



Mini Review

Motor neuron diseases and neurotoxic substances: A possible link?

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ABSTRACT

The motor neuron diseases (MNDs) are a group of related neurodegenerative diseases that cause the relative selective progressive death of motor neurons. Exploring the molecular mechanisms underlying MND phenotypes has been hampered by their multifactorial nature and high incidence of sporadic cases, although genetic factors are considered to play a considerable role at present. However, environmental factors, especial exposure to neurotoxic substances, could induce neurotoxicity with the same phenotypes of specific MNDs. Organophosphate-induced delayed neuropathy (OPIDN) is a neurodegenerative disorder characterized by ataxia and progression to paralysis, with a concomitant distal axonal degeneration and secondary demyelination of central and peripheral axons. The inhibition and subsequent aging of neuropathy target esterase (NTE) by organophosphate has been proposed to be the initiating event in OPIDN. NTE is characterized to be a lysophospholipase/phospholipase B mostly in the nervous system to regulate phospholipid homeostasis. Brain-specific deletion of mouse NTE contributes to the behavioral defects characterized by neuronal loss. Recently, mutations in human NTE have also been shown to cause a hereditary spastic paraplegia called NTE-related motor neuron disorder with the same characteristics of OPIDN, which supported the role of NTE abnormalities in OPIDN, and raised the possibility that NTE pathway disturbances contribute to other MNDs. Together with the identified association of paraoxonase polymorphisms with amyotrophic lateral sclerosis, there is a possibility that neurotoxic substances contribute to MND in genetically vulnerable people by gene–environment interactions.

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1. Introduction

The motor neuron diseases (MNDs) are a group of progressive neurological disorders that destroy motor neurons. For most of the MND patients, the cause of the disease is unknown. It is an important step to find out why these people develop the disease, which

causes muscles to weaken, atrophy, and dysfunction in order to develop therapy to treat or prevent MNDs. Genetic analysis methods, including a genome-wide scan for linkage and subsequent fine genetic mapping of MND families, are often used to identify specific MND-related gene locus, which may later be the target of genetic screening and gene therapy. In the meantime, environmental factors, especial exposure to neurotoxic substances, usually induce neurotoxicity and lead to some MNDs, such as amyotrophic lateral sclerosis (ALS), the most common adult-onset motor neuron disease characterized by weakness, muscle atrophy, and spasticity [1].

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Explaining the molecular mechanisms underlying MNDs phenotypes has been hampered by their multifactorial nature and high incidence of sporadic cases, although spinal muscular atrophy, an MND characterized by neuron loss and skeletal muscle atrophy, is caused by mutations in a single gene, the survival of motor neuron 1 (SMN1) gene [2]. ALS is the most common MND in humans and a good example of disorders that exemplify these challenges [3]. Most incidences (90%) of ALS are sporadic, that is without an obvious genetic component. Approximately 10% are inherited in a dominant manner (and referred to as familial ALS) [3]. Several genetic alterations may be involved in motor neuron injury in familial amyotrophic lateral sclerosis, in which approximately 20% are caused by dominantly inherited mutations in the Cu-, Zn-superoxide dismutase 1 (SOD1) gene [4,5]. To date, more than 100 mutations scattered throughout the SOD1 protein have been identified and it has been established that SOD1 mutants acquire toxic properties [6]. Although familial and epidemiologic data indicate that genetic factors contribute to ALS's pathogenesis [7,8], the etiology of sporadic ALS is largely unknown. Moreover, no single gene has been definitively shown to cause sporadic ALS, though a number of genes have been implicated [9].

2. Epidemiologic and toxicologic study of OPIDN

Exposure to neurotoxic organophosphorus compounds (OPs) of sufficient magnitude and duration causes neurodegeneration in humans, livestock, and laboratory animals. The most common OP-induced toxicity is an acute OP toxicity due to inhibition of acetylcholinesterase (AChE). The clinical signs of acute OP toxicity are apparent usually within minutes to hours of exposure and include lacrimation, salivation, muscular weakness, convulsions, respiratory depression, and death. A rare and delayed neurotoxicity induced by single or multiple doses of some OPs is so-called OP-induced delayed neuropathy (OPIDN), which is a neurodegenerative disorder characterized by a delayed onset of prolonged ataxia and upper motor neuron spasticity [10]. OPIDN often begins with sensory impairment, ataxia, weakness, muscle fasciculation, and hyporeflexia and may progress to complete flaccid paralysis followed by progressive spastic paraplegia [10].

The first and largest of OPIDN epidemics occurred in the southern United States during the prohibition era, and consumption of Jamaica ginger extract ("Ginger Jake") adulterated with tri-*ortho*-cresyl phosphate (TOCP) led to OPIDN in an estimated 50,000 Americans in 1930 [11]. Additional outbreaks of OPIDN causing paralysis of tens of thousands of individuals have occurred in Morocco, Fiji, and India due to the consumption of cooking oil contaminated with lubricating oil (containing TOCP) [12]. By the end of 20th century, there were many cases of OPIDN due to TOCP poisoning in other countries, such as Romania, Sri Lanka, Yugoslavia and China [13]. Besides TOCP, several other OPs, such as methamidophos, dichlorvos and leptophos, mainly used as insecticides have been reported to cause OPIDN in human [13]. These OPs are associated with OPIDN, of which TOCP is the most familiar organophosphorus ester because a single dose of TOCP may induce OPIDN in humans, hens, and other susceptible species.

The hen is the preferred model for the human OPIDN [14]. Significant electrophysiological deficits in peripheral nerves were observed as early as 24 h after dosing [15,16]. Leg weakness becomes apparent 8–10 days after dosing with TOCP and, by 14–16 days, the birds are severely paralyzed. Histopathological examination in the later stages reveals degeneration of distal regions of long axons in the spinal cord and peripheral nerves [14]. In contrast to hens, rodents are refractory to OPIDN. Although a few swollen axons in the brain stem of mice were detected 3 weeks after a single dose of TOCP, no clinical signs or axonal degeneration were observed [17].

However, dosing mice with TOCP daily for a longer time (9 months) induced axonal degeneration and hind-limb paralysis [18].

3. NTE: molecular characteristics and functions

Neuropathy target esterase (NTE, also known as neurotoxic esterase) was discovered over 30 years ago in chicken brain homogenates, which was selectively inhibited by OPs that can cause neuropathy [19]. However, not all covalent inhibitors of NTE are neuropathic, such as phenyl dipentyl phosphinate and related phosphinates. Moreover, certain organophosphinates protect the birds from a challenge with a neuropathic OP [20]. The key chemical feature of these organophosphinates is that they form a covalent adduct with NTE but, unlike neuropathic OPs, cannot undergo the aging reaction. In adult chicken, more than 70% inhibition of NTE activity (assayed in brain homogenate 24 h post-dose) and occurrence of the aging reaction will cause clinical neuropathy 1–2 weeks later [10]. Aging of NTE has classically been considered to involve the dealkylation of the OP adduct from phosphorylated NTE by nucleophilic attack and to generate a negatively charged group at its active site serine residue, which cause loss of a non-esterase function of NTE required by neurons and/or their axons [10]. However, the NTE esterase activity domain (NEST)–mipaflox conjugate ages by deprotonation rather than classical side-group loss [21].

Molecular cloning of the cDNA for NTE opened the door to understand the molecular characteristics and functions of NTE. NTE encode a polypeptide of 1327 amino acids [22]. NEST is a region that exists between the amino acids 727 and 1216 of NTE and it can react with both the ester substrates and covalent inhibitors in a manner very similar to NTE [23]. The modeled active site of NTE consists of a Ser966, Asp1086 catalytic dyad [24], although Asp960 is critical for catalysis [23]. Moreover, Asp1044 and Asp1004 have been demonstrated to act as an isopropyl group acceptor in the aging reaction of DFP-inhibited NEST [23]. NTE was anchored to the cytoplasmic side of endoplasmic reticulum (ER) by an amino terminal transmembrane segment in mammalian cells and neurons [25,26]. NTE displays potent lysophospholipase activity in mouse brain [27] and is responsible for converting phosphatidylcholine to glycerophosphocholine as a novel phospholipase B in mammalian cells [28]. In *Drosophila*, the swiss cheese protein (SWS) as the homolog of NTE regulates phosphatidylcholine homeostasis [29]. Therefore, NTE regulates phospholipid metabolism through deacylation.

Knockout of *NTE* gene revealed its important functions. In mice, constitutive deletion of the *NTE* gene resulted in death of the embryo by mid-gestation due to placental failure and impaired vasculogenesis [30,31]. Brain-specific inactivation of the *NTE* gene resulted in the development of neurodegeneration characterized by accumulation of intracellular aggregates and cytoplasmic vacuolation in the hippocampus and thalamus, as well as loss of neuronal cells, such as Purkinje cells and thalamic neurons [26]. Moreover, brain-specific *NTE* knockout mice also exhibit axonal degeneration and hind-limb ataxia between 6 and 12 months of age [32], which is similar to the clinical signs of OPIDN. In *Drosophila*, the SWS mutant shows age-dependent neurodegeneration detectable by the formation of spongiform lesions within the central nervous system, accompanied by neuronal apoptosis [33]. In addition, glial cells in mutant flies form multi-layered wrappings around neurons, which eventually form large whorls around degenerating neurons in adults [33]. Both the fly SWS and mouse NTE proteins can rescue the defects that arise in SWS mutant flies. Overexpression of catalytically active SWS caused formation of abnormal intracellular membranous structures and cell death [31]. Cell-specific expression revealed that not only neurons but also glia depend autonomously on SWS, and loss of SWS activity causes neuronal and glial death in adult flies [31]. These data indicated that NTE was involved in nervous system development.

4. NTE-related motor neuron disorder

Recently, Rainier et al. identified several NTE mutations in MND patients from a consanguineous family of Ashkenazi Jewish ancestry and a nonconsanguineous family of northern European ancestry by genome-wide linkage analysis [34]. All affected subjects developed childhood onset of insidiously progressive lower extremity spastic weakness and progressive wasting of distal upper and lower extremity muscles. The affected phenotype in each family conformed to both OPIDN and “Troyer syndrome,” the latter was an autosomal-recessive form of hereditary spastic paraplegia (HSP) associated with distal muscle wasting.

NTE coding sequence analysis showed that there was a homozygous mutation of NTE (substitution of guanine for adenine at NTE cDNA 3034) that disrupted an interspecies conserved residue (substitution of valine for methionine at amino acid position 1012) in NEST in the affected subjects in the consanguineous kindred. Affected subjects in the nonconsanguineous family were compound heterozygotes: one allele had a point mutation of substitution of guanine for adenine at NTE cDNA 2669, which disrupted an interspecies conserved residue in NEST, namely substitution of arginine for histidine at amino acid position 890, and the other allele had a four base pair CAGC insertion at NTE cDNA 2946, which led to a frameshift and protein truncation after residue 1019 missing the last 235 residues of NEST.

These NTE mutations are disease-specific and considered pathogenic based on the following reasons. First, NTE mutations were present in the affected subjects in two unrelated kindreds and absent in control subjects. Second, amino acid residues within NEST were affected. Third, NTE plays a central role in OPIDN, an upper and lower motor neuron disorder whose symptoms bear a striking resemblance to those exhibited by the affected subjects. Therefore, this disorder was referred to as NTE-related MND (NTE-MND) [34].

5. A possible link between MNDs and neurotoxic substances?

Although *SOD1* has been identified as a well-established causative gene for ALS, the identification of *SOD1* has not been followed up by the identification of other genes responsible for classic ALS [35]. This leads to the speculation that more complex genetic mechanisms are involved than initially assumed. While mutations in single genes are still likely to constitute a small proportion of ALS cases, the genes responsible for ALS in families with clusters of two or three affected individuals, and more particularly in sporadic cases, are far from being determined [9]. Therefore, multi-genic, somatic mutation, and gene–environment models may all contribute to the genetic etiology of ALS [35].

Although a number of genes have been implicated, no single gene has been definitively shown to cause sporadic ALS (SALS). Case reports have described ALS after exposure to OPs [36]. Several studies have suggested that risk of ALS is related to farming as an occupation, although not necessarily to living in rural areas [37]. Pesticide exposure has been considered in three case–control studies, which found some evidence for an association [38–40]. In addition, a cohort study found increased risk of ALS among workers exposed to the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) compared to other company employees [41]. Recently, the association of the polymorphisms of paraoxonases (PONs), the detoxifying enzymes involved in the metabolism of a variety of organophosphorus pesticides and chemical nerve agents, with sporadic ALS in several populations has been demonstrated [42–46]. Moreover, gene–environment interactions were identified at the allele level for PON1 promoter polymorphisms and pesticide exposure [46], which suggested that some PON1 promoter polymorphisms may

predispose to sporadic ALS, possibly by making motor neurons more susceptible to OPs. Therefore, environmental toxicity in a susceptible host may precipitate SALS.

In hens, aging of NTE has been to be a toxic gain of function which initiates events culminating in neuropathy [47]. Although classic OPIDN has not been observed in mice, brain-specific deletion 90% of mouse NTE activity leads to axonal degeneration and hind-limb ataxia [26]. However, NTE heterozygote mice (*NTE*^{+/-}) showed 40% decrease in esterase activity and did not develop neurodegeneration [30]. The relation between loss of NTE activity induced by gene knockout and neurodegeneration seems to be in accordance with the established threshold to the occurrence of OPIDN in adult chickens: over 70% inhibition of NTE by OPs [10]. Neuropathic syndrome in mice induced by genetic knockout of NTE and chronic TOCP dosing indicates that the OP is indeed acting by inactivating NTE [18,26,32].

The new study of NTE mutations in a previously unknown type of inherited MND raises several interesting and surprising possibilities about MNDs and neurotoxic substances [34]. OPIDN is characterized by distal degeneration of the long axons, which is also the primary neuropathological feature of HSP [48]. NTE-MND conformed to not only OPIDN but also “Troyer syndrome,” an autosomal-recessive form of HSP [34]. Disturbance of NEST by the identified NTE mutations in NTE-MND subjects may alter NTE activity *in vivo*. Association of NTE mutations with progressive MND indicates the importance of NTE in maintaining the integrity of corticospinal tract and peripheral motor axons. Disease-specific, nonconserved NTE mutations in unrelated MND patients also supports the role of NTE abnormalities in axonopathy produced by neuropathic OPs.

Observations that NTE mutations underlie corticospinal tract and peripheral motor axon degeneration raises the possibility that not only other NTE polymorphisms in the coding sequence and its promoter, but also other genetic variation in factors that regulate or interact with NTE could contribute to NTE-MND and other motor neuron disorders, such as ALS and primary lateral sclerosis (PLS). Moreover, these NTE mutations were sufficient to cause the disorder even in the absence of apparent exposure to neurotoxic OPs, which suggested that other polymorphisms in NTE and/or proteins with which it interacts influence the susceptibility to OP-induced neurologic disease. The findings support the possibility that toxic OPs contribute to MNDs in genetically vulnerable people, which is an exciting first step in uncovering a possible link between neurotoxic substances and MNDs.

Although the molecular mechanism leading from NTE inhibition to delayed neuropathy or neurotoxicity is not well understood, several new hypotheses based on the findings of NTE activity as a novel phospholipase B/lysophospholipase anchored to ER have been proposed including defective axonal transport leading to impaired nutrition of distal axons [32,49] and localized alterations in lysophosphatidylcholine metabolism or signal transduction pathways [27,50]. These putative molecular mechanisms for OPIDN may provide a new clue to reveal the mechanism of the related MNDs.

In summary, perturbation of the NTE pathway by chemical reaction (OP inhibition and aging) and genetic loss (gene knockout or gene mutations) of NTE activity lead to closely resembled neuropathy. Together with the association of paraoxonase polymorphisms with ALS, the role of abnormal NTE protein in inherited form of MND and in nerve damage caused by OP further supports a possibility that neurotoxic substances contribute to MNDs in genetically vulnerable people. However, the weight of evidence is insufficient for concluding that this is a causal relationship at present. In the future, to study the NTE mutations using animal models is necessary to elucidate the relationship of NTE and NTE-MND. In addition, to learn if mutations in the NTE gene happen in other types of MNDs, such

as ALS, will further support the role of NTE in MNDs. Furthermore, it will be important to determine whether other polymorphisms in NTE and other target proteins influence the susceptibility to neurotoxic substances-induced MNDs.

Conflict of interest

The authors declare that there are no conflicts of interest.

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