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Cytotoxicity of 17 tetrahydroisoquinoline derivatives in SH-SY5Y human neuroblastoma cells is related to mitochondrial NADH–ubiquinone oxidoreductase inhibition

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Abstract

Since the first report that 1-methyl-4-phenyl-l,2,3,6-tetrahydropyridine induces parkinsonism, various kinds of low-molecular-weight neurotoxins, such as tetrahydroisoquinoline derivatives, have been identified as possible Parkinson's disease-inducing substances. In the present study, we measured four parameters of 17 tetrahydroisoquinoline derivatives, i.e., cytotoxicity in SH-SY5Y human neuroblastoma cells, inhibitory activity towards mitochondrial NADH–ubiquinone oxidoreductase (complex I), affinity for dopamine transporter, and 1-butanol–H_2O partition coefficient (as an index of lipophilicity). Six of the derivatives showed comparatively strong inhibitory activity towards complex I (IC_{50} values < 100 μM) and five of them were cytotoxic to SH-SY5Y cells (TC_{50} values < 200 μM). Some of these compounds are endogenous. We found good correlations between cytotoxicity and complex I inhibitory activity, but not between cytotoxicity and affinity for dopamine transporter. Since cytotoxicity to SH-SY5Y neuroblastoma cells was related to inhibitory activity towards mitochondrial complex I, complex I inhibition is likely to be involved, at least in part, in the mechanism of TIQ derivative-induced cell death. Uptake of most of these compounds seems to be dependent on lipophilicity, rather than active transport *via* dopamine transporter.

Keywords: Tetrahydroisoquinoline; Neurotoxicity; NADH–ubiquinone oxidoreductase; Dopamine transporter; Lipophilicity

1. Introduction

Since the findings that 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) induces Parkinson's disease (PD)-like behavioral, biochemical, and anatomical abnormalities (Langston et al., 1983), various kinds of low-molecular-weight neurotoxins that structurally resemble MPTP have been identified as possible PD-inducing substances (Nagatsu, 1997). MPTP-injected experimental animals are widely used as models for PD, even though MPTP is an exogenous compound (Tipton and Singer, 1993). Various MPTP-like endogenous neurotoxins, such as 1,2,3,4-tetrahydroisoquinolines (TIQ) and β-carboline, derivatives also have parkinsonism-inducing effects, and TIQ-injected monkeys have also been used as a model for PD and for studies on chronic toxicity (Yoshida et al., 1990). TIQ derivatives have a range of pharmacological effects, in addition to their PD-related neurotoxicity.

Many researchers have been investigating the cause of nigral cell death in PD, and one hypothesis is mitochondrial respiratory failure with a defect in mitochondrial NADH–ubiquinone oxidoreductase (complex I, EC 1.6.5.3) (Mizuno et al., 1998). 1-Methyl-4-phenylpyridinium (MPP^+), an active metabolite of MPTP, has many potential biological effects, including release of dopamine from vesicles, and mitochondrial complex I is believed to be one of its main sites of action (Tipton and Singer, 1993). Complex I is the entry point for electrons into the electron transport chain, and is located in the inner membrane of mitochondria, where it catalyzes electron transfer from NADH to ubiquinone. NADH is generated from the TCA cycle in the mitochondrial matrix (Lardy and Ferguson, 1969). Complex I activities in multiple brain regions of PD patients are lower than those in controls (Parker et al.,

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1989; Schapira et al., 1990). Though some reports have suggested that a genetic defect in mitochondrial DNA causes complex I deficiency in familial PD patients, sporadic PD cannot be explained only in terms of genetic predisposition, and unknown environmental factors inhibiting complex I seem to be important (Kotake and Ohta, 2003). Complex I is an extremely large and complex enzyme system consisting of 41 different subunits in mammalians (Lardy and Ferguson, 1969). We cannot use gene knock-out models to study complex I, because the complex is essential in living cells.

In the present study, we measured the inhibitory activity of various TIQ derivatives towards complex I and examined the relationship of the inhibitory activity with cytotoxicity to human SH-SY5Y neuroblastoma cells, in order to investigate how chemical structure of TIQ derivative is suitable as PD-inducing substance. We also evaluated affinity for the dopamine transporter (DAT) and lipophilicity, and examined their relationships with cytotoxicity.

2. Materials and methods

2.1. Chemicals

Tetrahydroisoquinoline derivatives were synthesized according to the methods described previously (Gray et al., 1989; Kohno et al., 1986; Kotake et al., 1995; Kawai et al., 1998, 2000a; Yamato et al., 1966). Dulbecco's modified Eagle's medium (DMEM) powder was purchased from Nissui Pharmaceutical (Tokyo, Japan). Fetal bovine serum (FBS) and horse serum (HS) were purchased from JRH Biosciences (Lenexa, KS). Reserpine was purchased from Sigma (St. Louis, MO). Penicillin G and streptomycin were purchased from Meiji Seika (Tokyo, Japan). 2-(4-Iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2*H*-tetrazolium, monosodium salt (WST-1) and 1-methoxy-5-methylphenazinium methylsulfate (1-methoxy-PMS) were purchased from Dojindo (Kumamoto, Japan). Other chemicals and solvents were purchased from Wako Pure Chemicals (Osaka, Japan). All chemicals and solvents used were of the highest purity commercially available.

2.2. Cytotoxicity to SH-SY5Y human neuroblastoma cells

SH-SY5Y human neuroblastoma cells were purchased from American Type Culture Collection, and they were cultured to subconfluency in DMEM supplemented with 0.46% glucose, 0.24% $NaHCO_3$, 5% (v/v) heat-inactivated FBS, 5% (v/v) heat-inactivated HS, 32 mg/L penicillin G, and 80 mg/L streptomycin. Cells were kept at 37 °C in humidified 5% CO_2 and 95% air. The cells suspended in culture medium were seeded on 96-well microtiter plastic culture plates at a density of 2×10^4 cells/100 μL/well (ca. 6×10^4 cells/cm^2). Test compounds dissolved in the culture medium or dimethylsulfoxide were added for 48 h. Cell viability was assayed according to the WST-1 method of modified MTT assay. This colorimetric assay measures the metabolic activity of viable cells based on cleavage of the tetrazolium salt WST-1 to water-soluble formazan by mitochondrial dehydrogenase in living cells. The assay was performed by incubation with 0.43 mM WST-1 and 16.8 μM 1-methoxy-PMS (as an electron carrier) in the original conditioned media for 3 h. After thorough shaking, the formazan produced by metabolically active cells in each sample was measured at a wavelength of 415 nm with a Microplate Reader (Model 550, Bio-Rad Laboratories Inc., Hercules, CA). Absorbance readings were normalized against those of control wells with medium alone.

2.3. Measurement of mitochondrial complex I activity

The animals were handled in accordance with the guidelines for the care and use of experimental animals published by the Japanese Association for Laboratory Animal Science in 1987. Mitochondrial complex I activity was evaluated according to a modification of the method reported previously (Hatefi, 1978). Coenzyme Q_1 was selected as having the highest activity of complex I among ubiquinones having various isoprene lengths. Mitochondrial fraction was prepared from Slc:Wistar/ST rat brain because we were not able to obtain sufficient volume from SH-SY5Y cells, and it was sonicated 10 times for 10 s at intervals of 15 s. The assay sample contained 200 μg/mL protein of sonicated mitochondria, 10 mM sodium azide as a cytochrome *c* oxidase inhibitor, 2.5 mM Q_1, 0.6 mg/mL asolectin, and various concentrations of TIQ derivatives in 100 mM Tris–HCl (pH 7.4). The reaction was started by the addition of 1 mM NADH after preincubation for 5 min, and the decrease of absorbance at 340 nm derived from NADH was measured as complex I activity. A part of the mitochondrial fraction was used for protein determination by Lowry's method (Lowry et al., 1951).

2.4. Binding assay for dopamine uptake site

Synaptosomal fraction was prepared from Slc:Wistar/ST rat striatum, because we were not able to obtain sufficient volume from SH-SY5Y cells. Six-week-old male adult Slc:Wistar/ST rats were treated intraperitoneally with reserpine 5 mg/kg at 20 h and 2.5 mg/kg at 3 h before being killed, in order to deplete striatal dopamine (Amejdki-Chab et al., 1992). The animals were killed by decapitation and the striata were dissected out, and homogenized in 10 volumes of ice-cold 0.32 M sucrose with a Teflon-glass homogenizer. The nuclear fraction was removed by centrifugation at $900 \times g$ for 10 min, and the supernatant was again centrifuged at $11{,}500 \times g$ for 20 min. The pellets from the second centrifugation were resuspended in 0.32 M sucrose with a Teflon-glass homogenizer and this suspension was used for uptake assay as reserpinized rat striatal synaptosomes. A portion of this suspension was used for protein determination by a modification of the method of Lowry et al. (1951).

The following method was modified from that of Andersen (1987). The assay sample contained 80 μg protein of the striatal membrane fraction and various concentrations of test compound in 50 mM Tris–HCl buffer (pH 7.4) containing 120 mM NaCl and 4 mM $MgCl_2$. The binding reaction was initiated by addition