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Metabolic and Molecular Mechanisms of WGX-50 in Alzheimer's Disease through an Integrative in Vivo and in Silico Approach

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Abstract: *A mechanistic explanation of how WGX-50 mediates AD-plasm involves processes related to aging: metabolic disturbance, mitochondrial oxidative stress, and dysregulated proteostasis. This will determine whether WGX-50 coordinates a concerted in vivo metabolic remodeling of central carbon and redox metabolism and surface destabilization of early toxic amyloid-beta (Aβ1–42) in silico. Targeted LC–MS/MS metabolomics on the WT in the WGX-50 treated worms or vehicle will be performed, with n ≥5 biological replicates per group, for the quantification of glycolytic, tricarboxylic acid (TCA) cycle, pentose phosphate pathways, and redox/energy cofactor pool metabolites, normalized to total protein and assessed by quality control, univariate statistics, false discovery rate, principal component analysis, clustering, and pathway analyses. All-atom MD simulations of oligomers, whether unbound or bound by WGX-50, were set up for a total of at least three to four independent trajectories. The loss of oligomerization was estimated by the progressive decrease in inter-chain van der Waals contacts, beta-sheet content, structural parameters (RMSD, RMSF, SASA, and radius of gyration), and a detailed MM/GBSA energetic quantification. We anticipate that WGX-50 will induce a concerted metabolic shift in energy sensing and redox balance while disrupting inter-peptide interactions and increasing the oligomer conformational entropy. In such integrative experiments, metabolic profiling, organismal assays, and atomistic simulations are combined so that a mechanistic hypothesis can be built on putative anti-oxidative and anti-aggregative effects of WGX-50 relevant to AD proteotoxicity.*

Keywords: *WGX-50, Alzheimer's Disease, Targeted Metabolomics, Molecular Dynamics Simulation*

1. Introduction

Aging is the strongest risk factor for Alzheimer's disease (AD), and accumulating evidence links AD pathogenesis to metabolic dysregulation, mitochondrial dysfunction, oxidative stress, and impaired cellular stress responses. Small molecules from natural sources may modulate conserved energy-sensing pathways and redox homeostasis, thereby influencing organismal healthspan and proteostasis [1-3]. WGX-50 is a hydroxylamine-derived small molecule originally reported from Zanthoxylum with antioxidant and anti-inflammatory properties [4]. Prior studies suggest that WGX-50 may alleviate Aβ-associated phenotypes by reducing oxidative stress and inflammatory signaling and by potentially interfering with Aβ aggregation. However, two key mechanistic gaps remain: first, whether WGX-50 systematically reprograms central carbon metabolism and redox/energy cofactors in vivo; and second, whether WGX-50 can disrupt early toxic Aβ(1–42) oligomer interfaces at the molecular level, consistent with an anti-aggregation mechanism. C. elegans provides a tractable in vivo model for studying conserved metabolic regulation, stress responses, and organismal physiology. Targeted LC–MS/MS metabolomics enables sensitive, quantitative profiling of predefined metabolic pathways, including glycolysis, the TCA cycle, the pentose phosphate pathway (PPP), and redox/energy cofactor pools. In parallel, all-atom molecular dynamics (MD) simulations can generate mechanistic hypotheses about ligand effects on Aβ oligomer interface stability by quantifying inter-chain contacts, β-sheet persistence, and structural fluctuations. Integrating these two scales offers a rigorous and interpretable framework to evaluate whether WGX-50 influences energy-sensing metabolism and to test an AD-relevant molecular hypothesis in silico.

This paper will integrate targeted metabolomics in wild-type C. elegans using LC–MS/MS in multiple reaction monitoring (MRM) mode to quantify metabolites from glycolysis, the tricarboxylic acid (TCA) cycle, the pentose phosphate pathway (PPP), and redox/energy cofactors, and all-atom molecular dynamics (MD) simulations to test whether WGX-50 perturbs Aβ(1–42) oligomer interface stability.

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Together, these approaches aim to connect system-level metabolic modulation in vivo with atomistic hypotheses relevant to AD proteotoxicity.

2. Objective and Research Question

This study aims to evaluate coordinated changes in central carbon metabolism and redox/energy cofactors in C. elegans after application of WGX-50, test the hypothesis that WGX-50 weakens Aβ(1–42) oligomer interfaces in all-atom MD simulations, and provide mechanistic hypotheses relevant to AD proteotoxicity.

Are there significant changes between WGX-50 and vehicle control (VC) in a predefined targeted metabolite panel in C. elegans?

What are the most coordinated and internally consistent metabolic modules (glycolysis, TCA, PPP, redox and energy cofactors) that are affected by WGX-50?

Does WGX-50 affect/change the interface stability (e.g. decrease the number of persistent inter-chain contacts, decrease the number of β-sheets, change in interface SASA, etc) in oligomers in MD systems versus oligomer- only controls?

3. Hypothesis

H1 (in vivo): WGX-50 elicits reproducible, pathway-level changes in C. elegans metabolism, including co-regulated changes in the central carbon metabolism linked to the regulation of energy-sensing signaling and redox balance (TCA/PPP).

H2 (in silico): In MD simulations, WGX-50 disrupts the Aβ(1–42) oligomer peptide–peptide interface interactions (e.g., dimers/trimers/tetramers) that leads to decrease in the stability of the β-sheet, increase in the conformational heterogeneity and fewer inter-chain contacts than oligomer-only controls.

4. Experimental Design

This project integrates two coordinated components designed to address complementary mechanistic questions:

4.1. In Vivo Targeted Metabolomics in C. elegans

Controlled exposure study will be carried out using WGX-50 as the independent variable in a replicated exposure study. The main outcomes are quantitative changes in an a priori determined set of metabolites that includes glycolysis, TCA cycle, PPP and redox/energy cofactors (where applicable; e.g., NAD(H), NADP(H), glutathione pools). Worms treated with ethanol in a vehicle (0.1%) will be used as negative controls and will be handled in the same manner as the other worms. At least five biological replications will be included in the study for each group, and the sample processing and the data processing will be random and coded (blinded) to decrease bias. Where possible the design will involve dose-response (e.g., 10 μM and 50 μM) and/or time-course (e.g., 24 h and 120 h) endpoints to enhance causal inferences and distinguish early perturbations from longer-term adaptations.

4.2. All-Atom MD Simulations ofAβ(1–42) Oligomers ± WGX-50

MD systems will be simulated using identical protocols: (i) Aβ oligomer-only control and (ii) Aβ oligomer + WGX-50 treatment. Multiple independent trajectories per condition (≥3, different initial velocities) will reduce single-trajectory bias. Pre-specified endpoints will quantify oligomer stability and interface integrity, including inter-chain contacts, β-sheet content, RMSD, interface SASA, and compactness metrics. Relative energetic comparisons will be estimated with MM/GBSA on representative snapshots, interpreted as comparative trends rather than absolute binding free energies. These two components will be interpreted together to link in vivo metabolic modulation with an AD-relevant atomistic hypothesis about early Aβ oligomer stability.

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5. Materials and Methods

Wild-type C. elegans (N2 strain) will be acquired from the Caenorhabditis Genetics Center (CGC) and sustained on nematode growth medium (NGM) plates utilizing Escherichia coli OP50 as the nutritional source. Routine buffers will comprise M9 buffer and phosphate- buffered saline (PBS). WGX-50 will be solubilized in ethanol, with 0.1% ethanol utilized in the vehicle-control plates. LC–MS grade methanol, acetonitrile, and formic acid will be utilized for metabolite extraction and LC–MS/MS analysis, with ammonium acetate acting as the mobile-phase additive. Sample preparation will require liquid nitrogen, a centrifuge, and an ultrasonic homogenizer. Protein normalization will be conducted utilizing a BCA assay kit, and leftover protein pellets will be solubilized in SDT lysis buffer (4% SDS, 100 mM DTT, 100 mM Tris-HCl, pH 7.6). Targeted metabolomics data will be obtained using a Shimadzu Nexera X2 LC-30AD system linked to a QTRAP 6500+ triple quadrupole mass spectrometer and analyzed with MultiQuant, while further statistical analysis and route annotation will be conducted with Microsoft Excel and KEGG-based pathway tools.

5.1. OP50 Preparation

Freeze-dried OP50 beads will be cultured aseptically following the manufacturer's instructions. A single bead will be streaked onto LB agar and incubated at 37 °C overnight. A single colony will be inoculated into 50 mL LB medium, expanded, concentrated (target ~10 mg/mL), and seeded onto 6 cm NGM plates. Plates will be air-dried and used as standardized food sources.

5.2. C. elegans Culture and Synchronization

Wild-type N2 worms will be maintained on OP50-seeded NGM plates for ≥3 non-starved generations. Age synchronization will be achieved using sodium hypochlorite bleaching. Synchronized worms will be cultured on NGM plates supplemented with 10 μM nystatin at 15–20 °C until L4 stage.

5.3. WGX-50 Treatment

Age-synchronized L4 worms will be transferred to 10 cm NGM plates containing either: Age-synchronized L4 worms will be transferred to 10 cm NGM plates assigned toWGX-50 group (50 μM WGX-50 (with 0.1% ethanol vehicle) and vehicle control: 0.1% ethanol only). Worms will be incubated at 25 °C for 5 days (or additional timepoints as part of an expanded design). To minimize confounding from progeny and food depletion, worms will be transferred daily to fresh plates by M9 washing and brief low-speed centrifugation (1,000 rpm, 30 s), then placed onto fresh OP50-seeded plates.

At least five independent biological replicates will be prepared per group. Plates will be randomly allocated to treatment groups, and sample extraction and LC–MS/MS injection orders will be randomized. Sample identifiers will be encoded so that peak integration and downstream analysis are performed blinded to group labels.

5.4. Sample Collection and Bacterial Carryover Control

Worms will be washed off plates with M9 buffer, pelleted by low-speed centrifugation, and washed three times with M9 to minimize bacterial carryover. Excess liquid will be removed. Samples will be weighed (wet weight recorded) and snap-frozen in liquid nitrogen, then stored at −80 °C until extraction.

5.5. Metabolite Extraction

Samples will be thawed on ice and extracted using 1,000 μL of frigid 80% methanol. After vortexing, the samples will undergo sonication in an ice-water buffer for 20 minutes (80 W, alternating 10 seconds on and 10 seconds off), followed by incubation at −20 °C for 1 hour to promote protein precipitation. After centrifugation for 20 minutes, the supernatants will be harvested and subsequently subjected to vacuum desiccation. Dried samples will be reconstituted in 50% methanol before LC–MS/MS analysis.

Normalization approach: Metabolite abundances will be normalized relative to total protein content as determined by the BCA assay. Wet weight shall be documented as a secondary normalization reference to identify extraction outliers.

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5.6. Protein Quantification (for Normalization)

Protein particles remaining after extraction will be resuspended in SDT lysis buffer and subjected to heating at 95°C for 10 minutes. Following centrifugation at 20,000 × g at 4 °C for 15 minutes, the supernatants will be quantified utilizing a BCA assay.

5.7. MD simulations

To complement the in vivo metabolomics data with atomistic hypotheses relevant to AD, all- atom MD simulations will be performed to examine whether WGX-50 can destabilize Aβ(1– 42) oligomer interfaces. Simulations will be carried out using the AMBER software suite and AmberTools [5][6]. Representative WGX-50 binding configurations will be obtained through docking followed by clustering of ligand poses, and a subset of clustered poses will be selected as starting conformations for MD. Force-field parameters for WGX-50 will be generated using a restrained electrostatic potential (RESP) charge fitting approach, in which the electrostatic potential will be calculated at the HF/6-31G(d) level and bonded/nonbonded parameters will be assigned via Antechamber under the General Amber Force Field (GAFF). Protonation states of ionizable residues at physiological pH will be determined using the H++ server based on PROPKA3 algorithm [7], and missing hydrogen atoms and hydrogen-bonding networks will be optimized using PDB2PQR where applicable. All systems will be assembled using tleap in Amber22, with Aβ oligomers described by the ff14SB force field and WGX-50 described by GAFF with RESP charges(8). Each system will be solvated in an explicit cubic TIP3P water box with a minimum solute–box distance of 12 Å and neutralized by counterions.

MD simulations will be executed using the GPU-accelerated PMEMD.cuda engine with identical processes across conditions for energy minimization, equilibration, and production runs. Cpptraj will measure structural stability and flexibility from trajectories [8]. Root mean square deviation (RMSD) will assess global structural stability. Two matched systems will be simulated using the same technique to build a rigorous control framework: one with an Aβ oligomer-only control (no ligand) and another with an AỤ oligomer + WGX-50 treatment system. To enhance statistical robustness and prevent single-trajectory bias, it is suggested to use at least three distinct trajectories for each condition, each with a different starting velocity. To evaluate the packing of oligomers, quantitative endpoints will be measured, such as the number of inter-chain contacts, maps of interface contacts, time-resolved β-sheet content, solvent-accessible surface area (SASA), and compactness metrics: Rg, total SASA. The relative binding energetics will be calculated using MM/GBSA on average trajectory snapshots using MMPBSA.py. These data will be then used to compare different situations [9]. We will also use the Independent Gradient Model based on Hirshfeld partition (IGMH) analysis to support the mechanistic interpretation of WGX-50-oligomer interaction patterns, describing the dominant noncovalent interactions in representative conformations, such as hydrogen bonding and van der Waals contacts [10].

6. Data Analysis and Expected Results

In order to analyze focused metabolomics data, MRM chromatographic peaks will be found and measured using MultiQuant software. In order to account for changes in biomass and extraction efficiency between samples for each metabolite, the peak regions will be examined for integration consistency and normalized to the total protein level assessed by BCA assay. Analytical quality will be assessed through pooled QC injections. Metabolites that will not meet reproducibility criteria in QC samples, such as having a high coefficient of variation, will be excluded using a predetermined data-quality filter. As a result, downstream analysis will be limited to features that consistently demonstrated analytical performance.

Primary statistical analyses will be conducted to compare the WGX-50 treated group with the vehicle control group. Individual metabolite-level testing will employ two-tailed unpaired Student's t-tests for the two-group design, while analysis of variance (ANOVA) will be utilized for more intricate designs incorporating dose-response and/or time-course elements to assess main effects and interactions. To mitigate false positives from multiple hypothesis testing in the targeted metabolite panel, p-values will be corrected utilizing the Benjamini–Hochberg false discovery rate (FDR) approach, with statistical significance established as an adjusted q- value below 0.05. Alongside significance testing, effect sizes will be presented as fold change (FC) estimates, and confidence intervals will be included when possible to improve the understanding of biological magnitude, rather than depending exclusively on threshold-based significance.

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Principal component analysis (PCA) will be employed for multivariate analyses to delineate global metabolic patterns and assess technical stability. The close clustering of pooled QC samples demonstrated little analytical drift and reliable system performance, however the distinction between treatment and control samples will reveal treatment-related metabolic change. Hierarchical clustering will be employed to identify modules of metabolites that exhibit co-variation among biological replicates. Differentially abundant metabolites will be mapped to KEGG pathways and examined for pathway annotation and enrichment, with results will interpret within the framework of energy-sensing metabolic circuits such as glycolysis, the TCA cycle, the PPP, and oxidative phosphorylation-associated redox/energy cofactors.

Trajectory studies for the MD component will be conducted uniformly across both control (Aβ oligomer-only) and treatment (Aβ oligomer + WGX-50) systems. Time-resolved structural and interface parameters, including RMSD, β-sheet content, inter-chain contact counts, and interface solvent-accessible surface area (SASA), will be computed and summed across separate trajectories using uniform analytical windows to ensure comparability. Disparities concerning treatment will be examined by qualitative structural analysis and quantitative assessment of the established endpoints. Binding energetics will be evaluated by MM/GBSA calculations on representative data as a final-state, relative energetic comparison between circumstances, acknowledging that MM/GBSA provides comparative trends rather than definite absolute binding free energies.

6.1. Metabolomics Outcomes

Pooled QC samples should cluster strongly in PCA space, ensuring steady analytical performance with low drift. After normalization and QC filtering, WGX-50-treated worms should have a metabolic fingerprint different from vehicle controls. Differential metabolites should establish coherent pathway-level patterns consistent with systematic regulation, not random alterations.

WGX-50 may increase redox buffering, causing PPP-linked metabolites and cofactors to change synchronously (e.g., changed PPP intermediates aligned with NADPH needs) and TCA node modifications that may indicate mitochondrial remodeling. WGX-50 may cause metabolic stress, resulting in energy cofactor reductions or stress-related intermediate accumulation. These patterns are route disruption, not beneficial remodeling.

6.2. MD Outcomes

In MD simulations, if WGX-50 destabilizes Aβ oligomer interfaces, treatment systems may show fewer persistent inter-chain contacts, reduced β-sheet stability, increased structural fluctuations (RMSF), and altered interface SASA compared with oligomer-only controls. MM/GBSA comparisons may support relative weakening of interface-associated energetics in the presence ofWGX-50. If such effects are not observed, it may indicate that WGX-50 does not directly target oligomer interfaces under the tested configurations, or that additional sampling/alternative binding modes are required.

Together, these findings should link atomistic in silico evidence that suggests WGX-50 may disrupt early Aβ oligomer stability with system-level metabolic modulation in vivo (energy sensing, redox-related metabolism). The more general theory that WGX-50 may affect conserved pathways associated with neurodegeneration is supported by this integrated approach.

7. Safety and Risk Assessment

Biological Safety: Caenorhabditis elegans and Escherichia coli OP50 are classified as Biosafety Level 1 (BSL-1) organisms, posing minimal risk to researchers. All biological procedures shall adhere to standard microbiological practices. This will include the wearing of correct PPE, including gloves and lab coat, sufficient hand hygiene measures, periodic disinfection of surfaces, and proper disposal of biohazardous waste. All activities will follow the institutional biosafety requirements.

As for chemical hazards, WGX-50, organic solvents (such as methanol and acetonitrile), and metabolites can potentially create chemical hazards such as flammability and toxicity. The materials will be handled using relevant personal protective equipment, including gloves, protective eyeglasses, and laboratory coats. The chemical solutions will be prepared using an analytical balance and properly labeled containers. The chemical wastes and any solvent residue will be disposed of based on institutional chemical hazardous waste procedures.

As for safety for the instruments, the liquid chromatography-tandem mass spectrometry technique is

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comprised of high-pressure systems, electronics, and specialty instruments. The liquid chromatography-tandem mass spectrometry apparatus will be used only after proper training, possibly under the direction of an authority, according to the standard operating procedures prescribed by the manufacturer of the apparatus and the respective host lab.

Computational Safety: The simulation concerning the interaction involving WGX-50 and amyloid-beta or Aβ oligomers will be carried out only by using computational methods. This aspect of the proposed project does not pose any risk to the environment or to the researchers because there are no hazards involved, be it physical, chemical, or biological.

8. Conclusion

This proposal is going to test the dual role of WGX-50 in the emergence of aging-related Alzheimers at the systems level on metabolism and at the atomistic level on Aβ oligomer stability with high integration. In other words, targeted metabolomics of C. elegans and all-atom MD of Aβ(1–42) oligomers are seamlessly designed, making the mechanistic interpretation of how WGX-50 is viewed in the remodeling of central carbon and redox metabolism, as well as any destabilizing effects it may have on toxic oligomer complexes, feasible. In the ACC view, this would mean that WGX-50 is identified as a small molecule agent implicated in conserved energy-sensing and proteostasis pathways if WGX-50 acts in such a way as to coordinate the remodeling of redox-oriented metabolism with the destabilization of oligomer interfaces. Otherwise, it would be of an interpretable form due to the well-characterized limits of WGX-50 to its oxidative/anti-aggregatory rational evolution. This general explanatory initiative of the link between metabolic robustness and protein folding pursued above should be broadly cast in the mechanistic account of neurodegenerative diseases.

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