Preventative Effects of 1-Methyl-1,2,3,4-Tetrahydroisoquinoline Derivatives (*N*-Functional Group Loading) On MPTP-Induced Parkinsonism In Mice

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Abstract

1,2,3,4-Tetrahydroisoquinoline (TIQ) is endogenously present in human brain, and some of its derivatives are thought to contribute to the induction of Parkinson's disease (PD)-like symptoms in rodents and primates. In contrast, the endogenous TIQ derivative 1-methyl-TIQ (1-MeTIQ) is reported to be neuroprotective. In the present study, we compared the effects of artificially modified 1-MeTIQ derivatives (loading an *N*-propyl, *N*-propenyl, *N*-propargyl, or *N*-butynyl group) on 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced PD-like symptoms in mice. In a behavioral study, MPTP-induced bradykinesia was significantly decreased by all compounds. However, only 1-Me-*N*-propargyl-TIQ showed an inhibitory effect by blocking the MPTP-induced reduction in striatal dopamine content and the number of nigral tyrosine hydroxylase-positive cells. Western blot analysis showed that 1-Me-*N*-propargyl-TIQ and 1-Me-*N*-butynyl-TIQ potently prevented the MPTP-induced decrease in dopamine transporter expression, whereas 1-MeTIQ and 1-Me-*N*-propyl-TIQ did not. Induction of thiobarbituric acid reactive substances (TBARS) by MPTP in the substantia nigra was significantly suppressed by not only 1-Me-*N*-propargyl-TIQ and 1-Me-*N*-butynyl-TIQ, but also by 1-Me-*N*-propyl-TIQ; in contrast, 1-Me-*N*-propenyl-TIQ had no effect. These results suggest that although loading an *N*-propargyl group on 1-MeTIQ clearly enhanced neuroprotective effects, other *N*-functional groups showed distinct pharmacological properties characteristic of their functional groups. Thus, the number of bonds and length of the *N*-functional group may contribute to the observed differences in effect.

Introduction

Although more than 200 years have elapsed since the first report of Parkinson's disease (PD), its etiology remains unclear. Since exogenous 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) was found to cause PD-like behavioral disorder (Langston et al., 1983), many kinds of MPTP-like endogenous amines have been discovered. Among them, the 1,2,3,4-tetrahydroisoquinoline (TIQ) derivatives, such as (*R*)-1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline ((*R*)-salsolinol) and 1-benzyl-1,2,3,4-tetrahydroisoquinoline (1-BnTIQ), are presumed to be the compounds involved in the pathogenesis of PD (Niwa et al., 1987; Ohta et al., 1987; Kotake et al., 1995; Abe et al., 2005). TIQ derivatives have also been shown to exist in the brains of non-human animal species (Kohno et al., 1986; Kotake et al., 1995; Nakagawa et al., 1996), and TIQ and 1-BnTIQ have been reported to cause parkinsonism in rodents (Kotake et al., 1995; Tasaki et al., 1991). Salsolinol is also found in human and animal brains (Sjöquist B, and Magnuson, 1980; Sjöquist et al., 1982; Sjöquist and Ljungquist 1985), and *N*-methylated (*R*)-salsolinol was shown to induce locomotor disorders and significantly decreased dopamine content in the substantia nigra of rats (Naoi et al., 1996). These results indicate that TIQ derivatives may be substances associated with the development of PD.

On the other hand, it has been reported that behavioral disorders induced by TIQ and 1-BnTIQ were prevented by pretreatment of 1-methyl-1,2,3,4-tetrahydroisoquinoline (1-MeTIQ), one of the TIQ derivatives present in rodents (Kotake et al., 1995; Tasaki et al., 1991). We have also previously demonstrated that 1-

MeTIQ has stereoselective properties and prevents MPTP- and TIQ-induced parkinsonism in mice (Abe et al, 2001; Ishiwata et al., 2001).

Selegiline ((*R*)-*N*, α -dimethyl-*N*-2-propynyl-phenethylamine), an inhibitor of monoamine oxidase B (MAO-B), is similar in structure to TIQ derivatives, and has been used as a PD treatment (Gerlach et al., 1996). Selegiline decreases endogenous 1-BnTIQ content in the mouse brain (Kotake et al., 1998) and shows stereoselective and neuroprotective features in glutamate receptor-mediated toxicity of mesencephalic dopamine neurons (Mytilineou et al., 1997). Therefore, we expect that TIQ derivatives with structures similar to selegiline could be more effective as PD therapeutics. We have previously studied the pharmacological characteristics of several artificially synthesized TIQ derivatives. These results showed that the cytotoxicity of 1-alkyl-TIQs depended on the lipophilicity of the alkyl group (Kitabatake et al., 2009), and the neuroprotective effects of certain synthetic TIQ derivatives were enhanced by the loading of *N*-propargyl groups, which is a component of the selegiline chemical structure (Katagiri et al., 2010; Saito et al., 2013).

In the present study, we examined the neuroprotective effects of 1-MeTIQs loaded with a different *N*-functional groups (*N*-propyl, *N*-propenyl, *N*-propargyl, or *N*-butynyl) to estimate the role of bond number and carbon number in the functional groups. These functional groups possess three carbons with a single bond (*N*-propyl), one double bond (*N*-propenyl), one triple bond (*N*-propargyl), and four carbons with one triple bond (*N*-butynyl). The chemical structures of selegiline, 1-MeTIQ, and the four 1-MeTIQs loaded with *N*-functional groups are shown in Fig. 1.

Materials And Methods

Animals and drug administration

Seven-weeks-old male C57BL/6N strain mice were purchased from Charles River Japan Inc. All mice were handled in accordance with the guidelines for animal care and use provided by the National Institutes of Health and the code of ethics for laboratory animals of Ohi University (Fukushima, Japan). All possible means were used to eliminate pain for the experimental animals. The mice were kept in a temperature- and humidity-controlled environment where they could freely consume food and water. After an acclimation period, various doses of 1-MeTIQ (80 mg/kg), 1-Me-*N*-propyl-TIQ, 1-Me-*N*-propenyl-TIQ, 1-Me-*N*-propargyl-TIQ, and 1-Me-*N*-butynyl-TIQ (20, 40, and 80 mg/kg) were administered intraperitoneally (i.p.) twice a day, every 12 hours, for 4 consecutive days (days 1-4). Three days later, 30 mg/kg MPTP or saline was given intraperitoneally twice a day for 4 consecutive days (days 7-10). The hydrochloride salt of each compound was dissolved in physiological saline and used for administration. The volume used for administration was 10 mL/kg. Experiments were performed using 7-9 animals/group beginning on day 19. A diagram summarizing the drug administration is shown in Fig. 2.

Evaluation of bradykinesia

Bradykinesia is an indicator that is indicative of movement disorders. To assess bradykinesia, the pole test was performed on day 9 after completion of drug administration as described by Ogawa et al. (1985). Mice were placed on the top of a rough iron bar (10 mm in diameter and 55 cm in height) with their heads facing upward. We then measured the time it took for the animal's head to completely turn downward (defined as the turn time: T_{turn}) and the time it took for its limbs to completely touch the pole base (the locomotor activity time: T_{LA}). This measurement was performed five times consecutively, and the average value was used as the measurement value.

Measurement of monoamine content in the striatum

Nine days after the last dose of drug, animals were euthanized by decapitation under sodium pentobarbital (50 mg/kg, i.p.) anesthesia. The brain was quickly removed and the striatum was dissected from the both hemispheres. Monoamine content in the striatum was measured using the method of Kanthasamy et al. (1997).

The weight of the striatum was measured and homogenized by adding 1000 μL ice-cold 0.2 M perchloric acid solution (containing 0.05% disodium EDTA and 0.15% sodium metabisulfite) per 100 mg of tissue weight. The homogenate was centrifuged ($15,000 \times g$ for 15 min at 4°C) and the supernatant was filtered through a 0.45- μm pore size membrane filter. Striatal dopamine and its metabolites (3,4-dihydroxyphenylacetic acid, DOPAC; 3-methoxytyramine, 3-MT; and 3-methoxy-4-hydroxyphenylacetic acid, HVA) and serotonin (5-HT) and its metabolite, 5-hydroxyindoleacetic acid (5-HIAA) in the filtrate were quantitated.

Determination of analyte content in the filtrate samples was performed by reverse-phase high performance liquid chromatography (HPLC). The HPLC assay conditions were as follows: HTEC-500 system (Eicom, Kyoto, Japan); column, Eicompak SC-50DS (3.0-mm i.d. $\times$ 150 mm) with a precolumn. Mobile phase buffer content; 83% 0.1 M citric acid/0.1 M sodium acetate buffer (pH 3.5), 17% methanol, 0.023% sodium 1-octanesulfonate, including 5 mg/L disodium-EDTA; flow rate, 0.5 mL/min; electrode, Eicom WE-3G graphite electrode; reference electrode, Eicom RE-100 Ag-AgCl; applied voltage, 750 mV vs. Ag/AgCl.

Immunohistochemical detection of tyrosine hydroxylase (TH) positive cells

Perfusion fixation was performed by injecting 0.9% physiological saline into the cardiovascular vessels of mice (under sodium pentobarbital anesthesia; 50 mg/kg, i.p.), followed by perfusion with cold 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS, pH 7.4). The brain (including the brainstem) was quickly removed and embedded in OCT compound and rapidly frozen in liquid nitrogen. Twenty μm thin sections of the substantia nigra pars compacta (bregma -3.0 to -3.1 mm) were prepared according to the atlas of Franklin and Paxinos (1997). TH immunostaining was performed using a VECTASTAIN Elite ABC kit and a DAB Substrate kit for peroxidase (Vector Laboratories, CA, USA) according to the manufacturer's instruction manual. Sections were fixed with acetone and incubated with normal goat serum for 20 min at room temperature, then treated with anti-TH antibody (1:500, rabbit polyclonal

antibody raised against amino acids 1-196 of TH of human origin; sc-14007, Santa Cruz Biotechnology, CA, USA) and incubated overnight at 4°C.

Sections were washed with PBS and incubated with biotinylated secondary antibody (1:200) for 30 min at room temperature. After washing with PBS, an avidin-biotin complex was applied to the sections for 30 min. The sections were washed with PBS again and diaminobenzidine was applied until the appropriate staining intensity was obtained. The number of cells stained by the TH antibody was counted throughout the entire substantia nigra pars compacta area.

Western blot analysis of striatal dopamine transporter (DAT) levels

Dissection of the striatum was performed as described above. The striatum was weighed and homogenized in ice-cold 0.1 M PBS (containing 0.32 M sucrose, 0.2 mM PMSF, 2 mM EDTA, and protease inhibitor cocktail) at a ratio of 1000 µL/100 mg tissue weight. Sample preparation for western blot analysis was performed according to Kobayashi et al. (2012), with slight modifications. Tissue homogenates were centrifuged at $1,000 \times g$ for 5 min at 4°C, and the supernatant was transferred to a fresh tube. Ice-cold homogenization buffer was added to the resulting pellet, which was re-homogenized and centrifuged. Both supernatants were mixed and recentrifuged at $15,000 \times g$ for 20 min at 4°C. The resulting pellet was dissolved in ice-cold radio-immunoprecipitation assay lysis buffer (EzRIPA Lysis kit, ATTO, Tokyo, Japan) for sample determination. Samples were applied to 10% SDS polyacrylamide gel electrophoresis, and then transferred to polyvinylidene difluoride membranes and the blots were incubated with anti-DAT antibody (1:5000, MAB369, Merck Millipore, MA, USA). The membranes were subsequently incubated with peroxidase-conjugated secondary antibody. The densities of DAT protein (immunoreactive bands) were analyzed using image analysis software (ImageJ).

Thiobarbituric acid reactive substances (TBARS) levels in the substantia nigra

The animals were sacrificed by decapitation under sodium pentobarbital anesthesia, as above. The brains were removed and the substantia nigra was isolated. The substantia nigra was weighed and homogenized using ice-cold radio-immunoprecipitation assay lysis buffer (containing protease and phosphatase inhibitors, EzRIPA Lysis kit, ATTO). TBARS levels in the substantia nigra were assayed using a TBARS Assay Kit (Cayman Chemical, MI, USA) with malondialdehyde as the standard compound.

Chemicals

1-Me-TIQ, 1-Me-*N*-propyl-TIQ, 1-Me-*N*-propenyl-TIQ, 1-Me-*N*-propargyl-TIQ, and 1-Me-*N*-butynyl-TIQ were synthesized using the Pummerer reaction (Shinohara et al., 1997). MPTP hydrochloride was purchased from Sigma-Aldrich (St. Louis, MO, USA). Monoamines (Dopamine, DOPAC, 3-MT, HVA, 5-HT, and 5-HIAA) were purchased from Wako Pure Chemical Industries (Osaka, Japan).

Data analysis

Data were expressed as mean $\pm$ standard error (SE) for each group. Initially, determination of significant differences between the control (saline + saline) and MPTP-treated groups (saline + MPTP) was performed using the Student's or Aspin-Welch's t-test.

Where significant differences were identified between the control and MPTP-treated group, the MPTP-treated group was used as a control in a one-way analysis of variance (one-way ANOVA) with a subsequent Dunnett's multiple comparison test to identify significant differences. A value of $p < 0.05$ was considered statistically significant.

Results

General behavior

No change in general behavior was observed treated with 1-MeTIQ (80 mg/kg), 1-Me-*N*-propenyl-TIQ, 1-Me-*N*-propargyl-TIQ and 1-Me-*N*-butynyl-TIQ (20–80 mg/kg each). However, the high dose (80 mg/kg) of 1-Me-*N*-propyl-TIQ clearly induced tremors, head twitches, elevation of the tail and decreased locomotor activity (crouching) within 10 min after drug administration, and all animals died within 24 h ($n = 6$). Thus, this lethal, high dose of 1-Me-*N*-propyl-TIQ was eliminated from our study. No behavioral abnormalities were observed when mice were given the lower doses (20 and 40 mg/kg) of 1-Me-*N*-propyl-TIQ.

Effects of N-loaded 1-MeTIQs on MPTP-induced bradykinesia

The effect of 1-MeTIQ and 1-MeTIQ derivatives loaded with an *N*-functional group on inhibition of MPTP-induced bradykinesia was examined. The MPTP treated group (saline + MPTP) T_{turn} value (2.47 ± 0.11 s) was significantly prolonged relative to that of the control group (saline + saline, 1.33 ± 0.03 s, $p < 0.01$). 1-MeTIQ and its derivatives showed a significant effect on MPTP-induced bradykinesia (T_{turn} , $p < 0.01$). Similarly, the T_{LA} value in the saline + MPTP treated group (9.22 ± 0.37 s) was significantly extended compared to control (5.50 ± 0.13 s, $p < 0.01$). MPTP-induced bradykinesia (T_{LA}) was also significantly reduced ($p < 0.01$) by pretreatment with 1-MeTIQ and its derivatives. Dunnett's multiple comparison test identified individual differences relative to the MPTP treatment group. Pretreatment with 1-MeTIQ (80 mg/kg) significantly inhibited MPTP-induced bradykinesia. 1-Me-*N*-propenyl-TIQ, 1-Me-*N*-propargyl-TIQ, and 1-Me-*N*-butynyl-TIQ showed a dose-dependent and significant inhibitory effect on MPTP-induced bradykinesia. With 1-Me-*N*-butynyl-TIQ treatment, the inhibitory effect at the low dose was especially remarkable (Fig. 3). The low and medium doses of 1-Me-*N*-propyl-TIQ also produced a significant inhibitory effect on MPTP-induced bradykinesia (Fig. 3).

Effects of N-loaded 1-MeTIQs on striatal monoamine content

Striatal dopamine content in the saline + MPTP treatment group was significantly decreased compared to the saline + saline group at 9 days after completion of drug administration. Striatal dopamine content in the MPTP-treated group was decreased to 29.5% compared to the control group (MPTP: 3.7 ± 0.2 ng/mg

vs. control: 12.5 ± 0.4 ng/mg, $p < 0.01$). Although pretreatment with 1-MeTIQ showed a tendency to prevent the MPTP-induced decrease in dopamine content, a statistically significant difference was not observed (Table 1). Only 1-Me-*N*-propargyl-TIQ prevented the MPTP-induced decrease in dopamine content in a dose-dependent manner that was statistically significant. The other *N*-loaded 1-MeTIQs did not inhibit the effect of MPTP. In particular, the dopamine content in the high-dose 1-Me-*N*-propargyl-TIQ treatment group was nearly equal to that of the control group (11.5 ± 1.0 ng/mg tissue, Table 1). Preventative effects of 1-Me-*N*-propargyl-TIQ against the MPTP-induced decreases in other dopaminergic parameters (3,4-dihydroxyphenylacetic acid: DOPAC, 3-methoxytyramine: 3-MT, and 3-methoxy-4-hydroxyphenylacetic acid: HVA) were also observed, however, the other *N*-loaded 1-MeTIQs did not significantly alter these parameters (Table 1).

Striatal serotonin (5-HT) levels were not affected by repeated administration of MPTP. Pretreatment of 1-MeTIQ and *N*-loaded 1-MeTIQs also did not alter striatal 5-HT levels. In contrast, 1-Me-*N*-propargyl-TIQ tended to increase the levels of 5-hydroxyindoleacetic acid (5-HIAA) in the striatum at all doses, and 1-Me-*N*-propyl-TIQ significantly decreased 5-HIAA (Table 1).

Effects of *N*-loaded 1-MeTIQs on nigral TH-positive cells

At 9 days after completion of MPTP, the number of TH-positive cells in the substantia nigra was counted following immunohistochemical staining. The TH-positive cell count in the control group was 108.8 ± 8.3 cells/slice (10–13 slices/brain, $n = 5-8$, Table 2, Fig. 4A). MPTP significantly diminished TH-positive cells to 62.6 ± 7.0 cells/slice (57.5% of control, 11–14 slices/brain, $p < 0.01$, Table 2, Fig. 4B). Only 1-Me-*N*-propargyl-TIQ significantly attenuated the MPTP-induced reduction in TH-positive cells (102.2 ± 8.1 cells/slice, 10–15 slices/brain, $p < 0.01$, Table 2, Fig. 4E). None of the other *N*-loaded 1-MeTIQs prevented the MPTP-induced loss of TH-positive cells in the substantia nigra (Table 2, Fig. 4C, D, F).

Effects of *N*-loaded 1-MeTIQs on striatal DAT protein expression

Striatal DAT content was significantly decreased by MPTP to $37.7 \pm 3.8\%$ of control ($p < 0.01$, Fig. 5). Although 1-MeTIQ did not inhibit the decrease in DAT, the *N*-loaded 1-MeTIQ derivatives significantly antagonized the MPTP-induced decrease in striatal DAT, except for 1-Me-*N*-propyl-TIQ. In particular, 1-Me-*N*-butynyl-TIQ almost completely inhibited the MPTP-induced decrease in DAT at all doses evaluated (20–80 mg/kg, $p < 0.01$, Fig. 5).

Effects of *N*-loaded 1-MeTIQs on nigral TBARS content

MPTP treatment significantly increased nigral TBARS content (0.783 ± 0.059 nmol/mg) relative to the control group (0.479 ± 0.071 nmol/mg). *N*-loaded 1-MeTIQ derivatives showed a significant effect on MPTP-induced TBARS levels. Pretreatment with 1-MeTIQ revealed partial, non-significant inhibition of MPTP-induced TBARS levels (0.610 ± 0.065 μ mol/mg, Fig. 6). Although 1-Me-*N*-propenyl-TIQ did not alter the MPTP-induced increase in TBARS, *N*-propargyl- and *N*-butynyl-loaded 1-MeTIQs showed a dose-dependent inhibition of the increase in TBARS. In addition, both doses (20 and 40 mg/kg) of 1-Me-*N*-

propyl-TIQ potently blocked the MPTP induction of TBARS levels (Fig. 6). TBARS values were comparable to controls when 1-MeTIQ or *N*-loaded TIQs were administered in the absence of MPTP (data not shown).

Discussion

Because the structure of TIQ derivatives is similar to that of MPTP, which causes parkinsonism-like symptoms, they have been studied extensively as candidates for PD-inducing agents. Most TIQ derivatives, including TIQ, 1-BnTIQ, and *N*-methyl-(*R*)-salsolinol, have been reported to be parkinsonism-inducing compounds, while the TIQ derivative 1-MeTIQ prevents the development of bradykinesia induced by TIQ or MPTP (Nagatsu et al., 1988) and inhibits 1-methyl-4-phenylpyridinium ion (MPP⁺)-induced cell death (Parrado et al., 2000). Thus, 1-MeTIQ may be an endogenous PD-preventing substance (Okuda et al., 2006). In addition, selegiline, which has a structure similar to TIQ, is used for the treatment of PD. We have previously reported that the neuroprotective effects of certain synthetic TIQ derivatives are enhanced when a propargyl group (a chemical structure present in selegiline) is introduced to the nitrogen in their structure (Katagiri et al., 2010; Saito et al., 2013). In addition, *N*-propargyl groups play important roles in neuroprotection (Maruyama and Naoi, 1999) and cell viability (Kitabatake et al., 2009). Thus, we focused on the effects of 1-MeTIQs loaded with an *N*-functional group (*N*-propyl, *N*-propenyl, *N*-propargyl, or *N*-butynyl) to determine the role of the number of bonds and length of the carbon chain among the functional groups.

The propargylated drug selegiline exhibits a potently selective monoamine MAO-B inhibitory effect. In addition, the parkinsonism therapeutic drug rasagiline, which is a selective MAO-B inhibitor, has broad neuroprotective properties against a variety of neurotoxins in neuronal cell culture and *in vivo* models (Weinreb et al., 2004; Maruyama et al., 2002; Maruyama et al., 2004; Tabakman et al., 2004; Bonneh-Barkay et al. 2005). Furthermore, the propargylamine (the free propargyl moiety of rasagiline) showed neuroprotective effects against *N*-methyl(*R*)-salsolinol (Yi et al., 2006) and serum deprivation-induced cell death (Youdim et al., 2005; Bar-Am et al., 2005). Thus, these results suggest that the propargyl moiety is essential for the neuroprotection mediated by rasagiline. However, since this neuroprotective effect is also observed in propargylamine-containing molecules that do not inhibit MAO-B, we speculate that it may not be due to the inhibition of MAO-B. For example, TVP1022 (*S*-optical isomer of rasagiline) was shown to be neuroprotective, with its activity attributable to the propargyl moiety (Bar-Am et al., 2005; Youdim, 2013). Our previous study also suggested that 1-Me-*N*-propargyl-TIQ does not disturb MAO-B *in vitro* (Kitabatake et al., 2009). Thus, MAO-B inhibition may not be necessary for neuroprotection. In the present study, the order of neuroprotection efficacy according to the functional groups is *N*-propargyl (significant effects against all parameters) > *N*-butynyl (significant against bradykinesia, DAT, and TBARS) > *N*-propenyl (significant against bradykinesia and DAT) > *N*-propyl (significant against bradykinesia and TBARS, but lethal at high doses). These results suggest that the number of bonds in the *N*-functional group is crucial and plays a pivotal role in neuroprotection, and that the presence of a triple bond is the most effective in our study system.

Selegiline enters the active site cavity (core) in the center of MAO-B and inhibits monoamine oxidation. X-ray crystallography showed that when present in the active site, the position of the propargyl group of selegiline was very close to flavin adenine dinucleotide (FAD), which is a coenzyme of MAO-B (De Colibus et al., 2005). Thus, the propargyl group may affect FAD function. FAD is a co-factor of oxidation-reduction reactions, is an energy carrier, and is required for oxidative phosphorylation during metabolism. We speculate that the interaction between a propargyl functional group and FAD but not MAO-B may participate in mediating the neuroprotective effects of 1-Me-*N*-propargyl-TIQ. Further investigations will be required to determine whether this speculation reflects the mechanism of *N*-functional groups. In addition, the length of the *N*-functional group is also an important factor because *N*-butynyl, which has a triple bond and a long length compared to propargyl, showed decreased neuroprotective properties.

A possible mechanism by which selegiline slows the progression of PD symptoms is that it protects neurons from the effects of oxidative stress and various neurotoxins (Lahtinen et al., 1997; Przuntek et al., 1999). However, its neuroprotective effects may be diminished by the formation of neurotoxic metabolites such as amphetamine and methamphetamine (Bar-Am et al., 2004, 2007). Although the structures of the metabolites of 1-MeTIQ derivatives are unclear, the absence of the production of neurotoxic metabolites and/or the presence or production of neuroprotective metabolites may contribute to the protective function of 1-MeTIQ derivatives. In addition, TIQ derivatives are metabolized to *N*-methylated-TIQ by *N*-methyltransferase in the brain, and further oxidized to *N*-methylisoquinolium ions, which may exert neurotoxic effects (Naoi et al., 1989a, 1989b, 1993, Niwa et al., 1990). On the other hand, a possible neuroprotective mechanism of TIQ derivatives was reported by Antkiewicz-Michaluk et al. (2001), where 1-MeTIQ inhibits the *N*-oxidation catabolic pathway of dopamine. Therefore, a propargyl group attached to the *N*-position in the structure may interfere with *N*-methylation and may thus be involved in the neuroprotective effects of 1-MeTIQs loaded with an *N*-functional group.

In conclusion, our study suggests that the neuroprotective properties of 1-MeTIQ derivatives loaded with an *N*-functional group mainly depends on the number of bonds. The length of the functional group appears to also be partially involved. These results suggest that compounds intended to prevent MPTP-induced PD-like symptoms should include an *N*-propargyl group. Therefore, we should investigate how structural differences affect pharmacological properties in subsequent studies, which may reveal the reason that certain TIQ derivatives are neurotoxic and others are neuroprotective. Biochemical data regarding *N*-propargylated compounds is accumulating, including studies of its role in apoptosis and its neurotrophic mechanism. Some *N*-propargylated compounds are expected to be useful for PD treatment as well as in treating Alzheimer's disease (Youdim, 2013). In addition, although 1-MeTIQ did not prevent MPTP-induced decreases in dopamine content in our experimental system, it did prevent NMDA receptor antagonist (MK-801)-induced reduction in dopamine in the prefrontal cortex (Białoń et al., 2021), and 6-hydroxydopamine-induced striatal dopamine reduction (Wąsik et al., 2018). Because the pharmacological potential of TIQ derivatives is high and their actions are broad, combining their structurally similar skeletons with *N*-propargyl groups and various substituents may lead to the development of new PD therapeutics.

Abbreviations

1-MeTIQ – 1-methyl-1,2,3,4-tetrahydroisoquinoline, 3-MT – 3-methoxytyramine, 5-HT – 5-hydroxytryptamine, 5-HIAA – 5-hydroxyindoleacetic acid, HVA – 3-methoxy-4-hydroxyphenylacetic acid, DAT – dopamine transporter, DOPAC – 3,4-dihydroxyphenylacetic acid, MAO-B – monoamine oxidase B, MPTP – 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, PD – Parkinson's disease, TBARS – thiobarbituric acid reactive substances, TH – tyrosine hydroxylase, HPLC – high performance liquid chromatography

Declarations

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Author contribution statement

KT and KA conceived and designed research. HM, RI, TK and TC conducted experiments and analyzed data. TS contributed new reagents. HM, RI and TK wrote the original draft manuscript. KT and KA reviewed and edit manuscript. All authors read and approved the manuscript. The authors declare that all data were generated in-house and that no paper mill was used.

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Compliance with ethical standards

Conflict of interest The authors declare no conflicts of interest.

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Tables

Table 1 Effect of *N*-loaded 1-MeTIQ derivatives on the MPTP-induced decrease in striatal monoamine content (ng/mg tissue) in isolated striatum 9 days after completion of drug administration. Data are presented as mean $\pm$ standard error (SE, $n = 5-9$). Symbols show significant differences compared to the control group (saline + saline-treated group, * $p < 0.05$ and ** $p < 0.01$) and the saline + MPTP-treated group (# $p < 0.05$ and ## $p < 0.01$). DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; 3-MT, 3-methoxytyramine; HVA, 3-methoxy-4-hydroxyphenylacetic acid (homovanillic acid); 5-HT, serotonin; 5-HIAA, 5-hydroxyindoleacetic acid.

Effect of 1-Me-*N*-propyl-, *N*-propenyl-, *N*-propargyl and *N*-butynyl-TIQ on striatal monoamines

Treatment		dopamine	DOPAC	3-MT	HVA	5-HT	5-HIAA
1st	2nd						
saline	saline	12.5 ± 0.4	0.98 ± 0.07	0.81 ± 0.07	1.26 ± 0.10	0.56 ± 0.03	0.61 ± 0.12
saline	MPTP	3.7 ± 0.2**	0.30 ± 0.03**	0.45 ± 0.04**	0.58 ± 0.05**	0.56 ± 0.05	0.57 ± 0.06
1-MeTIQ (80 mg/kg)	MPTP	6.0 ± 1.0	0.53 ± 0.04 [#]	0.64 ± 0.02	0.80 ± 0.05	0.60 ± 0.05	0.75 ± 0.05
<i>N</i> -propyl	20 mg/kg	MPTP	3.6 ± 0.2	0.41 ± 0.02	0.65 ± 0.02	0.64 ± 0.03	0.54 ± 0.01
	40 mg/kg	MPTP	3.6 ± 0.6	0.38 ± 0.05	0.62 ± 0.07	0.59 ± 0.04	0.65 ± 0.06
<i>N</i> -propenyl	20 mg/kg	MPTP	3.6 ± 0.6	0.27 ± 0.07	0.41 ± 0.10	0.56 ± 0.12	0.70 ± 0.06
	40 mg/kg	MPTP	4.2 ± 0.8	0.32 ± 0.05	0.51 ± 0.05	0.63 ± 0.08	0.65 ± 0.03
	80 mg/kg	MPTP	4.8 ± 0.2	0.39 ± 0.03	0.58 ± 0.04	0.69 ± 0.05	0.71 ± 0.02
<i>N</i> -propargyl	20 mg/kg	MPTP	5.4 ± 0.6 [#]	0.41 ± 0.02	0.60 ± 0.02	0.81 ± 0.02	0.65 ± 0.05
	40 mg/kg	MPTP	9.7 ± 1.3 ^{###}	0.70 ± 0.06 ^{###}	0.81 ± 0.02 ^{###}	1.06 ± 0.06 ^{###}	0.63 ± 0.01
	80 mg/kg	MPTP	11.5 ± 1.0 ^{###}	0.72 ± 0.06 ^{###}	0.87 ± 0.07 ^{###}	1.11 ± 0.10 ^{###}	0.64 ± 0.02
<i>N</i> -butynyl	20 mg/kg	MPTP	4.6 ± 0.5	0.38 ± 0.05	0.43 ± 0.02	0.62 ± 0.04	0.68 ± 0.15
	40 mg/kg	MPTP	4.2 ± 0.7	0.40 ± 0.05	0.50 ± 0.06	0.65 ± 0.06	0.68 ± 0.07
	80 mg/kg	MPTP	5.0 ± 0.3	0.41 ± 0.03	0.59 ± 0.04	0.71 ± 0.06	0.74 ± 0.04

mean ± S.E.

Table 2 Brains were sectioned 9 days after completion of administration of saline or MPTP. The number of TH-positive cells in the substantia nigra are expressed as the mean ± standard error (SE, n = 5-8). Symbols show significant differences when compared to the saline + saline-treated group (**p < 0.01) and the saline + MPTP-treated group (###p < 0.01).

Effect of 1-MeTIQ derivatives on MPTP-induced reduction in TH-positive cells in the substantia nigra

Treatment		Number of TH-positive cells
1st	2nd	
saline	saline	108.8 $\pm$ 8.3
saline	MPTP	62.6 $\pm$ 7.0 **
1-Me- <i>N</i> -propyl-TIQ	MPTP	65.2 $\pm$ 1.4
1-Me- <i>N</i> -propenyl-TIQ	MPTP	64.5 $\pm$ 5.0
1-Me- <i>N</i> -propargyl-TIQ	MPTP	102.2 $\pm$ 8.1 ##
1-Me- <i>N</i> -butynyl-TIQ	MPTP	70.8 $\pm$ 9.2
		mean $\pm$ S.E.

Figures

Selegiline

1-MeTIQ

1-Me-N-propyl-TIQ

1-Me-N-propenyl-TIQ

1-Me-N-propargyl-TIQ

1-Me-N-butynyl-TIQ

Figure 1

Structural formula of selegiline, 1-MeTIQ, and 1-MeTIQ derivatives loaded with N-propyl, N-propenyl, N-propargyl, and N-butynyl.

Figure 2

A schematic illustration of the drug administration schedule.

Figure 3

Effect of 1-MeTIQ and N-loaded 1-MeTIQ derivatives on MPTP-induced bradykinesia. T_{turn} and T_{LA} were defined as the time for animals to turn completely downward at the top of the iron pole and the time required to arrive at the pole base, respectively. Data are the mean $\pm$ standard error (SE, n = 6-10). Symbols show significant differences when compared to the saline + saline-treated group (**p < 0.01) and the saline + MPTP-treated group (#p < 0.05, ## p < 0.01).

A: Saline + Saline

B: Saline +MPTP

C: 1-Me-N-propyl-TIQ +MPTP

D: 1-Me-N-propenyl-TIQ +MPTP

E: 1-Me-N-propargyl-TIQ +MPTP

F: 1-Me-N-butynyl-TIQ +MPTP

Figure 4

Protective effect of N-loaded 1-MeTIQ derivatives on MPTP-induced reduction in TH-positive cell numbers in the substantia nigra. A representative substantia nigra section is shown for each group. Mice were treated with (A) saline + saline, (B) saline + MPTP (20 mg/kg), (C) 1-Me-N-propyl-TIQ (40 mg/kg) + MPTP, (D) 1-Me-N-propenyl-TIQ (80 mg/kg) + MPTP, (E) 1-Me-N-propargyl-TIQ (80 mg/kg) + MPTP, and (F) 1-Me-N-butynyl-TIQ (80 mg/kg) + MPTP. Photomicrographs are shown at a magnification of x200.

Figure 5

Inhibitory effect of 1-MeTIQ and N-loaded 1-MeTIQ derivatives on the MPTP-induced decrease in DAT in the striatum. Data are presented as the mean $\pm$ standard error (SE, $n = 5-7$). Symbols show significant differences when compared to the saline + saline-treated group (** $p < 0.01$) and the saline + MPTP-treated group (# $p < 0.05$, ## $p < 0.01$).

Figure 6

Inhibitory effect of 1-MeTIQ and N-loaded 1-MeTIQ derivatives on the MPTP-induced increase in TBARS in the substantia nigra. Data are presented as the mean $\pm$ standard error (SE, $n = 5-9$). Symbols show significant differences when compared to the saline + saline-treated group (** $p < 0.01$) and the saline + MPTP-treated group (## $p < 0.01$).

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