from each transgenic line with mice homozygous for the *Uchl1*$^{gad/gad}$ allele (*gad* mice). Detergent-soluble (1% Triton X-100) fractions of mouse midbrain from H-hI93M/*gad* (*UCHL1*$^{I93M/-}$, *Uchl1*$^{gad/gad}$) at 2 and 15 weeks of age were subjected to SDS-PAGE and immunoblotted with anti-UCH-L1. We detected human UCH-L1 expression in H-hI93M/*gad* brains (Fig. 1B). Compared with endogenous mouse UCH-L1, which constitutes 1–2% of neuronal proteins, human UCH-L1 expression was substantially lower in H-hI93M/*gad* brains (~1% of endogenous UCH-L1 at 2 weeks of age; Fig. 1B). Interestingly, the level of transgenic human UCH-L1 was lower at 15 weeks than at 2 weeks of age (Fig. 1B). Although we could not detect human UCH-L1 in L-hI93M/*gad* and hWT/*gad* by standard immunoblotting methods, we were successful in detecting it by immunoprecipitation (Fig. 1C). These data suggest the expression of the human UCH-L1 in L-hI93M and hWT mice, which were much lower than in H-hI93M mice.

UCH-L1 is a cytosolic protein predominantly expressed in neuronal cells including dopaminergic neurons at substantia nigra with diffuse localization (data not shown). Thus, we next examined the immunohistochemical localization of the transgene products. In agreement with the data obtained by Western blotting analysis, UCH-L1-immunoreactive cells were not observed in any brain region, including the substantia nigra, of the L-hI93M/*gad* and hWT/*gad* mice (data not shown). In H-hI93M/*gad* mice, however, human UCH-L1^{I93M} was detected in the substantia nigra, the region that contains the central pathological lesions in PD, with relatively high intensities (Fig. 2A). Subthalamic nuclei, striatum, hippocampus CA3 and cerebellum also contained UCH-L1 immunoreactive cells in H-hI93M/*gad* mice (Fig. 2B). As with the previous report that CAT expression under control of the *PDGF-B* promoter in transgenic mice localizes to neuronal cell bodies (Sasahara et al., 1991), most UCH-L1-immunoreactive cells in H-hI93M/*gad* mice had a neuronal morphology (Fig. 2). Western blotting analysis of midbrain lysates showed a reduction of transgenic UCH-L1^{I93M} at 15 weeks of age as compared with that at 2 weeks in H-hI93M/*gad* mice (Fig. 1B). Thus, we also performed immunohistochemical analysis of UCH-L1 on substantia nigra from 2-, 7- and 20-week-old H-hI93M/*gad* mice. We found many UCH-L1-positive neurons at 2 weeks. The number of positive cells had decreased by 7 weeks, however, at which time small-sized and densely stained neurons were observed, and UCH-L1-positive cells were barely

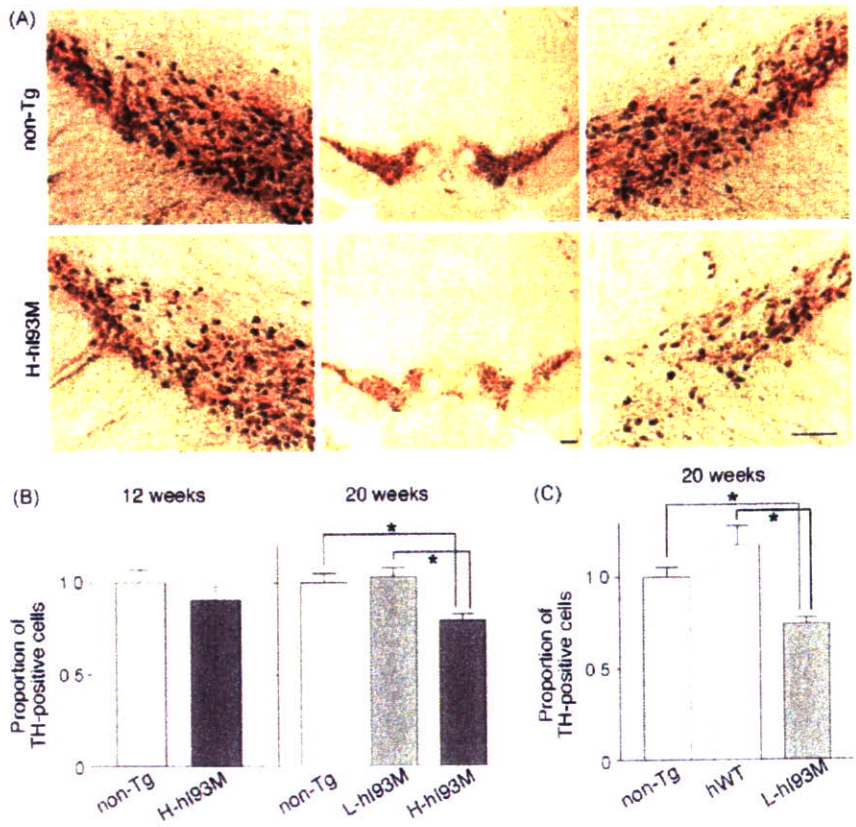

Fig. 3. TH-positive neurons of hI93M Tg mice were reduced as the animals aged. (A) Immunohistochemical staining of the substantia nigra with anti-TH in non-Tg (upper panels) and H-hI93M (lower panels) mice at 20 weeks of age. Scale bar: 1 mm. Left and right panels in the figure correspond to the left and right part of the middle panel, respectively. (B) Proportion of neurons stained with anti-TH in the substantia nigra from non-Tg and hI93M mice at 12 weeks (left panel) and 20 weeks (right panel) of age. Cell numbers were normalized to those for the non-Tg mice. Values are the mean ± S.E.M.; $n = 10$ Significance was examined by a one-way ANOVA. $^{*}p < 0.01$. (C) The number of TH-positive cells in the substantia nigra from 20-week-old non-Tg ($n = 5$). hWT ($n = 3$) and L-hI93M mice ($n = 5$) after treatment with MPTP. The cell numbers were normalized to those for non-Tg mice. Values are the mean ± S.E.M. Significance was examined by a one-way ANOVA. $^{*}p < 0.001$.

detectable at 20 weeks of age (Fig. 2A). Together, our results indicate that hUCH-L1^{I93M} is expressed in the neurons of the substantia nigra in H-hI93M mice, but the number of positive cells declines before 20 weeks of age. With the failure to detect hUCH-L1 protein in hWT/*gad* mice and L-hI93M/*gad* mice both in the Western blotting and the immunohistochemistry, we performed most of the analysis using H-hI93M mice with non-Tg mice as a control.

3.2. Loss of dopaminergic neurons in the substantia nigra of 20-week-old H-hI93M mice

We next determined whether the number of midbrain dopaminergic neurons was reduced in the substantia nigra of transgenic mice using TH immunohistochemistry. The number of TH-positive dopaminergic neurons in the substantia nigra at the same neuroanatomical level was compared and quantified for each transgenic mouse line. Surprisingly, we detected an ~30% reduction in TH-positive neurons in 20-week-old H-hI93M mice as compared with those in non-Tg control mice (Fig. 3A and B). This reduction was not seen in 12-week-old H-hI93M mice or 20-week-old L-hI93M mice. Together with the decrease in the level of UCH-L1^{I93M} (Fig. 1B) and the reduction in UCH-L1-positive neurons in the substantia nigra of H-hI93M/*gad* mice, our data indicate that UCH-L1^{I93M} expression in the dopaminergic neurons is sufficient to induce the degeneration of these neurons.

MPTP is a toxin used to induce an acute Parkinsonian syndrome that is indistinguishable from sporadic PD (Dauer and Przedborski, 2003). MPTP metabolite 1-methyl-4-pyridinium (MPP^+), an inhibitor of complex I of the mitochondrial respiration chain, is taken up by the terminals of dopaminergic neurons via the dopamine transporter (DAT), thereby causing the selective death of nigral neurons (Dauer and Przedborski, 2003). Although neuronal loss was not observed in L-hI93M mice at 20 weeks of age, we speculated that dopaminergic

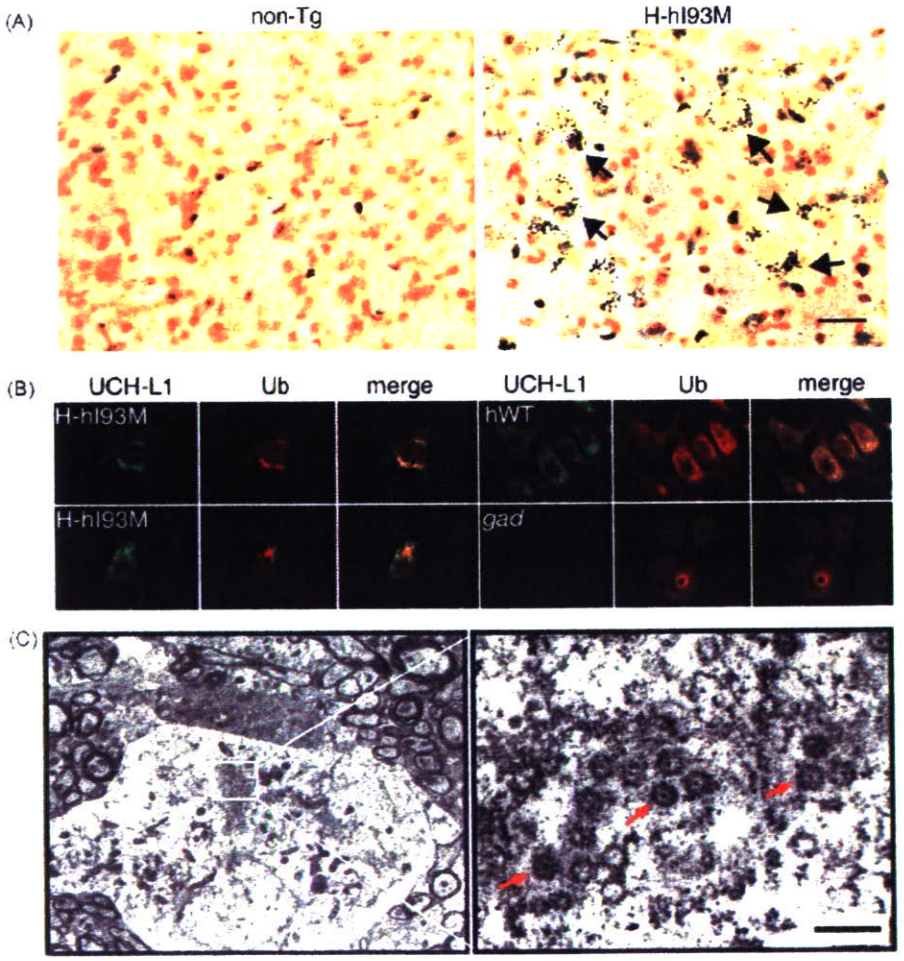

Fig. 4. Several neuropathological features reminiscent of PD are present in H-hI93M mice brains. (A) Silver staining of the substantia nigra at 12 weeks of age in non-Tg and H-hI93M mice. Note the presence of silver staining-positive argyrophilic grains in the cell bodies of some dopaminergic neurons in H-hI93M mice (arrows). This kind of abnormal structure was not seen in substantia nigra of non-Tg mice. Scale bar: 30 μm. (B) Confocal images of dopaminergic neurons from hWT, H-hI93M and *gad* mice. H-hI93M mice showed the formation of ubiquitin-positive cytoplasmic inclusions (red) co-localized with UCH-L1 staining (green) in the remaining nigral neurons at 20 weeks of age. Compared with the diffuse, reduced staining of ubiquitin in *gad* mice, nigral neurons from hWT mice also showed a diffuse pattern of staining but with fine small granular cytoplasmic staining (red) co-localized with UCH-L1 (green). (C) Electron micrographs of a nigral neuron from a 20-week-old H-hI93M mouse at the level of the cell body (left panel), and dense-core vesicles (red arrows) at higher magnification (right panel). Scale bar: 1 μm.

neurons of L-hI93M mice might be more susceptible to MPTP toxin compared to that of non-Tg mice or hWT mice. As expected, significantly fewer TH-positive neurons were observed in L-hI93M mice after MPTP treatment as compared with hWT or non-Tg control mice though hWT express higher *hUCHL1* compared to L-hI93M (Fig. 3C). The number of TH-positive neurons in MPTP-treated hWT mice was somewhat higher than that in non-Tg mice ($p < 0.001$). Taken together with the fact that expression of human UCH-L1 in L-hI93M is lower than that in hWT, these results suggest that the UCH-L1^{I93M} mutant, but not UCH-L1^{WT}, is specifically toxic to dopaminergic neurons.

3.3. Presence of neuropathology in dopaminergic neurons from H-hI93M mice

To evaluate the degenerative process of dopaminergic neurons, silver staining was used to indicate argyrophilic degenerating neurons (Lo Bianco et al., 2004). In non-Tg mice, no silver staining was observed, whereas scattered neurons containing grains that were silver staining positive were present in the substantia nigra of H-hI93M mice (Fig. 4A). The presence of intracellular inclusions called Lewy bodies and Lewy neurites are neuropathological characteristics of PD and are silver staining positive (Sandmann-Keil et al., 1999; Uchihara et al., 2005). It is also known that UCH-L1 and ubiquitin, as well as α-synuclein, are components of Lewy bodies (Lowe et al., 1990). Furthermore, UCH-L1 is tightly associated with mono-ubiquitin *in vivo* (Osaka et al., 2003). Thus, we expected that the silver staining-positive grains might have characteristic features of Lewy bodies. We therefore compared the immunohistochemical analysis of UCH-L1 and ubiquitin. Compared with reduced staining for ubiquitin in *gad* mice, strong and diffuse ubiquitin staining was observed in nigral neurons of hWT mice and non-Tg mice (data not shown), and this staining co-localized with UCH-L1, which is in agreement with our previous report (Osaka et al., 2003). In H-hI93M substantia nigra at 20 weeks of age, ubiquitin- and UCH-L1-positive cytoplasmic inclusions, a large aggregates with different morphology from small dots usually seen in hWT mice and non-Tg mice, were observed in a portion of the remaining nigral neurons (Fig. 4B). These inclusions were, however, α-synuclein or hematoxylin–eosin (HE) negative (data not shown). We could not observe UCH-L1- and ubiquitin-positive inclusions in L-hI93M mice (data not shown).

Another cellular characteristic of PD neuropathology is dense-core vesicles of about 80–200 nm in perikarya, which are frequently observed along with Lewy bodies in PD patients (Watanabe et al., 1977). We observed electron dense-core vesicles in the cytoplasm of ~30% of nigral neurons in H-hI93M mice using electron microscopy (Fig. 4C). In non-Tg mice, such vesicles with a similar shape were not detected in cell bodies but rather were seen in synaptic terminals. Taken together, our data indicate that degenerating dopaminergic neurons in the substantia nigra of H-hI93M mice are devoid of Lewy bodies but show some neuropathological features such as silver staining-positive argyrophilic grains, aggregates with UCH-L1 and ubiquitin, and dense-core vesicles in the perikarya.

3.4. Increased amount of SDS-insoluble but urea/SDS-soluble UCH-L1 in the midbrain of H-hI93M mice

UCH-L1^{I93M} has reduced α-helical content as compared with UCH-L1^{WT} (Nishikawa et al., 2003), and UCH-L1^{I93M} overexpression in COS7 cells results in more cells that contain cytoplasmic inclusions (Ardley et al., 2004). Thus, the presence of UCH-L1-positive inclusions in H-hI93M dopaminergic neurons led us to speculate whether UCH-L1^{I93M} would be less soluble than the wild-type protein *in vivo*. To biochemically characterize the changes in UCH-L1 deposited in the brains of H-hI93M mice, we sequentially extracted frozen midbrain tissues with 5% SDS (soluble fraction) and 8 M urea/5% SDS (insoluble fraction) and analyzed each fraction by immunoblotting with anti-UCH-L1. As expected, immunoblots of insoluble fractions showed a modest but statistically significant increase in UCH-L1 in the midbrains of H-hI93M mice as compared with those from a non-Tg mouse (Fig. 5A and B), indicating increased insolubility of UCH-L1^{I93M} *in vivo*, which might have resulted in dopaminergic neurotoxicity.

3.5. Decreased dopamine content in the striata of H-hI93M mice

Because the nigro-striatal pathway is severely affected in PD patients, and because our mice showed the degeneration of dopaminergic neurons in the substantia nigra, we evaluated the nerve terminals in the striatal pathway using

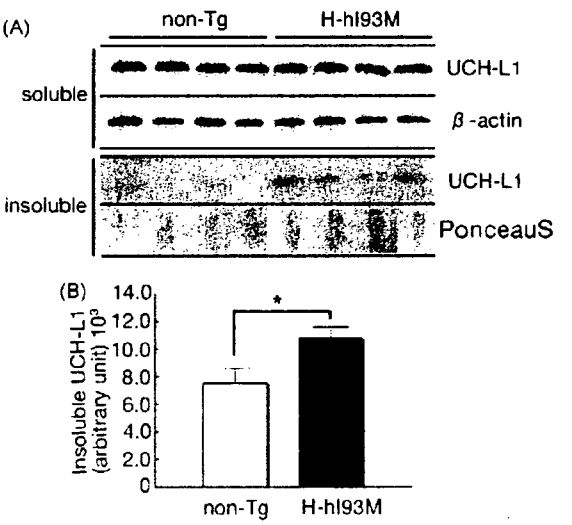

Fig. 5. Protein insolubility of UCH-L1 in H-hI93M Tg mice. (A) Immunoblotting analysis of UCH-L1 in soluble (5% SDS soluble) and insoluble (5% SDS insoluble and 8 M urea/5% SDS soluble) fractions from tissue containing the substantia nigra (11–13 weeks). Soluble fraction (5 μg for each) was probed with anti-UCH-L1 or anti-β-actin. Insoluble fraction (0.5 μg for each) was probed with anti-UCH-L1. One microgram of each insoluble fraction was applied to dot blotting and stained by Ponceau S to show that each fraction contained the same amount of total protein. A slight increase in the insolubility of UCH-L1 in the substantia nigra fraction from H-hI93M mice is seen as compared with that from non-Tg mice. (B) The experiment was done with H-hI93M mice and non-Tg littermates from five different litters, and the results of quantitative analyses in insoluble fraction is shown ($n = 5$ mice for each group).

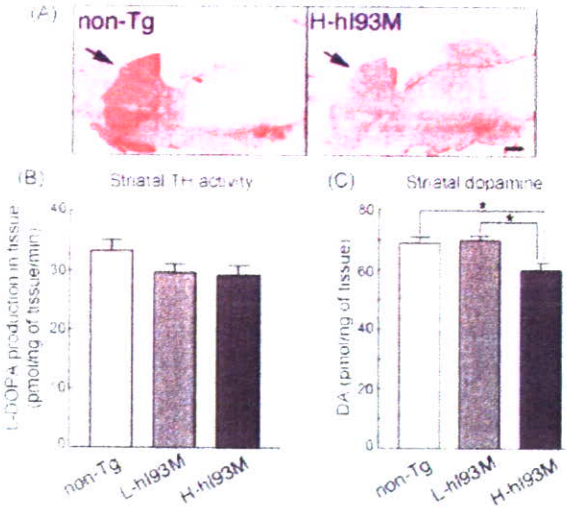

Fig. 6. H-hI93M mice show pathology in the striatum. Dopamine content and TH activity were lower in H-hI93M mice. (A) Sagittal sections from non-Tg and H-hI93M mice at 20 weeks of age were immunostained with the dopaminergic marker anti-TH. TH immunoreactivity is decreased in the nigro-striatal axons (arrows) of H-hI93M brains. Scale bar: 100 μm. (B) TH activity and (C) dopamine content were measured following extraction and homogenization of the mouse striatum of non-Tg, L-hI93M and H-hI93M mice at 20 weeks of age ($n = 4$; mean ± S.E). Significance was examined by a one-way ANOVA. $^{*}p < 0.05$.

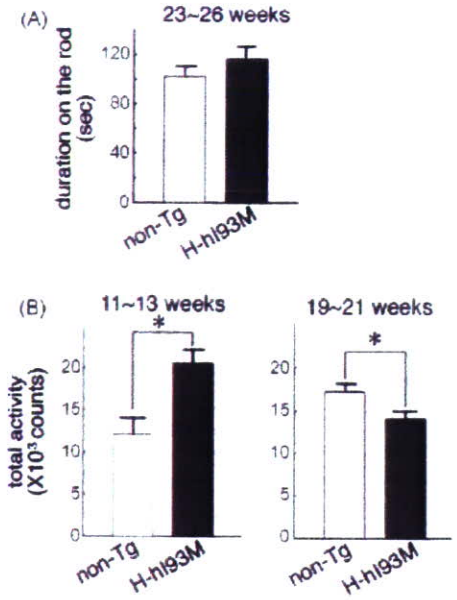

Fig. 7. H-hI93M transgenic mice show locomotor deficits. (A) Accelerated rota-rod analysis of H-hI93M and non-Tg mice ($n = 6$ for non-Tg and $n = 7$ for H-hI93M) at 23–26 weeks of age. Mice were placed on a rod, and their duration on the rod before falling off (mean value of three trials for each animal) was recorded. (B) Home cage monitor analysis of H-hI93M and non-Tg mice at 11–13 weeks of age (left; $n = 4$ for each line) and at 19–21 weeks of age (right; $n = 8$ for non-Tg and $n = 10$ for H-hI93M). Note the significant hypolocomotion of H-hI93M mice as compared with non-Tg mice at 19–21 weeks of age. Values are the mean ± S.E.M. Significance was examined using the unpaired Student's t-test. $^{*}p < 0.05$.

immunohistochemical and biochemical analyses. In agreement with the reduction of TH-positive dopaminergic neurons in the substantia nigra, nigro-striatal fibers in H-hI93M mice showed decreased immunoreactivity for TH as compared with that of non-Tg mice (Fig. 6A). TH activity, analyzed by determining L-DOPA production in the striatal tissues, also showed a tendency to decline in H-hI93M mice, although it was not significantly different (Fig. 6B). Loss of dopaminergic neurons in the substantia nigra and decreased TH activity in the striatum of H-hI93M mice prompted us to examine the concentration of striatal dopamine. Compared with non-Tg mice, H-hI93M mice showed a significant reduction of dopamine content in the striatum (Fig. 6C).

3.6. Decreased spontaneous, voluntary movements of H-hI93M mice

Given the prominent loss of dopaminergic neurons in the substantia nigra and the reduction in dopamine content in the striatum of H-hI93M mice, we next assessed the locomotor abilities of H-hI93M mice using a battery of well-established behavioral tests. Involuntary movement was analyzed by the rota-rod test (Goldberg et al., 2005) on 23–26-week-old mice. H-hI93M mice and non-Tg mice were similarly able to maintain their balance on the rotating rod during rod acceleration before falling off (Fig. 7A). We next analyzed spontaneous, voluntary movements with a locomotor activity test (Goldberg et al., 2005). Unexpectedly, 11–13-week-old H-hI93M mice showed significant hyperlocomotion during active periods (i.e., at night) as compared with non-Tg mice during home cage monitoring (Fig. 7B). However, 19–21-week-old H-hI93M mice showed a modest but significant reduction in locomotor activity during active periods as compared with non-Tg mice (Fig. 7B). These results indicate that, in addition to the neuropathological changes, H-hI93M mice exhibit mild behavioral deficits related to PD.

4. Discussion

In this study, we characterized transgenic mice expressing hUCH-L1^{I93M}, a mutation with presumptive association with familial PD, in the brain. Our previous attempt of making mouse UCH-L1^{WT} Tg mice under various higher expressing promoters, such as EF1α, resulted in an infertility of mice, thus it was impossible to maintain the lines. This failure resulted from the effect of overexpressing UCH-L1 in the testis/ovary leading to an increased apoptosis in these reproductive organs, although we did not find obvious morphological differences in the brain (Wang et al., 2006). Thus, we used *PDGF-B* promoter in this study to avoid massive expression of the transgene.

Two lines of hUCH-L1^{I93M} Tg mice and one line of hUCH-L1^{WT} Tg mice were viable and fertile without any predictable abnormalities. All of the three Tg lines expressed very limited levels of the human *UCHL1* gene with a maximum transcript ratio of about 1/100 as compared with the endogenous mouse *Uchl1*. However, immunohistological analysis indicated that higher level of hUCH-L1^{I93M} expression could be detected in the large number of neurons in the substantia nigra of

H-hI93M/gad mice at 2 weeks of age. In addition, there is a difference in the morphology of hUCH-L1^{I93M} expressing neurons, reminiscent of dying neurons, in the substantia nigra of H-hI93M/gad mice among 7 and 20 weeks of age. We also observed an eventual decline in the number of UCH-L1-positive neurons in H-hI93M/gad mice, as they age. Furthermore, the dopaminergic neurons in the substantia nigra of H-hI93M mice at 12 weeks of age showed silver staining-positive argylophilic grains, which represent neurons undergoing degeneration (Lo Bianco et al., 2004). Since we observed a loss of dopaminergic neurons in the substantia nigra and reduced dopamine content in the striatum of H-hI93M mice at 20 weeks of age, our results indicate the possibility that hUCH-L1^{I93M} expressing dopaminergic neurons degenerate with age.

In addition to cell loss, several neuropathological features were observed in the substantia nigra of H-hI93M mice. Dopaminergic neurons had (1) electron dense-core vesicles in the perikarya, and (2) cytoplasmic inclusions that were positive for both UCH-L1 and ubiquitin. Despite these features, we did not observe eosinophilic or α-synuclein-positive Lewy bodies at the substantia nigra in our morphological analyses. Thus, the mouse dopaminergic neurons expressing UCH-L1^{I93M} may die prior to the formation of Lewy bodies, or those mice might form these structures at stages beyond the period of our study.

The mechanisms responsible for dopaminergic cell loss in the substantia nigra of H-hI93M mice remain elusive. The I93M mutation in UCH-L1 reduces its hydrolase activity by about 50%, which has been suggested as a cause for the pathogenesis of PD (Nishikawa et al., 2003). However, we have not found clear evidence for nigro-striatal dopaminergic pathology in *gad* mice (data not shown). Since expression of UCH-L1 is not detected in gad mice, the reduction of hydrolase activity alone would not be the cause of PD. In light of our finding here that transgenic expression of UCH-L1^{I93M} results in dopaminergic pathology in mice, it would seem that this mutation elicits a gain of toxic function leading to the neuronal toxicity in the substantia nigra.

Our previous work using circular dichroism suggests that the I93M mutation reduces the α-helical content of UCH-L1 (Nishikawa et al., 2003). Recently, we had also showed, using small-angle neutron scattering, that wild-type or I93M mutant UCH-L1 exists as a dimmer in an aqueous solution. Moreover, their configuration differed; wild-type UCH-L1 has ellipsoidal shape where as I93M mutant has more globular shape (Naito et al., 2006). Cells expressing UCH-L1^{I93M} are more prone to form inclusions (Ardley et al., 2004). Proteomic analysis of autopsied brains from PD patients and AD patients shows that UCH-L1 is extensively modified by carbonyl formation, methionine oxidation and cysteine oxidation in the diseased brains (Choi et al., 2004). These modifications are shown to result from oxidative stress (Choi et al., 2004). We show here that I93M mutation in UCH-L1 increases its insolubility *in vivo*. From the very limited expression of human UCH-L1 I93M, it is possible to speculate that endogenous mouse UCH-L1 might become insoluble in the presence of I93M UCH-L1. In addition, L-hI93M neurons were more susceptible than hWT or non-Tg neurons to MPTP, an inhibitor of complex I. This observation suggests that UCH-L1^{I93M} easily gains toxicity under oxidative stress. The conformational change and/or the additional methionine oxidation in UCH-L1 caused by I93M mutation may cause increased insolubility and lead to the gain of a toxic function.

In addition, our behavioral analysis revealed that H-hI93M mice exhibit very slight defects in spontaneous, voluntary movement, as shown by their hyperlocomotion at 11–13 weeks of age and by their hypolocomotion at 19–21 weeks of age in the home cage monitor test. Patients with PD exhibit no clinical symptoms until 70–80% of dopaminergic neurons are lost (Dauer and Przedborski, 2003). Thus, the level of dopaminergic neuronal loss seen in H-hI93M mice might not be sufficient to produce severe clinical phenotypes. It is difficult to explain the hyperlocomotion detected at 11–13 weeks of age, by simple changes in the nigro-striatal pathway. Other brain areas might be related to the locomotor changes seen in H-hI93M mice. We will need further analysis to connect the dopaminergic cell loss and defects in spontaneous, voluntary movement in H-hI93M mice.

In attempts to replicate neuropathological aspects of PD, several of the familial PD genes have been altered in mice. Up to date, α-synuclein Tg mice with or without mutation (Fernagut and Chesselet, 2004), parkin knockout mice (Goldberg et al., 2003; Itier et al., 2003; Palacino et al., 2004; Perez and Palmiter, 2005; Von Coelln et al., 2004), and DJ-1 knockout mice (Chen et al., 2005; Goldberg et al., 2005; Kim et al., 2005) have been reported. Although these mice show some alterations in the function of dopaminergic neurons, none has dopaminergic neuron loss in the substantia nigra. Thus, we have developed the first mouse model with an alteration in a familial PD gene that leads to dopaminergic cell loss. Further analysis of these mice will help establish the role of UCH-L1 in PD, which may elucidate a common pathway for both familial and sporadic PD.

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Miho Murata

Pharmacokinetics of L-dopa

Special reference to food and aging

Abstract According to our data in rats, peripheral 3.4-dihydroxyphenylalanine (DOPA) kinetics are similar to striatal DOPA and dopamine kinetics. The measurement of plasma l-3.4-dihydroxyphenylalanine (L-dopa) concentration is thus useful to predict dopamine kinetics in the striatum and to treat the motor fluctuations of parkinsonian patients. In patients with Parkinson's disease (PD), long-term L-dopa therapy accelerated DOPA absorption and steepened features of L-dopa pharmacokinetics. In the senile-onset group, the pharmacokinetic pattern did not change even after long-term L-dopa therapy. The frequency of motor fluctuations is much lower in senile-onset patients with PD than in middle-onset patients. Differences in the pattern of L-dopa pharmacokinetics in the two groups may explain why the senile-onset group rarely develops 'wearing-off', even after long-term L-dopa therapy. L-dopa is transported by a saturable active transporter system, called the LNAA (large neutral amino acid) system, in the gut and blood brain barrier. L-dopa absorption is thus affected by food intake, especially a protein-rich diet. The slope of the time-concentration curve for L-dopa administered before a meal is steeper than if it is administered after a meal. Considering that pulsative stimulation of L-dopa may cause motor fluctuations, L-dopa should be given after meals whenever possible, even if it necessitates a higher L-dopa dose.

Key words Parkinson's disease · absorption · LNAA system · L-dopa · aging

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Introduction

L-dopa is the gold standard of antiparkinsonian pharmacotherapy; however, motor fluctuations, such as 'wearing-off', often develop during long-term L-dopa therapy. The definition of wearing-off is fluctuation of parkinsonian symptoms in line with L-dopa pharmacokinetics [1]. Therefore, the pharmacokinetics of L-dopa are very important in treating patients with Parkinson's disease (PD). The half-life ($T_{1/2}$) of L-dopa is short (1 h) and its absorption is greatly influenced by food intake and aging. Thus, these factors make PD therapy complicated.

Because L-dopa is used with a DOPA decarboxylase inhibitor (DCI), catechol-O- methyltransferase (COMT) is an important enzyme influencing peripheral L-dopa metabolism and L-dopa effects on parkinsonian symptoms. Therefore, nowadays, knowledge about L-dopa pharmacokinetics is increasingly important in treating PD.

In the present review, DOPA and dopamine kinetics in blood and brain, and food and aging effects on the pharmacokinetics of L-dopa are discussed. The correlation between DOPA and 3-O-methyl DOPA is also featured.

L-dopa and dopamine kinetics in peripheral blood and striatum

L-dopa pharmacokinetics are very important when treating PD, and the L-dopa concentration can be measured in blood. Dopamine kinetics in the brain are more

critical than peripheral DOPA kinetics; however, it is very difficult to measure dopamine in the brain of PD patients in vivo. Therefore, it is important to know how closely peripheral L-dopa kinetics reflect dopamine kinetics in the brain, especially in the striatum.

We measured DOPA and dopamine concentrations in the blood and striatum of normal rats after single and repeated L-dopa administration (Fig. 1) [2]. DOPA and dopamine kinetics in the striatum were well correlated with peripheral DOPA kinetics. We also showed that repeated L-dopa (L-dopa 50 mg/kg + benserazide 12.5 mg/kg) administration for 28 days increased the area under the concentration-time curve (AUC) and shortened $T_{1/2}$ and time to maximum plasma concentration (T_{max}) for both peripheral DOPA and central DOPA and dopamine in normal rats.

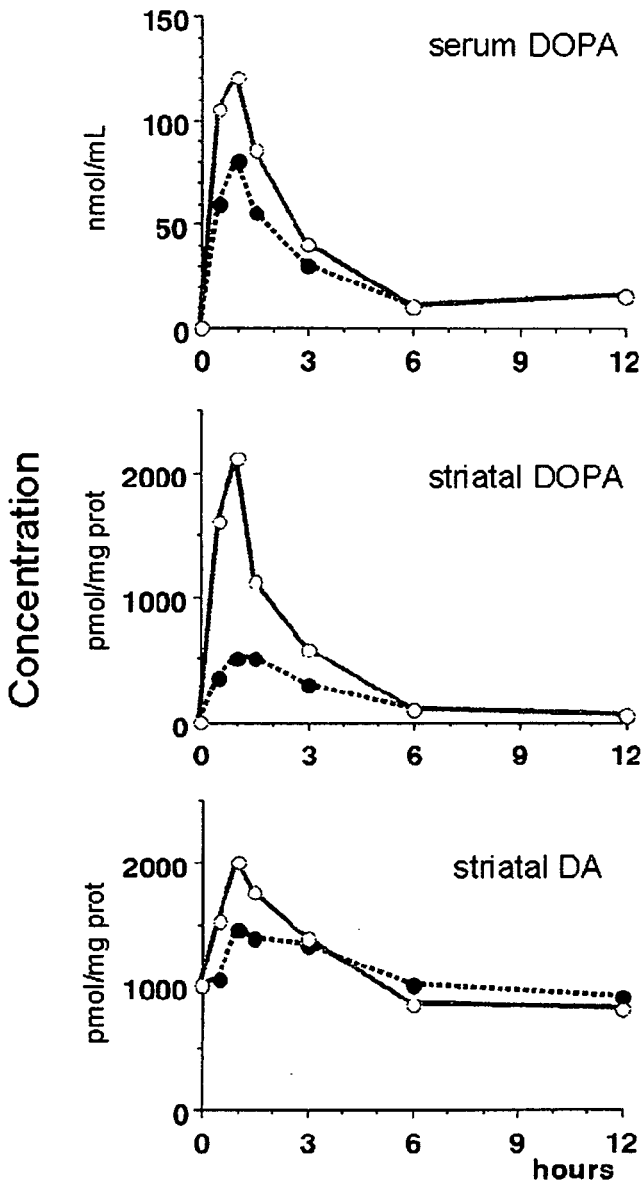

Fig. 1 Time course of serum L-dopa and striatal DOPA and dopamine (DA) after single and repeated administration of L-dopa in rats (-----●----- single administration, ——o—— repeated administration). Time course of serum L-dopa concentration is similar to that of striatal DOPA and dopamine. Repeated L-dopa administration increases C_{max} and AUC and shortens $T_{1/2}$

L-dopa pharmacokinetics in PD patients

PD patients were given L-dopa 100 mg plus benserazide 25 mg orally at 8:00 am after an overnight fast. Plasma DOPA concentrations were measured prior to treatment (baseline) and at 15 min, 30 min, 1 h, 2 h, 3 h and 4 h after medication using HPLC-ECD (L-DOPA test) [3]. In PD patients (onset age < 60 years old) who had received L-dopa therapy for longer than 5 years, the $T_{1/2}$ and T_{max} of L-dopa were much shorter than those measured in PD patients with a duration of L-dopa therapy of less than 5 years (Fig. 2a). In addition, the AUC was greater in the longer therapy duration group (Fig. 2a). These changes in the pharmacokinetics of L-dopa were significantly correlated with duration of L-dopa therapy and dose of L-dopa. A 4-year longitudinal study showed that four of five patients displayed an increased AUC and shortened $T_{1/2}$ and T_{max} at the second assessment [4]. Patients who demonstrated the wearing-off phenomenon had a significantly higher maximum plasma concentration (C_{max}) and greater AUC, and significantly shorter $T_{1/2}$ and T_{max} than those who did not display wearing-off. The pattern of L-dopa kinetics in those with wearing-off was obviously steeper than that of patients without wearing-off.

It is reasonable to suppose that these changes in pharmacokinetic features are due to changes in absorption or metabolism of L-dopa. Decreased metabolism of L-dopa can explain the increase in AUC and C_{max}, but cannot explain the shortening of T_{max} and $T_{1/2}$. Increased absorption, however, can explain the increase in AUC and C_{max} and the shortening of T_{max}. If the absorption system is saturable, increased absorption can also explain the shortening of $T_{1/2}$. L-dopa is transported by the saturable active transport system called the LNAA (large neutral amino acid) system in the gut and blood brain barrier (BBB) [5]. Furthermore, intravenous administration has demonstrated that the distribution and elimination of L-dopa was not changed after long-term L-dopa therapy [6]. Both monoamine oxidase (MAO) activity and COMT activity in the brain are unaffected by long-term L-dopa administration [2, 7]. Therefore, our results show that long-term L-dopa therapy alters its own kinetics by increasing the absorption of L-dopa. As early as 1971, Abrams et al. reported that long-term L-dopa therapy increases its own absorption [8]. At that time, L-dopa therapy involved L-dopa administered without a DCI, and liver DOPA decarboxylase (DDC) ac-

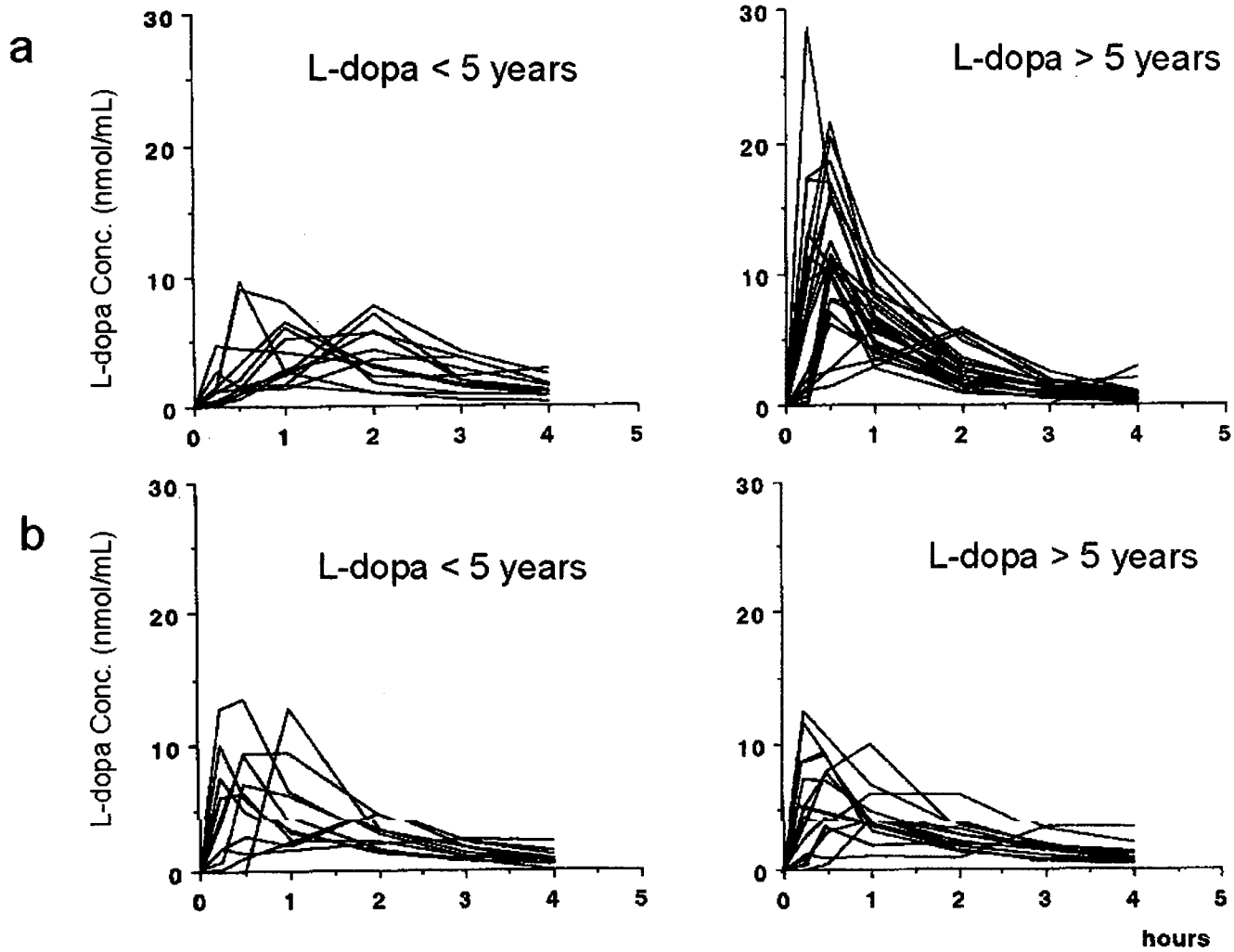

Fig. 2 L-dopa pharmacokinetics in parkinsonian patients with disease onset at < 60 years of age (**a**) and > 60 years of age (**b**) according to duration of L-dopa therapy. Long-term L-dopa therapy (> 5 years' duration) increases C_{max} and AUC and shortens $T^1/_2$. Pharmacokinetic changes after long-term L-dopa therapy are not seen in the senile-onset group

tivation was suggested as a cause of this phenomenon [9]. In fact, long-term L-dopa administration activates DDC in the liver but not in the brain, and no data has been published in the gut [10]. Our data was obtained using L-dopa with a DCI. It has been reported that plasma DDC is induced by administration of L-dopa with a DCI [11]. Therefore, the DDC activation theory cannot explain our results. We propose that long-term L-dopa therapy may induce the LNAA transporter system.

Aging and L-dopa pharmacokinetics

Although long-term L-dopa therapy steepened L-dopa kinetics, this change was less marked in senile-onset patients (onset age > 60 years old) than in younger onset patients (Fig. 2b). The frequency of wearing-off is much lower in senile-onset patients than in younger onset patients [12]. This suggests that changes in peripheral L-dopa pharmacokinetics after long-term therapy certainly contribute to the clinical expression of wearing-off.

Food and acidity effects on L-dopa absorption

L-dopa shares a saturable transporter system with other LNAA such as phenylalanine. Therefore, competitive inhibition of L-dopa absorption occurs with rising concentrations of neutral amino acids derived from food (Fig. 3). The L-dopa pharmacokinetic profile is steeper when intake occurs before a meal than after a meal. Considering that pulsative stimulation of L-dopa may cause motor fluctuations, L-dopa should be given after meals

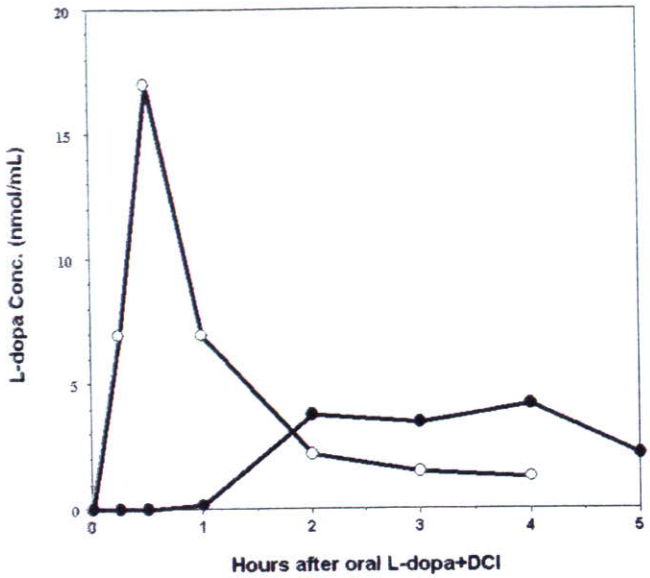

Fig. 3 Effects of a meal on L-dopa kinetics in a 55-year-old female patient with Parkinson's disease (○ L-dopa administration before meal, ● L-dopa administration after meal). C_{max} and AUC were markedly decreased and T_{max} was increased by L-dopa administration after a meal

whenever possible, even if it necessitates a higher L-dopa dose.

L-dopa is known to be easily soluble in acid environments and the pH of gastric juices affects the absorption of L-dopa. Fig. 4 shows the results of an L-DOPA test from a 65-year-old male patient with PD. The first test was performed using the ordinary method and, 1 year later, the second test was performed using duodenal infusion of L-dopa. Although the pH of duodenal juice is high, absorption was not impaired because L-dopa was administered dissolved in water. When it is dissolved in water, L-dopa is absorbed rapidly and adequately, even in alkaline duodenal juice.

L-dopa and 3-O-methyl DOPA

The main metabolite of DOPA is dopamine, formed by decarboxylation, and the COMT pathway is usually a rather minor pathway in the metabolism of DOPA. However, when L-dopa is used with a DCI, the COMT pathway is activated and a large amount of 3-O-methyl DOPA (3OMD) is synthesized. The $T_{1/2}$ of 3OMD (16 h) is much longer than that of DOPA; thus, the plasma concentration of 3OMD increases according to long-term L-dopa therapy (Fig. 5). Although the plasma concentration of 3OMD is usually closely correlated to daily L-dopa dose (Fig. 6), some patients show very low 3OMD concentrations relative to the L-dopa dose and plasma L-dopa concentration. These patients may obtain a good response with a COMT inhibitor. As COMT inhibitors will be approved for PD therapy in Japan this year, it will be important to assess this hypothesis soon.

3OMD also uses the LNAA transporter system in the gut and BBB. After protein-rich meals, competition between L-dopa, dietary LNAA and 3OMD for gut and BBB transport may further contribute to motor fluctuations.

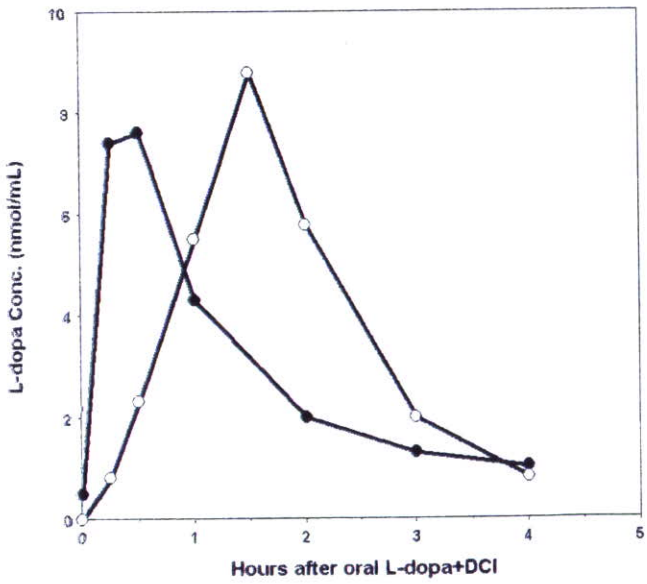

Fig. 4 Effects of duodenal infusion of L-dopa in a 65-year-old male patient with Parkinson's disease (○ L-dopa administration by tablet orally, ● L-dopa administration by duodenal infusion in water suspension). L-dopa concentration is rapidly and adequately increased by duodenal infusion

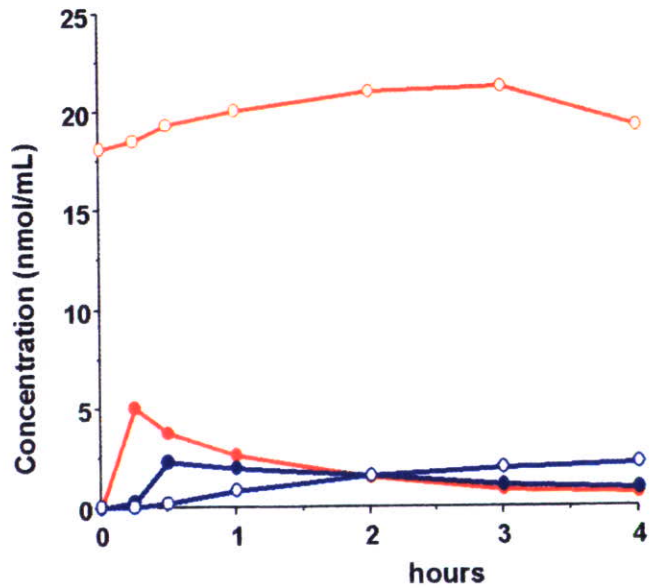

Fig. 5 Plasma concentration of dopa and 3OMD in PD patients (○ concentration of 3OMD, ● concentration of dopa, red line: long-term L-dopa therapy, blue line: L-dopa initial use)

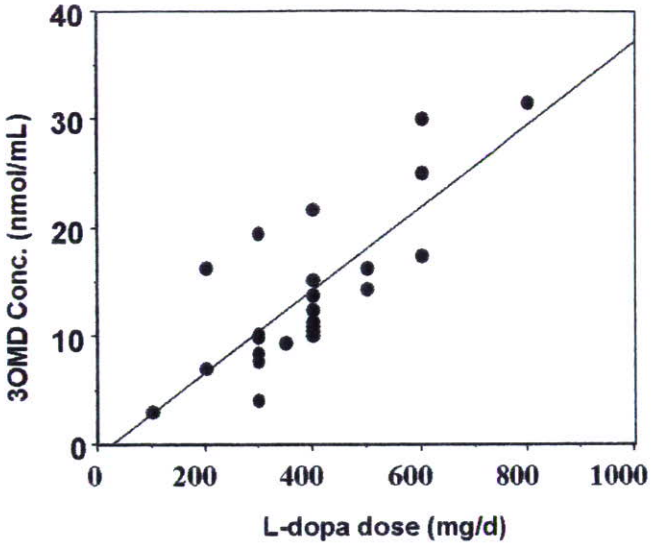

Fig. 6 Relationship between L-dopa daily dose and plasma concentration of 3OMD in PD patinets

Following L-dopa administration without a DCI, the plasma dopamine concentration is greatly increased and the 3OMD concentration is very low; however, L-dopa administration with a DCI results in synthesis of a large amount of 3OMD in plasma (Fig. 7) by activating the COMT pathway. If L-dopa is administered in combination with both a DCI and a COMT inhibitor, new metabolic pathways may be activated such as enhanced quinine formation [13].

Conclusion

Peripheral L-dopa kinetics closely reflects dopamine kinetics in the striatum so that measurements of L-dopa kinetics are useful for treating patients with PD. L-dopa is transported by a saturable active transporter system (LNAA system) in the gut and BBB. Onset age of PD, treatment duration, and food are greatly influence peripheral L-dopa kinetics. Food and onset age decrease C_{max} and prolong T_{max} and $T_{1/2}$. When L-dopa is administered with a DCI, the COMT pathway is activated and the COMT inhibitor becomes an important factor for peripheral L-dopa kinetics.

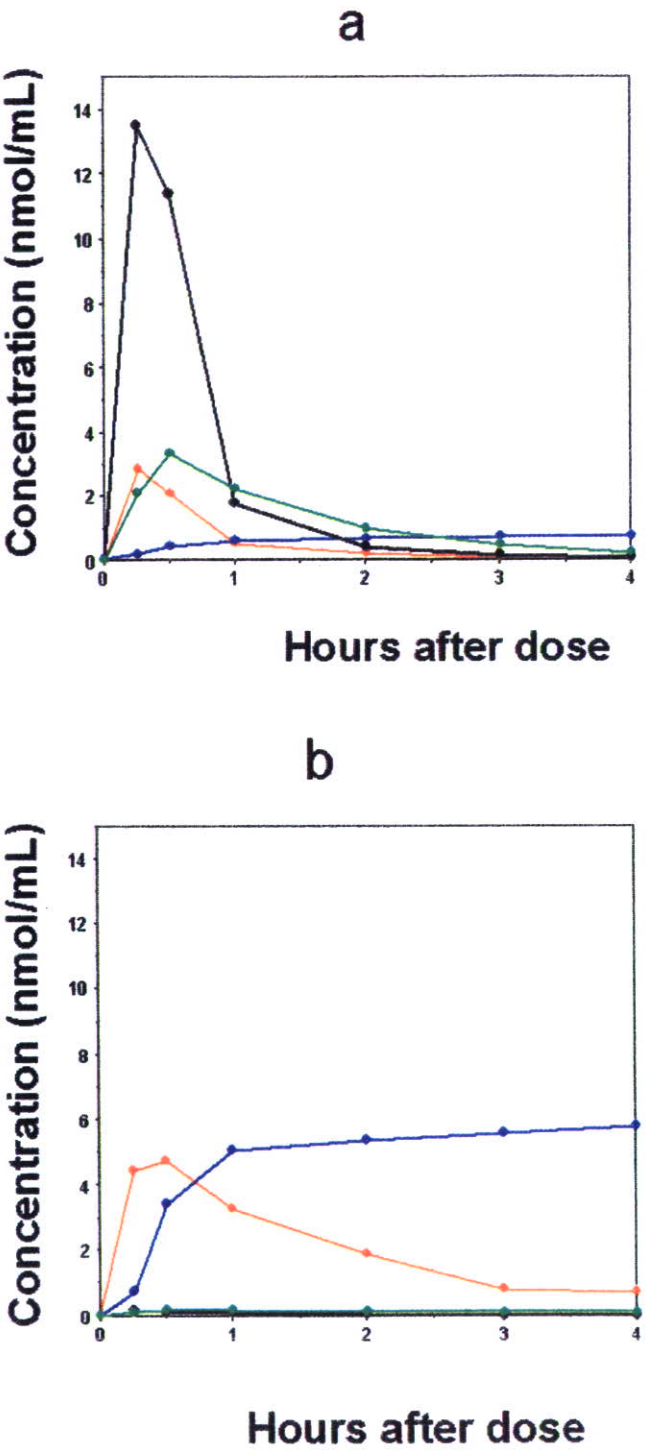

Fig. 7 Plasma concentration of DOPA and its metabolites after oral administration of L-dopa (100 mg) without DCI (**a**), and oral administration of L-dopa (100 mg) with DCI (benserazide 25 mg) (**b**) of the same PD patient (red: DOPA; black: dopamine; blue: 3OMD; green: homovanillic acid (HVA))

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CME

Zonisamide improves motor function in Parkinson disease

A randomized, double-blind study

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Abstract—*Objective:* To evaluate the efficacy, safety and tolerability of daily doses of 25, 50, and 100 mg of zonisamide (ZNS) administered as adjunctive treatment in patients with Parkinson disease (PD). *Methods:* We conducted a multicenter, randomized, double-blind, parallel-treatment, placebo-controlled study in Japan. Patients with PD who showed insufficient response to levodopa treatment were given placebo for 2 weeks and then treated for 12 weeks with 25, 50, or 100 mg/day of ZNS or placebo, in addition to levodopa, followed by a 2-week dose-reduction period. The primary endpoint was change from baseline in the total score of the Unified Parkinson's Disease Rating Scale (UPDRS) Part III at the final assessment point. Secondary endpoints included changes from baseline in total daily "off" time; total scores of UPDRS Parts I, II, and IV; and Modified Hoehn and Yahr Scale score. Safety analysis was based on the incidence of adverse events. *Results:* There was significant improvement in the primary endpoint in the 25-mg and 50-mg groups vs placebo. The duration of "off" time was significantly reduced in the 50-mg and 100-mg groups vs placebo. Dyskinesia was not increased in ZNS groups. The incidence of adverse effects was similar between the 25-mg, 50-mg, and placebo groups but higher in the 100-mg group. *Conclusions:* Zonisamide is safe, effective and well tolerated at 25 to 100 mg/day as an adjunctive treatment in patients with Parkinson disease.

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Zonisamide (ZNS) (1,2-benzisoxazole-3-methanesulfonamide) is an antiepileptic drug with a long-half life ($T_{1/2}$ = 62 hours) that was originally synthesized in Japan.[1] ZNS has been used to treat epilepsy in Japan for more than 10 years; is currently approved for marketing in the United States, Europe, and Korea; and is generally well tolerated. We reported previously that ZNS has beneficial effects on PD in one patient with convulsion attacks.[2] Based on this finding, we subsequently performed an open trial in nine patients with PD and found that ZNS improved the main symptoms of PD, with particular benefits on motor fluctuation, known as "wearing-off."[2] Then, we conducted a small double-blind study that showed a daily dose of 50 to 100 mg of ZNS as an adjunct therapy significantly improved limb rigidity, tremor, and postural instability in patients with advanced PD and was well tolerated.[3]

In this study, we sought to confirm ZNS effectiveness as an adjunctive treatment for PD by evaluating the efficacy, safety, and tolerability of daily oral doses of 25, 50, and 100 mg of ZNS (once a day) in a large population of patients with PD who showed insufficient response to levodopa treatment.

Additional material related to this article can be found on the *Neurology* Web site. Go to www.neurology.org and scroll down the Table of Contents for the January 2 issue to find the title link for this article.

Methods. This was a multicenter, randomized, double-blind, parallel-treatment, placebo-controlled study of ZNS as adjunctive treatment in patients with PD who showed insufficient response to levodopa (including dopa decarboxylase inhibitor: DCI combination drugs). Fifty-eight institutions throughout Japan participated in the study during the study period of January 15 to December 1, 2004.

Patients with PD of both sexes between ages 20 and 80 years were enrolled in the study. Patients who exhibited any problems based on levodopa therapy, such as wearing-off phenomena, "on"–"off" phenomena, and freezing phenomena, no-"on" and delayed-"on," or in whom the suboptimal dose of levodopa had been administered because of side effects or therapeutic strategy were not excluded from the study. Patients had received individual dosages of levodopa (plus a DCI) and were stable for at least 28 days before study initiation. Patients who fulfilled the above criteria were enrolled into the study by the investigators at each participating institution. Patients who met the above criteria and provided informed consent were randomized into the treatment groups of 25, 50, or 100 mg/day ZNS or placebo.

The study consisted of a 2-week run-in period of single-blind treatment with placebo, a 12-week double-blind treatment period, and a 2-week double-blind dose-reduction period (figure E-1 on the *Neurology* Web site at www.neurology.org), with the exception

*See the appendix for a full list of study participants.

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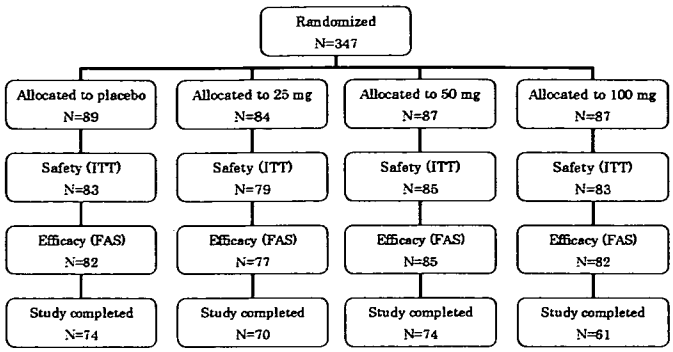

Figure 1. Patient disposition. ITT = intent-to-treat; FAS = full analysis set.

that the 25-mg group did not undergo dose reduction. Baseline assessment was conducted after a 2-week run-in period to reduce placebo effects. Clinical assessment including the Unified Parkinson's Disease Rating Scale (UPDRS) and Hoehn and Yahr staging was conducted at "on" state every 2 weeks.

The daily dosage was administered orally once a day in the morning as four tablets in the run-in and treatment periods and two tablets in the dose-reduction period. Study medication was dispensed as ZNS 25 mg tablets or matching placebo. Patients were randomized to one of the four treatment groups in blocks of size 8 (2 patients per group) during the run-in period using a randomization code generated by the study sponsor or designee. Study medication, indistinguishable by appearance, packaging, and labeling, was provided to each institution, and 1-week supplies were dispensed to patients according to the randomization code.

Patients were required to have concomitant administration with levodopa preparations including DCI combination drugs and were allowed to continue with other anti-Parkinson medications, such as dopamine receptor agonists (DAs), monoamine oxidase type B (MAO-B) inhibitors, anticholinergics, amantadine, or droxydopa, during the study. The dose regimens of these concomitant medications were to be maintained from 4 weeks before study initiation until the end of the dose-reduction period, except as required to alleviate dyskinesia or psychotic symptoms that were likely caused by dopaminergic-receptor hyperstimulation due to concomitant medication.

The primary endpoint was a change from baseline in the total score of UPDRS Part III (motor examination score) at the final assessment point. Secondary endpoints included a change from baseline in total daily "off" time as determined from patients' diaries, and changes from baseline in UPDRS Part I, II, and IV scores and Modified Hoehn and Yahr Scale score. Changes from baseline at the assessment point were analyzed by analysis of covariance using treatment group as a factor, and baseline value and treatment group scores were compared with placebo using the Dunnett test. A significance level of 0.05 (two-sided) was used for intergroup comparison, except for homogeneity assessment, when a significance level of 0.15 (two-sided) was used. The planned sample size of 80 patients per group (320 patients in total) was selected based on a requirement of 69 patients per group to achieve 80% power for comparison between placebo and each of the ZNS groups, assuming a between-group difference of 5.5 and an SD of 10.0 on the primary endpoint as seen in a preliminary study.[3] Multiplicity was taken into consideration in the primary analysis, but not in the secondary analysis or assessment of the dose–response relationship. Subgroup subset analysis was performed for the primary endpoint. Safety assessment was based on the incidence of adverse events including abnormalities of clinical/laboratory examinations and the incidence compared between the treatment groups by χ^2 test. Demographic and efficacy analyses were performed on the full analysis set (FAS), and safety assessments were performed on the intent-to-treat (ITT) population.

Results. Patient disposition is summarized in figure 1. Of the 347 screened and randomized patients, 279 patients (80.4%) completed the protocol as planned. There were no major differences between groups except that markedly fewer patients in the ZNS 100 mg group completed the study. The ITT population consisted of 330 patients (95.1%), with 83 patients in the placebo group, 79 in the 25-mg group, 85 in the 50-mg group, and 83 in the 100-mg group. A total of 6 patients in the placebo group, 5 in the 25-mg group, 2 in the 50-mg group, and 4 in the 100-mg group were not included in the ITT population because of withdrawal of consent or dosing regimen violation. The FAS consisted of the ITT minus 4 patients: 2 in the 25-mg group and 1 in each of the placebo and 100-mg groups because of no efficacy data during and after treatment period. Of the 326 patients (FAS), 279 patients completed the therapy period, and 47 patients discontinued therapy prematurely (8 patients in the placebo group, 7 in the 25-mg group, 11 in the 50-mg group, and 21 in the 100-mg group). The most common reason for discontinuation was adverse events (4 patients in the placebo group, 5 in the 25-mg group, 4 in the 50-mg group, and 9 in the 100-mg group). There were no Good Clinical Practice deviations in this study.

Table 1 shows the demographic background of patients in the placebo and ZNS treatment groups. There were no major differences between groups with respect to patients' background, including disease and treatment histories. The mean morbidity period was 8.6 years, and the mean modified Hoehn and Yahr Scale score ("on") was 2.5. The mean number of concomitant anti-Parkinson medicines was 3.2. The most common concomitant medications were DAs, which were used by 91.7% of the patients, and MAO-B inhibitors, which were used by 51.5% of the patients.

The changes (least-squares mean ± SE) in UPDRS Part III total score from baseline at final assessment were as follows: placebo group, −2.0 ± 0.8; 25-mg group, −6.3 ± 0.8; 50-mg group, −5.8 ± 0.8; and 100-mg group, −4.6 ± 0.8 (figure 2). All treatment groups showed decreases of UPDRS Part III total scores from baseline, but the improvement was significant for the 25-mg ($p = 0.001$, Dunnett test) and 50-mg ($p = 0.003$, Dunnett test) groups, vs the placebo group.

The proportions of responders, defined as patients with ≥30% reduction in UPDRS Part III total score from baseline at final assessment, were as follows: placebo group, 22.0% (18/82); 25-mg group, 35.1% (27/77, $p = 0.067$, χ^2 test vs placebo group); 50-mg group, 38.8% (33/85, $p = 0.018$, χ^2 test vs placebo group); and 100-mg group, 31.7% (26/82, $p = 0.158$, χ^2 test vs placebo group).

The degree of change for the primary endpoint were similar in the 25-mg and 50-mg groups, and these were greater than in the 100-mg group and significantly greater than in the placebo group. Subgroup analyses indicated no significant effects in subject baseline characteristics including with or without MAO-B inhibitor (table E-1) on the primary endpoint.

The mean decrease in total "off" time from baseline at final assessment is shown in figure 3. The mean changes in "off" time (hours) from baseline were as follows: placebo group, −0.20 (n = 61); 25-mg group, −0.22 (n = 58); 50-mg group, −1.30 (n = 68); and 100-mg group, −1.63 (n = 52). The duration of daily "off" time decreased for all treatment groups with improvement in the 50-mg ($p = 0.014$, Dunnett test) and 100-mg ($p = 0.013$, Dunnett test) groups compared with the placebo group.

Table 1 *Demographic and baseline characteristics of patients according to the dose of zonisamide*

		ZNS		
	Placebo	25 mg/day	50 mg/day	100 mg/day
n	82	77	85	82
Age, years	65.3 (7.5)	65.1 (8.5)	63.9 (9.4)	65.7 (8.6)
Older than 65 years	47 (57.3%)	42 (54.5%)	46 (54.1%)	53 (64.6%)
Men	41 (50.0%)	42 (54.5%)	51 (60.0%)	47 (57.3%)
Duration of PD, years	8.9 (5.8)	8.5 (4.6)	8.6 (6.0)	8.5 (5.6)
Dose of l-dopa, mg/day	351.2 (138.8)	355.5 (115.6)	363.9 (177.4)	327.7 (118.2)
Wearing-off	67 (81.7%)	64 (83.1%)	74 (87.1%)	62 (75.6%)
Dyskinesia	28 (34.1%)	18 (23.4%)	33 (38.8%)	22 (26.8%)
+ Dopamine agonist	80 (97.6%)	76 (98.7%)	85 (100.0%)	80 (97.6%)
+ MAO-B inhibitor	42 (51.2%)	38 (49.4%)	43 (50.6%)	45 (54.9%)
UPDRS Part III	22.9 (10.7)	26.5 (13.0)	22.5 (13.1)	22.7 (11.6)
H-Y ("on")	2.60 (0.72)	2.68 (0.76)	2.49 (0.80)	2.60 (0.77)
H-Y ("off")	3.52 (0.80)	3.64 (0.80)	3.49 (0.90)	3.40 (0.77)
"Off" time, hours	7.13 (3.45)	6.76 (3.13)	6.51 (2.30)	7.62 (3.03)

Data are mean (SD) or number (%).

ZNS = zonisamide; PD = Parkinson disease; H-Y = Modified Hoehn and Yahr Scale score.

There were no significant differences between the ZNS and placebo groups with respect to changes from baseline in UPDRS Parts I, II, and IV scores and in the Modified Hoehn and Yahr Scale score.

Some patients showed increased duration of dyskinesia with increase of "on" time; however, the frequency of dyskinesia was not increased in the entire ZNS group compared with the placebo group. Further analysis showed a decrease in disabling dyskinesia (UPDRS Part IV, No. 33) in the 50-mg group (table 2). In addition, the basal dose of levodopa did not correlate with worsening or improvement of dyskinesia.

There was no significant difference in incidence of adverse events between the 25-mg (a total of 164 adverse events reported by 70.9% [56/79] of the patients) and 50-mg (195 adverse events reported by 72.9% [62/85] of the patients) groups, compared with the placebo group (153 adverse events by 65.1% [54/83] of the patients). However, the incidence of adverse events was significantly higher in the 100-mg group (204 adverse events reported by 79.5% [66/83] of the patients) compared with the placebo group ($p = 0.037$, χ^2 test). Adverse events with an incidence of greater than 5% in the ZNS group are presented in table 3. Adverse events for which the incidence was greater in the total ZNS than in the placebo group were somnolence (10.9%), apathy (8.5%), decrease in body weight (6.9%), and constipation (6.5%). Adverse events for which the incidence in the total ZNS was less than that of the placebo

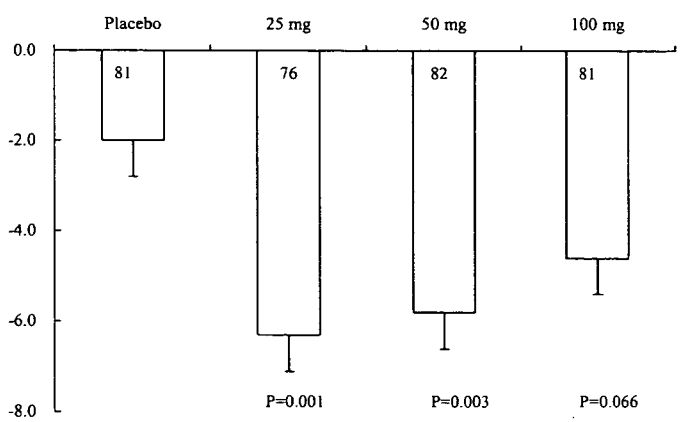

Figure 2. Changes in Unified Parkinson's Disease Rating Scale (UPDRS) Part III total score induced by zonisamide (ZNS) treatment from baseline to end of study (least-squares mean ± SE). Numbers indicate patient numbers. The total score of UPDRS Part III decreased after treatment in the 25-mg/day ($p = 0.001$) and 50-mg/day ($p = 0.003$) ZNS groups compared with the placebo group.

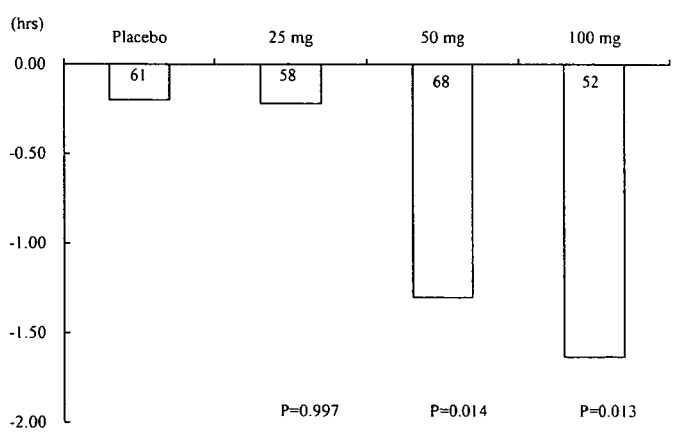

Figure 3. Changes from baseline in mean daily "off" time (hours) induced by treatment with zonisamide (ZNS). Numbers indicate patient numbers. "Off" time decreased after treatment with ZNS in the 50-mg/day (−1.3 hours, $p = 0.014$) and 100-mg/day (−1.63 hours, $p = 0.013$) groups compared with the placebo group.

Table 2 Changes in dyskinesia during 12-week treatment of zonisamide combined with anti-Parkinson disease drugs

		ZNS		
	Placebo	25 mg/day	50 mg/day	100 mg/day
Dyskinesia				
Baseline	28	18	33	22
Final assessment	28	21	29	22
Post-ZNS improvement	4	4	8	0
Post-ZNS worsening	1	2	5	4
Appearance during ZNS	1	5	0	1
Disabling dyskinesia				
Baseline	19	7	17	11
Final assessment	17	9	12	11
Post-ZNS improvement	5	1	7	3
Post-ZNS worsening	3	0	0	0
Appearance during ZNS	1	2	0	3

Data are number of patients. Dyskinesia (UPDRS Part IV, No. 32), Disabling dyskinesia (UPDRS Part IV, No. 33).

ZNS = zonisamide.

group were dizziness (5.7%), decrease in appetite (10.1%), and increase in serum creatinine phosphokinase (7.3%).

Discussion. In this study, ZNS adjunctive therapy significantly improved PD symptoms vs placebo, as indicated by the significant improvement in the UDPRS Part III total score for the primary endpoint in the 25-mg and 50-mg groups and significant mean decrease in total "off" time in the 50-mg and 100-mg treatment groups. The improvement in "wearing-off" was similar to the effects seen with rasagiline and entacapone, although neither drug improved the UPDRS Part III total score.[4] Although the randomized patients of our study used many anti-Parkinson concomitant medicines, they did not meet the requirements of adequate treatment of PD because of the attenuation of the beneficial effects. ZNS treatment improved all main PD symptoms in these patients, including tremor, similarly to previous reports.[2,3,5]

Interestingly, administration of ZNS did not increase the frequency of dyskinesia, and the frequency of both dyskinesia and disabling dyskinesia improved in the 50-mg group. The reason for the improvement in parkinsonian symptoms and disabling dyskinesia is not known at present. ZNS is not a glutamate antagonist, but it reduces glutamate release[6] and increases neuronal transporter excitatory amino acid carrier 1.[7] These actions of ZNS on the glutamate system may mediate the improvement of dyskinesia seen in our patients.

In this study, the mean basal levodopa dose was approximately 350 mg/day, although it is lower than that used in Western countries. In Japan, many physicians are using lower doses from therapeutic strategy and patients' preference for not having troublesome side effects. Cultural difference between Japan and Western countries may also affect the maintenance dose. Furthermore, the effective dopa plasma level in Western PD patients is 2,000 to

Table 3 Adverse effects associated with zonisamide treatment with an incidence of ≥5%

		ZNS			
	Placebo	All patients	25 mg/day	50 mg/day	100 mg/day
Somnolence	4.8	10.9	1.3	15.3	15.7
Apathy	6.0	8.5	7.6	7.1	10.8
Dizziness	7.2	5.7	3.8	5.9	7.2
Reduced appetite	14.5	10.1	5.1	8.2	16.9
Weight loss	4.8	6.9	7.6	3.5	9.6
Constipation	3.6	6.5	6.3	8.2	4.8
Increased in serum CK	8.4	7.3	8.9	8.2	4.8

Data are presented as percentage of incidence.

ZNS = zonisamide; CK = creatinine phosphokinase.

4,000 ng/mL[8] but that of Japanese patients is around 500 (max 1,000) ng/mL (unpublished data; data from 200 Japanese PD patients). Race, amount of protein intake, and physique may explain the difference in the effective levodopa dose between Western countries and Japan. The above data indicate that our patients were not undertreated with anti-PD drugs. In fact, our patients, like other Japanese patients with PD, developed treatment-related adverse effects during maintenance therapy using levodopa with or without other drugs. Nevertheless, further studies are necessary to evaluate ZNS in patients with PD treated with anti-PD drugs at doses commonly used in Western countries.

We started the study with a run-in period of single-blind treatment with placebo to minimize placebo effects. To the best of our knowledge, this is the most rigorous study design used to date for the evaluation of anti-Parkinson effects. Our study design may explain the lower response rate in the ZNS groups (although still significantly higher than placebo in the 25-mg and 50-mg study groups) than that of previous reports for pramipexole.[9]

Although there was a higher incidence of adverse events in the 100-mg group than in the other treatment groups, the incidence of hallucination and dyskinesia, which are typically of concern with anti-Parkinson drugs, was the same across all treatment groups, indicating that a once-daily dose of 25 to 100 mg of ZNS is well tolerated.

Although the present study was only of 12 weeks' duration, our preliminary data showed that the benefits observed at 12 weeks were maintained for more than 1 year in all 17 patients in a study on the long-term effects of ZNS on PD. Another study that was designed to assess the long-term (up to 1 year) effects of ZNS on PD (n = 100) also showed that 12-week course of ZNS improved parkinsonian symptoms and that such effects were maintained for up to 1 year (manuscript in preparation).

It is notable that the typical dose of ZNS is 300 to 600 mg/day for epilepsy, but a significant improvement in motor symptoms was noted in our patients with PD with only 50 mg/day of ZNS. This suggests that the mechanism of action of ZNS in PD may be different from those in epilepsy. In this regard, ZNS has multiple mechanisms of action. The major effect of ZNS in epilepsy is modification of neuronal firing at high frequency through enhancement of sodium channel inactivation and reduction of T-type calcium current.[10-13] ZNS has no affinity to γ-aminobutyric acid (GABA) type A receptor or glutamate receptors[11] but is known to increase GABA[6] and glutamate[7] release. In the dopaminergic system, therapeutic doses of ZNS (20 and 50 mg/kg) increase intracellular and extracellular dopamine levels in the rat striatum.[14-16] Conversely, supratherapeutic doses of ZNS reduce intracellular dopamine. Thus, ZNS has biphasic effects on the dopamine system. We reported previously that at therapeutic levels, ZNS increased dopamine synthesis by increasing tyrosine hydroxylase (TH) activity and TH messenger RNA.[14] ZNS also affects MAO-B activity. The IC_{50} (50% inhibitory concentration) value of MAO-B in liver microsomal fraction was 600 μM, and that in striatal membrane fraction was 28 μM.[14,17] These data suggest ZNS inhibits striatal MAO-B activity but not peripheral MAO-B activity, and therefore ZNS may have little effect on peripheral MAO-B inhibition of functions such as blood pressure.

Zonisamide has no affinity to dopamine receptors (D1–D5) or dopamine transporter. ZNS also has no direct effects on glutamate receptors, adenosine receptors, or serotonin receptors, which have been suggested as possible sites of action for anti-PD drugs, other than the dopaminergic system.[14] We proposed previously that activation of dopamine synthesis and moderate inhibition of MAO-B are the main mechanisms that mediate the effects of ZNS in PD.[14] However, the present finding of lack of change in the efficacy of ZNS when coadministered with an MAO-B inhibitor suggests that MAO-B inhibition is not a main factor. We consider that the primary mechanism of action of ZNS in PD is to increase dopamine synthesis. Whether sodium channel inactivation or T-type calcium channel inhibition is involved in ZNS effects has not been elucidated yet. Further investigation is needed to clarify the mechanism of the beneficial actions of ZNS on PD.

Appendix

The Japan Zonisamide on PD Study Group Investigators included the following members: H. Aizawa, MD, Asahikawa Medical College, Asahikawa; T. Kimura, MD, National Dohoku Hospital, Asahikawa; S. Kikuchi, MD, Hokkaido University, Sapporo; M. Baba, MD, Hirosaki University, Hirosaki; K. Chida, MD, National Iwate Hospital, Iwate; K. Hisanaga, MD, National Miyagi Hospital, Sendai; I. Toyoshima, MD, Akita University, Akita; K. Kurita, MD, Yamagata University, Yamagata; Y. Suzuki, MD, Nihonkai Hospital, Yamagata; K. Yoshizawa, MD, Mito Medical Center, Ibaraki; S. Shoji, MD, Tsukuba University, Ibaraki; I. Nakano, MD, Jichi Medical School, Tochigi; K. Hirata, MD, Dokkyo University School of Medicine, Tochigi; K. Kamakura, MD, National Defense Medical College, Saitama; T. Shimizu, MD, Teikyo University, Tokyo; S. Nogawa, MD, Keio University, Tokyo; H. Utsumi, MD, Tokyo Medical University, Tokyo; H. Mizusawa, MD, Tokyo Medical and Dental University, Tokyo; F. Yokochi, MD, Tokyo Metropolitan Fuchu Hospital, Tokyo; K. Hirabayashi, MD, Tokyo Metropolitan Ebara Hospital, Tokyo; K. Hasegawa, MD, National Sagamihara Hospital, Kanagawa; Y. Takahashi, MD, St. Marianna University, Kawasaki; Y. Kuroiwa, MD, Yokohama City University, Yokohama; S. Kameyama, MD, Nishi-Niigata Chuo National Hospital, Niigata; K. Komai, MD, Kanazawa University, Kanazawa; T. Hashimoto, MD, Shinsyu University, Matsumoto; K. Mizoguchi, MD, National Epilepsy Center Shizuoka, Shizuoka; S. Mitake, MD, Tosei General Hospital, Aichi; T. Yasuda, MD, Toyota Memorial Hospital, Aichi; Y. Washimi, MD, National Center for Geriatrics and Gerontology, Aichi; Y. Tatsuoka, MD, Tatsuoka Neurology Clinic, Kyoto; S. Matsumoto, MD, Kitano Hospital, Osaka; K. Abe, MD, Osaka University, Osaka; H. Fujimura, MD, Toneyama National Hospital, Osaka; H. Hashiguchi, MD, Nippon Steel Hirohata Hospital, Himeji; K. Nakashima, MD, Tottori University, Tottori; K. Takamatsu, MD, Brain Attack Center Oota Memorial Hospital, Hiroshima; T. Yamada, MD, Yamada Neurosurgery Hospital, Hiroshima; M. Nomoto, MD, Ehime University, Ehime; T. Yuhi, MD, University of Occupational and Environmental Health, Fukuoka; T. Yamada, MD, Fukuoka University, Fukuoka; K. Ikezoe, MD, Kyusyu University, Fukuoka; A. Sato, MD, Nagasaki Kita Hospital, Nagasaki; H. Matsuo, MD, National Hospital Organization Nagasaki Medical Center, Nagasaki; K. Tsuruta, MD, Koga General Hospital, Miyazaki; K. Arimura, MD, Kagoshima University, Kagoshima; T. Yuasa, National Center of Neurology and Psychiatry, Kohnodai Hospital, Ichikawa; N. Kawashima, MD, Kawashima Neurology Clinic, Kanagawa; A. Ishikawa, MD, Agano Hospital, Niigata; N. Yoshikawa, MD, Yoshikawa Clinic, Kobe; Y. Higashi, MD, Himeji Central Hospital, Himeji; H. Ohnishi, MD, Ohnishi Neurosurgical Center, Akashi; J. Yoshinaga, MD, City Hospital Hiroshima, Hiroshima; H. Fujita, MD, Murakami Memorial Hospital, Ehime; R. Katagi, MD, Katagi Neurological Surgery, Ehime; H. Miyajima, MD, Hamamatsu University School of Medicine, Hamamatsu; K. Ojika,

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Leucine-Rich Repeat kinase 2 G2385R variant is a risk factor for Parkinson disease in Asian population

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To assess the effect of genetic factors on sporadic Parkinson disease, we performed a case–control study of a variant (G2385R) in *Leucine-Rich Repeat kinase* 2 among the Japanese population. The G2385R (c.7153G>A) variant was reported as a risk factor for sporadic Parkinson disease in the Chinese population from Taiwan and Singapore. Genotyping was conducted in 448 Parkinson disease patients and 457 healthy controls. The frequency of A allele in Parkinson disease was significantly higher than in the control ($P=1.24\times10^{-4}$, odds ratio 2.63, 95% confidence interval 1.56–4.35). Our results suggest that the G2385R variant is a risk factor for sporadic Parkinson disease in the Asian population. *NeuroReport* 18:273–275 © 2007 Lippincott Williams & Wilkins.

Keywords: *Leucine-Rich Repeat kinase 2*, risk factor, single nucleotide polymorphisms

Introduction

Parkinson disease (PD) is one of the most frequent neurodegenerative diseases characterized by resting tremor, rigidity, bradykinesia, and postural instability. PD is thought to be a multifactorial disease caused by a combination of aging, environmental, and genetic factors. Although the majority of patients of PD are of sporadic type, some genes have been identified as a monogenic causative gene by molecular genetic studies for familial PD [1–6]. *Leucine-Rich Repeat kinase 2* (*LRRK2*) has been identified as a causative gene associated with autosomal dominant familial PD [7,8]. To date, many pathogenic substitutions in *LRRK2* have been identified in familial and sporadic PD [9]. The G2385R variant (c.7153G>A) in *LRRK2* was reported recently as a risk factor for sporadic PD in the Chinese population from Taiwan and Singapore [10,11]. This variant was identified originally as putative pathogenic mutation in a small Taiwanese PD family and was not found in Caucasians [12]. Thus, it is possible that the G2385R variant is a risk factor in Asian sporadic PD. To test this hypothesis, we conducted a case–control study to evaluate the association between the G2385R genotype and the risk for PD in the Japanese population.

Methods

Subjects and genomic DNA

Genomic DNA was isolated from 448 sporadic PD patients and 457 controls of the Japanese population by a standard protocol (Table 1). All PD patients had no family history of PD. PD patients with *parkin* or *PTEN-induced putative kinase 1 (PINK1)* mutation were not included in the study. Diagnosis of PD was adopted by the participating neurologists and was established on the basis of the United Kingdom Parkinson's Disease Society Brain Bank criteria [13]. This study was approved by the ethics committee of Juntendo University School of Medicine. All individuals gave an informed and signed consent form.

Genotyping

Exon 48 of *LRRK2* from each individual was amplified by polymerase chain reaction (PCR) using the primers and protocol described by Zimprich *et al.* [8]. The PCR products were sequenced directly using the BigDye Terminators v1.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, California, USA). The reverse PCR primer was used as sequencing primer.

Statistical analysis

Statistical analysis included the Hardy–Weinberg equilibrium test, χ^2 test, Fisher's exact test, odds ratio and its 95% confidence interval (95% CI), using SNPAlyze v5.1 software (Dynacom, Chiba, Japan). The *t*-test was performed using JMP 6.0 (SAS Institute Japan, Tokyo, Japan). In all statistical analyses, *P* values of 0.05 or less were considered statistically significant.