

Neurotoxicity Induced by Environmental Low-molecular-weight Substances

Yaichiro Kotake^{*,a,b}

^aGraduate School of Biomedical Sciences, Hiroshima University, 1–2–3 Kasumi, Minami-ku, Hiroshima 734–8553, Japan and ^bCenter for Quantum Life Sciences, Hiroshima University, 1–2–3 Kasumi, Minami-ku, Hiroshima 734–8553, Japan

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The neurotoxicity of Parkinson's disease (PD)-related tetrahydroisoquinolines (TIQs) and organotins is reviewed. PD is one of the most common neurodegenerative diseases among aged people and characterized by the selective death of nigrostriatal dopaminergic neurons, but its pathological mechanism remains unknown. 1-Benzyl-1,2,3,4-tetrahydroisoquinoline (1BnTIQ), an endogenous brain amine, was proposed as a possible PD-inducing neurotoxin, and its characteristics relevant to PD were evaluated. 1BnTIQ was detected in the mouse brain and human cerebrospinal fluid (CSF), and 1BnTIQ content was found to increase in the patients with PD. Repeated administration of 1BnTIQ induced PD-like symptoms in monkey and mice. 1BnTIQ induced dopaminergic cell death and subsequent dopamine decreases in organotypic slice co-culture, an *in vitro* culture system similar to the *in vivo* physiological environment. 1BnTIQ treatment also inhibited NADH-ubiquinone oxidoreductase in the mitochondrial respiratory chain. Thus, 1BnTIQ might be a causative factor for PD, although the concentration required for neurotoxicity is higher than that found in the parkinsonian cerebrospinal fluid. We propose that tributyltin is another neurotoxin that acts through the glutamatergic pathway. Glutamate is one of the most abundant neurotransmitters in the central nervous system (CNS), but excessive release of glutamate causes prolonged stimulation of NMDA receptors, inducing calcium overload and neuronal death that is collectively referred to as excitotoxicity. Glutamate-mediated toxicity is selective for the CNS because only the CNS possesses this system. We clarified that tributyltin induces extracellular glutamate release and that tributyltin-induced neuronal death is mediated by glutamate excitotoxicity in cultured rat cortical neurons.

Key words — neurotoxicity, tetrahydroisoquinoline, tributyltin, Parkinson's disease, excitotoxicity

INTRODUCTION

It is important to evaluate neurotoxicity induced by substances we take in from the environment. Most toxic actions against neurons are irreversible and can lead to serious disorders because neurons are post-mitotic difficult to reproduce. Chemicals penetrate especially easily into the brain during the fetal and neonatal periods because the blood-brain barrier is not completely formed. Neurotoxins induce various disorders, occasionally may be related to neurological diseases such as Parkinson's disease (PD). In this review, the neurotoxicity of PD-

related tetrahydroisoquinolines (TIQs) and organotins is discussed.

PD-RELATED TIQ DERIVATIVES

PD is one of the most common neurodegenerative diseases among aged people and characterized by the selective death of nigrostriatal dopaminergic neurons.^{1,2)} Its pathological mechanism still remains unknown but widely investigated. Recently, striking progress has been made using genetic analyses of familial PD and functional analyses of proteins coded from causative genes. However, the population of familial PD patients is at most a few percent; more than 90% of PD is sporadic PD whose pathogenesis is believed to rely on environmental factors rather than genetic factors. Low-molecular-weight neurotoxins such as

*To whom correspondence should be addressed: Graduate School of Biomedical Sciences, Hiroshima University, 1–2–3 Kasumi, Minami-ku, Hiroshima 734–8553, Japan. Tel.: +81-82-257-5326; Fax: +81-82-257-5329; E-mail: yaichiro@hiroshima-u.ac.jp

metals or agrochemicals have long been postulated to be PD-inducing substances.^{3,4)} More than twenty years ago, it was found that the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) induces PD-like biochemical, behavioral, and anatomical features.⁵⁾ MPTP is metabolized to 1-methyl-4-phenylpyridinium ion (MPP⁺), which inhibits mitochondrial NADH-ubiquinone oxidoreductase (complex I) and exerts neurotoxicity. However, MPTP is a by-product of a synthetic heroin analog and does not exist in ordinary people. Therefore, neurotoxins that structurally or functionally resemble to MPTP have been examined as possible PD-inducing compounds.^{6,7)} They include endogenous TIQs, β -carbolines, and analogous amines.

We proposed 1-benzyl-1,2,3,4-tetrahydroisoquinoline (1BnTIQ) derived from β -phenethylamine as an MPTP analog. We tried to detect 1BnTIQ in the cerebrospinal fluid (CSF) using gas chromatography-mass spectrometry and compare the 1BnTIQ content in PD patients with that in neurological controls. 1BnTIQ was detected as a novel endogenous amine in the mouse brain and Parkinsonian CSF.⁸⁾ The mean level of 1BnTIQ was 3-fold higher in the CSF of Parkinsonian patients when compared to controls with other neurological diseases. Repeated administration of 1BnTIQ induced PD-like behavioral abnormalities in mice and monkey.^{8,9)} We also examined 1BnTIQ toxicity in organotypic slice co-cultures of the ventromedial midbrain and striatum in order to elucidate the neurotoxic mechanism *in vitro* culture system that more closely reflects the *in vivo* physiological environment.¹⁰⁾ 1BnTIQ treatment irreversibly decreased dopamine content at the early stage, leading to dopaminergic death. Dopamine decrease was also induced by low concentrations of 1BnTIQ. 1BnTIQ inhibited mitochondrial complex I in the same manner as MPP⁺.¹¹⁾ We also reported correlations between the neurotoxicity of TIQ derivatives, including 1BnTIQ, and their complex I inhibitory activity in SH-SY5Y neuroblastoma cells.¹²⁾ 1BnTIQ at lower concentrations was reported to increase alpha-synuclein expression.¹³⁾

In addition to MPTP and its analogues, rotenone has been noted as another PD-related environmental factor. Rotenone is a potent complex I inhibitor. Betarbet *et al.* reported that chronic and systemic rotenone exposure in rats causes selective nigrostriatal dopaminergic degeneration.¹⁴⁾ Rotenone-treated rats showed behavioral abnormalities such as hypokinesia and rigidity, and nigral

neurons in rotenone-treated rats accumulated fibrillar cytoplasmic inclusions that contain ubiquitin and alpha-synuclein. These features are correspondent with those of PD.

PD-RELATED DISEASES IN THE FRENCH WEST INDIES

It remains difficult to prove a link between these chemicals and human PD, however. We therefore searched for approachable diseases in which it is easier to analyze the causative factors and found an endemic tropical disease. In Guadeloupe, the French West Indies, there is a high incidence of atypical parkinsonism or progressive supranuclear palsy,¹⁵⁾ which are both PD-related diseases for which potent genetic factors have not been found. All patients investigated by Caparros-Lefebvre *et al.* had consumed herbal tea or tropical fruits of the *Annonaceae* family.¹⁶⁾ We used liquid chromatography-tandem mass spectrometry with multiple reaction monitoring to detect low-molecular-weight neurotoxic benzyloquinoline derivatives in the *Annonaceae* family.¹⁷⁾ We detected reticuline and *N*-methylcoculaurine in every *Annona muricata* sample examined. These compounds were toxic to SH-SY5Y neuroblastoma cells and inhibited mitochondrial respiratory complex I (Kotake *et al.*, unpublished data). It is possible that uptake of the benzyloquinoline derivatives reticuline and *N*-methylcoculaurine and their accumulation in the brain may be related to the pathogenesis of the local endemic disease. It has also been reported that neurotoxic acetogenins are present in these fruits.¹⁸⁾

ORGANOTIN COMPOUNDS AND GLUTAMATE EXCITOTOXICITY

We have also noted the neurotoxicity of organotin compounds, which have been used as agricultural fungicides, wood preservatives, and disinfecting agents as well as antifouling paints for marine vessels although the antifouling application has been recently restricted. Organotin compounds have broad toxicity in a wide variety of species including mammals. Endocrine disruption induced by organotins is thought to lead to abnormalities in sexual development and reproduction collectively referred to as imposex.¹⁸⁻²¹⁾ Among organotin com-

pounds, tributyltin chloride (TBT) has been most widely used, even though its residues represent an environmental and health hazard. TBT and its degradation products dibutyltin and monobutyltin remain in marine sediments for many years,²²⁾ and food and water can be contaminated via industrial effluents and leaching from polyvinyl chloride water pipes.²³⁾ Indeed, Tsuda *et al.* reported that the daily intake of TBT in Japan was 2.2 to 6.9 μg ,²⁴⁾ and Whalen *et al.* reported the presence of butyltin compounds, including TBT, at concentrations between 50 nM and 400 nM in human blood.²⁵⁾

In addition to reproductive toxicity and hepatotoxicity, TBT also has neurotoxicity.^{26,27)} It was reported that TBT caused significant changes in rat behavior.²⁸⁾ Exposure of mammals to organotin compounds can induce epilepsy, amnesia, and memory defects,²⁹⁾ and it is toxic to the developing central nervous system (CNS).³⁰⁾ The mechanism of TBT neurotoxicity remains unknown, but TBT is reported to cause an increase in intracellular calcium in various cells such as catecholaminergic PC12 cells.³¹⁾ In the CNS, glutamate is one of the most abundant neurotransmitters and is widely distributed throughout the whole brain. Glutamate is associated with various brain functions such as synaptic plasticity, learning, and long-term potentiation.³²⁾ Its physiological and pathological functions in the CNS are mainly carried out by NMDA and non-NMDA receptors. NMDA receptor activation allows influx of extracellular Ca^{2+} and neuronal signaling, but excessive glutamate release causes prolonged stimulation, inducing calcium overload and neuronal death collectively referred to as excitotoxicity. Glutamate-mediated toxicity is selective for the CNS because the glutamatergic system is only found in the CNS. Glutamate excitotoxicity is speculated to be involved in many neurodegenerative diseases. Moreover, it has been reported to play an important role in the neurotoxicity of environmental pollutants such as methyl mercury.³³⁾ We examined whether TBT induces extracellular glutamate release and whether TBT-induced neuronal death is mediated by glutamate excitotoxicity in cultured rat cortical neurons.³⁴⁾ We found that TBT (500 nM) induced cell death in a time-dependent manner, and cortical neurons were sensitive to TBT insult. Measurement of extracellular glutamate concentrations showed that glutamate release was induced by TBT treatment. Two types of the glutamate receptor antagonists MK-801 and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) res-

cued TBT-induced neuronal death. These results suggest that glutamate release and its subsequent activation of glutamate receptors are involved in TBT-elicited excitotoxicity. TBT treatment did not affect nuclear morphology during cell death suggesting that the tributyltin-mediated glutamate excitotoxicity did not occur by apoptosis. We have reported that TBT induces cell death through calpain activation in PC12 cells, and that intracellular Ca^{2+} increase and caspase-3 activation are required for calpain activation by TBT.³⁵⁾ We have also reported concentration dependence for TBT-induced cell death in PC12 cells.³⁶⁾

CONCLUSION

Though there are many reports about the neurotoxicity of various chemical substances, most of their mechanisms remain unknown. Further studies may lead to understanding the role of environmental chemicals in some kind of neurodegenerative diseases.

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