiment to check for validation or further expand upon research.
- Because SYBR Green binds to any double-stranded DNA, it can also bind to nonspecific double-stranded DNA sequences resulting in an overestimation of the target.

Future Directions

In this experiment, SYBR Green was used to identify how much the DNA was amplified during the qRT-PCR process. Better alternatives to this product that can be used in future experiments are EvaGreen and SYTO dyes because they are more efficient and less susceptible to PCR inhibition compared to SYBR Green. To expand upon the current findings related to wild type *C. elegans*, a new experiment could be conducted in which mutant strains of *C. elegans* are exposed to the same conditions as the original experiment. The results can be compared to gain a deeper understanding of the effects of HFPO-DA on target genes in wild and mutant types.

Bibliography

"BLAST: Basic Local Alignment Search Tool." *National Center for Biotechnology Information*, U.S. National Library of Medicine,

Chase, R. (2006, March 20). Dupont grapples with legacy OF Benlate. Retrieved February 05, 2021,

Conley, J., Lambright, C., Evans, N., McCord, J., Strynar, M., Hill, D., . . . Gray, L. (2020, October 27). Hexafluoropropylene Oxide-dimer Acid (HFPO-DA Or Genx) ALTERS maternal and fetal glucose and lipid metabolism and Produces Neonatal mortality, low Birthweight, and Hepatomegaly in the Sprague-Dawley rat. Retrieved February 05, 2021,

Shabalina, I., Panaretakis, T., Bergstrand, A., & Depierre, J. (1999, December 01). Effects of the rodent peroxisome proliferator and hepatocarcinogen, perfluorooctanoic acid, on apoptosis in human hepatoma hepg2 cells. Retrieved February 05, 2021,

Sir-2.1 (gene) - Wormbase : Nematode information resource. (n.d.). Retrieved February 05, 2021,

Smith, Cindy J., and A. Mark Osborn. "Advantages and Limitations of Quantitative PCR (Q-PCR)-Based Approaches in Microbial Ecology." *OUP Academic*, Oxford University Press, 1 Jan. 2009,

Takara Bio Blog Team. "One-Step vs. Two-Step RT-QPCR-Tips for Choosing the Right Protocol." *Takara Bio-Home*, 13 Aug. 2018,