Use of Selenium Nanoparticles for the Therapy of Huntington's Disease: A New Era

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I affirm that I have identified all my sources and that no part of my dissertation paper uses unacknowledged materials

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Contents

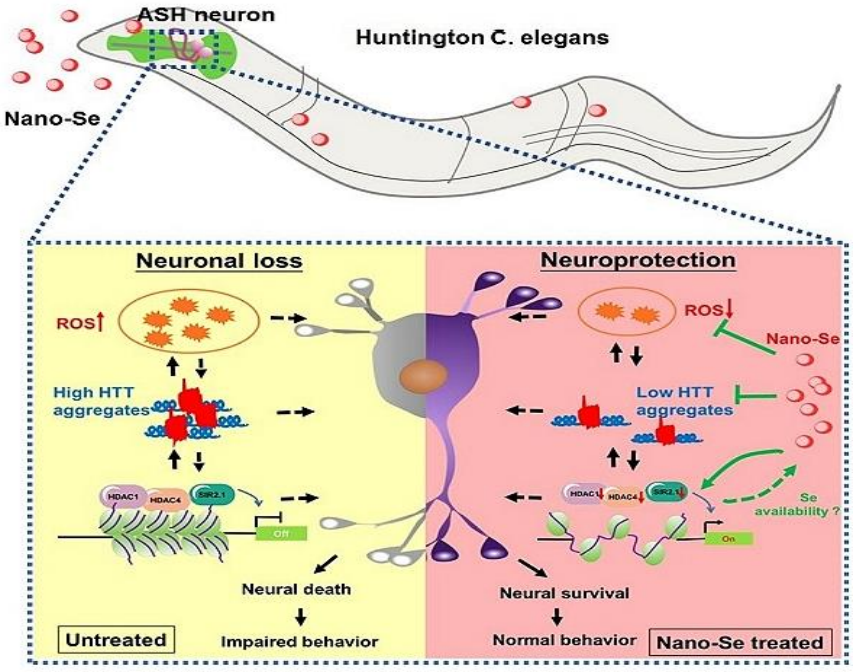
Abstract

Nanoparticles (NPs) are used as drug carriers in delivery of therapeutic molecules. Incorporation of NPs in treatment, diagnosis and monitoring of diseases has opened a new discipline called nanomedicine. This review illustrates the use of selenium nanoparticles (Se NPs) as a potential nanomedicine for the therapy of Huntington's disease (HD). As soon as Huntingtin protein (HTT) was discovered in 1993, scientists started targeting inhibition of HTT aggregation in order to cure HD, but the effort proved to be futile.

HD is a rare, inherited condition that causes widespread neurodegeneration in the brain which disrupts thinking, behaviour, emotion and movement. HD usually begins in midlife and as it progresses, it may develop into dementia and an inability to speak or move. The pathology of HD can be traced back to a dominant mutation in a single gene called the huntingtin (HG). The HG contains a DNA sequence of a trinucleotide repeat of Cytosine, Adenine and Guanine. When HG is mutated, an excess number of repeats lead to a mutated form of HTT which has a tendency to form clusters within neurons that are not easily removed by the brain enzymes. It has been hypothesized that these aggregations lead to neurodegeneration in the basal ganglia and other regions of the brain.

HD patients have insufficient levels of Se in their brain. Delicate toxicity margins of Se compounds provide a very narrow therapeutic window. So, Se NPs with its remarkable reduced toxicity comes into the forefront. In this study, Se NPs were exposed to transgenic HD models of *Caenorhabditis elegans* (*C. elegans*) by regulating HD-related neurodegeneration and cognitive decline. The study outlines the mechanism by which Se NPs protects and repairs neural functions from damages in stress conditions and eventually puts forward the importance of use of NPs to improve HD therapy in the future.

Graphical Abstract



1. Introduction

1.1 Huntington's disease

Huntington's disease is believed to affect 3 to 7 per 100,000 people of European ancestry. History has shown that it is an easy problem to define, for which it is surprisingly difficult to find solutions. More than 20 years of research since the gene discovery in 1993 has revealed a lot about huntingtin protein and its toxic twin. However, the disease still remains to be known as the most curable incurable brain disorder.

It is an autosomal dominant and inherited neurodegenerative disorder that causes cell death in the brain. It attacks areas of the brain that help to control voluntary movement and affects movement, mood and thinking skills. Symptoms of HD typically appear in middle-aged people (adult HD). They can also appear in children (juvenile HD), but this is rare. The disease gets worse over time. The time from the first symptoms to death is often about 10 to 30 years. Juvenile HD usually results in death within 10 years after symptoms develop. In recent years, HD has shown up in various countries (Fig. 1)

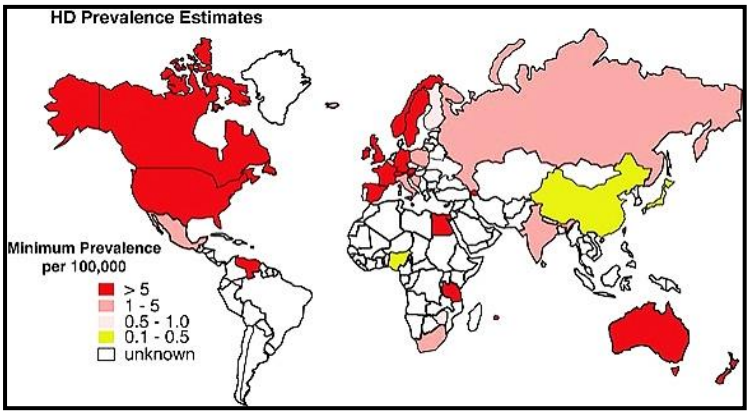


Fig. 1: Prevalence of HD around the world as of 2011

HD is a complex multi factorial disorder converging to epigenetic factors and oxidative stresses. There are evidences linking epigenetic alterations to the development of HD. Decreased acetylation of histone and increased oxidative damage has been regarded as a key factor in the development of neurodegenerative disorders.

1.1.1 Main causes of Huntington's disease

The pathology of HD can be traced back to a dominant mutation in a single gene called the huntingtin (HG). So, if one of the parents suffers from the disease, then the child has a 50% chance of developing it too. The disease may occur without a family history too, and then it is called sporadic HD.

The huntingtin gene contains a DNA sequence of a trinucleotide repeat of Cytosine, Adenine and Guanine. When HG is mutated, an excess number of repeats occur and a mutated form of the huntingtin protein (HTT) is created where excess CAG repeats encode for polyglutamines at the amino terminal end of the protein. An increase in the size of the CAG segment leads to the production of an abnormally long version of the HTT. The elongated protein is cut into smaller, toxic fragments. These fragments have a tendency to group together to form clusters within neurons that are not easily removed by brain enzymes. It has been hypothesized that these aggregations lead to neurodegeneration in the basal ganglia and other regions of the brain.

- Normal human beings have fewer than 27 CAG repeats in their HD gene
- People with 36 or more CAG repeats suffers from HD
- People who have CAG repeats in the middle range (27 to 35) are not likely to develop the disease, but they could still pass it on to future generations

HTT is expressed during embryonic development and throughout life. Studies in animals have shown that the normal HD gene is vital for brain development. Adults who carry the mutant HD gene but have not yet displayed symptoms show measurable changes in the structure of their brain, even up to 20 years before clinical diagnosis.

Transcriptional dysregulation follows after the mutation and is an important pathological mechanism linking nutritional availability to cellular processes in HD. Aggregates of mutant HTT may cause significantly decreased acetylation of histone that links to neuronal damage and loss in HD. It is thought that cognitive processes like learning and memory depend on histone acetylation.

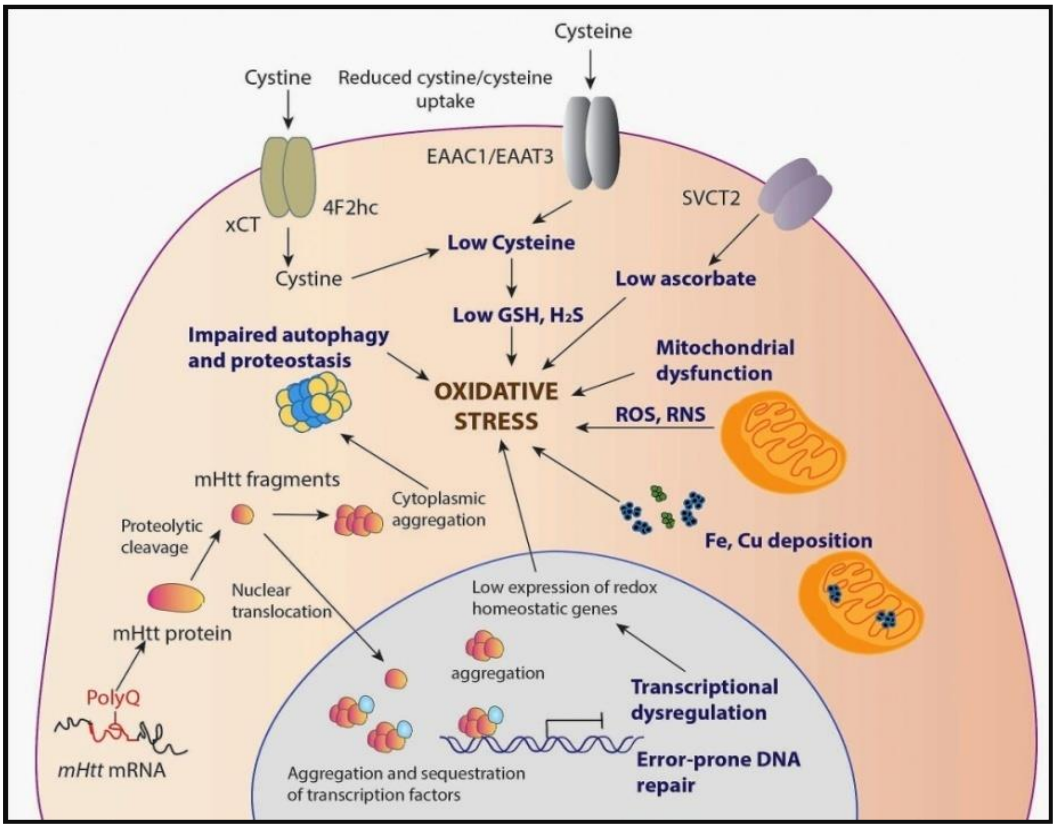


Fig.2: Pathology of HD

Oxidative stress refers to elevated intracellular levels of reactive oxygen species (ROS) that cause damage to lipids, proteins and DNA. Studies from the postmortem brains of HD patients have demonstrated increased oxidative damage, which has been regarded as a key factor in the development of neurodegenerative disorders.

Cumulative oxidative stress can damage cellular structures, impair the DNA repair system, and induce mitochondrial dysfunction (Fig. 2).

HD is characterized by a general shrinkage of the brain and degeneration of the striatum (caudate nucleus and putamen), with specific loss of efferent medium spiny neurons (MSNs) as proposed by Reiner et al. 1988 (Fig. 3A). Rosas et al. 2002 saw that although the striatum appears to be the most affected region of the brain, a regionally specific thinning of the cortical ribbon was found in patients with HD. Such loss of cortical mass is an early event in the pathology of HD and proceeds from posterior to anterior cortical regions with disease progression. Motor dysfunction correlates with the extent of cell loss

in the primary motor cortex whereas mood changes are associated with cell loss in the cingulate cortex (Thu et al., 2010).

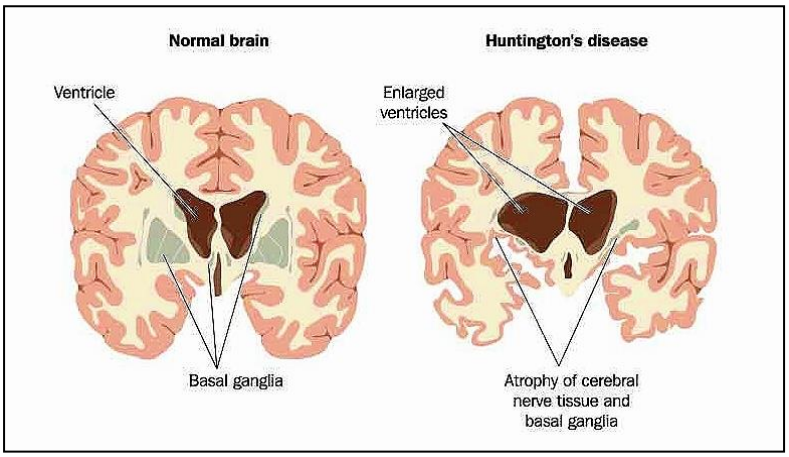


Fig. 3A: Shrinkage of the brain in HD patients when compared to normal human.

1.1.2 Selenoprotein in the brain

A selenoprotein is any protein that includes a selenocysteine (Sec, U, Se-Cys) amino acid residue. There are 25 selenoprotein genes in human genome. Among functionally characterized selenoproteins are five glutathione peroxidases (GPX) and three thioredoxin reductases, (TXNRD) and selenoprotein P (SELENOP). In humans, it contains 10 Sec residues, which are split into two domains, a longer N-terminal domain that contains 1 Sec, and a shorter C-terminal domain that contains 9 Sec. The longer N-terminal domain is likely an enzymatic domain, and the shorter C-terminal domain is likely a means of safely transporting the very reactive selenium atom throughout the body.

Selenoproteins may be helpful in preventing:-

- Neurodegeneration in Alzheimer's disease (AD)
- Parkinson's disease (PD) is associated with impaired function of glutathione peroxidase selenoenzymes

- In HD, selenium deters lipid peroxidation by increasing specific glutathione peroxidases
- Selenium deficiency increases risk of seizures in epilepsy, whereas supplementation may help to alleviate seizures

There are five GPx proteins in humans that are selenoproteins. These include –

1) The ubiquitous cytosolic GPx (GPx1)
2) The gastrointestinal GPx (GPx2)
3) The plasma GPx (GPx3)
4) The phospholipid hydroperoxides glutathione peroxidase (GPx4)
5) The olfactory epithelium and embryonic tissue GPx (GPx6).

GPxs use the antioxidant glutathione to reduce peroxides and other reactive oxygen species (ROS) that could potentially destroy cells and tissues.

GPx1–3 are involved in the reduction of hydrogen peroxide and organic hydroperoxides while GPx4 directly reduces phospholipids and cholesterol hydroperoxides. GPx4 has an additional structural role in sperm maturation as an important component in the sperm's helical mitochondrial capsule that is responsible for sperm motility.

GPx 1 and 4 are major forms of the GPx in the brain. GPx1 is a ROS scavenger that is expressed in both neurons and astrocytes . **Previous work reported that Se serves as an important constituent of selenoproteins such as GPX and TXNRD systems that scavenge reactive oxygen species (ROS) such as $OH^{\cdot}$, O_2^{-},and H_2O_2.** GPx4 functions in various locations of the neuron including the cytosol, mitochondria, and nucleus. It destroys phospholipid hydroperoxides and can work with vitamin E to suppress lipid peroxidation in various cell membranes and lipoproteins . Glutathione peroxidase has been localized in glial cells (non-neuronal cells in the brain and spinal cord that do not produce electrical impulses) and its expression is increased surrounding the damaged area in Parkinson's disease and occlusive cerebrovascular disease, consistent with its

protective role against oxidative damage. **Therefore, Selenoproteins possess antioxidant activities and the ability to promote neuronal cell survival.** Hence, Se deficiency is associated with cognitive decline (Fig. 3B). For instance, HD mice's brain levels of oxidized glutathione and mutant HTT aggregation may be reduced by sodium selenite.

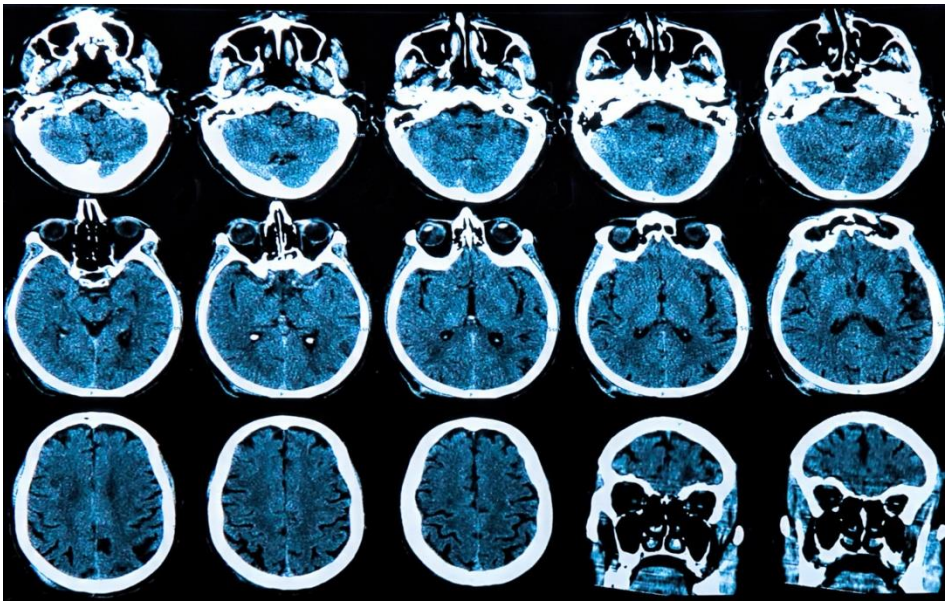


Fig. 3B : Effect of neural loss over the course of HD

1.1.3 Conventional treatment and its limitations

At this time, there is no effective treatment that can either stop the progression of HD or cure it. But medications can lessen some symptoms of movement and psychiatric disorders. Multiple interventions can help a patient adapt to changes in abilities for a certain amount of time.

Drugs like haloperidol, tetrabenazine, and amantadine are especially helpful for controlling the unusual movements caused by Huntington disease. Haloperidol and tetrabenazine can also help offset hallucinations and delusional thoughts. Depression and suicide are common among those with Huntington disease. Antidepressants and antianxiety medications may be prescribed to treat these symptoms. Medications will

likely evolve over the course of the disease, depending on overall treatment goals. Also, drugs that treat some symptoms may result in side effects that worsen other symptoms. HD affects many systems in the body which makes it difficult to target and treat only one symptom.

The majority of HD treatments in clinical trials have focused on targeting HTT, such as AAV-delivered RNAi therapies, CRISPR-based genome editing, HD-SNP targeting, and so forth. **However, the efficacy of these therapeutic approaches is subpar, and some of their limitations include off-target cleavage, the possibility of adverse effects on safety, and long-term gene interference.** Genetic disorders like HD are rare and, consequently, human sample material is limited and submitted to strict ethics committees.

2.1 Nanotechnology

Nanoparticles (NPs) are used as drug carriers in the delivery of the therapeutic molecules. NPs serve to reduce the toxicity, enhance bioactivity, improve targeting, and provide versatile means to control the release profile of the encapsulated moiety. Selenium (Se) is an essential trace element and its deficiency causes a disruption to selenium homeostasis which deters normal brain functioning.

Se has both beneficial and harmful effects, despite its neuroprotective effects. Chemical form of Se influences its biological activities whereas Se NPs possess remarkably reduced toxicity. Se exists in different oxidation states like 2^+, 4^+, 6^+, and 2^-. It has "zero" oxidation state which is colorless, non-toxic, and biologically inert material. High doses of Se may cause toxic effects. The key Se ion behind it's toxic effects is selenite (Se^{+4}) which is required to be reduced to selenium (Se°) by biogeochemical cycles.

Thus, Se is a double edged sword. It is antioxidant at sub nutritional doses and becomes pro-oxidant at supranutritional doses.

In comparison to other forms of Se, Se NPs, has recently been found to be less toxic, more bioavailable, and highly effective at preventing oxidative damage. At concentrations below 0.5 mM, Se NPs can scavenge free radicals more effectively than Na_2SeO_3. Se NP is also less toxic than methylselenocysteine as it doesn't affect compromising selenoenzymes as much. **So, the main challenge is to find a safe dosage window for the application of Se NP to treat HD patients effectively.**

2.2 Model organism: Caenorhabditis elegans

One of the most well-known models for studying the structures and functions of the nervous system is Caenorhabditis elegans (C. elegans) which has a brain consisting of 302 neurons. It's neuronal heredity has been chalked out making it a powerful model to investigate physiological elements of neurological frameworks and neurological illnesses. Se NPs can be ingested by C. elegans by introducing it to its food source because selenium is not present in the nematode growth medium (NGM).

According to the introduced Se NP in the food, the intake of selenium is primarily responsible for the accumulation of selenium in the worms as a dietary supplement. The therapeutic efficacy of Se NPs for HD disease was evaluated using wild-type and transgenic C. elegans (HA759 and AM141, respectively).

2.3 Experimental Set-up

L. Wang et al. synthesized Se NPs in the laboratory and noted its zeta potential in aqueous solution. The zeta potential value suggested that the surface charge of Se NPs was negative and the NPs have a good dispersity in the solution.

The dispersion solution of Se NPs was stable that exhibited brown color and remained clear without sediments even after 180 days' storage (Figure 4C). Moreover, DLS

measurements also show that the average size of Se NPs did not change at 0 h, 24 h, and 72 h post-preparation (Figure 4D), which suggested the stable condition.

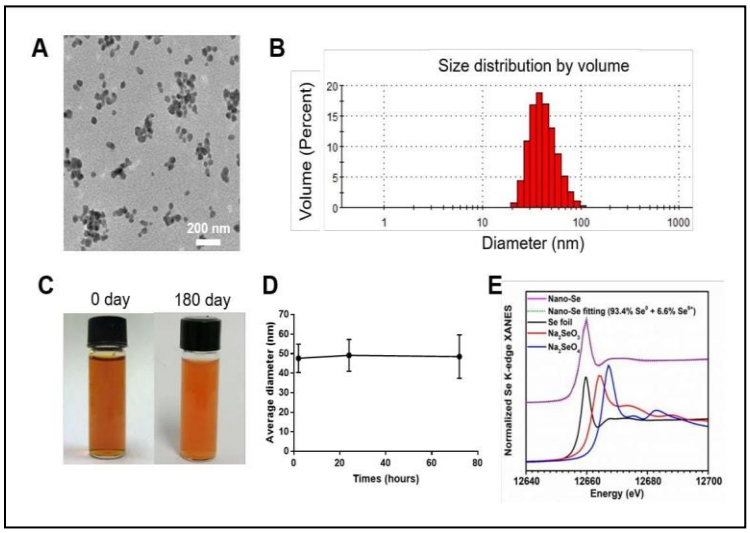


Fig. 4: Characterization of Nano-Se. (A) TEM image of Se NPs. (B) Size distribution of Se NPs by dynamic light scattering (DLS). (C, D) Stability of Se NPs in the aqueous solution during the storage for 180 days. (C). The average diameter of Se NPs at 0 h, 24 h, and 72 h post the preparation of dispersion (D). (E) Chemical speciation of Se for Se NPs solution as determined by Se K-edge XANES. With respect to Se NPs sample, the least squares fitting of XANES was performed based on the spectra of the reference samples including Se foil, Na_2SeO_3, and Na_2SeO_4.

In addition, XANES is powerful to reveal electronic structures of elements that is capable of characterizing the chemical forms of interested elements in liquid and solid phase, as well as chemical information for the bulk sample or the surface region. Herein, Se K-edge XANES spectra were collected for reference samples and Se NPs. The fitting XANES result indicated that Se NPs includes two compositions: 93.4% Se0 and 6.6% Se^{6+}. It meant that most Se existed as the reduced state and a small part of Se was the oxidative state (+6) which may reside on the surface of NPs. XANES results show Se NPs mainly exist as the elemental Se, as the major chemical form for their application.

The steps to monitor any change in the model organism after exposure of Se NPs :-

- First, Se NPs are prepared and characterized before exposing it to wild-type and transgenic C. elegans in normal, MeHg-, or juglone-stressed, and stressed conditions.
- Then, survival rates of the worms are noted before and after treatment with Se NPs under each condition. Chemotaxis tests are used to investigate defensive impacts

of Se NPs on the endurance of debris and physiological elements of tactile reaction in transgenic C. elegans strain HA759.

- Chemosensory conduct and mechanical tactile way of behaving are assessed at the aggregate and quality levels.
- In addition, a molecular mechanism is formulated at the epigenetic (heritable changes) level and investigated whether Se NPs reduced the burden of mutant huntingtin (HTT) aggregates and oxidative stress in C. elegans strain AM141.

The worms must be maintained under standard conditions on the nematode growth medium (NGM) and seeded with *Escherichia coli* strain OP50. The toxicity of Se may increase with the elevated temperature at 25 °C on upon exposure; the study is usually carried out at 20 °C.

Survival Rate and Growth: Worms were exposed to Se NPs at different concentrations for 72 h. Then, the survival rate and growth rate of worms were assessed.

Neurodegeneration Analysis: Synchronized L1 HA759 worms were grown in NGM plate in the presence of 0, 0.02, 0.2, and 2 μM Se NPs for 3 days. To assess ASH neuronal viability, HA759 strain worms were counted to calculate the fraction of live ASH neurons per group.

Chemosensory Behavior Assay: Chemosensory assay was performed. Prior to that, young adult stage worms were washed three times with S-basal buffer to remove bacteria. NGM plates were divided into two identical areas (A and B). Glycerol was spread along region A and the midline of the plate. When glycerol penetrated into chemotaxis agar, 1% butanedione (2 μL) and 200 mM NaN_3 (2 μL) were spotted on A side of the plate, approximately 1 cm-distant from the edge. Worms placed on B region approached butanedione that were driven by attraction and then were paralyzed by NaN_3 during 90 min. Afterwards, the number of nematodes in areas A and B was counted and chemosensory index was calculated as B/(A+B).

Touch tests: After L1 HA759 worms were synchronized, sensitivity to mechanical sensations was observed and scored after the tails of nematodes were touched 10 times and analyzed as the ratio of response. When a normal animal is moving forward and is tapped on the head, it will halt forward locomotion initiating backing. Worms with such behavior were scored as normal. When the head was tapped and the body paused, the continued forward of worms displays a backing deficit. In this case, the movement was scored as backing deficit/failure. The ratio of response to mechanical touch was counted as times scored as normal per 10 touch times.

Huntingtin protein aggregates measurement: AM141 worms expressing a muscle-specific polyglutamine (Q40)::YFP were grown in NGM plate in the presence of 0, 0.02, 0.2, and 2 μM Se NP for 3 days after synchronization from the L1 onward. Levamisole (1%) was used to anesthetize the worms before the observation. The expression of polyQ aggregates labeling with green fluorescent protein (GFP) were used to monitor PolyQ40::YFP aggregates in muscle cells. In each group, at least 30 worms were counted under fluorescence microscope.

ROS Level Measurement: Some worms were pre-treated with 2 μM Se NPs for 3 days while some were not. They were then exposed to 300 mM Juglone for 1 h in order to induce oxidative stress. The worms were homogenized by sonication and centrifugation. The supernatants were transferred to a 96-well plate containing 25 mM H2-DCF-DA in dark. Fluorescence intensity was measured with an excitation at 488 nm and an emission at 520 nm after incubation with H2-DCF-DA. Fluorescence intensities were normalized to time. ROS level could be described as related intensity of probe fluorescence by dividing that of control. At least 30 nematodes should be examined per test.

Stress Resistance Assays: For Juglone stress resistance assay, Juglone was dissolved to a final concentration of 300 mM in a solution of 0.23% (v/v) ethanol in M9.Prior to Juglone stress resistance assay, the wildtype N2 worms were pre-treated with 2 μM Se NPs or not for 3 days and then were transferred to fresh NGM plates with Juglone for 8 h. Finally, number of dead worms was counted by touch-provoked movement. Prior to

MeHg stress resistance assay, the wildtype N2 worms were pre-treated with 2 μM Se NPs or not for 2 days and then transferred to S medium containing *E. coli* OP50 bacteria for 36 h. Meanwhile, these *E. coli* OP50 was pre-treated by 40 μM of MeHg (LD50) for 30 h. At last, the number of dead worms was counted by touch provoked movement .

Determination of Selenium *in vivo*: To quantify intake of selenium by the worms, inductively-coupled plasma mass spectroscopy (ICP-MS) was used to determine content of selenium accumulated in about 20 nematodes. At first, 2 μM Se NPs was exposed to the L1 stage *C. elegans* for 3 days. To remove bacteria outside of the worm, young adult worms were rinsed three times with S-basal buffer. Then, the nematodes were broken up by sonication, and then the supernatant was collected for ICP-MS analysis. Total protein mass in 20 worms was determined by BCA. A blank without worms was used to measure Se content at background levels. Selenium isotopes of ^{77}Se and ^{78}Se were quantified in triplicate.

Statistical Analysis: Data were described as mean value and standard errors which relied on the averaging results obtained from all the plates of each strain or population type (*e.g.* treated or untreated) counted. Significant difference in different populations was determined using one-way ANOVA and LSD post hoc test. Except of specific statement, all of the experiments were repeated three times. Probability levels at 0.05 and 0.01 represent significant and very significant difference.

4.1 Role of Se NPs in Huntington's disease therapy

4.1.1 Se NPs therapy promoting the intake of Selenium by C. elegans

L. Wang et al. study revealed that the Se content under normal conditions was significantly lower in transgenic HA759 *C. elegans* than that in wild type worms (Figure 5A). **This result was consistent with previous reports that the Se content in human HD brains at advanced stage decreases compared to normal brains.**

After the therapy, the level of selenium in both N2 and HA759 worms increased in dose-dependence (Figures 5B and 5C). Se content of transgenic HA759 worms reached normal level as wildtype ones below 0. 2 μM of Se NP, while Se content in N2 worms changed much less after Se NP supplementation (Figures 5B and 5C). The difference in relative absorption capacity of Se suggested the selectivity of nutrient requirements for Se in HD worms. The reason might be that intestinal structures and function change in mouse HD model that influence the intake and metabolism of Se. Finally, upon exposure to Se NPs, increasing uptake and accumulation of Se in HD model of *C. elegans* largely improve the level of Se *in vivo*, which can explain how Se NPs eliminates ROS and play protective roles in recovering physiological functions of neurons in HD disease. However, further study will be addressed in the future to reveal how Se NP works and behaves *in vivo* to regulate the therapy of HD at molecular and cellular aspects.

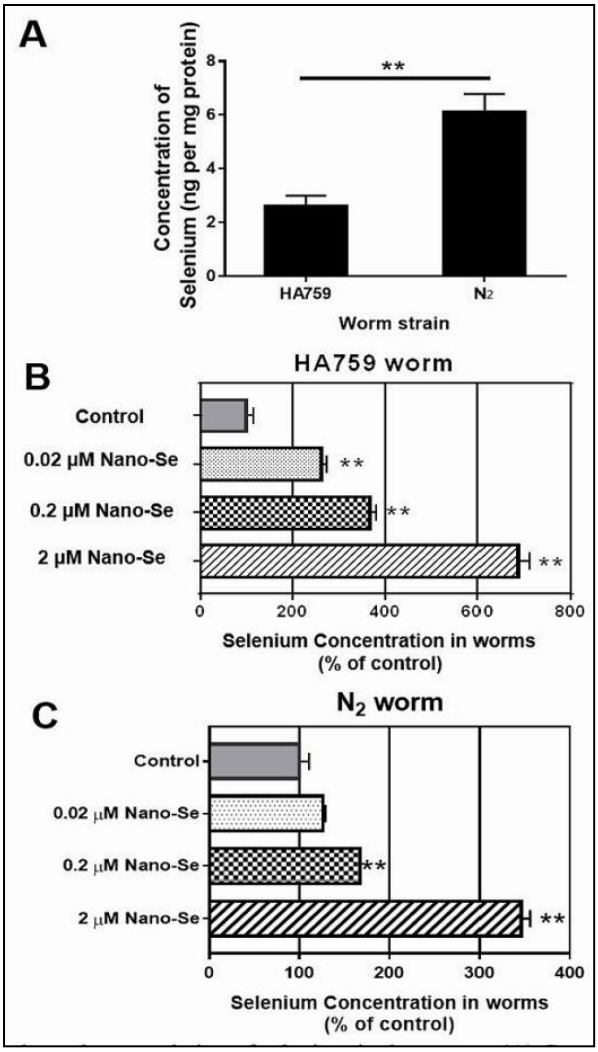


Fig. 5: Intake and accumulation of Se in the worms. (A) Content of Se levels in the body of N2 and HA759 *C. elegans*. The data was described as the mass of Se within 1 mg proteins of worm (ng Se/mg protein). (B) Accumulated Se level in HA759 worm with selenite supplementation. (C) Accumulated Se level in N2 worm with selenite supplementation. Data represent mean value± standard errors. ** $p<0.01$.

4.1.2 Se NPs protecting C. elegans survival under neurotoxic stress

Survival rate was taken as a parameter to describe the protective effects of Se NP on the viabilities whiles the body length of *C. elegans* were taken as a parameter to describe the growth of *C elegans*. Both these parameters were chosen because the threshold values may vary with two evaluation modes and the parameter of body length was more sensitive than the viability under the stress stimuli. According to the findings of this study, juglone and methylmercury (MeHg) can cause neurodegenerative disorders by

triggering oxidative stress in the brain. **Concentration is one of the critical determinants for the therapeutic activity in case of micronutrient such as Se**.

L. Wang et al. noted that Se NP under 20 μM did not induce toxic effect on *C. elegans* under normal situations (Figure 6A and 6D) and it played a dosage-dependent protective effect on the viability after the worms are exposed to both stress stimuli (Figure 6B and 6C). After further investigation for the appropriate dosage window, Se NP under 2 μM was found to display protective effects on the growth of the worms when they are exposed to the same stress (Figure 6E and 6F). In conclusion, according to the viability and body length, Se NP below 2 μM produced significantly protection to *C. elegans* after their exposure to neurological stress such as Juglone and MeHg. So, the concentrations of Se NPs were set to 0.02, 0.2, and 2 μM as shown in Figure 6E.

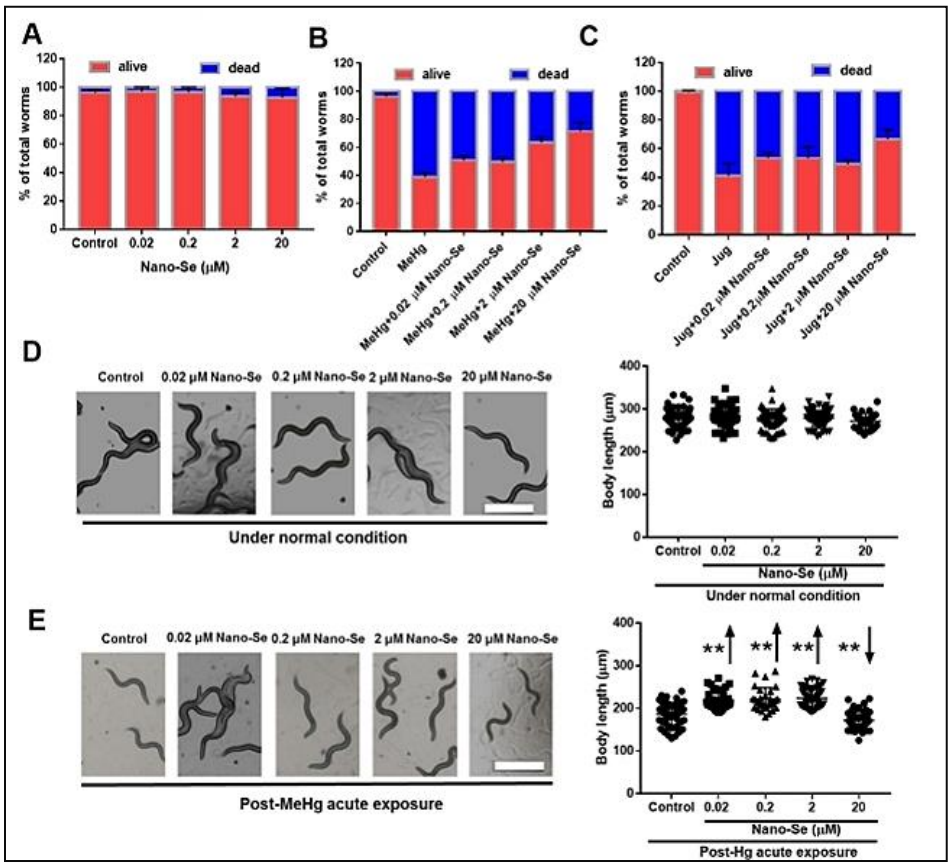


Fig. 6: Influence of Se NPs treatment on survival and body growth of C. elegans under normal and stressed situations. (A-C) Effects of Se NPs on worms' survival rate under normal, MeHg and juglone stressed situations. (D-E) Effects of Se NPs on the body growth of worms under normal and MeHg-stressed situations. Scale bar represents 500 μm.

4.1.3 Protective effects of Se NPs on ASH neuronal survival

Detection of aversive sensory stimuli in the environment is an essential feature of animal nervous systems that allows them to avoid toxic chemicals and dangerous conditions. Animals make use of specialized neurons and sensory structures called nociceptors to detect a wide range of aversive and painful stimuli, including toxic chemicals.

C. elegans contains at least 11 bilaterally symmetric pairs of sensory neurons in the head and two in the tail that function primarily as chemosensors. ASH is one of these bilateral pairs in the head which plays a central role in mediating avoidance behaviors in response to chemical repellents as well as several different noxious stimuli. It is specifically required for behavioral avoidance of water-soluble (e.g. copper, quinine and SDS) and volatile (octanol) chemical repellents, osmotic shock and mechanical stimulation on the tip of the animal's nose.

In transgenic HA759 C. elegans, huntingtin fragments Htt-Q150 (a polyQ150 tract derived from human Huntington) were highly expressed in ASH neuron and lead to neuronal death, but were lowly expressed in other neurons.

C. elegans was treated with Se NPs from early L1 stage of the nervous system. L1 stage is the most sensitive and vulnerable and detrimental to external environmet effects. Before and after Se NP treatment, survival rate of ASH neurons was evaluated by the number of GFP (Fig. 7A). L. Wang et al. found that Se NPs reduced neuronal death significantly in a dose-dependent manner. Only 21% of ASH neurons survived in untreated nematodes, while 2 μM of Se NPs improved the neuronal survival rate to 44% after treatment with Se NPs for three days (Fig. 7B).

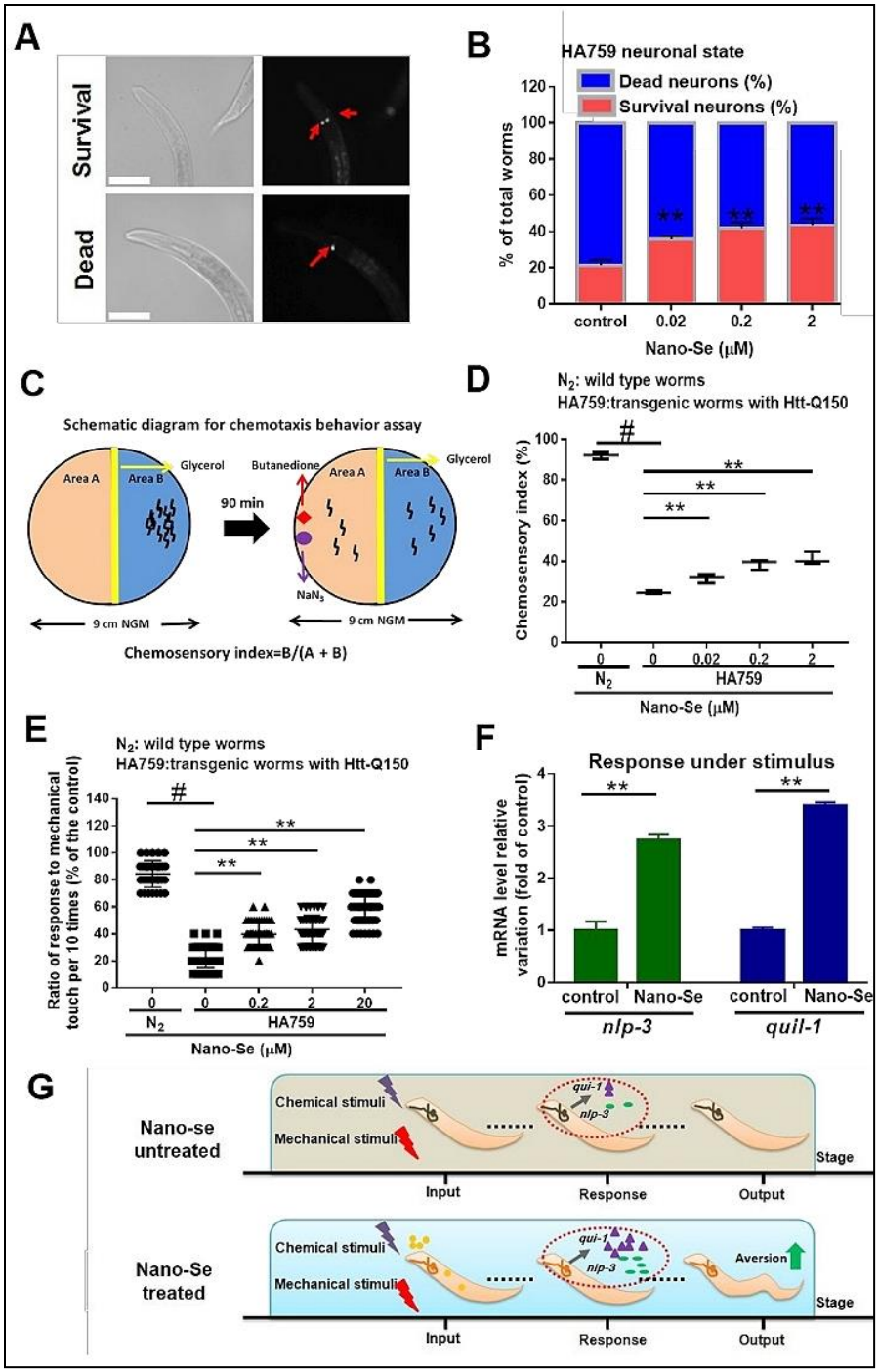


Fig. 7: Effect of Se NPs on ASH survival and sensory response function in Transgenic C. elegans strain HA759. (A) Representative live fluorescence imaging of ASH neurons of HA759 nematodes. Death of ASH neurons was assessed by the loss of Bilateral GFP fluorescence. (B) Survival rate of ASH neurons after Se NPs treatment. Schematic diagram for chemotaxis behavior assay system. The worms at the Bregion can be attracted to the A region by

butanedione during 90 min. After the worms approached the A region, they will be paralyzed by NaN3. The number of nematodes in areas A and B were counted and calculated for chemosensory index as B/(A+B). (D, E, F) Effect of on (D) chemosensory behavior, (E) mechanical sensory behavior in C. elegans. Among all the worms, the ratio of worms that responded to the mechanical touch were counted upon the touch every ten times. (F) Quantitative real-time PCR results of nlp-3 and qui-1 under chemo stimulus in HA759 after treatment Se NPs. (G) Schematic diagram for the protective effect of Se NPs on behavioral dysfunction in HA759 C. elegans. Data represent mean value±standard errors. The double asterisks (**) indicate very significant difference ($p<0.01$) between untreated control and Se NPs treated ones for HA759 worms. The pound key (#) represents very significant difference ($p<0.01$) between N2 wildtype and the untreated HA759 worms.The scale bar represents 100 μm.

4.1.4 Se NPs preventing C. elegans from behavioral dysfunction

Elimination of ASH sensory neuron pair impairs the avoidance behavior in *C. elegans*. The worm's sensation and motion behaviors are simple and they are used to investigate integrated responses to different sensory stimuli.

The sensory and mechanical responses of ASH neurons were observed when HA759 nematodes were treated with Se NPs at 0, 0.02, 0.2, and 2 μM for 3 days. The majority of transgenic HA759 nematodes lost the ability to sense noxious stimuli due to ASH neuronal death with a chemosensory index of 0.25, as shown in Figure 7D. In comparison to the N2 wildtype, approximately 90% of C. elegans' ASH neurons were in a healthy condition.

The chemosensory index increased to 0.4 following treatment with 2 μM of Se NPs, and the number of HA759 worms and improved functions for ASH neurons were dose-dependent. In addition, in terms of mechanosensory measurement, less than 20% of HA759 C. elegans and less than 80% of N2 nematodes normally responded to physical touch (Figure 7E). **Intriguingly, the percentage of HA759 worms with a normal response to mechanical disturbance increased to up to 60% following treatment**

with 2μM of Se NPs (Figure 7E), indicating that Se NPs played a significant role in reducing neuronal destruction.

The results concluded the protective effects of Se NP against neuronal dysfunction (Fig. 7G). L. Wang et al.'s experiment Se NPs induces opposite effects compared to Se-containing compounds that decreased the movement and accelerated the paralysis of *C. elegans* in previous reports. The reason could be that a higher concentration of other Se compounds such as $(PhSe)_2$ and Na_2SeO_3 were adopted that caused deleterious effects. In addition, the forms of Se may affect physiological functions of *C. elegans* distinctly.

4.1.5 Se NPs alleviating the aggregation degree of Huntingtin proteins and the ROS level

In HD, mutations in the HTT gene lead to the expansion of glutamine (CAG) repeats and conformational rearrangements of proteins that form insoluble aggregates and disrupt normal functions of neurons. The mutations and subsequent aggregates are involved in the pathological processes including oxidative stress and ultimately cell death. **In this study, transgenic strain AM141 *C. elegans* was adopted because the body wall muscle cells specifically express polyQ40::YFP fusion proteins that underwent the formation of discrete aggregates with fluorescent and the degeneration of the axonal processes when the worms reach adulthood.**

In the muscle of body wall, a highly punctate pattern with fluorescence is visualized and can be used to quantify polyQ aggregation (Fig. 8A). The level of aggregation can reflect the number of formed huntingtin fragments into discrete foci. After treatment with 0.02, 0.2, and 2 μM of Se NPs the aggregation level was significantly reduced by 18%, 22%, 30% compared with control, in a dose-dependent mode (Fig. 8B and 8C).

Consistently, more fluorescent proteins (YFP) appeared as soluble and the continuous fluorescent signals along the body wall after Se NPs therapy compared to strong

fluorescence signals for aggregation in untreated AM141 worms (30%) (Fig. 8A-8C). The decreasing number of discontinuous punctate along body wall in Se NPs group indicated its role in decreasing axonal degeneration. For simplification, Se NPs treatment could effectively decrease HTT aggregation and reduce axonal degeneration in *C elegans*.

Previous reports showed that the surface of Se NPs could easily form protein corona in biological systems. When NPs interact with proteins in biological environments, they are surrounded by a layer of biomolecules, mainly proteins adsorbing to the surfaces. This protein rich layer formed around NPs is called the "protein corona". Consequential interactions between NPs and proteins are governed due to the characteristics of the corona.

The features of NPs such as the size, surface chemistry, charge are the critical factors influencing the behavior of protein corona. **Molecular properties and protein corona composition affect the cellular uptake of NPs. The proteins surrounding the Se NPs causes inhibitory effects on HTT aggregation.** L. Wang,b, * and C. Chena et. al verified the protective effects of Se NPs supplement in *C. elegans* based on anti-oxidative capacity of Se NPs. The ROS level of Huntington nematodes significantly decreased following treatment with 2 μM of Se NPs, indicating that Se NPs can clear ROS (Fig. 8D). Se NPs in vivo antioxidant activity may play a significant role in reducing HTT aggregation and neural degeneration. In contrast, under normal culture conditions, Se NPs had no effect on the wildtype N2 worms' basal level of ROS.

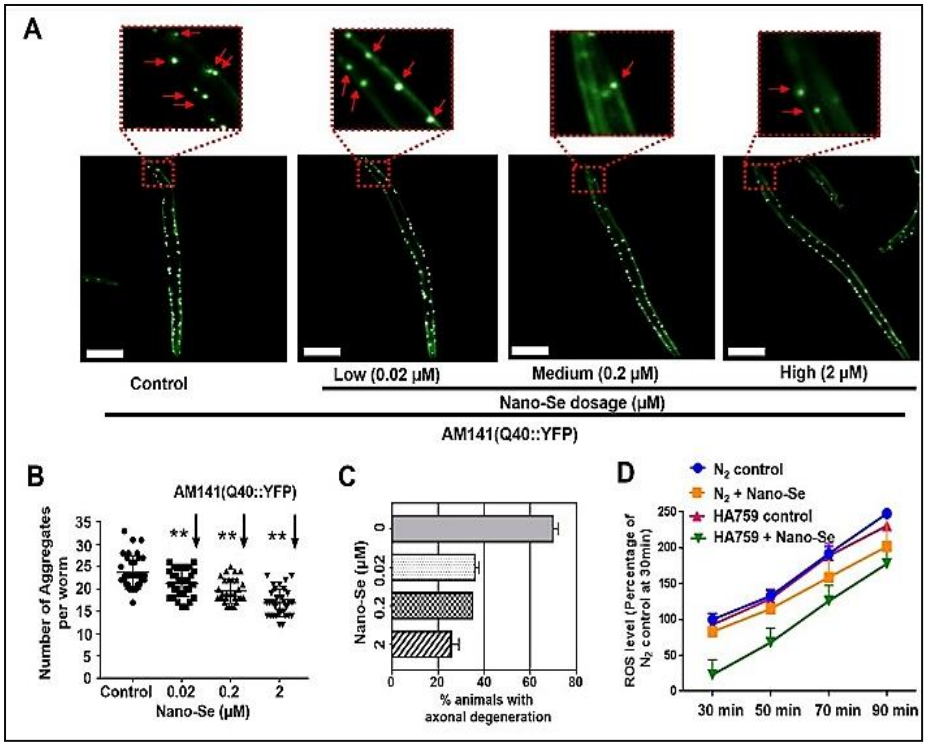


Fig. 8: Decreased mutant HTT aggregates burden and oxidative stress in *C. elegans* strain AM141 by Se NP. (A) Representative fluorescence microscopic images of polyQ 40::YFP aggregates in Se NP treated and control groups. As a control group, untreated AM141 worms expressing polyQ 40::YFP display discontinuous and punctate fluorescent signals in the muscle cell of the body wall. (B,C) Quantitation of polyQ 40::YFP fluorescent aggregates in the groups after the exposure to three dosages of Se NP or not. The representativeness of the aggregates was described as the average value and standard errors. The fluorescent points along the worm body stands for the muscle-specific polyglutamine (Q40)::YFP aggregates that are labelled with green fluorescent protein (GFP). The number of fluorescent aggregates in the whole worm was calculated for the quantitation. Totally, 20 nematodes were randomly selected and scored for each tested group. (D) Quantitative ROS level in Se NP treated and control groups. Data show mean value± standard errors. Double asterisks (**) mean the very siginicant difference between control and the Se NP treated samples. $^{**}p<0.01$. Scale bar represents 100 μm.

5. Conclusion

Se NPs are smaller, faster and highly sensitive diagnostic tools which has the ability to reduce the accumulation of HTT aggregation in *Caenorhabditis elegans*. This might seem like small information in the field of science but this was proven after a lot of hard work put in by various scientists from all around the world. NPs when compared to drugs produce less negative effects due to greater target specificity. Conventional methods for treatment of HD do not include surgical procedures and the use of NPs for is also of non-invasive type. Treatments for HD usually are very costly as the disease spans for about the patient's half life time. Cost effectiveness of medicines and disease management procedures as a whole may be more efficient in treatment by NPs. Se NPs below 2 μM can reduce neuronal death by decreasing ROS levels, relieving behavioral dysfunction, and offering protection to *C. elegans* under stress. A cause-effect relationship lies between oxidative stress, epidemic factors and HTT proteins in the pathogenesis of neurodegenerative disorders. Therefore, Se NPs play antioxidant roles in regulating the expression of *hda* family and is capable in reducing the degree of polyQ aggregation. In conclusion, the findings suggest that diet-based treatment of human HD with Se NP may be appealing and promising. Understanding how Se NPs alleviates HD disease in humans and the connection between physicochemical properties and dosage dependent therapeutic efficacy will be of great benefit to HD disease treatment in the future.

Future Perspectives

Nanotechnology applied to HD therapy has led to a new era of HD treatment. Genetic disorders like HD are rare and, consequently, human sample material is limited and submitted to strict ethics committees. However, SeNPs have craved the path for scope of diet-based treatment for HD in humans. In no time, nanoparticles will be used for treatment of most hereditary diseases in today's world. But, the cost effectiveness for such treatment is debatable when compared to traditional ways.

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