

Application of *Caenorhabditis elegans* in Neurotoxicology

Zixuan Zhao

Abstract:

Neurotoxicological studies usually use mammals similar to humans as model animals for research. Although the high similarity with humans makes it a good choice, the complicated nervous system and the difficulty of feeding and operation have become unfavorable conditions in the research. And the longer life span greatly extends the research cycle to study long-term toxicity. The problem can be solved using *Caenorhabditis elegans* (*C.elegans*) as experimental animals. *C.elegans* have a fast growth rate, short research period, and easy culture and preservation compared with mammals used to study Neurotoxicology. It has the advantages of strong genetic stability, gene conservation, small individual differences, simple structure, and easy observation and operation. So, *C. elegans* is an ideal model for long-term toxicity study.

Keywords: *Caenorhabditis elegans*; Neurotoxicological; model organism

1. Biological characteristics of *C. elegans*

C. elegans is a non-parasitic small nematode that can survive independently in the soil, belonging to rhabditia, rhabditida, and rhabditoidea¹. It mainly feeds on *E. coli* in the soil. The adult is about 1mm long, 70 μm wide, and symmetrical on both sides. *C.elegans*, the Bristol strain N2 is the common *C.elegans* in the study. Its structure is simple; its body is transparent and easy to observe. The anatomy of *C. elegans* consisted of a layer of mouth, pharynx, intestine, gonads, and collagen keratin. Nematodes have two sexes: hermaphrodite and male. The vast majority of nematodes in nature are hermaphrodites, and only 0.05% of nematodes are male. The male has a single-leaf gonad, vas deferens, and a specialized mating tail. The hermaphrodite has a uterus, two ovaries, fallopian tubes, and sperms. The hermaphrodite nematode can produce both sperm and eggs for autosemination. Males can mate with females and give birth to fertilized eggs. Unmated hermaphrodite nematodes can produce about 300 fertilized eggs during reproduction.

2. Studies on the use of *C. elegans*

C. elegans are widely used in toxicological studies. When studying the neurotoxicity effect of copper in Yang Huimin, Han Yan studied the toxicity effect of copper on *C.elegans*, the RC Cu-resistant *C.elegans*, and WT nematodes were used as experimental animals in studying copper neurotoxicity². Add copper to rc medium only. The head pendulum, body bending, and the number of basic movements within 20 s of nematodes were taken as reference indexes, and the distance of radiation movement in the center of the culture plate with a diameter of 9 cm

was calculated for 5,10,15 minutes. The RC is significantly higher than the WT head pendulum frequency ($P < 0.01$); body bending and basic movement are not seriously affected. During the three radiation motion periods, severe dyskinesia results appeared in RC. Conclude that Cu will cause damage to the nervous system, and the decline of the perception and judgment ability of the environment makes the nervous system function decline.

Yang Chunju, Xu Jianxin, in the study of formaldehyde toxicity effect³ 10% formaldehyde solution after 5,20,80 μmol /l of exposure to toxicity for 1 hour, the number of head wobbles in a minute and the number of bending of the body in 20 seconds as a reference item. It is concluded that formaldehyde can significantly decrease the frequency of head swing and body bending. It is concluded that formaldehyde poisoning will cause damage to the nervous system of nematodes, leading to defects in their motor behavior and decreased motor ability. And speculated that its synaptic function and neuromuscular connection may be disrupted.

Lu Weiwei, Hu Yu, and others used *C.elegans* as model organisms when studying the neurotoxicity of methylmercury. the Bristol strain N2 and transgenic plants were used as experimental animals, such as BZ555 (*dat-1::GFP*), LX929 (*unc-17::GFP*), DA1240 (*eat-4::GFP*), EG1285 (*unc-47::GFP*), GR1366 (*tph-1::GFP*). In addition to the Bristol strain N2, fluorescent proteins in the nerves of other mutants can emit fluorescence under excitation light. The L1 and L4 phase nematodes were exposed to 24 degrees constant temperature for 30 minutes, then cultured for 24,48,72 hours, then the mutant was observed by fluorescence microscope, and the neuron morphology was evaluated to observe the percentage of nematodes in the total number of nematodes injured

by neurons to quantify the damage level of nematodes. Behavioral experiments were performed to record the number of body bends within 30 seconds, the number of forward, backward, and ω movements of the inner nematode in 3 minutes, the number of head swings in one minute, and the number of backward reactions of the nematode were observed by touching the head and tail of the nematode with fine hair five times each. We conclude that methylmercury can cause structural damage in bz555, eg1285 nematode neurons, and dopamine neurons are more sensitive to methylmercury damage than γ -aminobutyric acid neurons. The toxicity of stage L1 nematodes to methylmercury was more sensitive than that of stage L4 nematodes.

Jin Siyi, Zhang Fan, and others used *C.elegans* as model organisms to study the toxic effects of potassium sorbate, methyl Nipagin, and sodium benzoate⁴. The concentration of sodium benzoate in one g/L plate, potassium sorbate in 1 g/L plate, and nipagin methyl ester in 0.25 g/L plate were measured in a cm medium with *Escherichia coli*. After 24 hours, 48 hours, and 72 hours of treatment, the number of body bends in 20 seconds, the number of head swings in 20 seconds, the number of times touching the head of the nematode, and the number of times each of the tail of the nematode retreats were detected. The results showed that the conventional dosage of sodium benzoate, potassium sorbate, and methyl nipagin had some neurotoxicity to nematodes.

Pu Zhijun, A study on the inhibitory effect and mechanism of $A\beta_{(1-42)}$ neurotoxicity by Dauricine⁵, the paralysis rate of $Ap(1-42)$ transgenic nematodes and wild-type nematodes treated with different concentrations of bat puerarin was observed. The effects of bat ge based on $Ap(1-42)$ protein expression in nematodes were analyzed using western blot techniques. qRT-PCR method was used to detect the gene level changes of the protein homeostasis network in nematodes. The results showed that the $A\beta_{(1-42)}$ secretion of APPsw cells could be significantly reduced. Moreover, we also found that Batskyine can delay the paralysis of the CL2120 of $A\beta_{(1-42)}$ transgenic nematodes and reduce the levels of $A\beta$ oligomeric proteins in them. It suggests that bat garnet has pharmacological value in reducing $A\beta_{(1-42)}$ aggregation and delaying ad progression, and bat garnet reduces $A\beta_{(1-42)}$ aggregation and toxicity, presumably associated with activating UPR ire/xbp-1 pathways and enhancing endoplasmic reticulum-related protein degradation.

Wu Tianshu and Ho Keyu in the study of cdte quantum dots on neurotoxicity and regulatory mechanisms of *C. elegans*⁶, using *C. elegans* as experimental animals to pre-expose them to different concentrations of cdte medium for 24 and 72 hours. After observing the situation of

nematode intake sub-points by fluorescence microscope, the body bending, head swing, frequency of pharyngeal pump movement, defecation cycle time, chemical tendency learning coefficient, and percentage of isothermal tracking behavior motion nematodes in the range of 20 degrees were detected. Fluorescent quantitative PCR was also used to determine the effects of quantum dots on the expression of 15 genes related to three neurotransmitters: penthydroxytryptamine, glutamate, and dopamine. It is concluded that quantum dots are mainly in the head and intestine, and the defecation time of the experimental group is prolonged; the exercise, chemical, and temperature tendency learning ability are all decreased. The gene expression of the transporter and molecular target of three neurotransmitters decreased significantly in the 24-hour group, but the results were reversed in 72 hours. It is concluded that CdTe quantum dots have certain.

It is not difficult to see that *C. elegans* are suitable for various neurotoxicological experiments. In the study of Neurotoxicology, it can be used not only for kinematic detection but also for the detection of neuromorphology and gene and protein levels, as well as for chemical and temperature tendency experiments and learning ability.

3. The Advantage of *C. elegans*

According to the research done by *C.elegans*, its advantages can be summarized from the following aspects and compared with the commonly used neurotoxicological model organisms.

3.1 Fast growth rate, short experimental study period

First of all, *C. elegans* grow quickly and easily. Many experimental materials can be obtained quickly, and it is easy to study the influence on the whole life course of the organism. It takes only 3-4 days to develop from fertilization to spawning at 20 degrees, with a short life cycle of about three weeks. The average fecundity is 300-350, But mating with males produces more than 1400 offspring. Other experimental animals commonly used in neurotoxicological studies do not have more than ten children per child, and the average life span of rats is 3 to 4 years. To 60 days of age before self-mating. The average life span of rabbits was 5-12 years. Females 7-8 days old, males 8-9 days old to mate independently. The average life span of a crab-eating monkey is 20 years, and it is only five years old before sexual maturity. It takes a long time to observe the long-term effects of toxicity. For example, Li Qing, Sun Lin, and others were studying the effects of long-term low-dose ketamine on the behavior and nerve cells of adolescent crab-eating monkeys⁷. The

36 crab-eating monkeys were randomly divided into 1,3, and 6 months groups according to the duration of administration and were tested on the 30th,90,180th day of administration. Nematodes could have a group in 3 days, greatly reducing the study cycle. At the same time of administration, nematodes are equivalent to a longer life course, helping to study the effects of long-term toxicity. For example,72 hours of exposure to the same agent, equivalent to 15 percent of the entire life course of nematodes, corresponds to 0.164% of rabbits with a life span of five years. Therefore, in studying the long-term effects of neurotoxicity, using *C. elegans* can greatly shorten the study time.

3.2 Easy to cultivate and preserve

The adult length of *C. elegans* was about 1 mm; *E. coli* was used as food and could be cultured on NGM medium⁸. *E. coli* is easy to culture, and gum medium is easy to configure. The culture of nematodes requires only one medium or small medium. In the process of poisoning, there is no need to add drugs every day; just add drugs in the configuration medium. *C. elegans* are also easy to preserve; they can be stored in minus 80°C degrees 30% glycerol or liquid nitrogen for a long time; 15 °C seals can also be stored for several months. Facilitate the storage of experimental results. Rats, rabbits, and other experimental animals need to use a certain size of the cage, daily special feeding, and cleaning feces. It can not preserve the living body for a long time; it can only preserve its tissue sections for a long time.

3.3 Genetic stability, high degree of conservation

The main sex of *C. elegans* is hermaphrodite; it can be self-crossed and purebred in a natural state, and some males can perform genetic tests. As a rule, the hermaphroditic nematodes can be self-crossed in a medium to ensure genetic stability. While mammals have no hermaphrodite and can only mate with other individuals, genetic stability is difficult to guarantee. At the same time,40% of the genes of *C. elegans* are the same as those of humans and are highly conserved with human genes. Therefore, the research results can be applied to higher animal studies. Many neurobiological processes are highly conserved with nematodes. The effect of drugs on the nerve of nematodes can be observed by judging the behavior of nematodes and then judging the effect on human nerves. The effects of *c.elegans* on the tactile and motor functions of human dopamine *c.elegans* serotonin neurons can be assessed by measuring *c.elegans* pharyngeal frequency and touch response and by observing *c.elegans c.elegans* body sinusoidal activity

(amplitude and frequency of body bending) and foraging behavior to assess γ -aminobutyric acid and excited choline-like activity.

3.4 Easy to observe

The structure of nematodes is simple; the whole body is transparent and easy to observe. Its cell lineage is clear; the number of whole-body cells is nearly 1000. *c.elegans* consists of 302 neurons forming 118 neuronal subtypes,¹⁰ of each neuron assigned a number by location. This relatively “simple” nervous system comprises 6393 chemical synapses, 890 potential points, and 1410 muscle nerve contacts¹¹. Nematodes can be used as models for multidisciplinary studies. Because of the body’s transparency, the number of nerves is small and simple, easy to observe. The neural lineage and the complete circuit diagram of the nervous system have been determined and fully described, which makes it a controllable in vivo experimental model for studying the mechanism of nerve injury using a single nerve cell¹².

The animals used in the general neurotoxicological study are rats, mice, guinea pigs, rabbits, hamsters, dogs, ferrets, and monkeys. The nerve structure of these animals is complex and inconvenient to observe, and when observing the nerve, it needs to cut off the skin, which has damage to the subjects. The nematode is transparent; the observation only needs to be in vivo vision, using the neurons expressed fluorescent protein transgenic lines so that their nerves can be visible under a fluorescence microscope. Mammals need to slice their nerves before being observed in a complicated process. When judging the area of concentration of the substance ingested by nematodes, if the substance can emit fluorescence, for example, CdTe, nematode nematodes can be directly observed by stereoscope or fluorescence microscope because of their transparent body, and fluorescence can occur where there is CdTe deposition. Mammals can not do this because their bodies are opaque, making it more difficult to determine the distribution of drugs.

3.5 Simplicity of operator

The experiment operation of *C.elegans* is very simple. The method of poisoning is to add the material to the medium. Because *C. elegans* are transparent, they do not need to be dissected. It can be observed only under the stereoscope or fluorescence anatomic microscope. However, the mammals headed by crab-eating monkeys and rats have complex operations and strong activity abilities. When performing anesthesia, it will often resist, resulting in increased experimental difficulty and accidental injury, such as mice, crab monkey bite, resulting in infection, and so on. The nematode operation

requires only one platinum wire and one eyebrow to complete most of the physical stimuli, much simpler than in mammals, such as the determination of the rat tail thermal pain threshold (TFL) using halogen lamps as a radiation source and glass panels as support. To determine the tail-switching reaction of mice. Von Frey filaments determined claw mechanical pain threshold measurement (PWT) mechanical pain sensitivity. Rats were placed on a metal mesh, covered with a transparent plexiglass cover, and stimulated with a series of standardized von Frey fibers in a certain order in the middle of the plantar of the hindlimb of rats. Foot contraction reaction was observed¹³. Most of the behavioral indicators of nematodes do not require stimulation, and the learning ability of mechanical stimulation is only to touch the head of nematodes with eyebrows.

In addition to the existing research, the following aspects can be studied.

When the individual ingests the drug, it reaches the body with the body fluid circulation. The drug may also arrive if the individual has a developing fertilized egg or embryo. Studies have shown that in addition to affecting individuals, drugs can also affect offspring of ontogeny¹⁴. In addition to studying the effects of short-term and long-term toxicity on individuals, *C. elegans* can also be used to study the effects of neurotoxicity on offspring. Using mammals to study the effects of a drug on the neurodevelopment of offspring and cognitive ability can significantly slow the study's progress over long periods of pregnancy and the growth of the young. The use of *C. elegans* only needs to expose parents to drug poisoning to the L4 stage of the transfer of parents to the drug-free medium; after the parents lay eggs, the parents will be picked away, leaving only eggs. Detecting L1 and L3 kinematic indicators, learning ability, and neural morphology after egg hatching. Can analyze the effects of drugs on offspring legacy.

References

- [1] Pu zhijun. Study the inhibitory effect and mechanism of bat-
going on A (1-42) neurotoxicity. Guilin Medical College, no. 01,
pp: 98,2018
- [2] Golden master;Made;Cao yan. Toxicity of three common
preservatives on *Caenorhabditis elegans*. Chinese Journal
of Food Safety and Quality Testing, Vol. 9, no. 04, pp. 905-
909,2018
- [3] Chamomile; Xu jianxin. Toxic effects of formaldehyde on
Caenorhabditis elegans. Journal of Chuxiong Normal University,
Vol. 27, no. 06, pp. 41-44,2012
- [4] Yang Huimin; Han Yan; Shao-Juan Song; Mei-ling Yang; Ma
Enbo; Guo y p. toxicity of copper on *Caenorhabditis elegans*.
Sichuan Animals, Vol. 31, no. 02, pp. 236-239,2012. (in Chinese
with English abstract
- [5] Zhao Qing; Jiang tian tian. A review of studies on
Caenorhabditis elegans. *Science of agriculture in Anhui*, Vol. 38,
no. 19, pp. 10092-10093 + 10095,2010
- [6] Wu tianshu; He Keyu; Should beauty; Leon sang joon;
Zhang Shihan; May; Xue Yuying; Tang meng. Neurotoxicity of
CdTe quantum dots on *Caenorhabditis elegans* and its regulatory
mechanism. 7th National Toxicology Conference and 8th Hubei
science and Technology Forum, pp. 2, October 2015
- [7] Li qing;Have;Wang li.Jiang Hong;Tak cheung lau.Li3 qi2;
Pan f. abnormal behavior and apoptosis of hippocampal nerve
cells in adolescent cynomolgus monkeys induced by long-
term low-dose ketamine. 17th national academic conference on
Psychology, pp. 2, October 2014
- [8] Zhao Guangxi, Zhao Baisong, Gao Linlin, Ding Pudong.
Changes of neural behavior and spinal nerve structure in rats
after intrathecal injection of lidocaine - bupivacaine mixture in
different proportions. *Journal of Practical Clinical Medicine*, Vol.
18, no. 16, pp. 1-6 + 10,2014
- [9] Wang Yunbiao; *Journal of Toxicology*, Vol. 27, no. 05, pp.
382-386,2013
- [10] Wang Yang;Wang da-yong. Defects in the transmissibility
of *Caenorhabditis elegans* from generation to generation due to
copper poisoning. *Journal of Safety and Environment*, no. 02,
pp. 10-14,2017
- [11] Panlinhai; Du Aifang; Li Xiaojun; Yu-hui hou; Gao Xiang.
Study on culture characteristics and preservation methods of *C.*
elegans. *Acta agriculturae zhejiang*, no. 01, pp. 34-36,2017