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The Antiepileptic Drug Zonisamide Inhibits MAO-B and Attenuates MPTP Toxicity in Mice: Clinical Relevance

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Abstract

Zonisamide is an FDA-approved antiepileptic drug that blocks voltage-dependent Na⁺ channels and T-type Ca²⁺ channels and improves clinical outcome in Parkinson's disease (PD) patients when used as an adjunct to other PD therapies. Zonisamide also modifies dopamine (DA) activity, provides protection in ischemia models and influences antioxidant systems. Thus, we tested it for its ability to protect DA neurons in a mouse model of PD and investigated mechanisms underlying its protection. Concurrent treatment of mice with zonisamide and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) attenuated the reduction in striatal contents of DA, its metabolite DOPAC and tyrosine hydroxylase (TH). We also discovered that zonisamide inhibited monoamine oxidase B (MAO-B) activity *in vitro* with an IC₅₀ of 25 μM, a concentration that is well within the therapeutic range used for treating epilepsy in humans. Moreover, the irreversible binding of systemically administered selegiline to MAO-B in mouse brain was attenuated by zonisamide as measured by *ex vivo* assays. Zonisamide treatment alone did not produce any lasting effects on *ex vivo* MAO-B activity, indicating that it is a reversible inhibitor of the enzyme. Consistent with the effects of zonisamide on MAO-B, the striatal content of 1-methyl-4-phenylpyridinium (MPP⁺), which is derived from the administered MPTP via MAO-B actions, was substantially reduced in mice treated with MPTP and zonisamide. The potency and reversibility with which zonisamide blocks MAO-B may contribute to the ability of the drug to improve clinical symptoms in PD patients. The results also suggest that caution in its use may be necessary, especially when administered with other drugs, in the treatment of epilepsy or PD.

Keywords

zonisamide; epilepsy; MPTP; MAO-B; mice; Parkinson's disease

Introduction

Zonisamide is an FDA-approved drug for use in treating epilepsy. It has many actions which include blocking voltage-dependent sodium channels, inactivating T-type calcium channels

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and reducing calcium influx during membrane depolarization, actions involved with preventing seizure spread (Biton, 2007, Kito, et al., 1996, Meldrum, et al., 2007). It is also reported that zonisamide improves clinical outcome when used as an adjuvant in therapy for Parkinson's disease (PD) (Murata, et al., 2007) possibly due to its ability to modify dopamine (DA) neurotransmission.

Zonisamide exerts neuroprotection in several experimental animals of neuronal injury including seizure models (Asanuma, 2007, Ueda, et al., 2005) and ischemia models (Minato, et al., 1997). A preliminary report indicates it protected against damage to the nigrostriatal DA system induced by the dopaminergic neurotoxin, 6-hydroxydopamine (Asanuma, 2007). It is proposed that mechanisms by which zonisamide may exert neuroprotection is via free radical scavenging, elevating brain glutathione content and reducing oxidative stress (Asanuma, et al., 2008, Tokumaru, et al., 2000, Ueda, et al., 2005). Taken together, these actions suggest that zonisamide may provide neuroprotection by reducing the burden of oxidative stress which is increased in the brains of PD patients and experimental PD models.

Presently, there is no therapy for slowing the progression of neuronal loss in PD. The possibility that an FDA-approved drug, which improves clinical symptoms in PD, might also slow the progression of the disease, prompted us to consider the possibility that zonisamide would protect DA neurons in an animal model of PD. The purposes of the study were to examine the effects of zonisamide on neurotoxicity induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), on MPTP metabolism, on the activity of monoamine oxidase B (MAO-B) and on DA homeostasis. Our findings indicate that zonisamide is an effective inhibitor of MAO-B at concentrations that are within the plasma therapeutic concentrations achieved in humans.

Materials and Methods

Animals and Treatment

Male Swiss Webster mice (10-12 wks of age, Taconic Farms, Germantown, NY) were used in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory animals* and as approved by the University's Institutional Animal Care and Use Committee. Mice were treated with MPTP (free base, 30 mg/kg s.c., Sigma-Aldrich, St. Louis, MO), zonisamide (various doses, i.p., Sigma-Aldrich) or 2.5 mg/kg selegiline i.p. (Sigma-Aldrich) and killed at the times as indicated in the figure legends. The doses of zonisamide administered were those in the low to moderate dose range for blocking seizure activity in mice (Borowicz, et al., 2007, Nagatomo, et al., 1996, Nagatomo, et al., 2000). The dose of selegiline used provides selective and near complete inhibition of MAO-B and protects against MPTP toxicity in mice (Sonsalla, et al., 1987). Striata were rapidly removed from the brain, frozen on dry ice, and stored at -60°C until assayed.

Determination of striatal TH content

TH was measured with ELISA as previously described (Alfinito, et al., 2003). Briefly, striata were homogenized in phosphate buffered saline and aliquots of the homogenates were incubated in 96-well microplates which had been coated with monoclonal anti-TH and blocked with 5% non-fat dry milk, followed by co-incubation with rabbit polyclonal anti-TH (Calbiochem, San Diego, CA) and polyclonal anti-rabbit horseradish peroxidase (Amersham Biosciences, Piscataway, NJ). Reaction products from exposure to Amplex Red (Molecular Probes, Eugene, OR) and horse radish peroxidase substrate were measured fluorometrically (excitation/emission ratio of 530/580 nm).

Measurement of DA and DOPAC

An aliquot of the striatal homogenate was immediately removed after homogenization and added to 0.2N perchloric acid (PCA) containing 100 μ M EDTA. After centrifugation, the supernatant was analyzed for DA and DOPAC with HPLC and electrochemical detection (Antec Leyden, The Netherlands) as previously described (Moy, et al., 2000).

Monoamine oxidase (MAO) activity

MAO-B activity in homogenates from mouse striata or cerebral cortices was determined with the Amplex Red Monoamine Oxidase Assay kit (Molecular Probes, Eugene, OR) as previously described (Rocha, et al., 2008). Briefly, homogenates were incubated with the MAO-B substrate benzylamine in the presence or absence of zonisamide (concentrations from 10 – 300 μ M) or selegiline (1 μ M; MAO-B inhibitor) for 30 min. For MAO-A activity, homogenates were incubated with tyramine and selegiline (the latter was required to block the MAO-B metabolism of tyramine) or clorgyline (1 μ M; MAO-A inhibitor) for 30 min. All drugs and substrates were dissolved in deionized water. Activity was measured as the reaction product of H_2O_2 generated with 10-acetyl-3,7-dihydroxyphenoxazine in a horseradish peroxidase-coupled reaction. Fluorescence was measured using a multiplate reader (CytoFluor 4000, PerSeptive Biosystems, Framingham, MA) with excitation/emission at 530 nm/580 nm, respectively. GraphPad Prism Software (LaJolla, CA) was used to determine the IC₅₀ values for MAO-B inhibition. In some experiments striata were used whereas in other experiments the cortex was used for evaluating activity. In preliminary studies we found no differences in MAO-B activity or in the extent of selegiline inhibition between striata and cortex and thus data obtained from the two different brain regions were combined.

Glutathione (GSH) Assay

Striatal levels of total glutathione (GSH and GSSG) were measured by a kinetic assay according to kit directions (Sigma-Aldrich). Briefly, PCA extracts used for HPLC measurements were incubated with 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB), glutathione reductase, and NADPH. The rate of formation of the reduction product of DTNB was monitored at 412 nm with a spectrophotometric plate reader (SpectraMax, Molecular Devices, Sunnyvale, CA). Concentrations of unknown samples were determined from standard curves generated with known amounts of GSH.

MPTP and MPP⁺ Analysis

Striata were dissected from the brain, frozen and stored at -20°C until analysis. Striata (10-15 mg weight) were homogenized in 150 μ l of 12% acetic acid by sonication (20 -30 min). The homogenates were extracted in a Mars X microwave sample digester (Mathews, North Carolina) for 30 min using 50% of the 300W power setting. After extraction, both solid and liquid phases were transferred onto 10 kDa cut off Microcon centrifuge filters and filtered using centrifugation (4°C, 12,000 g, 90 min). Filtrates were stored at -20 °C until analyzed. Sample analysis. Samples were analyzed by liquid chromatography mass spectrometry in an assay analogous to one for paraquat (Prasad, et al., 2007). Separation was carried out on a Waters Alliance Separation Module 2690 (Waters Corporation, Milford, MA) with a gradient elution carried with a three-solvent system : (A) 0.1 % formic acid in water, (B) 0.1 %, formic acid in methanol (C) 0.1 % formic acid in acetonitrile on a ZORBAX RX-C8, 4.6mm $\times$ 15 cm, 5 μ m column (Agilent Technologies, Santa Clara, CA). MPP⁺ was eluted at 9.4 min and MPTP at 10.1 min monitored by both UV detector and Mass Spectrometer. The analytes were quantified on an LCQ ITMS mass spectrometer (Thermo Finnegan, San Jose, CA) equipped with an electro spray ionization source (ESI) operated using Xcalibur1.3 software. Mass spectrometric settings were optimized using a standard solution (1 μ g/ml) and electro spray ionization in the positive mode with sheath gas flow rate 1.4 L/min, heated capillary temperature 275°C, and

spray voltage 5.0 kV. Data acquisition was performed in Selective Ion Monitoring (SIM) mode for MPTP and MPP⁺ at 174 m/z and 170 m/z respectively, with an isolation width 1 m/z. Concentrations were determined from a calibration curve using integrated peak areas. Integration was performed using Xcalibur software.

Data Analysis

Data were analyzed using GraphPad Prism and are presented as the mean $\pm$ SD. Treatment effects on the neurochemical measures were compared with one-way ANOVA followed by Tukey's multiple comparison test. All values at $P < 0.05$ were considered statistically significant.

Results

Zonisamide protects against MPTP-induced reductions of striatal DA measures

The administration of MPTP (one injection of 30 mg/kg/day for 4 days) produced profound reductions in the content of striatal TH (-84%), DA (-92%) and DOPAC (-65%) in mice killed 7 days after the last MPTP injection; see Figure 1. The pretreatment of mice with zonisamide significantly attenuated these losses in a dose-dependent manner. At 20 mg/kg zonisamide, the reductions in TH, DA and DOPAC were only -57%, -58% and -37%, respectively. Significant protection was also seen with the 5 and 10 mg/kg doses but not the 1 mg/kg dose.

Zonisamide pretreatment reduces content of striatal MPP⁺ in mice treated with MPTP

The next studies were designed to examine if the neuroprotective effect observed with zonisamide was possibly due to its ability to alter MPTP metabolism. Thus, mice were treated with or without zonisamide (20 mg/kg, i.p.) and MPTP (30 mg/kg, s.c.) and killed 90 min later. The 90 min time point was selected because MPP⁺ levels in the mouse brain peak at this time (Giovanni, et al., 1991). The amount of striatal MPP⁺ in mice treated with zonisamide and MPTP was significantly lower than that seen in mice treated with vehicle and MPTP (0.8 ng/mg tissue vs. 3.1 ng/mg tissue, respectively); see Table 1. MPTP levels were similar in both groups at this time point. In previous studies in which measurements were made at the 90 min time point, MPTP levels in mice treated with the MAO-B inhibitor selegiline and MPTP were not different from those in mice treated with vehicle/MPTP although MPP⁺ levels were reduced by 65% and were similar to the reduction observed in the present studies (Heikkila, et al., 1988). These data are consistent with an inhibitory action of zonisamide on MPTP metabolism.

Zonisamide inhibits MAO-B activity in vitro and in vivo

Because MAO-B is the enzyme that converts MPTP to MPP⁺, we next examined the inhibition profile for zonisamide on MAO-B activity in mouse brain homogenates. Zonisamide was discovered to be a fairly potent inhibitor of MAO-B and inhibited enzyme activity by 50% (IC₅₀) at 24.8 ± 4.3 μ M (mean $\pm$ SD, 3 experiments; see Figure 2). The well-characterized MAO-B inhibitor selegiline (1 μ M) completely blocked MAO-B activity (data not shown). No inhibition of MAO-A was seen up to the highest concentration tested (300 μ M) although the MAO-A inhibitor clorgyline (1 μ M) blocked product formation (data not shown). *Ex vivo* studies were also conducted to determine if zonisamide were a reversible or irreversible inhibitor of MAO-B and whether it could attenuate the irreversible binding of the well-known inhibitor selegiline *in vivo*. For these studies, mice received an injection of zonisamide (20 mg/kg, i.p.) followed by an injection of selegiline (2.5 mg/kg) and *ex vivo* measurements were performed. There was no inhibition of *ex vivo* MAO-B activity in brain homogenates derived from mice treated with zonisamide, indicating that it was a reversible inhibitor (Figure 3). However, in mice treated with zonisamide followed by selegiline treatment, there was a significant prevention of the irreversible binding of selegiline in the brain. Selegiline treatment

significantly reduced *ex vivo* MAO-B activity and this reduction was significantly prevented in zonisamide/selegiline treated mice (Figure 3). These findings indicate zonisamide is a reversible inhibitor of MAO-B *in vitro* and *in vivo* at doses that exert anti-seizure activity in mice.

Zonisamide does not alter DA transport, DA turnover or GSH levels in striatum

Protection of DA neurons by drugs can occur by modifying DA transport, DA activity or by substances that increase antioxidant systems. Zonisamide increases DA synthesis and DA release in rats as measured by microdialysis (Okada, et al., 1995). However, in our studies, zonisamide treatment of mice (20 mg/kg) did not alter the striatal contents of DA, DOPAC, TH, or GSH nor did it modify DA turnover as calculated for the DOPAC/DA ratio in mice killed 6 h after drug administration (see table 2). Moreover, selegiline administration did not affect DA metabolism or turnover. In the mouse, DA is metabolized by MAO-A. Thus these data indicate that neither zonisamide nor selegiline had any effect on MAO-A activity at the doses tested. We also examined if zonisamide had any effect on the dopamine transporter by measuring 3H-DA uptake into striatal synaptosomal preparations. No appreciable effect of zonisamide was found at concentrations up to 100 μ M and are in agreement with previously published findings (Okada, et al., 1995). Thus, it is unlikely that zonisamide prevented MPP⁺ uptake into DA neurons or exerted any long-lasting effect on DA function.

Discussion

Zonisamide is an FDA-approved drug used for seizure therapy and is also being used as an adjunct to improve clinical outcome in PD patients (Murata, et al., 2007). Several properties of zonisamide suggested that it might exert neuroprotection towards DA neurons via its ability to block sodium channels, T-type calcium channels and enhance antioxidant systems (Asanuma, et al., 2008, Asanuma, 2007, Czapinski, et al., 2005, Murakami, et al., 2001, Okada, et al., 1995, Sobieszek, et al., 2003), actions which would be beneficial to the survival of stressed DA neurons.

In our studies, zonisamide treatment protected against the MPTP-induced decrements in DA nerve terminal measures in a dose-related manner. Furthermore, our findings indicate that this protection is mostly, if not completely, due to the drug's inhibition of MAO-B and a reduction in MPTP metabolism rather than to any intrinsic action on DA turnover or antioxidant systems. Evidence in support of this conclusion comes from both *in vitro* and *in vivo* studies.

Zonisamide was found to be a potent inhibitor of MAO-B in mouse brain homogenates with an IC₅₀ ~25 μ M, a concentration well within the recommended plasma therapeutic range for seizure control in humans. *In vivo*, two separate experiments demonstrated that zonisamide was an efficient blocker of MAO-B activity in mouse brain. First, zonisamide markedly reduced the *in vivo* formation of MPP⁺ from MPTP, a process that is dependent on MAO-B. Second, zonisamide treatment attenuated the irreversible binding of systemically administered selegiline. The greater effect of zonisamide in inhibiting MPTP metabolism than in preventing irreversible selegiline binding is likely due to the differences in affinities of MPTP and selegiline for MAO-B with selegiline having a much higher affinity than MPTP. The K_m for MPTP metabolism by MAO-B is approximately 100 μ M whereas MAO-B is completely inhibited by selegiline at concentrations less than 1 μ M (Sonsalla, et al., 1987). Thus, zonisamide (with a K_m ~25 μ M) would be more able to compete with MPTP than selegiline for the enzyme and thus exert a greater effect in blocking MPTP metabolism than in preventing selegiline binding. In addition, because selegiline binds irreversibly to MAO-B, access by zonisamide to its binding site is markedly diminished as compared to other reversible substances which exhibit the dynamic pharmacological “on/off” rate of a compound at its site of action.

The doses of zonisamide used in these studies were selected based on literature reports of their anti-seizure efficacy in mice and their ability to produce plasma concentrations within the therapeutic range for humans. It was not expected that these doses would provide brain concentrations that would interfere with MAO-B. In mice, brain levels of zonisamide are nearly 2-fold higher than plasma levels (approximately 70 μM vs 40 μM , respectively) after a 20-25 mg/kg dose in mice or rats (Nagatomo, et al., 1996, Nagatomo, et al., 2000, Okada, et al., 1995). Okada et al (1995) reported that zonisamide inhibited MAO-B activity with an IC_{50} value of approximately 700 μM which is 10 fold higher than the expected brain concentration in our mice treated with a 20 mg/kg dose of zonisamide. However, with the discovery that zonisamide is more potent than previously reported in inhibiting MAO-B (IC_{50} of 25 μM) and with brain concentrations approaching 70 μM after a 20 mg/kg dose, it might be expected that significant MAO-B inhibition would occur. That it did is documented by the *in vivo* inhibition of MPTP metabolism and selegiline binding. The reason why zonisamide was much more potent as an MAO-B inhibitor in our studies vs the Okada studies is not clear, but may reflect species and/or tissue differences in the source of MAO-B (rat liver vs mouse brain) or in the preparation or storage of the drug solutions (e.g., use of methanol for drug dissolution in the *in vitro* experiments of Okada et al.). We found that refrigeration or freezing of solutions of zonisamide dissolved in water caused a precipitation of the drug. Thus, we used only fresh drug solutions for testing.

Our data indicate that zonisamide had little effect on the striatal content of DA or DOPAC or on DA turnover. These observations differ from previous findings in which tissue DA content was slightly, but significantly, increased and DOPAC content slightly decreased with zonisamide treatment of rats at the same dose that we administered to mice (Okada, et al., 1995). The differences in our findings vs previous studies may reflect the species used (rat vs mouse) and/or the time of measurement (2 h vs 6 h) after drug administration. It is important to note that in rodents, DA metabolism and turnover is primarily a function of MAO-A and not of MAO-B as it is in humans (Shih, et al., 1999). Thus, the lack of an effect of zonisamide treatment on DA turnover in mice is consistent with the lack of an effect of zonisamide on MAO-A activity. Given the species differences in DA metabolism by MAO, the effect of zonisamide treatment in our mouse studies does not exclude the possibility, and indeed our studies indicate a high likelihood, that zonisamide exerts DA-enhancing effects in humans by inhibition of MAO-B.

The doses of zonisamide used in seizure disorders range from 200-600 mg/day and produce plasma concentrations in humans ranging from 43-171 μM (Mimaki, 1998, Peters and Sorkin, 1993). If concentrations of zonisamide are higher in brain than in plasma in humans, as they are in rodents, then doses that produce blood levels within the therapeutic range would also likely produce substantial inhibition of brain MAO-B. Targeted steady state serum concentrations of zonisamide for humans approximate 85 μM (Mimaki, 1998) and are close to 3-fold above the IC_{50} we determined for MAO-B inhibition. Thus, there is likely to be substantial MAO-B inhibition in humans taking therapeutic doses of zonisamide for the treatment of seizures. This may require caution in its use, particularly when used in conjunction with other drugs.

The doses of zonisamide administered to PD patients were lower than those used to control for seizures and ranged from 25-100 mg/day (Murata, et al., 2007). Although plasma zonisamide concentrations were not determined in the PD patients, it seems likely that these doses, particularly the higher doses, would produce brain concentrations capable of significant inhibition of MAO-B. As such, this action of zonisamide would be beneficial for enhancing DA actions in the brains of PD patients.

Our discovery that zonisamide is a potent and effective *in vivo* inhibitor of MAO-B was surprising but adds to the list of drugs serendipitously found to be substrates or inhibitors of this enzyme. One of the first surprises was the discovery that MPTP was a substrate for MAO-B. Subsequently, several other structurally unrelated compounds have been found to be substrates or inhibitors of MAO-B and include MPTP analogs, caffeine analogs, plant alkaloids and pioglitazone (a peroxisome proliferator-activated receptor- γ agonist) (Chen, et al., 2001, Khalil, et al., 2006, Kong, et al., 2004, Quinn, et al., 2008, Yang, et al., 2007, Youngster, et al., 1987, Zhou, et al., 2001). These discoveries underscore the need to examine any potential neuroprotective drug for possible interference with the pharmacokinetic or pharmacodynamic properties of the toxicant being used to create the animal model of PD.

After completion of the studies and during the writing of this paper, we became aware of a very recent publication in which it was reported that zonisamide did not protect against MPTP toxicity in mouse or marmoset although it increased DA turnover in the MPTP-treated animals (Yabe, et al., 2009). Reasons for these differences between our studies and those of Yabe et al. are not clear but may reflect differences in mouse strain used, doses and dosing protocols. We used Swiss Webster mice whereas Yabe and colleagues used C57bl mice. We dosed once per day (30 mg/kg MPTP s.c. and ≤ 20 mg/kg zonisamide i.p.) whereas Yabe and colleagues dosed 4 times in 1 day at 2 h intervals (MPTP, 15 mg/kg/inj s.c. for a total dose of 60 mg/kg). Zonisamide was also administered 4 times at 2-h intervals at a dose of 40 mg/kg/inj s.c. for a total dose of 160 mg/kg. While it might be expected that better protection would have been seen with a larger dose of zonisamide, the dose and dosing protocol with zonisamide alone produced a significant decrease in striatal DA content 1 week later as compared to controls which indicates the drug may have had an adverse effect on the DA system which was compounded in mice also treated with MPTP. Previous studies showed that low doses of zonisamide increase DA release whereas high doses inhibit release (Okada, et al., 1995). Further studies are needed to more completely characterize the dose-effect of zonisamide on DA neurotransmission, particularly under the stressed conditions which arise following a toxic insult.

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Abbreviations used

DA	dopamine
DOPAC	dihydroxyphenylacetic acid
GSH	glutathione
MAO-B	monoamine oxidase-B
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MPP ⁺	1- methyl-4-phenylpyridinium
TH	tyrosine hydroxylase

Figure 1. Zonisamide protects against MPTP-induced reductions in striatal TH, DA and DOPAC
Mice were treated with zonisamide (Zonis) at 20, 10, 5, or 1 mg/kg i.p. or vehicle 15 min before each MPTP injection. Mice received 4 daily injections of MPTP (30 mg/kg/day s.c.) and were killed 7 days after the last treatment. Results are the mean $\pm$ SD from 3-5 mice/group (control $n=4$, zonisamide $n=3$, all other groups $n=5$). Analysis of the data by one-way ANOVA with post hoc testing revealed a significant effect for TH (F_6 $df_{5,20} = 52$), DA (F_7 $df_{6,24} = 64$) and DOPAC (F_7 $df_{6,24} = 23$). ^a $P < 0.05$ from control, ^b $P < 0.05$ from MPTP group.

Figure 2. Regression line for inhibition of MAO-B by zonisamide

MAO-B assay was performed on mouse brain homogenates as described in Methods. Concentrations of zonisamide ranged from 10-300 μM and are expressed in log units. Points plotted are the mean $\pm$ SD from three experiments.

Figure 3. Zonisamide reduces *in vivo* selegiline binding: Ex vivo measurement of MAO-B activity Mice were treated with zonisamide (20 mg/kg i.p.) or vehicle approx. 30 min before selegiline (2.5 mg/kg i.p.) and killed 5.5 h later. Results are the mean MAO-B activity $\pm$ SD from mice treated with vehicle (control, n = 9), zonisamide (n=3), selegiline (n=8) or zonisamide plus selegiline (n=8). Analysis of the data by one-way ANOVA with post-hoc testing revealed a significant effect ($F_{3,df_{3,24}} = 157.5$) ^a $P < 0.001$ from control, ^b $p < 0.01$ vs selegiline group.

Table 1
Zonisamide reduces striatal MPP⁺ formation

Treatment	Striatal MPP ⁺ (ng/mg tissue)	Striatal MPTP (ng/mg tissue)
MPTP	3.1 ± 0.6	0.60 ± 0.09
Zonisamide + MPTP	0.8 ± 0.1 ^a	0.45 ± 0.11

Mice were treated with vehicle or zonisamide (20 mg/kg, i.p.) 15 min before MPTP (30 mg/kg, s.c.) and killed 90 min later. Results are the mean striatal content of MPP⁺ ± SD (n = 4 mice/group).

^aP<0.005 from group treated with vehicle/MPTP.

Table 2

Acute zonisamide treatment does not alter DA turnover or GSH content in striata

	Control (n=7)	Zonisamide (n=3)	Selegiline (n=8)	Zonisamide + Selegiline (n = 7)
DA (ng/mg tissue)	12.9 ± 2.0	13.5 ± 2.0	13.5 ± 2.0	13.4 ± 2.8
DOPAC (ng/mg tissue)	1.11 ± 0.27	0.95 ± 0.55	0.98 ± 0.27	1.01 ± 0.31
DOPAC/DA ratio	0.11 ± 0.04	0.07 ± 0.03	0.07 ± 0.03	0.10 ± 0.04
HVA (ng/mg tissue)	1.9 ± 0.6	1.7 ± 0.8	1.7 ± 0.4	1.4 ± 0.2
TH (ng/mg tissue)	135 ± 24	113 ± 21	152 ± 21	143 ± 29
GSH (nmol/mg tissue)	1.23 ± 0.09	1.40 ± 0.11	1.22 ± 0.13	1.22 ± 0.10

Mice were treated with zonisamide (20 mg/kg i.p.) approximately 30 min before selegiline (2.5 mg/kg i.p.) and killed approximately 5.5 h later. Results are the mean ± SD from the number of mice indicated in the table.