# *Caenorhabditis elegans* as a model for drug-induced peripheral neuropathy

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**Abstract**. Drug-induced peripheral neuropathy (DIPN) may not be life-threatening. However, it poses a problem in the quality of life of a patient, especially for those who undergo antiretroviral medication and chemotherapy. Nucleoside reverse transcriptase inhibitors interrupt viral replication; however, it also halts mitochondrial DNA replication, which causes dysfunction and leads to axonal damage. Only a few studies attempted to explore drug-induced peripheral neuropathy using non-mammalian organisms. Moreover, the understanding of DIPN in *Caenorhabditis elegans* remains elusive. Hence, this paper provides theoretical evidence that *C. elegans* is a potential model for DIPN. Studies show that drugs can damage the axon of *C. elegans*, which may lead to peripheral neuropathy. Peripheral neuropathy in *C. elegans* may be observed through various physiological, morphological, and biochemical parameters. Furthermore, the cost-effectiveness of *C. elegans* as a model for preliminary studies may help improve interventions on functional recovery through understanding various factors, such as age and health, which affect the quality of life of an individual.

**Key Words**: drug, axons, peripheral neuropathy, *in vivo*.

**Introduction**. Peripheral neuropathy affects the function of peripheral nerves in transmitting signals from different parts of the body to the brain (Torpy et al 2010). Typically, individuals suffering from human immunodeficiency virus (HIV) infection, tuberculosis, diabetes, and even cancer have a high risk of developing neuropathy (Mafukidze et al 2016). Some studies stated that it is due to the pathogen or disease itself (Ferrari et al 2006), but more recent studies claim that it is a side effect of the medication (Mafukidze et al 2016). Drug-induced peripheral neuropathy (DIPN) can be caused by several drugs, such as antimicrobial, antitumor, and others. Alarmingly, HIV treatments, specifically nucleoside reverse transcriptase inhibitors (NRTI), lead to permanent disability in individuals in the long run (Jones et al 2020).

The combination of antiretroviral therapy (ARV), NRTI, and HIV-1 protease inhibitors are considered in managing HIV infections. However, ARV-associated treatments have corresponding side effects, such as peripheral neuropathy (Jones et al 2020). Another study associates NRTI medications, like dideoxy-NRTIs, zalcitabine, didanosine, and stavudine, to peripheral neuropathy (Hammer et al 2008). All these references suggest that ARV leads to peripheral neuropathy.

NRTI and HIV proteins cause axonal damage resulting from mitochondrial injury (Robinson et al 2007). ARV drugs disrupt the production of new proviral DNA by supplying NRTI as a nucleotide source during the replication process. The absence of a chemical bond in NRTI signals the halting of viral replication (Birkus et al 2002). This exact mechanism happens during mitochondrial replication. The decline in mitochondrial copy number leads to mitochondrial dysfunction and cell death (Lewis et al 2006). Mitochondrial dysfunction is common to HIV-infected individuals taking ARV treatment, and it is evident in the decrease in their mitochondrial DNA copy number (Apostolova et al 2011). Neurons rely on their mitochondria for their fundamental functions; hence, they are vulnerable to unintended damages caused by ARV drugs (Hasegawa et al 2016).

Mitochondria perform a pivotal role in the maintenance and integrity of the axon, both in primates and in *Caenorhabditis elegans*. This organelle houses the nicotinamide

mononucleotide adenylyltransferase (NMNAT) protein. Besides, this protein is essential during axonal degeneration and regeneration (Cirrincione & Rieger 2020). *C. elegans* is a non-parasitic nematode from the family *Rhabditidae*, notable for its rod-like appearance. The microbivory feature of *C. elegans* allows them to thrive in a bacteria-rich environment like decaying organic matter. *C. elegans* are grown in a nematode growth medium in a typical laboratory environment and are fed with *Escherichia coli* OP50 strain (Nas et al 2019).

Studies on DIPN, primarily on axonal regeneration and degeneration, use mice or rats, *in vitro*, and *in vivo*, as model organisms. The use of these model organisms has some disadvantages, such as high maintenance, high cost, and limitations on large-scale screening. In this paper, it is suggested to consider a non-mammalian model for DIPN, which is advantageous during preliminary screening.

The use of non-mammalian organisms for DIPN may have several advantages. *C. elegans*, for instance, is commonly studied for neurodegenerative diseases, aging, cancer, alcohol withdrawal, etc. (Manalo et al 2017; Nas et al 2019; Nas et al 2020a; Nas et al 2020b). The availability of the genome of *C. elegans* makes it beneficial for genetic screening studies. The silencing of genes in *C. elegans* through RNA interference is very efficient in studying the regulation of genes during neuronal injury and regeneration. In addition, using *C. elegans* as an *in vivo* model is cost-efficient due to its low maintenance and high propagation rate. This model is also helpful for large-scale pharmacological screenings. Additionally, the influence of age on DIPN may also be studied in *C. elegans* due to its short lifespan of about 20 days at 20°C (Nas et al 2019). Lastly, one gleaming advantage of *C. elegans* in the DIPN study is its transparency, making it easier to observe axonal degeneration and regeneration using fluorescent markers. However, DIPN in *C. elegans* is not yet extensively demonstrated and well established.

With this knowledge, the researcher proposes that *C. elegans* can demonstrate DIPN by supporting evidence that: (1) drugs can cause axonal damage; and (2) DIPN can be assessed by various morphological, physiological, and biochemical parameters.

## Axonal Damage in *C. elegans*

***A. C. elegans as an injury model***. Two injury models were used to study axon regeneration in *C. elegans*, namely laser axotomy and β-spectrin mutants. Laser axotomy cuts the axon using a pulsed laser to observe axon regeneration since axon regeneration only occurs during nerve injury. Pulsed lasers are precise in controlling injury at a single axon resolution by creating a free electron to create nanoscale bubbles and cavitation bubbles that result in the collapse of axons (Bourgeois & Ben-Yakar 2008). On the other hand, β-spectrin mutants are *C. elegans* strains that lack β-spectrin through the deletion of the unc-70 gene. These mutant strains are morphologically abnormal due to continuous axon breakage caused by a lack of β-spectrin, a valuable component of the membrane skeleton (Hammarlund et al 2007).

***B. Paclitaxel leads to axonal degeneration in C. elegans***. One study examined the effects of a drug on the axons of *C. elegans* (Mao et al 2016). This study observed that paclitaxel leads to axonal fragmentation. Studies show that short axons are vulnerable to fragmentations (Maxwell et al 1993). There was an observation that after the fragmentation, the axon retracts and develops a bulb (Kerschensteiner et al 2005). The study also reveals that paclitaxel results in axonal beading (Mao et al 2016). During this scenario, the axonal transport mechanism in *C. elegans* is impaired, which leads to the build-up of axoplasmic organelles and disturbs axonal transport (Peng et al 2011).

## Different Parameters to Assess Neuropathic Axons in *C. elegans*

***A. Morphological parameters***. As previously mentioned, paclitaxel leads to the beading and fragmentation of the axon in *C. elegans* (Mao et al 2016). In humans, axonal damage is graded in terms of demyelination, endoneurium, and epineurium damage (Menorca et al 2013). However, this may not be applicable in *C. elegans* due to the

absence of myelination. One simple way to determine the severity of the damaged neuron is to measure the distance of the commissure from the dorsal cord for GABAergic and cholinergic motor neurons (Gabel et al 2008; Hammarlund et al 2009); and from the ventral cord for mechanosensory touch neurons (Gabel et al 2008). This idea comes from the principle that the regenerating axons tend to grow towards the dorsal cord or the ventral cord. Thus, if the drug causes extensive damage to a neuron, a larger area of the axon will most likely be impaired, which affects its interaction with the dorsal or ventral cord.

***B. Physiological parameters***. The mechanosensory axon of *C. elegans* is more developed, in terms of the number of protofilament microtubules, compared to a human (Bounoutas et al 2009). These extra protofilaments affect the cytoskeletal assembly in *C. elegans*, making the touch receptor neurons sensitive (Bounoutas et al 2009; Cirrincione & Rieger 2020). The DIPN inflicted on nematodes may lead to insensitivity due to the impaired axonal transport system. Insensitivity in *C. elegans* can be assessed by measuring the distance traveled by the nematode from the point of origin after a gentle touch, crude touch, or plate tap (Chalfie & Sulston 1981). Another applicable way to measure sensitivity is through recording the number of body bends and omega turns of the nematode (Manalo & Medina 2020).

***C. Biochemical parameters***. After an injury, various signaling pathways are involved in axon regeneration, which are targets for therapeutic studies. In the presynaptic region, the initiation of dlk-1 leads to the activation of MAP kinase kinase (mkk-4) in the cytoplasm through phosphorylation (Hammarlund et al 2009). After a series of phosphorylations, phosphorylated p-38 MAP kinase (pmk-3) and MAP kinase activated kinase (mak-2) are activated and gain functionality through bZip-containing protein (cbep-1) (Yan et al 2009). Also, mlk-1 regulates the JNK-like kinase (kgb-1) through MAPKK (mek-1) (Gallo & Johnson 2002). The levels of dlk-1 and mlk-1 should be measured to determine whether there is actual axonal damage. These proteins are upregulated after an injury to initiate innate axonal regeneration. On the contrary, the indication of axonal regeneration termination may be detected by high levels of rpm-1. In *C. elegans*, rpm-1 is a negative regulator of both dlk-1 and mlk-1, preventing neurite outgrowth (Grill et al 2007).

**Future Directions**. With the advances in pharmacogenomics, designing a drug that will not adversely affect a non-target entity remains tenuous. Hence, several studies are needed to build knowledge on existing gaps. The use of non-mammalian organisms is an efficient, non-invasive, and simple way to study the side effects of drugs, medications, or interventions. Detection of DIPN using morphological, physiological, and biochemical parameters in C. elegans may lead to developing treatment interventions that may minimize the side effects of a drug administered to an individual. Nonetheless, it is ideal for mitigating the consequences of DIPN by discerning several factors, such as age and health. The short lifespan of C. elegans may give insight into the prevalence of risk factors for developing DIPN at different stages. Importantly, monitoring several health indicators in C. elegans, like feeding behavior, infection, and fecundity may explain how DIPN affects an individual's overall quality of life.

**Conflict of Interest**. The author declares that there is no conflict of interest.

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