Keywords:
Iron
Iron regulatory protein
Oxidative stress
Mitochondria
Parkin
Parkinson's disease

perturbed the integrity of neurons in the substantia nigra and provoked motor symptoms. These results suggest that a subtle, but chronic increase in IRP2 induces mitochondrial oxidative insults and accelerates neurodegeneration in a mouse model of Parkinson's disease. Thus, the IRP2 Tg may be a useful tool to probe the roles of iron-induced mitochondrial damages in neurodegeraration research.

1. Introduction

Iron is an essential nutrient but can also be toxic because iron can readily cycle between ferrous (Fe^{2+}) and ferric (Fe^{3+}) in physiological settings and oxidizes proteins and nucleic acids via generation of free radicals. Dysregulation of iron metabolism causes some neurodegenerative diseases [21] and iron progressively accumulates in the lesions of sporadic neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease [17,18]. Therefore, tight regulation of iron metabolism appears to be critical for maintenance of neuronal cells [2,21].

Iron homeostasis is mainly regulated by coordinated expression of molecules involved in iron uptake and storage. Iron availability is regulated at the post-transcriptional level through the interactions between the iron-responsive elements (IREs) on mRNAs encoding proteins involved in iron metabolism and mRNA-binding proteins called iron regulatory proteins (IRPs) [14]. Binding of IRPs to IREs on the mRNA of the iron uptake protein, transferrin receptor1 (TfR1) enhances translation of TfR1, whereas binding of IRPs to the IRE on the mRNA of the iron storage protein, ferritin suppresses its production. Iron stored in ferritin is not toxic, because Fe^{3+} stored in ferritin cannot be converted to Fe^{2+}. Therefore, augmented expression of IRPs leads to an increase in iron uptake and a decrease in iron storage, which result in an increase of iron that cannot be stored safely and able to oxidize and damage cellar components [10]. There are two IRPs (IRP1 and IRP2) and IRP2 is abundant in brain as compared to other organs [9].

To examine the effects of iron in the integrity of neurons in mice, we generated transgenic (Tg) mice that express IRP2 in neurons [4]. We show increase in IRP2 induces mitochondrial oxidative insults and accelerates neurodegeneration.

2. Material and methods

2.1. Antibodies

The anti-myc (4A6 and 9E10) were purchased from Millipore and Roche, respectively. The following antibodies were obtained as indicated: 4-hydroxynonenal (4-HNE) (JaICA and Alpha diagnostic); β-actin, Tom20 (Santa Cruz Biotechnology); PINK1 (Novus); HA (Covance); COX III core1 (Invitrogen); and tubulin, Tyrosine hydroxylase (TH) (Cedarlane). Anti-IRP2 has been described [1].

2.2. Plasmids and cell culture

HA- and GFP-human Parkin was subcloned into pDNA3.1 (invitrogen) and pTRE2 (Clontech), respectively. p220-IRP2-myc has been described previously [6]. pNSE-IRP2-myc was generated by subcloning the human IRP2-myc cDNA into pNSE [4]. pcDNA3.1-HA- or pTRE2-GFP-Parkin were stably introduced in HEK293 or HeLa cells, respectively using Lipofectamine 2000 (Invitrogen). Parkin expression was induced by addition of 1 μg/ml doxycycline (DOX) for 48 h in HeLa cells that expressed GFP-Parkin in a DOX-dependent manner. IRP2-myc under the control of dexamethasone (DEX) (p220-IRP2-myc) was induced by treatment with 80 nM DEX for 48 h.

2.3. Immunoblotting, immunoprecipitation and fluorescence microscopy

These analyses were performed as described previously [19]. Quantifications were performed by Fluoview (Olympus) and BZ-II Analyzer (Keyence).

2.4. Assessment of mitochondrial membrane potential

Cells were treated with 25 nM MitoTracker Orange for 10 min at 37 °C.

2.5. Generation of NSE-IRP2 Tg mice

NSE-IRP2 transgenic mice were generated by microinjection of pNSE-IRP2-myc into E0.5 mouse embryos from a C57BL/6J × DBA2/J F1 background. Parkin KO mice have been described [15]. These mice were backcrossed to C57BL/6J mice (Charles River Japan) more than ten times. All the experiments using mice were carried out according to the Guidelines for Animal Experimentation, Juntendo, Osaka, and Kyoto University.

2.6. Southern blotting

Southern blotting was performed as previously described using human IRP2 cDNA as a probe [19].

2.7. RNA electrophoretic mobility shift assay (EMSA)

EMSA was performed as described previously [6].

2.8. Histochemical and morphological analyses

Brain sections were stained with the appropriate primary antibodies, followed by development using HISTOFINE (Nichirei) and a metal-enhanced diaminobenzidine (DAB) substrate kit (Pierce). Toluidine blue staining and electron microscopy were performed as described previously [7].

2.9. Fe^{2+} staining

Brains were perfused consecutively with 50 mM hydrogen sulfide and 4% paraformaldehyde, embedded in paraffin. Sections were immersed in a solution of 5% $K_3[Fe(CN)_6]$ and 5% HCl followed by immersion in 0.05% DAB and in 1% H_2O_2 plus 0.05% DAB.

2.10. Measurement of striatal dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA)

Dissected striata were analyzed using a reverse-phase C18 column (150 × 4.6 mm; Tosoh) on an HPLC system (ESA Biosciences) with a coulometric 8-electrode electrochemical detection system.

2.11. Behavioral analyses

A 40 × 40 cm^2 open field with 10 cm × 10 cm grids was used for the open-field test.

2.12. Administration of desferrioxamine (DFO)

Saline containing 300 mg/kg DFO was injected intraperitoneally into mice once a day for 10 consecutive days.

2.13. Statistical analysis

Statistical significance was determined using a one-way ANOVA. Data are shown by mean ± SEM.

3. Results

3.1. A subtle increase in IRP2 induces oxidative insults in neurons

To probe the effect of iron in the integrity of neurons, we generated Tg mice expressing IRP2-myc using the rat neuron specific enolase (NSE) promoter because induced expression of IRP2 increases TfR1 expression and decreases ferritin expression, thereby increases the amount of iron that are not stored safely (Fig. 1A). Two lines of Tg mice exhibited identical phenotypes and Line 26 was analyzed here (Fig. 1B and C). IRP2 is stabilized in iron-depleted condition [14]. IRP2-myc was expressed in all areas in the brain examined, including the SN of NSE-IRP2 Tg mice treated with an iron-chelator, DFO (Fig. 1D). IRP2-myc expressed in the brain exhibited IRE-binding activity (Fig. 1E). The amount of exogenous IRP2 (Fig. 1C, top panel) was very small compared to endogenous IRP2 and an increase in total IRP2 levels could not be detectable in NSE-IRP2 Tg mice brain using conventional immunoblotting (Fig. 1C, bottom panel), which might be attributed to the observation that mice expressing a large amount of IRP2 might not be viable [10]. To probe the amount of iron that is potentially harmful to cells, levels of Fe^{2+}, which can cycle between Fe^{2+} and Fe^{3+}, were probed. Increase of Fe^{2+} was observed in all regions of the brain of 12-month-old NSE-IRP2 Tg mice, including the SN (Fig. 1F). The activity of TH, a critical enzyme for catecholamine synthesis, is enhanced by iron [12]. The amount of TH in the striatum increased in NSE-IRP2 Tg mice (Fig. 1G), confirming an increase in available iron in NSE-IRP2 Tg neurons. Modification of proteins by 4-HNE, a major product of lipid peroxidation, is one of the most reliable markers of oxidative insults to proteins [20]. Anti-4-HNE immunoreactivity which represents 4-HNE-modified proteins [20], was increased in all areas of the brain examined in 18-month-old NSE-IRP2 Tg mice (Fig. 1H). These data suggest that although subtle, chronic expression of exogenous IRP2 can provoke oxidative insults in neurons possibly by increasing the amount of iron that are not stored safely.

3.2. Induced expression of IRP2 augments mitochondrial oxidative damage

Since 4-HNE modification seems to provoke dysfunction of mitochondrial proteins [13], subcellular localization of 4-HNE-modified proteins induced by iron was probed using HEK293 cells that expressed IRP2 in an inducible manner. 4-HNE immunoreactivity detected in IRP2-expressing cells treated with iron source, ferric ammonium citrate (FAC) was co-localized with the mitochondria marker Tom20 (Fig. 2A–C). Mitochondrial membrane potential is an important indicator of mitochondrial integrity [11]. A substantial fraction of mitochondria did not stain with MitoTracker, which is known to accumulate to mitochondria with intact membrane potential, in IRP2-expressing cells treated with iron (Fig. 2D). These results indicate that enhanced IRP2 expression decreases its membrane potential.

Decrease in mitochondrial membrane potential triggers stabilization of PINK1 kinase in mitochondria and subsequent recruitment of the Parkin ubiquitin ligase to mitochondria, which

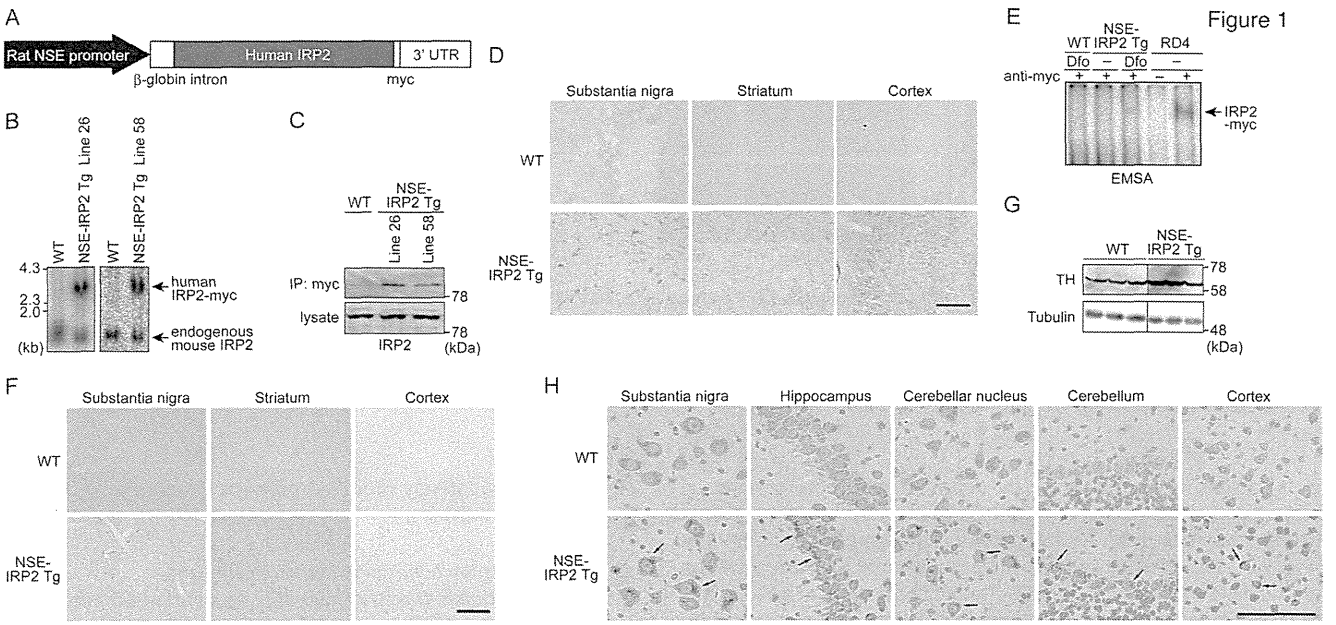

Fig. 1. Generation of NSE-IRP2 Tg mice. (A) Schematic of the NSE-IRP2 transgene. (B) Genomic DNA was analyzed by Southern blotting. (C) Brain lysates from mice that were administered DFO were used for immunoprecipitation with anti-myc and probed with anti-IRP2. (D) Sections from 12-month-old mice were immunostained with anti-myc. Bar 50 μm. (E) Lysates, as in (C) and lysates of RD4 cells expressing IRP2-myc were incubated with ^{32}P-labeled IRE and anti-myc, and the mixture was then separated by PAGE and analyzed by autoradiography. (F) Sections from12-month-old mice were stained using Turnbull's blue and DAB to detect ferrous iron. Bar 50 μm. (G) Lysates from the striatum of 12-week-old mice were probed with anti-TH. (H) Sections from 18-month-old mice were immunostained with anti-4-HNE. Arrows indicates 4-HNE-modificated proteins. Bar 200 μm.

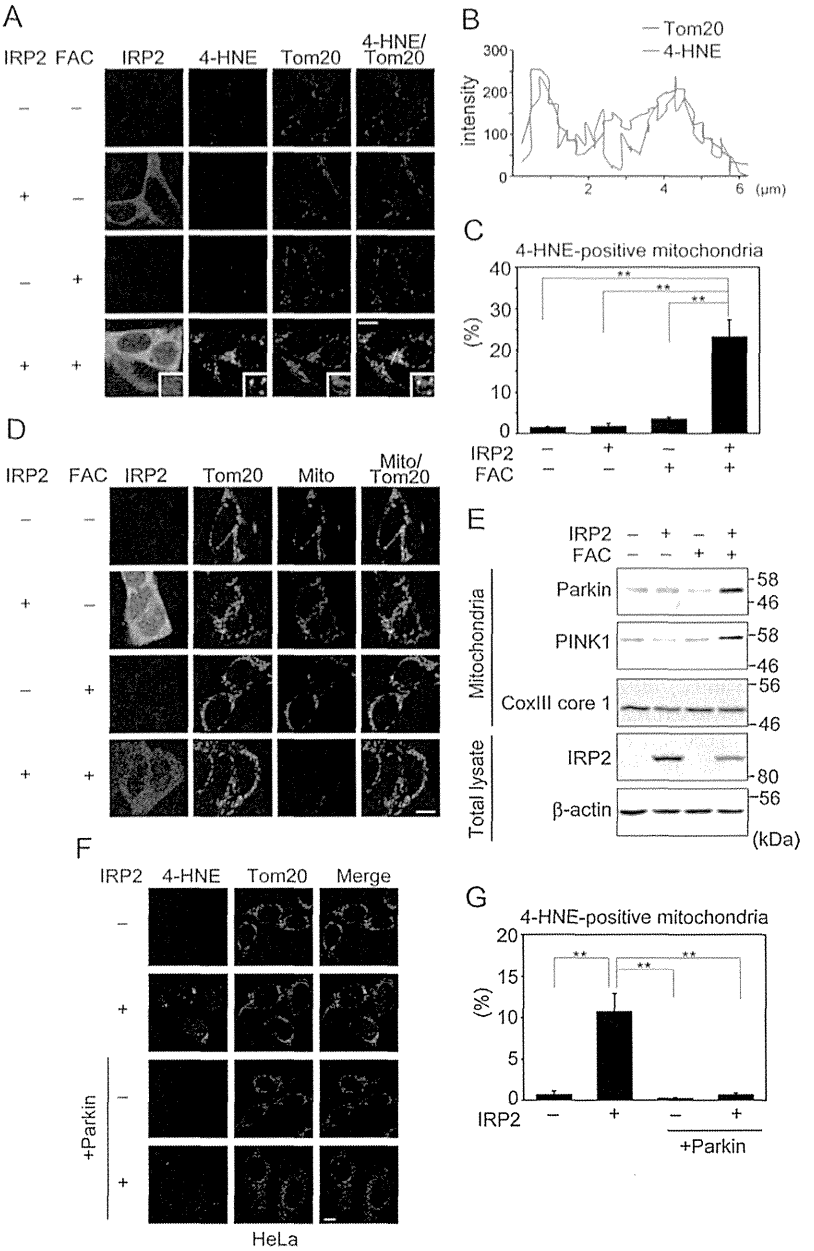

Fig. 2. Induced expression of IRP2 augments iron-mediated mitochondrial damage. (A-D) HEK293 cells in which IRP2 expression was induced, or un-induced cells, were treated with the 100 μg/ml FAC for 12 h. Cells were analyzed by immunostaining as indicated (A), and line scan plot in FAC-treated cells expressing IRP2 and the percentage of 4-HNE-positive mitochondria were shown in (B) and (C), respectively ($n = 10$). Cells were analyzed by MitoTracker staining (D). (E) Parkin-overexpressing HEK293 cells in which IRP2 expression was induced or un-induced, were incubated in the presence or absence of 100 μg/ml FAC for 9 h. Lysates were probed by immunoblotting. (F and G) HeLa cells expressing IRP2 and/or Parkin were treated with 50 μg/ml FAC. Cells were analyzed by immunofluorescence staining (F) and the percentage of 4-HNE-positive mitochondria was shown (G) ($n = 10$). Bars, 10 μm. **, $P < 0.01$.

is suggested to be crucial for the maintenance of mitochondrial integrity [8,11]. PINK1 was stabilized in and Parkin was recruited to mitochondria in FAC-treated cells expressing exogenous IRP2 (Fig. 2E). We then examined the involvement of Parkin in the removal of mitochondrial 4-HNE-modifed proteins using HeLa cells that lack Parkin expression [8]. Although 4-HNE-immunoreactivity was not detected in HeLa cells treated with FAC alone, exogenous IRP2 induced 4-HNE signals that co-localized with Tom20 in FAC-treated HeLa cells. The mitochondrial 4-HNE signal induced by exogenous IRP2 and iron was eliminated by introduction of Parkin (Fig. 2F and G). These results suggest that Parkin is involved in the clearance of oxidatively modified proteins generated by IRP2 and iron in mitochondria.

3.3. *Increased mitochondrial oxidative insults and neurodegeneration in the SN of NSE-IRP2 Tg × Parkin KO mice*

Although 4-HNE modification could not be detected without adding exogenous iron in cultured cells (Fig. 2A), 4-HNE modification could be observed in mouse neurons with enhanced expression of IRP2 alone (Fig. 1H). Iron is essential nutrient for cell proliferation, whereas, neurons are regarded as quiescent cells [5]. Then the

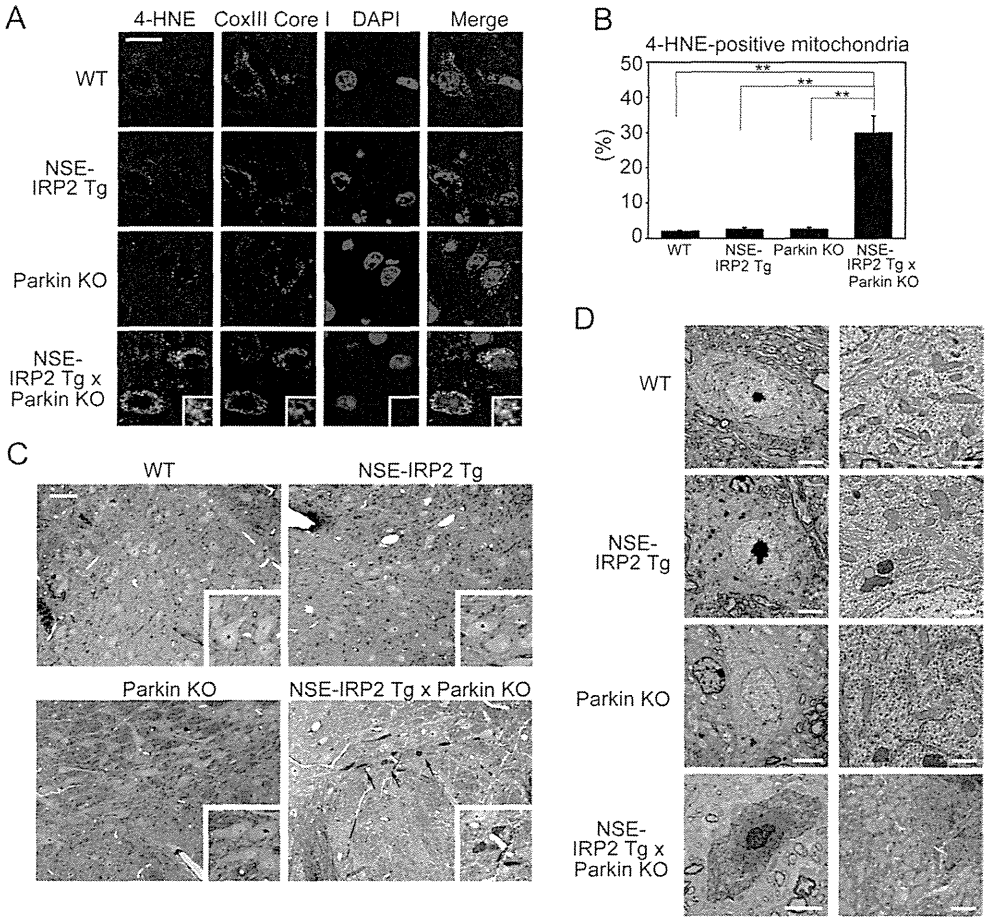

Fig. 3. IRP2 increased mitochondrial oxidative insults and neurodegeneration in the SN. (A and B) Sections of the SN from 6-month-old mice were immunostained with as indicated antibodies. The percentage of 4-HNE-positive mitochondria was shown (B) ($n = 10$). Bar 20 μm. **, $P < 0.01$. (C) Semi-thin sections of the SN from 6-month-old mice of were stained with toluidine blue. In the double-mutant mice, dying neurons that show shrinkage are intensely stained with toluidine blue (arrows). Bar 50 μm. (D) Electron micrographic analysis of neurons in the SN from 6-month-old mice. In the double-mutant mouse, a dying neuron with increased electron density is shrunken and contains round swollen mitochondria with an electron-lucent matrix and a nucleus with a large peculiarly shaped nucleolus. Bar 5 μm (left) and 1 μm (right).

effect of IRP2 expression on mitochondrial oxidative damages in neurons was probed in 6-month-old mice. As shown in Fig. 3A and B, no overt 4-HNE immunoreactivity could be detected in the SN of NSE-IRP2 Tg mice. However, the 4-HNE immunoreactivity, which was co-localized with a mitochondrial protein, COX III, was prominently detected in the SN neurons of NSE-IRP2 Tg × Parkin KO mice [15]. These results suggested that IRP2 expression provokes mitochondrial oxidative insults and Parkin is involved in the removal of the mitochondrial insults in the SN. Consistent with these results, degenerated neurons, which stain intensely with toluidine blue, were observed in the SN of 6-month-old NSE-IRP2 Tg × Parkin KO mice (Fig. 3C). Electron microscopic analyses revealed the presence of neurons with condensed nuclei and round swollen mitochondria in the SN of 6-month-old NSE-IRP2 Tg × Parkin KO mice (Fig. 3D). These results indicate that the subtle increase of IRP2 perturbs the integrity of the neurons by increasing mitochondrial oxidative insults and that Parkin is involved in the clearance of iron-induced oxidized mitochondrial proteins.

3.4. Decrease in dopaminergic neurons in the SN and locomotor dysfunctions in NSE-IRP2 Tg × Parkin KO mice

Selective loss of dopaminergic neurons in the SN is involved in the pathogenesis of Parkinson's disease and Parkin is a familial Parkinson's disease-related protein [3]. Consistent with neuronal degeneration in the SN (Fig. 3), the number of TH-positive cells was significantly lower in the SN of 5-month-old NSE-IRP2 Tg × Parkin KO mice (Fig. 4A and B). The amounts of dopamine and its metabolites were significantly lower in the striatum of 5-month-old NSE-IRP2 Tg × Parkin KO mice (Fig. 4C–E). Motor conditions are major symptoms of Parkinson's disease [3]. Consistent with loss of dopaminergic neurons in the SN, both horizontal activity and rearing scores in open-field tests were significantly lower in 5-month-old NSE-IRP2 Tg × Parkin KO mice (Fig. 4F and G). Collectively, these results clearly indicate that the subtle increase in exogenously expressed IRP2 and loss of Parkin synergistically accelerates the loss of dopaminergic neurons in the SN and provokes motor symptoms.

4. Discussion

Since induced expression of IRP2 increases the amount of iron that cannot be stored safely, we dissected the role of iron in neuronal damages using neuron-specific IRP2 Tg mice. Only subtle increase of IRP2 was enough for mitochondrial 4-HNE modification in mouse neurons (Fig. 1H) although IRP2 expression alone could not provoke mitochondrial 4-HNE modifications in transformed cells (Fig. 2A and F). Expression of IRP2 is subtle in neurons in NSE-IRP2 Tg mice, but it is continuous throughout their lifetime. Moreover, 4-HNE signals could be detected in 18-month-old,

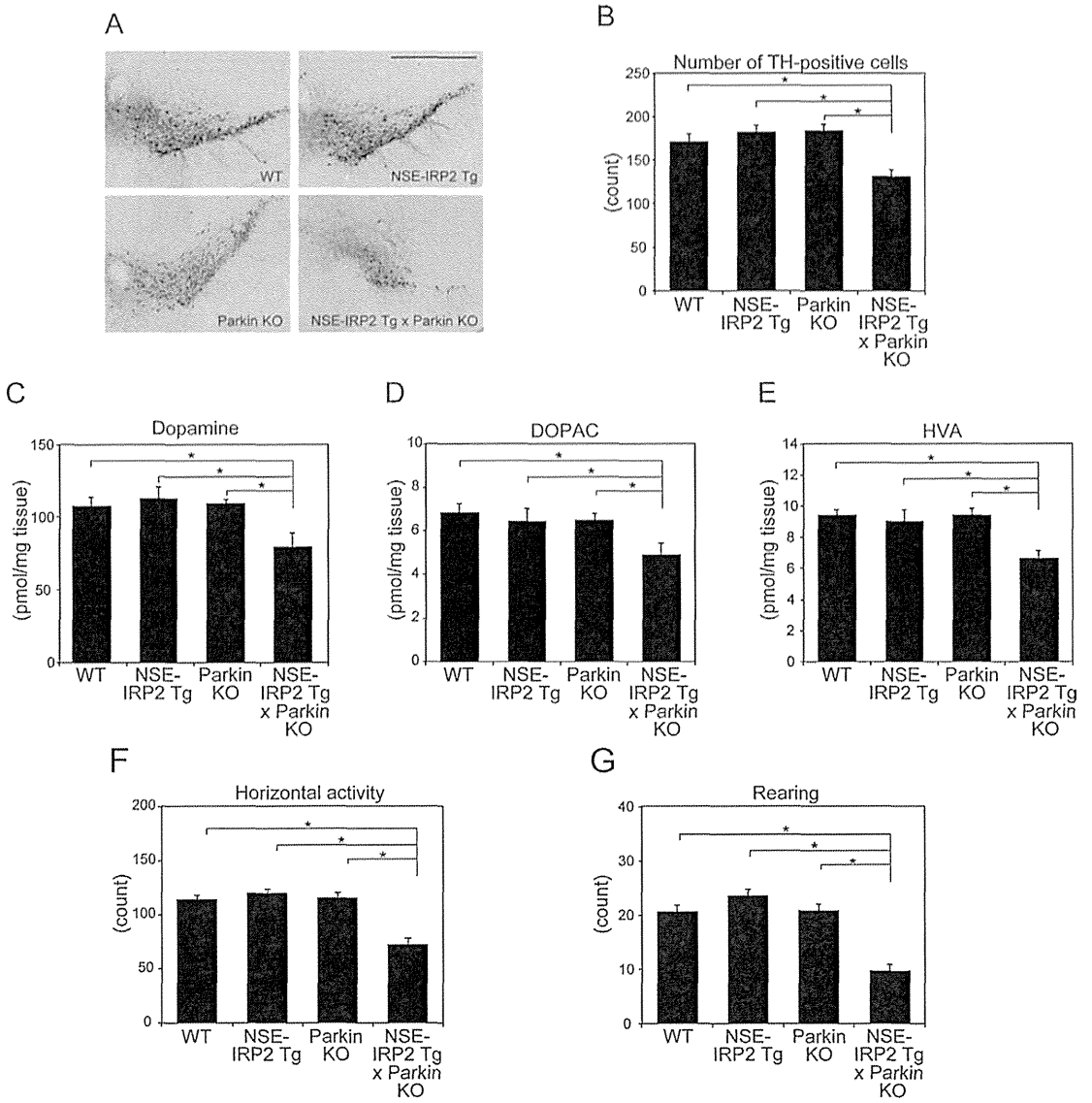

Fig. 4. IRP2 accelerates the progression of Parkin-induced symptoms. (A and B) Sections of the SN from 5-month-old mice were immunostained with anti-TH (A) and the number of TH-positive cells was quantified (B) ($n = 10$). Bar 1 mm. (C–E) Concentrations of dopamine (C) DOPAC (D), and HVA (E) in the striata of 5-month-old mice were measured ($n = 15$). (F and G) The horizontal activity (F) and rearing activity (G) of 5-month-old mice were measured using the open-field test scored over a 5 min period ($n = 15$). *, $P < 0.05$.

but not in 6-month-old in NSE-IRP2 Tg mice (Fig. 1H and Fig. 3A), which suggests that duration of IRP2 expression in quiescent neuronal cells may be a critical factor for iron-induced mitochondrial 4-HNE modifications. Since cultured cells proliferated rapidly, we evaluated the effect of iron on 4-HNE modifications within two days. Thus, we suspect that despite very trace, transgenic expression of IRP2 can induce the subtle, but chronic increase of iron that cannot be stored safely and result in mitochondrial oxidative insults and the deterioration of neurodegeneration in quiescent neurons.

Loss of Parkin enhanced accumulation of mitochondrial 4-HNE-modified proteins and degeneration of neurons of the SN in NSE-IRP2 Tg mice. Impaired clearance of oxidized mitochondrial proteins in the SN neurons appeared involved in Parkinson's disease-like phenotypes in NSE-IRP2 Tg × Parkin KO mice (Fig. 4). Additionally, Parkin is involved in the clearance of oxidatively modified proteins in mitochondria (Fig. 2F). However, mechanism underlying Parkin-mediated removal of mitochondrial 4-HNE modification is currently unknown because in iron-treated IRP2-expressing cultured cells, we could not detect mitophagy in that Parkin is shown to be involved [8,11] (unpublished observation). Thus, the mitochondrial quality control, in which Parkin is involved, but possibly in a different mechanism from mitophagy, may play critical roles protecting dopaminergic neurons from iron-induced mitochondrial oxidative damages. Mitochondrial damages are suggested to function as triggering and accelerating factors in Parkinson's disease and accumulation of iron in the lesions of the disease is known [2,16]. Iron-induced mitochondrial oxidative damages might be involved in the development of Parkinson's disease.

Our results show that subtle increase of IRP2 is sufficient to induce mitochondrial oxidative insults. Thus, NSE-IRP2 Tg mice may be useful to probe the roles iron-induced mitochondrial oxidative insults in neurodegenerative disorders.

5. Conclusions

Expression of even trace amounts of IRP2 increased mitochondrial oxidative changes in neurons. The increase in IRP2 accelerated the loss of dopaminergic neurons in the SN and provokes motor symptoms of Parkin KO mice. NSE-IRP2 Tg mice may be suitable to probe the role of iron-induced mitochondrial oxidative damages in neurodegenerative disorders.

Acknowledgments

We thank Dr. Forss-Petter for pNSE. This work was partly supported by grants and a Grant-in-Aid for Scientific Research on Innovative Areas (Comprehensive Brain Science Network) from the Ministry of Education, Science, Sports and Culture of Japan to K.I. and M.K., respectively.

References

[1] M. Ashizuka, T. Fukuda, T. Nakamura, K. Shirasuna, K. Iwai, H. Izumi, K. Kohno, M. Kuwano, T. Uchiumi, Novel translational control through an iron-responsive element by interaction of multifunctional protein YB-1 and IRP2, Mol. Cell Biol. 22 (2002) 6375–6383.
[2] R.R. Crichton, D.T. Dexter, R.J. Ward, Brain iron metabolism and its perturbation in neurological diseases, J. Neural. Transm. 118 (2011) 301–314.
[3] D.W. Dickson, H. Braak, J.E. Duda, C. Duyckaerts, T. Gasser, G.M. Halliday, J. Hardy, J.B. Leverenz, K. Del Tredici, Z.K. Wszolek, I. Litvan, Neuropathological assessment of Parkinson's disease: refining the diagnostic criteria, Lancet Neurol. 8 (2009) 1150–1157.
[4] S. Forss-Petter, P.E. Danielson, S. Catsicas, E. Battenberg, J. Price, M. Nerenberg, J.G. Sutcliffe, Transgenic mice expressing β-galactosidase in mature neurons under neuron-specific enolase promoter control, Neuron 5 (1990) 187–197.
[5] K. Herrup, Y. Yang, Cell cycle regulation in the postmitotic neuron: oxymoron or new biology? Nat. Rev. Neurosci. 8 (2007) 368–378.
[6] K. Iwai, R.D. Klausner, T.A. Rouault, Requirements for iron-regulated degradation of the RNA binding protein, iron regulatory protein 2, EMBO J. 14 (1995) 5350–5357.
[7] M. Koike, M. Shibata, M. Tadakoshi, K. Gotoh, M. Komatsu, S. Waguri, N. Kawahara, K. Kuida, S. Nagata, E. Kominami, K. Tanaka, Y. Uchiyama, Inhibition of autophagy prevents hippocampal pyramidal neuron death after hypoxic-ischemic injury, Am. J. Pathol. 172 (2008) 454–469.
[8] N. Matsuda, S. Sato, K. Shiba, K. Okatsu, K. Saisho, C.A. Gautier, Y.S. Sou, S. Saiki, S. Kawajiri, F. Sato, M. Kimura, M. Komatsu, N. Hattori, K. Tanaka, PINK1 stabilized by mitochondrial depolarization recruits Parkin to damaged mitochondria and activates latent Parkin for mitophagy, J.Cell Biol. 189 (2010) 211–221.
[9] E.G. Meyron-Holtz, M.C. Ghosh, K. Iwai, T. LaVaute, X. Brazzolotto, U.V. Berger, W. Land, H. Ollivierre-Wilson, A. Grinberg, P. Love, T.A. Rouault, Genetic ablations of iron regulatory proteins 1 and 2 reveal why iron regulatory protein 2 dominates iron homeostasis, EMBO J. 23 (2004) 386–395.
[10] T. Moroishi, M. Nishiyama, Y. Takeda, K. Iwai, K.I. Nakayama, The FBXL5-IRP2 axis is integral to control of iron metabolism in vivo, Cell Metabol. 14 (2011) 339–351.
[11] D. Narendra, A. Tanaka, D.F. Suen, R.J. Youle, Parkin is recruited selectively to impaired mitochondria and promotes their autophagy, J. Cell Biol. 183 (2008) 795–803.
[12] W.D. Rausch, Y. Hirata, T. Nagatsu, P. Riederer, K. Jellinger, Tyrosine hydroxylase activity in caudate nucleus from Parkinson's disease: effects of iron and phosphorylating agents, J. Neurochem. 50 (1988) 202–208.
[13] J.R. Roede, D.P. Jones, Reactive species and mitochondrial dysfunction: mechanistic significance of 4-hydroxynonenal, Environ. Mol. Mutagen. 51 (2010) 380–390.
[14] T.A. Rouault, The role of iron regulatory proteins in mammalian iron homeostasis and disease, Nat. Chem. Biol. 2 (2006) 406–414.
[15] S. Sato, T. Chiba, S. Nishiyama, T. Kakiuchi, H. Tsukada, T. Hatano, T. Fukuda, Y. Yasoshima, N. Kai, K. Kobayashi, Y. Mizuno, K. Tanaka, N. Hattori, Decline of striatal dopamine release in parkin-deficient mice shown by ex vivo autoradiography, J. Neurosci. Res. 84 (2006) 1350–1357.
[16] E.A. Schon, S. Przedborski, Mitochondria: the next (neurode) generation, Neuron 70 (2011) 1033–1053.
[17] M.A. Smith, P.L. Harris, L.M. Sayre, G. Perry, Iron accumulation in Alzheimer disease is a source of redox-generated free radicals, Proc. Natl. Acad. Sci. U. S. A. 94 (1997) 9866–9868.
[18] M. Takanashi, H. Mochizuki, K. Yokomizo, N. Hattori, H. Mori, Y. Yamamura, Y. Mizuno, Iron accumulation in the substantia nigra of autosomal recessive juvenile parkinsonism (ARJP), Parkinsonism Relat. Disord. 7 (2001) 311–314.
[19] F. Tokunaga, S. Sakata, Y. Saeki, Y. Satomi, T. Kirisako, K. Kamei, T. Nakagawa, M. Kato, S. Murata, S. Yamaoka, M. Yamamoto, S. Akira, T. Takao, K. Tanaka, K. Iwai, Involvement of linear polyubiquitylation of NEMO in NF-κB activation, Nat. Cell Biol. 11 (2009) 123–132.
[20] N. Zarkovic, A. Cipak, M. Jaganjac, S. Borovic, K. Zarkovic, Pathophysiological relevance of aldehydic protein modifications, J. Proteomics 92 (2013) 239–247.
[21] L. Zecca, M.B. Youdim, P. Riederer, J.R. Connor, R.R. Crichton, Iron, brain ageing and neurodegenerative disorders, Nat. Rev. Neurosci. 5 (2004) 863–873.

Parkinsonism and Related Disorders 20 (2014) 1388–1393

Contents lists available at ScienceDirect

Parkinsonism and Related Disorders

journal homepage: www.elsevier.com/locate/parkreldis

Rotigotine vs ropinirole in advanced stage Parkinson's disease: A double-blind study

Yoshikuni Mizuno [a,*], Masahiro Nomoto [b], Kazuko Hasegawa [c], Nobutaka Hattori [a], Tomoyoshi Kondo [a], Miho Murata [d], Masahiro Takeuchi [e], Masayoshi Takahashi [f], Takayuki Tomida [f], on behalf of the Rotigotine Trial Group

[a] Department of Neurology, Juntendo University School of Medicine, Tokyo, Japan
[b] Department of Neurology, Ehime University School of Medicine, Matsuyama, Japan
[c] Department of Neurology, National Hospital Organization, Sagamihara National Hospital, Sagamihara, Japan
[d] Department of Neurology, National Center Hospital, National Center of Neurology and Psychiatry, Tokyo, Japan
[e] Department of Biostatistics, Kitasato University School of Pharmacy, Tokyo, Japan
[f] Department of Clinical Research and Development, Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan

ARTICLE INFO

Article history:
Received 19 May 2014
Received in revised form
22 September 2014
Accepted 5 October 2014

Keywords:
Parkinson's disease
Treatment
Randomized trial
Rotigotine
Ropinirole

ABSTRACT

Objective: To confirm the superiority of transdermal rotigotine up to 16 mg/24 h over placebo, and non-inferiority to ropinirole, in Japanese Parkinson's disease (PD) patients on concomitant levodopa therapy.
Methods: This trial was a randomized, double-blind, double-dummy, three-arm parallel group placebo- and ropinirole-controlled trial. Four-hundred and twenty PD patients whose motor symptoms were not well controlled by levodopa treatment were randomized 2:2:1 to receive rotigotine, ropinirole (up to 15 mg/day) or placebo during a 16-week treatment period followed by a 4-week taper period. The primary variable was change in the Unified Parkinson's Disease Rating Scale (UPDRS) Part III (ON state) sum score from baseline to the end of the treatment period.
Results: The difference in the change in the UPDRS Part III (ON state) sum score from baseline to the end of treatment between rotigotine and placebo groups was -6.4 ± 1.2 (95% CI: -8.7 to -4.1; $p < 0.001$), indicating superiority of rotigotine over placebo. The difference between rotigotine and ropinirole groups was -1.4 ± 1.0 (95% CI: -3.2 to 0.5), below the non-inferiority margin, indicating the non-inferiority of rotigotine to ropinirole. Application site reaction was seen in 57.7% of the patients in the rotigotine group and in 18.6% in the ropinirole group ($P < 0.001$). No other safety issue was noted.
Conclusions: Rotigotine was well tolerated at doses up to 16 mg/24 h and showed similar efficacy to ropinirole except that the application site reaction was much higher in the rotigotine group.

1. Introduction

Long-term treatment of Parkinson's disease (PD) with levodopa are frequently complicated by motor fluctuations [1–3]. Ahlskog and Muenter reported 42.1% motor fluctuations and 38.5% dyskinesia in 4–6 years of treatment with levodopa; these figures rose up to 69.6% and 87.8%, respectively, with more than 9 years of treatment [2]. The use of dopamine agonists is associated with lower frequencies of wearing off and dyskinesia compared to levodopa in early stage PD [3,4]. Rotigotine is a non-ergot dopamine agonist, which has been developed as a patch with high selectivity for D2 and D3 receptors [5]. Rotigotine is superior to placebo in patients with early-stage [6–9] and advanced PD patients [10–12]. In addition, rotigotine was non-inferior to ropinirole [8] and pramipexole [10]. A clinical trial conducted in Japan showed superiority of rotigotine over placebo in patients with PD on concomitant levodopa therapy in the dose range of 2–16 mg/24 h [12]. We conducted a randomized, double-blind trial to see the efficacy and safety of transdermal rotigotine in Japanese advanced PD patients. We selected ropinirole as an active comparator drug, as it has been proved to be efficacious both in early stage [13–15] and advanced stage PD patients [16,17]. As the maximum daily dose for rotigotine has been set at 15 mg/24 h in Japan; we used this dose.

* Corresponding author. Department of Neurology, Juntendo University School of Medicine, 2-1-1 Hongo, Bunkyo-ku, Tokyo 113-8421, Japan. Tel.: +81 3 3813 3111.
E-mail address: y_mizuno@juntendo.ac.jp (Y. Mizuno).

http://dx.doi.org/10.1016/j.parkreldis.2014.10.005
1353-8020/

2. Methods

2.1. Design

The study is a randomized, double-blind, double-dummy, three-arm parallel group, placebo- and ropinirole-controlled trial of rotigotine in Japanese PD patients on levodopa. The study was conducted in compliance with ethical principles in accordance with the Declaration of Helsinki, the Pharmaceutical Affairs Law, and the "Ordinance on Good Clinical Practice." The protocol was approved by the institutional review boards of each center, and written informed consent was obtained from all patients participating in the trial. The study has been registered with Clinicaltrials.gov (identifier: NCT01628926) and in the Japan Primary Registries Network (identifier: Japic CTI-090888). The study was financially supported by Otsuka Pharmaceutical Company.

2.2. Patients

Sixty-two sites in Japan participated in the trial, with the patient enrollment commencing in June 2009. We enrolled patients aged 30–79 years and with a diagnosis of PD, Hoehn & Yahr stage of 2–4, and Unified Parkinson's Disease Rating Scale (UPDRS) Part III sum score of ≥10 at screening (ON state), who were experiencing motor fluctuations or whom levodopa could not be increased to an optimal level because of side effects or other reasons. The levodopa doses were not changed from the period 28 days before starting treatment. Diagnosis of PD was made according to the UK Brain Bank criteria [18].

We excluded patients with psychiatric symptoms; orthostatic hypotension; a history of epilepsy or convulsion; a history of serious cardiac disease, arrhythmia, or QT prolongation; abnormal liver function; or a history of allergy to topical agents; and female patients who were pregnant or lactating from the trial. Concomitant use of drugs that may affect the symptoms of PD, cause QT prolongation, or interact with ropinirole was prohibited. Levodopa, selegiline and entacapone could be used concomitantly, provided there was no change in the dose from 28 days before the first dose of the study drug until the end of the treatment period. Anticholinergic drugs, amantadine, droxidopa, and zonisamide could be used concomitantly, provided there was no change in the doses for 14 days before the first dose of the study drug or during the treatment period.

2.3. Randomization and treatment

Eligible patients were randomized 2:2:1 to receive rotigotine, ropinirole, or placebo using a dynamic allocation procedure designed to balance the UPDRS Part III (ON state) sum score, the presence/absence of OFF time, the presence/absence of dystonia in the early morning, and responsiveness to prior dopamine receptor agonists. A double-dummy technique was used to maintain blinding with placebo patches or tablets.

We evaluated the enrolled patients every week until the maintenance dose is determined and every two weeks thereafter. The treatment period consisted of a maximum of 12 weeks of titration and at least 4 weeks of maintenance, and a dose taper period of up to 4 weeks. Rotigotine or placebo patches were applied once daily and ropinirole or placebo tablets were administered three times daily. Rotigotine was delivered at an initial dose of 2 mg/24 h, and the dose was increased to 16 mg/24 h in weekly increments of 2 mg/24 h. Ropinirole was administered at an initial dose of 0.75 mg/day. The dose was increased to 3.0 mg/day in weekly increments of 0.75 mg/day and then was increased to 15 mg/day in weekly increments of 1.5 mg/day. One level of back titration was allowed for rotigotine and ropinirole during the titration period. Dose increments for either drug could be stopped if the optimal dose or the maximally tolerated dose was reached, if adverse events resolved after back titration, or if the maximum dose level was attained. The maintenance dose of rotigotine and ropinirole was determined for each patient considering their efficacy and safety.

2.4. Efficacy measurement

The primary variable was the change in the UPDRS Part III (ON state) sum score from baseline to week 16 of the treatment period (end of treatment, EOT). Secondary variables included changes from baseline to EOT for the time spent in OFF, ON, and ON with troublesome dyskinesia and changes from baseline to EOT for the score in UPDRS Part II (ON), UPDRS Part II (OFF), UPDRS Part II (average ON and OFF state), sum of UPDRS Part II (average ON and OFF state) + UPDRS Part III scores, and PD sleep scale-2 (PDSS-2) [19]. Additional secondary variables were the responder rate sum score (patients with a ≥20% or ≥30% reduction in the UPDRS Part III sum score) (ON state), and the responder rate in terms of the UPDRS Part II (average ON and OFF state) sum score. Patient diaries were utilized, in which each patient described his or her condition as off time, on time, on time with troublesome dyskinesia or sleep in every 30 min every day starting seven days prior to the initial drug administration to EOT. Examination of the patients was done at the ON state.

2.5. Safety

Safety was assessed in all randomized patients who received at least one dose of the test drugs. Safety variables were the frequency of the onset of adverse events, laboratory values, blood pressure/pulse rate, electrocardiogram parameters, skin irritation assessment score, physical and neurologic examination, and frequencies of compulsive disorder and impulse control disorder as assessed by the translated Jay Modified Minnesota Impulsive Disorder Interview [20]. Regurgitation of the cardiac valve and drug dependency were assessed separately by the specialist committees.

2.6. Sample size calculations

Based on the results of the late phase 2 trial of rotigotine in Japanese advanced PD patients on levodopa [12] and the Japanese clinical trial of ropinirole [17], we assumed effect sizes of 5.4 for the rotigotine and 5.0 for the ropinirole group and a standard deviation (SD) of 9.0 for each group. The sample size required to show superiority of rotigotine over placebo was calculated to be 88 and 44 patients for the rotigotine and placebo groups, respectively, with a two-tailed significance level of 5% and 90% power. The margin for non-inferiority of rotigotine to ropinirole was set to 2.5 based on the range of effect size in clinical trials of rotigotine and other non-ergot dopamine agonists [4,21,22]. The number of patients required to achieve 80% power and an upper limit of the 95% confidence interval (CI) for the difference between rotigotine and ropinirole being lower than the non-inferiority margin was 152 per group. Therefore, the target sample size was set as 160 patients each for the rotigotine and ropinirole groups and 80 patients for the placebo group.

2.7. Statistical analyses

The primary analysis of the primary variable was conducted using analysis of covariance (ANCOVA) with treatment group as a fixed factor. The different null hypotheses were tested in a pre-assigned order (closed testing principle). The test procedure started with a two-sided test between rotigotine and placebo with $\alpha = 5\%$. If the *P*-value was significant (i.e., rotigotine was superior to placebo), a non-inferiority test was conducted to compare rotigotine with ropinirole. Non-inferiority was accepted if the 95% CI for the difference between rotigotine and ropinirole was within the pre-defined non-inferiority margin of 2.5. For secondary analyses of the primary variable, ANCOVA was applied with treatment group as a fixed factor and the corresponding baseline value as a covariate. Changes from baseline to EOT in the secondary variables were assessed using ANCOVA. Responder rates were compared between each group using χ^2 tests. Safety variables were summarized using descriptive statistics and between-group comparisons were done using χ^2 tests.

3. Results

We obtained responses from 546 patients. However, 126 patients were not randomized; 36 from consent withdrawal, 59 not meeting the enrollment criteria, 31 from other reasons. Thus 420 patients were randomized (rotigotine 168, ropinirole 167, placebo 85). The full analysis set (FAS) included 414 patients because of three not meeting the enrollment criteria and three not having any valid post-baseline assessment of UPDRS Part III (ON state) sum score, and the safety set 420 patients including all randomized patients who received at least one dose of the test drugs (Fig. 1). The baseline characteristics of the 414 patients are shown in Table 1. There were no differences between groups, except for PDSS-2, which was higher in the placebo group than in the rotigotine and ropinirole groups ($p = 0.023$), and the patients receiving previous treatment with entacapone was higher in the ropinirole group than in the placebo and rotigotine groups ($p = 0.03$).

3.1. Treatment

After the start of the study, 26 patients in the rotigotine group, 23 in the ropinirole group and 17 in the placebo group discontinued the study. The most common reason for discontinuation was adverse events (AE) (13, 13, and 8 patients in the rotigotine, ropinirole, and placebo groups, respectively). None of these patients were seriously ill after the discontinuation of the test drugs.

Of the 420 patients in the safety analysis set, 381 (153, 153, and 75 patients in the rotigotine, ropinirole, and placebo groups, respectively) entered the dose maintenance period. Of these patients, 24.8% (38 patients), 28.8% (44 patients), and 41.3% (31 patients) in the rotigotine, ropinirole, and placebo groups, respectively, received dose increases up to the maximum maintenance dose. The mean maintenance doses were 12.9 mg/24 h and 9.2 mg/day in the rotigotine and ropinirole groups, respectively.

3.2. Efficacy variables

The change in the UPDRS Part III (ON state) sum score from baseline to EOT in the FAS was −10.9 ± 8.1, −9.5 ± 8.7, and −4.5 ± 9.7 (mean ± SD) in the rotigotine, ropinirole, and placebo groups, respectively. The difference between the rotigotine and the placebo group was −6.4 (95% CI: −8.7 to −4.1; $p < 0.001$), and that between the ropinirole and the placebo group was −5.1 (95% CI: −7.4 to −2.8; $p < 0.001$), showing superiority of rotigotine and ropinirole over placebo. The difference between the rotigotine and the ropinirole group was −1.4 (95% CI: −3.2 to 0.5, $p = 0.156$) showing the non-inferiority of rotigotine to ropinirole.

Regarding motor fluctuations (110/164 = 67.1% in the rotigotine,113/166 = 68.1% in the ropinirole, and 57/84 = 67.9% in the placebo group, no statistical difference), off period decrease was 1.4 h in the rotigotine, 1.9 h in the ropinirole, and 0.4 h in the placebo group. The differences between the rotigotine and the placebo and the ropinirole and the placebo group were significant ($p = 0.009$ and $p < 0.001$, respectively). The difference between the rotigotine and the ropinirole group was not significant ($P = 0.148$).

The comparisons between groups for other efficacy variables are shown in Table 2. The difference in the UPDRS Part II (average ON and OFF state) sum score between the rotigotine and the placebo group was −2.4 (95% CI: −3.3 to −1.5; $p < 0.001$) and that between the ropinirole and the placebo group was −1.8 (95% CI: −2.7 to −0.8; $p < 0.001$), while the difference between the rotigotine and the ropinirole group was −0.6 (95% CI: −1.4 to 0.1; $p = 0.106$). The difference in the UPDRS Part II (OFF state) sum score between the rotigotine and the placebo group was −2.4 (95% CI: −3.9 to −0.9; $p = 0.002$) and that between the ropinirole and the placebo group was −1.4 (95% CI: −2.9 to 0.0; $p = 0.058$), while the difference between the rotigotine and the ropinirole group was −1.0 (95% CI: −2.2 to 0.2; $p = 0.114$).

Significantly more patients in the rotigotine group were classified as responders for UPDRS Part III (ON state), UPDRS Part II (average ON and OFF state), and the sum of UPDRS Part II (average ON and OFF state) + UPDRS Part III compared with the placebo group (Table 2). The ropinirole group also showed similar results compared with the placebo group. More patients in the rotigotine group were classified for 20% responder rate on UPDRS Part III (ON state), 30% responder rate on UPDRS Part II (average ON and OFF state), and 20% responder rate on the sum of UPDRS Part II (average ON and OFF state) + UPDRS Part III compared to the ropinirole group.

3.3. Safety outcomes

Adverse events occurred in 88.7% (149/168 patients), 77.8% (130/167 patients), and 69.4% (59/85 patients) in the rotigotine, ropinirole, and placebo groups, respectively. Adverse events with an incidence of ≥3% are shown in Table 3. Most adverse events were mild to moderate in severity, and the proportion of patients with severe adverse events was similar in all three groups (8% in both rotigotine and placebo groups, and 7% in the ropinirole group). Only application site reaction was higher in the rotigotine than in the ropinirole and the placebo group (57.7%, 18.6% and 15.3%, respectively). All application site reactions were mild or moderate in intensity. Skin irritation was evaluated using a six-grade skin irritation assessment (−, ±, +, ++, +++, ++++). Only 2.4% of patients in the rotigotine group and none in the ropinirole and placebo groups had a score of +++ (concurrent erythema, edema and papule; serous papule; and vesicle) during the dose titration period. The proportion of patients in the rotigotine group with a score of +++ during the dose maintenance period was 0.7%. No patients had skin irritation with a score of ++++ (large blisters). Three subjects in the rotigotine group discontinued the trial from skin irritation.

Dyskinesias occurred in 16.1% (27/168), 13.8% (23/167), and 1.2% (1/85) of patients in the rotigotine, ropinirole and placebo groups, respectively. The difference between the rotigotine and the ropinirole group was not significant. Adverse events leading to treatment discontinuation occurred in 7.7% (13/168), 7.8% (13/167), and 9.4% (8/85) of patients in the rotigotine, ropinirole, and placebo groups, respectively. Sudden onset of sleep was observed in one patient each in the rotigotine and ropinirole groups. Neither case required treatment discontinuation or dose reduction.

Serious adverse events, which required hospitalization, occurred in seven patients in the rotigotine, five in the ropinirole, and six in the placebo group. Among them, serious adverse events related to the test drugs include gastric ulcer, torticollis, and spinal compression fracture and posture abnormality in three patients in the rotigotine group, worsening of PD in one in the ropinirole group, and angina pectoris and worsening of PD in the placebo group.

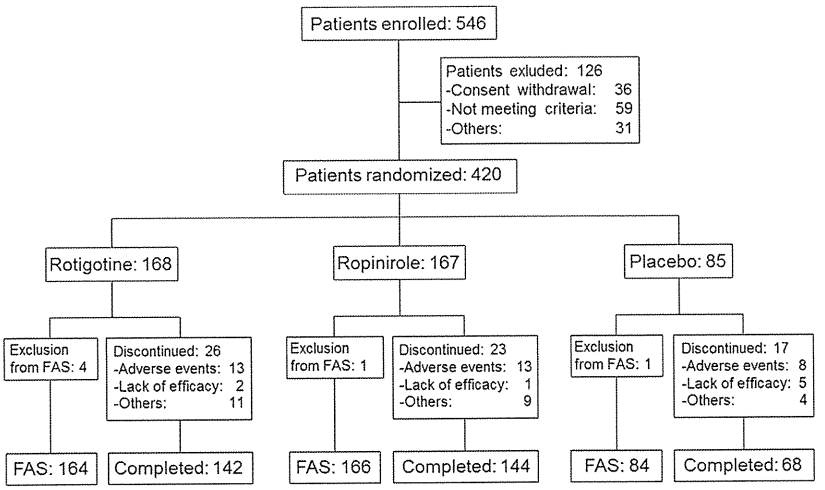

Fig. 1. Disposition of patients. The numbers indicate the number of patients in each category in FAS (full analysis set).

Table 1
Baseline patient characteristics (full analysis set, $n = 414$).

	Rotigotine ($n = 164$)	Ropinirole ($n = 166$)	Placebo ($n = 84$)	p-Value
Gender				
Male	61 (37.2%)	68 (41.0%)	42 (50.0%)	0.152[a]
Female	103 (62.8%)	98 (59.0%)	42 (50.0%)	
Age (years)	64.8 (8.8)	67.0 (7.9)	65.3 (7.9)	0.066[b]
Duration of PD (years)	7.0 (4.9)	6.8 (4.2)	7.0 (4.2)	0.880[b]
Wearing off	107 (65.2%)	110 (66.3%)	57 (67.9%)	0.918[a]
Dyskinesias	42 (25.6%)	43 (25.9%)	15 (17.9%)	0.319[a]
Levodopa dose (mg)	367.7 (151.9)	350.6 (125.3)	370.5 (146.6)	0.764[b]
Previous concomitant anti-Parkinson's medication				
Entacapone	40 (24.4%)	57 (34.3%)	33 (39.3%)	0.033[a]
Anticholinergic drugs	33 (20.1%)	32 (19.3%)	16 (19.0%)	0.973[a]
Amantadine	39 (23.8%)	40 (24.1%)	27 (32.1%)	0.306[a]
Selegiline	60 (36.6%)	69 (41.6%)	35 (41.7%)	0.594[a]
Droxidopa	12 (7.3%)	11 (6.6%)	8 (9.5%)	0.709[a]
Zonisamide	16 (9.8%)	13 (7.8%)	12 (14.3%)	0.271[a]
Hoehn & Yahr average	2.7 (0.6)	2.8 (0.6)	2.8 (0.6)	0.204[b]
UPDRS Part III (ON state)	25.8 (10.6)	25.8 (11.0)	25.6 (10.4)	0.970[b]
UPDRS Part II (average ON and OFF state)	11.0 (6.2)	10.6 (5.6)	11.1 (7.0)	0.978[b]
UPDRS Part II (ON state)	8.5 (5.9)	7.8 (5.7)	7.9 (6.7)	0.357[b]
UPDRS Part II (OFF state)	14.9 (8.4; $n = 110$)	15.3 (6.9; $n = 114$)	15.8 (9.4; $n = 58$)	0.562[b]
Sum of UPDRS Part II (average ON and OFF state) + UPDRS Part III	36.9 (15.2)	36.4 (15.2)	36.7 (16.0)	0.909[b]
PDSS-2	12.3 (8.9)	14.3 (9.2)	15.0 (9.2)	0.023[b]
OFF time (hr)	4.5 (3.4; $n = 111$)	5.0 (3.6; $n = 113$)	4.9 (3.0; $n = 57$)	0.359[b]
ON time (hr)	13.1 (3.6)	12.5 (3.8)	12.6 (3.7)	0.375[b]
ON time with troublesome dyskinesias (hr)	2.4 (2.6; $n = 23$)	1.6 (1.5; $n = 16$)	0.7 (1.2; $n = 5$)	0.079[b]

Data are means (SD) or number (%).
UPDRS: unified Parkinson's disease rating scale; PDSS: Parkinson's disease sleep scale.
In this clinical trial, we defined FAS as follows; Those who were given the trial drugs at least once and at least one evaluation for the efficacy was made. However, those patients who violated GCP, those who do not fulfill the enrollment criteria, and those who meet the exclusion criteria are not included in the FAS. According to this criteria, three patients met the exclusion criteria, and in three patients there was no efficacy evaluation after enrollment to the study.
[a] χ^2 test.
[b] Kruskal–Wallis test.

QTc prolongation (>500 ms) in ECG was noted in two patients in the ropinirole group, but none in the rotigotine and placebo groups. The committee's assessment of results was of no clinically significant worsening of cardiac valve regurgitation in any patients. Non-significant difference was found regarding drug dependency. Impulse control disorder rates were non-significantly higher for ropinirole (6.6%) than rotigotine (3.5%), or placebo (3.5%).

Table 2
Efficacy variables at end of treatment (full analysis set, last observation carried forward).

	Change from baseline (least squares (LS) mean or %)			Comparison for rotigotine vs placebo		Comparison for rotigotine vs ropinirole	
	Rotigotine ($n = 164$)	Ropinirole ($n = 166$)	Placebo ($n = 84$)	Difference	p-Value (95% CI)	Difference	p-Value (95% CI)
Changes form baseline							
UPDRS Part III (ON state)	−10.9	−9.5	−4.5	−6.4	<0.001 (−8.6, −4.2)	−1.4	0.137 (−3.2, 0.4)
UPDRS Part II (average ON and OFF state)	−3.6	−3.0	−1.2	−2.4	<0.001 (−3.3, −1.5)	−0.6	0.106 (−1.4, 0.1)
UPDRS Part II (ON state)	−2.8	−2.3	−0.6	−2.2	<0.001 (−3.1, −1.3)	−0.5	0.201 (−1.2, 0.3)
UPDRS Part II (OFF state)	−4.9; $n = 109$	−3.9; $n = 111$	−2.4; $n = 57$	−2.4	0.002 (−3.9, −0.9)	−1.0	0.114 (−2.2, 0.2)
Sum of UPDRS Part II (average ON and OFF state) + UPDRS Part III	−14.6	−12.5	−5.7	−8.8	<0.001 (−11.7, −6.0)	−2.0	0.091 (−4.4, 0.3)
PDSS-2	−3.7	−3.0	−1.1	−2.6	<0.001 (−4.1, −1.1)	−0.7	0.277 (−1.9, 0.6)
OFF time (hr)	−1.4; $n = 110$	−1.9; $n = 113$	−0.4; $n = 57$	−1.1	0.009 (−1.9, −0.3)	0.5	0.148 (−0.2, 1.2)
ON time (hr)	1.4	1.6	0.2	1.2	<0.001 (0.6, 1.8)	−0.2	0.426 (−0.7, 0.3)
ON time with troublesome dyskinesias (hr)	0.3; $n = 22$	0.2; $n = 16$	−1.2; $n = 5$	1.5	0.166 (−0.7, 3.7)	0.1	0.860 (−1.3, 1.5)
Responder analysis							
UPDRS Part III (ON state)							
20% responder	80.5	69.1	56.6	23.9	<0.001 (11.6, 36.1)	11.4	0.017 (2.1, 20.7)
30% responder	69.5	60.6	39.8	29.8	<0.001 (17.1, 42.4)	8.9	0.090 (−1.4, 19.2)
UPDRS Part II (average ON and OFF state)							
20% responder	65.2	56.7	47.0	18.2	0.006 (5.2, 31.2)	8.5	0.116 (−2.1, 19.1)
30% responder	55.9	43.3	28.9	27.0	<0.001 (14.6, 39.4)	12.6	0.023 (1.8, 23.4)
Sum of UPDRS Part II (average ON and OFF state) + UPDRS Part III							
20% responder	78.3	66.5	51.8	26.5	<0.001 (14.0, 38.9)	11.8	0.017 (2.2, 21.4)
30% responder	68.3	57.9	37.3	31.0	<0.001 (18.3, 43.6)	10.4	0.052 (−0.0, 20.8)

Change from baseline to EOT was assessed using analysis of covariance with baseline value as covariate.
Adjusted LS means were calculated. Inter-group comparisons for responder rate were performed using the χ^2 test.
UPDRS: Unified Parkinson's Disease Rating Scale; PDSS: Parkinson's disease sleep scale.

4. Discussion

We showed superiority of rotigotine and ropinirole to placebo and non-inferiority of rotigotine to ropinirole up to 15 mg/day for the primary efficacy variable (UPDRS Part III sum score) in this study. In addition, we showed reduction in off time in patients with motor fluctuations treated with rotigotine and ropinirole compared with placebo. There was no difference between rotigotine and ropinirole treatment.

As the maximum dose of ropinirole (15 mg/day) is lower in this study compared to those reported in the western literature (24 mg/day) [3,13–17], whether or not this difference might have resulted in non-inferiority of rotigotine to ropinirole should be discussed. First of all, 15 mg/day of ropinirole is the maximum approved dose in Japan. Although the maximum administered dose of ropinirole in this study was lower than those in the western literature, the magnitude of improvement as measured by UPDRS Part III sum score are similar between western and Japanese patients [13–17]. This may in part be due to the difference in the body weight. As none of the previous studies have addressed the question as to the dose–response relationship on ropinirole, we compared the average dose of ropinirole and efficacy in the previous studies. In the present study, average daily maintenance dose of ropinirole was 9.2 mg/day and the average motor UPDRS score decreased from 25.8 to 16.3 (9.5 points difference) after 16 weeks and off time decreased by 1.9 h (34% reduction) in the ropinirole group. In the study by Korczyn et al. the final dose of ropinirole was 12.0 mg at three years' treatment [14]. The motor UPDRS reduced from 23 to 14 at 24 weeks after the randomization. In the study by Rascol et al. the average daily dose of ropinirole was 16.5 mg and the UPDRS motor score decreased from 23 points to 14 points at 24 weeks [3]. In the study by Lieberman et al. [16], there was no description in the final average dose of ropinirole. Therefore, the magnitude of the motor UPDRS decrease is about the same in these studies. We wanted to compare the improvement in wearing off with different doses of ropinirole; however, this was difficult because the total number of patients who showed improvement in wearing off was not described [16].

Rotigotine is a patch formulation, which provides stable and continuous stimulation of dopamine receptors. Continuous dopaminergic drug delivery was thought to be an effective strategy for PD patients. Rotigotine was well tolerated, and there were no significant safety issues with doses up to 16 mg/24 h compared to ropinirole up to 15 mg/day except the high incidence of application site reactions in the rotigotine group, which may limit the use of rotigotine. In conclusion, once-daily administration of the rotigotine patch is a favorable option for the treatment of PD patients on levodopa.

Author contributions

YM: Coordinating investigator. Conception of study design; organization of the study; review and critique of the statistical analysis; writing of the first draft; review and critique of all drafts.

NH: Coordinating investigator. Conception of study design; organization of the study; execution of the study; review and critique of the statistical analysis; review and critique of all drafts.

TK: Coordinating investigator. Conception of study design; organization of the study; execution of the study; review and critique of the statistical analysis; review and critique of all drafts.

KH: Coordinating investigator. Conception of study design; organization of the study; execution of the study; review and critique of the statistical analysis; review and critique of all drafts.

MM: Coordinating investigator. Conception of study design; organization of the study; execution of the study; review and critique of the statistical analysis; review and critique of all drafts.

M Takeuchi: Statistical advisor. Conception of study design; organization of the study; design, execution, review and critique of the statistical analysis; review and critique of all drafts.

M Takahashi: Conception of study design; organization of the study; execution of the study; review and critique of the statistical analysis.

TT: Design of the statistical analysis; execution of the statistical analysis; review and critique of the statistical analysis.

MN: Medical expert. Conception of study design; organization of the study; review and critique of the statistical analysis; writing of the first draft; review and critique of all drafts.

Financial disclosure concerning the research related to the manuscript

Funding for this study was provided by Otsuka Pharmaceutical Co., Ltd.

YM is an advisory board member for Otsuka Pharmaceutical Co., Ltd. and has received personal compensation for attending advisory board meetings.

Table 3
Treatment-emergent adverse events occurring with an incidence of ≥3% in at least one group (safety analysis set). *n* (%).

	Number of patients (%)			*P* value	
	Rotigotine (n = 168)	Ropinirole (n = 167)	Placebo (n = 85)	Rotigotine vs placebo	Rotigotine vs ropinirole
Any adverse event	149 (88.7)	130 (77.8)	59 (69.4)	<0.001	0.008
Application site reactions[a]	97 (57.7)	31 (18.6)	13 (15.3)	<0.001	<0.001
Nasopharyngitis	28 (16.7)	24 (14.4)	13 (15.3)	0.78	0.562
Dyskinesia	27 (16.1)	23 (13.8)	1 (1.2)	<0.001	0.555
Nausea	25 (14.9)	23 (13.8)	7 (8.2)	0.133	0.772
Vomiting	11 (6.5)	11 (6.6)	2 (2.4)	0.153	0.988
Somnolence	11 (6.5)	9 (5.4)	2 (2.4)	0.153	0.655
Contusion	7 (4.2)	2 (1.2)	6 (7.1)	0.325	0.093
Orthostatic hypotension	5 (3.0)	7 (4.2)	4 (4.7)	0.483	0.549
Blood creatine kinase increased	5 (3.0)	6 (3.6)	1 (1.2)	0.374	0.752
Hallucination[b]	3 (1.8)	6 (3.6)	0	0.215	0.306
Back pain	3 (1.8)	5 (3)	2 (2.4)	0.759	0.469
Cystitis	3 (1.8)	3 (1.8)	4 (4.7)	0.181	0.994
Upper respiratory tract inflammation	3 (1.8)	1 (0.6)	3 (3.5)	0.389	0.317
Peripheral edema	0	2 (1.2)	3 (3.5)	0.014	0.155

Comparisons were made using the χ^2 test.
The safety set (420 patients) includes all randomized patients who received at least one dose of the test drugs and the safety evaluation is done.
[a] Corresponds to the MedDRA term "Application and instillation site reactions".
[b] Corresponds to the MedDRA terms "Hallucination", "Hallucination, visual", "Hallucination, auditory".

MN has received speaker's honoraria from Otsuka Pharmaceutical Co., Ltd.

TK has received honoraria, support for travel to meetings, and fees for participation in review activities from Otsuka Pharmaceutical Co., Ltd.

KH received personal compensation for attending advisory board meetings from Otsuka Pharmaceutical Co., Ltd.

MM has received honoraria for consulting and/or lecturing from Otsuka Pharmaceutical Co., Ltd.

MT received honoraria for consulting from Otsuka Pharmaceutical Co., Ltd.

NH is an advisory board member for Otsuka Pharmaceutical Co., Ltd.

MT and TT are employees of Otsuka Pharmaceutical Co., Ltd.

Full financial disclosures of all authors for the past year

YM was a Professor of Neuroregenerative Medicine at Kitasato University School of Medicine, a position donated by Nippon Boehringer Ingelheim Co., Ltd and Medtronic Japan Co., Ltd. YM was also an advisory board member for Nippon Boehringer Ingelheim Co., Ltd.. YM is an advisory board member for FP Pharmaceutical Corporation, Otsuka Pharmaceutical Co., Ltd., Abbott Japan Co., Ltd., and Kyowa Hakko Kirin Co., Ltd., and he has received personal compensation for attending advisory board meetings.

NH is an advisory board member for Novartis Pharma K.K., Otsuka Pharmaceutical Co., Ltd., GlaxoSmithKline K.K., Kyowa Hakko Kirin Co., Ltd., and MSD K.K., and has received honoraria from Nippon Boehringer Ingelheim Co., Ltd., GlaxoSmithKline K.K., Novartis Pharma K.K., FP Pharmaceutical Corporation, Takeda Pharmaceutical Company Limited., Janssen Pharmaceutical K.K., Daiichi Sankyo Co., Ltd., Kyowa Hakko Kirin Co., Ltd., and Dainippon Sumitomo Pharma Co., Ltd.

TK has received honoraria, support for travel to meetings, and fees for participation in review activities from FP Pharmaceutical Corporation, Novartis Pharma K.K., GlaxoSmithKline K.K., Nippon Boehringer Ingelheim Co., Ltd., Dainippon Sumitomo Pharma Co., Ltd., Kyowa Hakko Kirin Co., Ltd., and Otsuka Pharmaceutical Co., Ltd.

KH received personal compensation for attending advisory board meetings from Otsuka Pharmaceutical Co., Ltd.

MM has received honoraria for consulting and/or lecturing from GlaxoSmithKline K.K., Nippon Boehringer Ingelheim Co., Ltd., Dainippon Sumitomo Pharma Co., Ltd., Novartis Pharma K.K., and Otsuka Pharmaceutical Co., Ltd.

M Takeuchi received honoraria for consulting from GlaxoSmithKline K.K., and UCB Japan Co. Ltd.

MT and TT are employees of Otsuka Pharmaceutical Co., Ltd.

MN has received speaker's honoraria from Dainippon Sumitomo Pharma Co., Ltd., Kyowa Hakko Kirin Co., Ltd., Nippon Boehringer Ingelheim Co., Ltd., Novartis Pharma K.K., and Otsuka Pharmaceutical Co., Ltd.

Acknowledgments

The authors wish to thank the following additional members of the Rotigotine Trial Group, who participated in this study as principal investigators (all MDs): Takashi Kimura, Sanae Honma, Kazunori Ito, Masayuki Baba, Chigumi Ohtsuka, Takashi Abe, Atsushi Takeda, Souichi Katayama, Kazuo Yoshizawa, Minoru Kotera, Shuzo Shintani, Kenichi Fujimoto, Koichi Hirata, Daisuke Uematsu, Kimihito Arai, Hideki Shimura, Norihiro Suzuki, Satoshi Orimo, Taku Hatano, Fusako Yokochi, Hiroshi Kurisaki, Masayuki Yokochi, Shinichiro Kubo, Fumihito Yoshii, Noriko Kawashima, Yusaku Shimizu, Toshihiko Ohashi, Tetsushi Atsumi, Akira Inukai, Hideyuki Sawada, Ryousuke Takahashi, Kyoko Ozawa, Yoshihisa Tatsuoka, Hiroshi Sugiyama, Sadako Kuno, Kazuto Nishinaka, Takanori Hazama, Sadayuki Matsumoto, Harutoshi Fujimura, Satoshi Kaneko, Yoshiyuki Mitsui, Noriko Hayashi, Takashi Naka, Tamotsu Kubori, Nobuo Kouhara, Nobuyoshi Yoshikawa, Kazuo Toda, Hiroshi Tokinobu, Toshio Inui, Mitsutoshi Yamamoto, Masahiro Nagai, Tatsuo Yamada, Naokazu Sasagasako, Youichi Hokezu, Toshio Okazaki, Mikio Shoji, Itaru Toyoshima, Tetsuya Ishihara, Mieko Ogino. The authors also wish to thank Nicholas D Smith, PhD, for providing editorial support during the preparation of this manuscript.

References

[1] Lang AE, Lozano AM. Parkinson's disease. First of two parts. New Engl J Med 1998;339:1044–53.
[2] Ahlskog JE, Muenter MD. Frequency of levodopa-related dyskinesias and motor fluctuations as estimated from the cumulative literature. Mov Disord 2001;16:448–58.
[3] Rascol O, Brooks DJ, Korczyn AD, De Deyn PP, Clarke CE, Lang AE. A five-year study of the incidence of dyskinesia in patients with early Parkinson's disease who were treated with ropinirole or levodopa. N Engl J Med 2000;342:1484–91.
[4] Holloway RG, Shoulson I, Fahn S, Kieburtz K, Lang A, Marek K, et al. Pramipexole vs levodopa as initial treatment for Parkinson disease. A 4-year randomized controlled trial. Arch Neurol 2004;61:1044–53.
[5] Pfeiffer RF. Potential of transdermal drug delivery in Parkinson's disease. Drugs Aging 2002;19:561–70.
[6] Parkinson Study Group. A controlled trial of rotigotine monotherapy in early Parkinson's disease. Arch Neurol 2003;60:1721–8.
[7] Watts RL, Jankovic J, Waters C, Rajput A, Boroojerdi B, Rao J. Randomized, blind, controlled trial of transdermal rotigotine in early Parkinson disease. Neurology 2007;68:272–6.
[8] Giladi N, Boroojerdi B, Korczyn AD, Burn DJ, Clarke CE, Schapira AHV. Rotigotine transdermal patch in early Parkinson's disease: a randomized, double-blind, controlled study versus placebo and ropinirole. Mov Disord 2007;22:2398–404.
[9] Mizuno Y, Nomoto M, Kondo T, Hasegawa K, Murata M, Ikeda J, et al. Transdermal rotigotine in early-stage Parkinson's disease: a randomized, double-blind, placebo-controlled trial. Mov Disord 2013;28:1447–50.
[10] Poewe WH, Rascol O, Quinn N, Tolosa E, Oertel WH, Martignoni E, et al. Efficacy of pramipexole and transdermal rotigotine in advanced Parkinson's disease: a double-blind, double-dummy, randomized controlled trial. Lancet Neurol 2007;6:513–20.
[11] LeWitt PA, Lyons KE, Pahwa R, SP 650 Study Group. Advanced Parkinson disease treated with rotigotine transdermal system: PREFER Study. Neurology 2007;68:1262–7.
[12] Nomoto M, Mizuno Y, Kondo T, Hasegawa K, Murata M, Takeuchi M, et al. Transdermal rotigotine in advanced Parkinson's disease: a randomized, double-blind, placebo-controlled trial. J Neurol 2014. in press.
[13] Adler CH, Sethi KD, Hauser RA, Davis TL, Hammerstad JP, Bertoni J, et al. Ropinirole for the treatment of early Parkinson's disease. The Ropinirole Study Group. Neurology 1997;49:393–9.
[14] Korczyn AD, Brunt ER, Larsen JP, Nagy Z, Poewe WH, Ruggieri S, For the 053 Study Group. A 3-year randomized trial of ropinirole and bromocriptine in early Parkinson's disease. Neurology 1999;53:364–70.
[15] Schrag A, Keens J, Warner J. Ropinirole for the treatment of tremor in early Parkinson's disease. Eur J Neurol 2002;9:253–7.
[16] Lieberman A, Olanow CW, Sethi K, Swanson P, Waters CH, Fahn S, et al. A multicenter trial of ropinirole as adjunctive treatment for Parkinson's disease. Ropinirole Study Group. Neurology 1998;51:1057–62.
[17] Mizuno Y, Abe T, Hasegawa K, Kuno S, Kondo T, Yamamoto M, et al. Ropinirole is effective on motor function when used as an adjunct to levodopa in Parkinson's disease: STRONG study. Mov Disord 2007;22:1860–5.
[18] Hughes AJ, Daniel SE, Kilford L, Lees AJ. Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinico-pathological study of 100 cases. J Neurol Neurosurg Psychiatry 1992;55:181–4.
[19] Trenkwalder C, Kohnen R, Högl B, Metta V, Sixel-Döring F, Frauscher B, et al. Parkinson's disease sleep scale—validation of the revised version PDSS-2. Mov Disord 2011;26:644–52.
[20] Christenson GA, Faber RJ, deZwaan M. Compulsive buying: descriptive characteristics and psychiatric comorbidity. J Clin Psychiatry 1994;55:5–11.
[21] Barone P, Lamb J, Ellis A, Clarke Z. Sumanirole versus placebo or ropinirole for the adjunctive treatment of patients with advanced Parkinson's disease. Mov Disord 2007;22:483–9.
[22] Kulisevsky J, Pagonabarraga J. Tolerability and safety of ropinirole versus other dopamine agonists and levodopa in the treatment of Parkinson's disease. Drug Saf 2010;33:147–61.

J Neurol (2014) 261:1887–1893
DOI 10.1007/s00415-014-7427-3

ORIGINAL COMMUNICATION

Transdermal rotigotine in advanced Parkinson's disease: a randomized, double-blind, placebo-controlled trial

Masahiro Nomoto · Yoshikuni Mizuno · Tomoyoshi Kondo · Kazuko Hasegawa · Miho Murata · Masahiro Takeuchi · Junji Ikeda · Takayuki Tomida · Nobutaka Hattori

Received: 26 September 2013 / Revised: 27 June 2014 / Accepted: 27 June 2014 / Published online: 15 July 2014

Abstract Rotigotine, a non-ergot dopamine receptor agonist, offers potential for continuous dopaminergic stimulation that could avoid the fluctuations observed with traditional treatments. We conducted a randomized, double-blind, placebo-controlled trial in Japanese patients with advanced Parkinson's disease (PD) to investigate the efficacy and safety of rotigotine. Inclusion criteria included the presence of motor complications, such as wearing off, on–off, delayed-on/no-on, any circumstances that could interfere with levodopa dose escalation because of side effects, or declining levodopa efficacy. The enrolled patients received once-daily applications of rotigotine transdermal patches or matched placebo patches. A total of 174 patients were randomly assigned to rotigotine (87 patients) or placebo (87 patients). The full analysis set included 172 patients (86 for the rotigotine group and 86 for the placebo group). The maximum maintenance dose of rotigotine was set at 16 mg/24 h. The changes in unified PD rating scale Part III scores from baseline to the end of the trial were -10.1 ± 9.0 (mean ± standard deviation) in the rotigotine group and -4.4 ± 7.4 in the placebo group ($p < 0.001$). There was a significantly greater reduction in the off-time ($p = 0.014$) in the rotigotine group. Rotigotine was well tolerated, with serious adverse events being reported in only three patients in each group. Rotigotine at doses of up to 16 mg/24 h is efficacious and safe in Japanese patients with advanced PD.

Keywords Rotigotine · Randomized controlled trial · Advanced Parkinson's disease · Wearing off · Dyskinesia

Introduction

Levodopa, a dopamine precursor that is converted to dopamine in the brain, has been the mainstay treatment for Parkinson's disease (PD) for over 40 years, and is still the

On behalf of the Rotigotine Trial Group. Members of the Rotigotine Trial Group are listed in Appendix.

M. Nomoto (✉)
Department of Neurology and Clinical Pharmacology, Ehime University Graduate School of Medicine, Shitsukawa, Toon, Ehime 791-0295, Japan
e-mail: nomoto@m.ehime-u.ac.jp

Y. Mizuno · T. Kondo
Department of Neurology, Juntendo University School of Medicine, Tokyo, Japan

K. Hasegawa
Department of Neurology, National Hospital Organization, Sagamihara National Hospital, Kanagawa, Japan

M. Murata
Department of Neurology, National Center Hospital of Neurology and Psychiatry, Tokyo, Japan

M. Takeuchi
Department of Biostatistics, Kitasato University School of Pharmacy, Tokyo, Japan

J. Ikeda · T. Tomida
Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan

N. Hattori
Department of Neurology, Juntendo University School of Medicine, Tokyo, Japan

most effective treatment for the disease [1]. However, long-term treatment of PD patients with levodopa causes motor complications such as wearing off and dyskinesia [2–8]. Indeed, wearing off and dyskinesia were observed in 45 and 34 % of patients who were treated with levodopa for 5 years [5]. It has been suggested that the intermittent stimulation of dopamine receptors by levodopa, which is administered orally and has a short half-life, may play a role in the generation of the motor fluctuations seen in PD patients treated with the drug; therefore, continuous dopaminergic stimulation (CDS) is emerging as a key therapeutic strategy for the treatment of PD [9–12].

Rotigotine (Neupro®), a non-ergot dopamine receptor agonist with activity across the D1 to D5 receptors [13, 14], is a new formulation that ensures continuous dopaminergic stimulation for the treatment of PD [15]. It is a silicone-based once-daily transdermal patch that maintains a stable plasma rotigotine level over a period of 24 h [16–18]. Approximately 44 % of the rotigotine delivered via transdermal patch is systemically available, and systemically absorbed rotigotine is metabolized rapidly [16].

It has been reported that rotigotine (delivered at up to 16 mg/24 h) and pramipexole (up to 4.5 mg/day orally) both reduced off-time compared with placebo [19]. In another randomized, double-blind, placebo-controlled trial, the patients given rotigotine (delivered at up to 12 mg/24 h) experienced significant decreases in off-time [20].

The present randomized, double-blind, placebo-controlled, two-arm parallel group trial was performed to determine the safety and efficacy of rotigotine transdermal patches delivering up to 16 mg of rotigotine per day in patients with advanced-stage PD. Since wearing off is not observed in all patients with advanced PD, we sought to examine the effects of rotigotine at doses of up to 16 mg/24 h in combination with levodopa on changes in the unified PD rating scale (UPDRS) Part III scores as the primary variable.

Patients and methods

The present trial was a randomized, double-blind, placebo-controlled, two-arm parallel group trial. The trial was registered at Clinicaltrials.gov (identifier: NCT01628848), and was conducted in accordance with the International Conference on Harmonisation Guidelines for Good Clinical Practice and the Declaration of Helsinki.

Patients

The trial was approved by the institutional review boards of the 38 centers where the trial was conducted. Informed consent was obtained from all patients before enrollment. Two-hundred and fourteen patients with advanced PD, aged 30–79 years, and with Hoehn and Yahr stage II–IV and a UPDRS Part III sum score of ≥10 ("on" period), were enrolled. Patients had to have received a stable levodopa dose for ≥28 days before starting the trial, and had to show problematic motor complications such as wearing off, on–off phenomenon, or delayed-on/no-on circumstances that could interfere with levodopa dose escalation because of side effects, or declining levodopa efficacy. Subjects were considered to have been on the optimal L-dopa treatment when they were enrolled in the study, even though the dose of L-dopa was low in many of them.

Patients were excluded if they had met any of the following criteria: previous surgery for PD; psychiatric symptoms (for example, confusion, hallucination, delusion, excitation, delirium, and abnormal behavior); orthostatic hypotension; a history of epilepsy or convulsion; clinically relevant hepatic, renal or cardiac disorders; a prolonged QTc interval (QTc interval >450 ms twice during screening or a mean QTc interval of two electrocardiograms of >450 ms in males or >470 ms in females at baseline); a history of skin sensitivity to adhesives or other transdermal medications; or if they were pregnant, nursing, or a woman of child-bearing potential. Patients who had previously received other dopamine agonists or neuroleptics were also excluded. Anti-PD agents such as levodopa, selegiline, amantadine, and anti-cholinergic agents were permitted if the patients were on a stable dose for >28 days before baseline and providing the dose was maintained throughout the trial period.

Trial design

The trial consisted of a 4-week screening period, a maximum 8-week dose-titration period, a 4-week maintenance period, a 2-week tapering period, and a 1-week safety follow-up period. Eligible patients were randomly assigned to receive rotigotine or placebo in a 1:1 ratio using a dynamic allocation procedure designed to balance the distribution of UPDRS Part III sum scores and the off-time between the groups. Subjects received either once-daily rotigotine or placebo transdermal patch. Patients were instructed to rotate the application site (abdomen, thigh, hip, flank, shoulder, upper arm) on a daily basis to minimize application site reactions. The starting rotigotine was 2 mg/24 h. The dose was increased with a weekly increment of 2 mg/24 h to a maximum of 16 mg/24 h during the dose-titration period. If the drug was not tolerated, dose adjustment was allowed. However, further dose adjustment was not permitted after the end of the dose-titration period. The subjects, investigators and all trial personnel remained blinded to the treatments throughout the trial period.

Outcome variables

The primary efficacy variable was the absolute change in UPDRS Part III sum score from baseline to the end of treatment (EOT). Secondary variables included the absolute changes in off-time, UPDRS Part II (average ON and OFF state) sum score, UPDRS Part II (ON state) sum score, UPDRS Part II (OFF state) sum score, and the Hoehn and Yahr scale.

The 20 and 30 % responder rates (defined as the percentage of subjects who achieved ≥20 and ≥30 % reductions in UPDRS Part III scores from baseline to EOT, respectively) were calculated based on patient diaries. In a subgroup analysis, the changes from baseline to EOT in the UPDRS Part III and Part II (average ON and OFF state) scores and the off-time were assessed for their possible associations with age, baseline UPDRS sum score, and Hoehn and Yahr stage.

Safety and tolerability were assessed based on adverse events and changes in vital signs, body weight, electrocardiogram findings, and laboratory parameters.

Statistical methods

The sample size in the present trial was selected based on the hypothesis that rotigotine treatment would improve UPDRS Part III scores better than placebo. Assuming a difference in the change in the UPDRS Part III score from baseline between the two groups of 5 and a standard deviation of 11, 85 patients in each treatment group was considered sufficient to detect a significant difference between the two groups with a power of >80 %. For the primary efficacy analysis, Student's t test with a two-sided type 1 error rate of 5 % was used to compare the changes in UPDRS Part III scores from baseline to EOT between the placebo and rotigotine groups. The responder rates were compared between the two groups using two-sided χ^2 tests. For secondary variables, the groups were compared using the Kruskal–Wallis test for continuous variables or χ^2 tests for categorical variables [21]. Efficacy variables were evaluated using the full analysis set (FAS), which was defined as all randomized patients who received at least one dose of trial medication. If there were any missing values, the last observation was carried forward. All randomized patients were included in the safety analysis set (SAS).

Results

Trial subjects

The trial was conducted between August 2006 and September 2007. Figure 1 shows the patient disposition and flow through the trial. A total of 214 patients were enrolled and 174 were randomly assigned to rotigotine or placebo groups. The FAS comprised 172 patients after exclusion of two patients; one who fulfilled an exclusion criterion and another who had deviated from the trial protocol (Fig. 1).

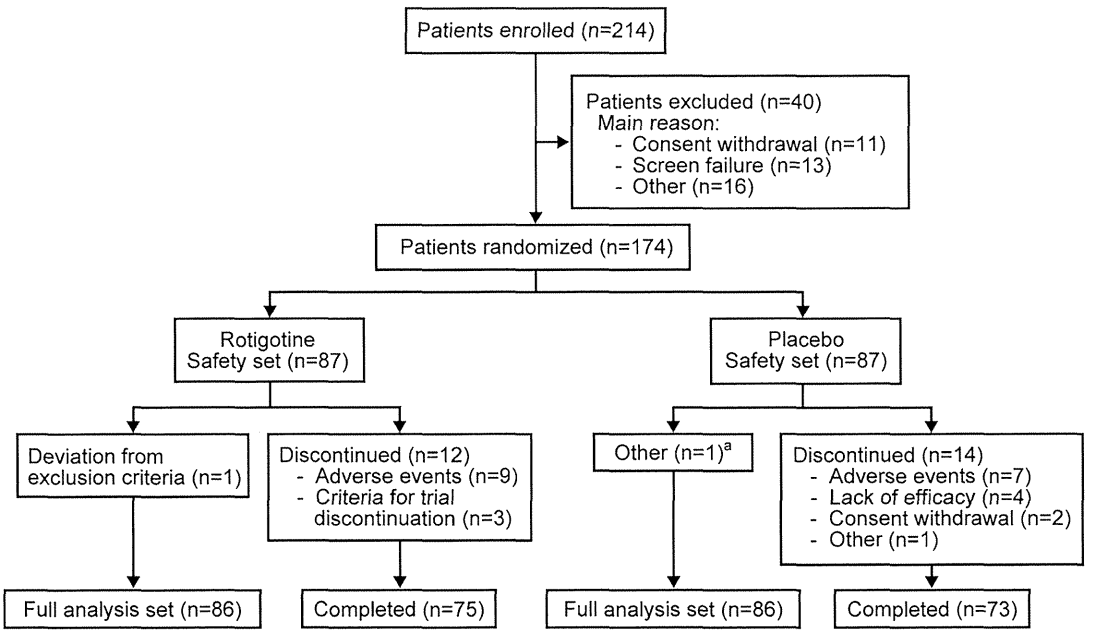

Fig. 1 Patient disposition and flow of patients through the trial. [a]The subject was excluded from the full analysis set and discontinued the trial because of a significant protocol deviation (accidental prolactin measurement in week 2)

Table 1 Baseline characteristics according to treatment group (full analysis set)

	Rotigotine ($n = 86$)	Placebo ($n = 86$)	p value[a]
Sex			
Male	34 (39.5 %)	44 (51.2 %)	0.126[c]
Female	52 (60.5 %)	42 (48.8 %)	
Age (years)	67.0 (6.8)	66.8 (8.3)	0.658[d]
Duration of PD (years)	7.5 (6.0)	5.4 (3.0)	0.037[d]
Hoehn and Yahr stage			
2	11 (12.8 %)	22 (25.6 %)	0.200[c]
2.5	22 (25.6 %)	20 (23.3 %)	
3	45 (52.3 %)	38 (44.2 %)	
4	8 (9.3 %)	6 (7.0 %)	
UPDRS Part II (average ON and OFF state) sum score	11.8 (6.1)	10.3 (4.6)	0.132[d]
UPDRS Part II (ON state) sum score	8.8 (5.6)	8.1 (4.8)	0.322[d]
UPDRS Part II (OFF state) sum score	16.9 (9.3) ($n = 55$)	14.0 (5.7) ($n = 59$)	0.193[d]
UPDRS Part III sum score	28.1 (12.2)	26.2 (10.4)	0.365[d]
Daily off-time (h)	6.6 (3.5)[b] ($n = 54$)	6.0 (3.4)[b] ($n = 57$)	0.383[d]
>0	56 (65.1 %)	59 (68.6 %)	0.627[c]
0	30 (34.9 %)	27 (31.4 %)	
Levodopa (mg/day)	348.8 (170.3)	329.1 (132.5)	0.756[d]
Concomitant medications			
Anticholinergics	19 (22.1 %)	11 (12.8 %)	0.108[c]
Amantadine	36 (41.9 %)	31 (36.0 %)	0.434[c]
Selegiline	42 (48.8 %)	41 (47.7 %)	0.879[c]

Data are means (SD) or number (%)

PD Parkinson's disease, *UPDRS* Unified Parkinson's Disease Rating Scale

[a] Rotigotine versus placebo

[b] Average for 7 days prior to baseline

[c] Chi-square test

[d] Wilcoxon two-sample test

There were no significant differences in baseline demographics or clinical variables between the treatment groups (Table 1). At the end of the dose-titration period, 50.6 % of the rotigotine group entering the maintenance period had reached the maximum dose of 16 mg/24 h.

Efficacy

The change in UPDRS Part III score from baseline to EOT was significantly greater in the rotigotine group than in the placebo group ($p < 0.001$). Patients treated with rotigotine showed a mean reduction in UPDRS Part III score of 10.1 points, while those in the placebo group showed a mean reduction of 4.4 points (Table 2). The mean difference (rotigotine − placebo) between the two groups for the change in UPDRS Part III score was −5.7 [95 % confidence interval (CI) −8.2 to −3.2]. Significantly more patients in the rotigotine group were classified as responders, with improvements in UPDRS Part III scores of ≥20 % (73.3 vs. 43.0 %) or ≥30 % (64.0 vs. 29.1 %) compared with the placebo group, corresponding to mean differences (rotigotine − placebo) of 30.2 % (95 % CI 16.2–44.3 %, $p < 0.001$) and 34.9 % (95 % CI 20.9–48.9 %, $p < 0.001$), respectively.

In terms of other secondary variables, the between-group differences for the changes in UPDRS Part II (average ON and OFF state) score, UPDRS Part II (OFF state) score, and off-time were −2.2 (95 % CI −3.1 to −1.2, $p < 0.001$), −2.6 (95 % CI −4.2 to −1.1, $p < 0.001$) and −1.4 (95 % CI −2.5 to −0.3, $p = 0.014$), respectively.

In subgroup analyses, the differences in the change in UPDRS Part III scores from baseline to EOT between the rotigotine and placebo groups were −1.7 and −7.3 in subjects with baseline scores of <20 and ≥20. The between-group differences in the changes in UPDRS Part II (average ON and OFF state) scores were −0.5 and −3.3 in subjects with baseline scores of <10 and ≥10. The between-group differences in the changes in off-time were

Table 2 Changes in outcomes from baseline to the end of the maintenance period (full analysis set with last observation carried forward)

	Rotigotine[a]	Placebo[b]	Treatment comparison (rotigotine − placebo)		
	Mean (SD)	Mean (SD)	Mean (SE)	95 % CI	*p* value
UPDRS Part III (change from baseline values)	−10.1 (9.0)	−4.4 (7.4)	−5.7 (1.3)	−8.2, −3.2	<0.001[c]
UPDRS Part III					
20 % responder rate[e]	73.3 %	43.0 %	30.2 %	16.2 %, 44.3 %	<0.001[d]
30 % responder rate[e]	64.0 %	29.1 %	34.9 %	20.9 %, 48.9 %	<0.001[d]
UPDRS Part II (average ON and OFF state) (change from baseline)	−3.8 (3.6)	−1.6 (2.6)	−2.2 (0.5)	−3.1, −1.2	<0.001[c]
UPDRS Part II (ON state) (change from baseline)	−3.0 (3.7)	−1.2 (2.6)	−1.9 (0.5)	−2.8, −0.9	<0.001[c]
UPDRS Part II (OFF state) (change from baseline)[f]	−4.6 (4.5)	−1.9 (3.6)	−2.6 (0.8)	−4.2, −1.1	0.001[c]
Off-time (change from baseline)[f]	−2.1 (3.1)	−0.7 (2.8)	−1.4 (0.6)	−2.5, −0.3	0.014[c]

CI confidence interval

[a] $n = 86$ for UPDRS Parts III and II (ON state); $n = 82$ for UPDRS Part II (average ON and OFF state); $n = 51$ for UPDRS Part II (OFF state); $n = 54$ for off-time

[b] $n = 86$ for UPDRS Parts III and II (average ON and OFF state) and II (ON state); $n = 57$ for UPDRS Part II (OFF state); $n = 56$ for off-time

[c] *t* test

[d] Chi-square test

[e] 20 and 30 % responder rates were defined as the percentages of subjects who achieved ≥20 and ≥30 % reductions in UPDRS Part III scores from baseline to EOT

[f] UPDRS Part II (OFF state) and off-time are shown for patients in whom "off" time was observed

−2.3, −0.5, −2.2 and −1.4 in subjects with baseline time of ≤1, 1–2, 2–3, and >3 h, respectively. In subjects stratified by baseline Hoehn and Yahr scores of 2, 2.5, 3, and 4, the between-group differences were as follows: −3.8, −4.8, −6.2 and −7.2, respectively, for the change in UPDRS Part III sum scores; −1.3, −0.9, −2.5, and −4.9, respectively, for the change in UPDRS Part II (average ON and OFF state); and 0.0, −1.8, −1.4, and −0.9, respectively, for change in off-time.

Safety

Overall, 94.3 % (82/87) of the subjects in the rotigotine group and 88.5 % (77/87) of the subjects in the placebo group reported at least one adverse event (AE) during the treatment period. A summary of the treatment-emergent AEs with an incidence of ≥5 % in either group is shown in Table 3.

The most common treatment-emergent AEs that occurred more frequently in the rotigotine group than in the placebo group were application site reactions (50.6 vs. 18.4 %), nausea (19.5 vs. 5.7 %), somnolence (13.8 vs. 1.1 %), constipation (10.3 vs. 1.1 %), vomiting (10.3 vs. 1.1 %), postural dizziness (8.0 vs. 1.1 %), and anorexia (6.9 vs. 0.0 %). All application site reactions were mild or moderate in intensity.

AEs that led to discontinuation were reported by 12.6 % of the rotigotine-treated patients and 8.0 % of the placebo-

Table 3 Treatment-emergent adverse events with an incidence of ≥5 % in any group

Adverse event by preferred term	Rotigotine ($n = 87$)			Placebo ($n = 87$)		
	n[a]	%[a]	AEs[b]	n[a]	%[a]	AEs[b]
Any system organ class	82	94.3	333	77	88.5	194
Nausea	17	19.5	17	5	5.7	8
Constipation	9	10.3	9	1	1.1	1
Vomiting	9	10.3	10	1	1.1	1
Application site reaction	44	50.6	46	16	18.4	21
Application site erythema	8	9.2	14	4	4.6	5
Application site pruritus	5	5.7	5	4	4.6	4
Nasopharyngitis	18	20.7	20	13	14.9	19
Fall	6	6.9	6	7	8.0	7
Bruise	5	5.7	5	3	3.4	4
Increased blood creatine phosphokinase	7	8.0	7	3	3.4	3
Anorexia	6	6.9	6	0	0	0
Dyskinesia	12	13.8	13	7	8.0	7
Dizziness	7	8.0	8	2	2.3	2
Postural dizziness	7	8.0	7	1	1.1	2
Headache	5	5.7	5	2	2.3	2
Somnolence	12	13.8	12	1	1.1	1
Hallucination, visual	8	9.2	10	2	2.3	2

[a] Number of subjects reporting at least one adverse event

[b] Number of individual adverse events occurring among the subjects in that group

treated patients. Six serious AEs (anemia, malaise, increased blood creatine phosphokinase, neuroleptic malignant syndrome, delusion, and auditory hallucination) occurred in three patients in the rotigotine group. A causal relationship to the trial medication was ruled out for all of the events, except for anemia. Four serious AEs (inguinal hernia, gastroenteritis, bacterial arthritis, and loss of consciousness) occurred in three patients in the placebo group and a causal relationship to the trial medication was not ruled out for any event, except for loss of consciousness. These AEs occurred across multiple body systems with no obvious trends. No deaths occurred in this trial.

No subject in the rotigotine group had a QTc interval $\geq$500 ms or a change from baseline QTc interval of $\geq$60 ms. Rotigotine had no clinically significant effect on QTc interval.

Discussion

In the 172 subjects in the FAS (86 subjects treated with rotigotine and 86 subjects treated with placebo), rotigotine significantly reduced UPDRS Part III scores compared with placebo, demonstrating superiority of rotigotine over placebo ($p < 0.001$). Rotigotine also reduced UPDRS Part II (average ON and OFF state) sum scores and the off-time between baseline and EOT. The reduction in UPDRS Part II off-state was particularly noteworthy, and suggests that rotigotine may be effective throughout the day by improving symptoms in the off-state, as well as the on-state. Although dyskinesia was more common in the rotigotine group, the risk/benefit profile of rotigotine, particularly the improvement in off-state, may outweigh the problems. Taken together, the results of the present trial show that rotigotine improves motor functions, activities of daily living, and off-time in advanced PD patients treated with levodopa, and that continuous dopaminergic stimulation with rotigotine is an important treatment option for advanced PD.

In an earlier phase III trial performed in the United States (CLEOPATRA-PD trial), the mean difference between rotigotine (with doses of up to 16 mg/24 h) and placebo for the change in UPDRS Part III scores from baseline to EOT was −4.4 [19]. In a phase III trial conducted in Europe (PREFER trial), the between-group differences were −3.4 and −5.3, when rotigotine was administered at doses of up to 8 mg/24 h and 12 mg/24 h, respectively [20]. In the present trial, the between-group difference was −5.7, with rotigotine administered at doses of up to 16 mg/24 h. The mean reductions in off-time were −1.58 h in the CLEOPATRA-PD trial, −1.8 (8 mg/24 h) and −1.2 (12 mg/24 h) h in the PREFER trial, and −1.4 h in the present trial. From this comparison, the improvement in UPDRS Part III was greater in the present trial compared with the trials in the United States and Europe, while the reduction in off-time was similar in all three trials.

It is worth noting that the administered doses of concomitant medications differed in each trial [19, 20]. The mean administered doses of levodopa in the phase III trials conducted in Europe and the United States ranged from 600 to 700 mg [19, 20], while that in the present trial was 380 mg. Monoamine oxidase-B inhibitors were used in 10–20 % of patients in the trials performed in Europe and the United States, compared with 50 % of patients in the present trial. When the present trial was conducted, marketing authorization for the catechol *O*-methyltransferase inhibitor entacapone had not been granted in Japan; therefore, this drug was not used by any of the patients in the present trial.

The International Conference on Harmonization E14 guidelines require a thorough QT/QTc study for all new drugs, including at supratherapeutic doses—doses well above the maximum clinically expected dose [22]. Therefore, Malik et al. performed a thorough assessment of QT/QTc in patients with advanced PD treated with rotigotine. They found that application of supratherapeutic doses of up to 24 mg/24 h did not affect the QTc interval, indicating that rotigotine did not adversely affect cardiac repolarization in patients with a QTc >500 ms. Furthermore, they found that rotigotine did not cause any change in QTc of >60 ms from baseline [17]. Thus, rotigotine is well tolerated and safe at doses up to 16 mg/24 h.

Conclusion

In Japanese patients with advanced PD, rotigotine improved activities of daily living and motor symptoms, and greatly reduced both off-time and UPDRS Part II score (OFF state) compared with placebo. The rotigotine transdermal patch was efficacious and safe with once-daily application within a dose range of 4–16 mg/24 h in this trial. No significant safety issues were observed with doses of up to 16 mg/24 h.

Acknowledgments This trial was supported by Otsuka Pharmaceutical Co., Ltd., Japan.

Conflicts of interest YM is a Professor of Neuroregenerative Medicine, a position donated by Nippon Boehringer Ingelheim Co., Ltd. and Medtronic Japan Co., Ltd., at Kitasato University School of Medicine. YM is also an advisory board member for Nippon Boehringer Ingelheim Co., Ltd., FP Pharmaceutical Corporation, Otsuka Pharmaceutical Co., Ltd., Abbott Japan Co., Ltd., and Kyowa Hakko Kirin Co., Ltd., and he received personal compensation for attending advisory board meetings. MN received speaker's honoraria from Dainippon Sumitomo Pharma Co., Ltd., Kyowa Hakko Kirin Co., Ltd., Nippon Boehringer Ingelheim Co., Ltd., Novartis Pharma K.K., and Otsuka Pharmaceutical Co., Ltd. TK received honoraria, support for travel to meetings, and fees for participation in review activities

from FP Pharmaceutical Corporation, Novartis Pharma K.K., GlaxoSmithKline K.K., Nippon Boehringer Ingelheim Co., Ltd., Dainippon Sumitomo Pharma Co., Ltd., Kyowa Hakko Kirin Co., Ltd., and Otsuka Pharmaceutical Co., Ltd. KH received personal compensation for attending advisory board meetings from Otsuka Pharmaceutical Co., Ltd. MM received honoraria for consulting and/or lecturing from GlaxoSmithKline K.K., Nippon Boehringer Ingelheim Co., Ltd., Dainippon Sumitomo Pharma Co., Ltd., Novartis Pharma K.K., and Otsuka Pharmaceutical Co., Ltd. MT received honoraria for consulting from Otsuka Pharmaceutical Co., GlaxoSmithKline K.K., and UCB Japan Co. Ltd. NH is an advisory board member for Novartis Pharma K.K., Otsuka Pharmaceutical Co., Ltd., GlaxoSmithKline K.K., Kyowa Hakko Kirin Co., Ltd., and MSD K.K., and has received honoraria from Nippon Boehringer Ingelheim Co., Ltd., GlaxoSmithKline K.K., Novartis Pharma K.K., FP Pharmaceutical Corporation, Takeda Pharmaceutical Company Limited., Janssen Pharmaceutical K.K., Daiichi Sankyo Co., Ltd., Kyowa Hakko Kirin Co., Ltd., and Dainippon Sumitomo Pharma Co., Ltd. JI and TT are employees of Otsuka Pharmaceutical Co., Ltd.

Appendix: Members of the SPM 962 Rotigotine Trial Group

The authors wish to thank the following additional members of the SPM 962 Rotigotine Trial Group, who participated in this trial as investigators: Takashi Kimura, Hidenao Sasaki, Mikio Shoji, Takashi Abe, Atsushi Takeda, Itaru Toyoshima, Kazuo Yoshizawa, Toshiaki Kamitani, Kimihito Arai, Shigeki Tanaka, Sadako Kuno, Fusako Yokochi, Hiroshi Kurisaki, Noriko Kawashima, Shinji Ohara, Kouichi Mizoguchi, Toshihiko Ohashi, Tetsushi Atsumi, Akira Inukai, Tatsuya Hattori, Hideyuki Sawada, Harutoshi Fujimura, Nobuyoshi Yoshikawa, Sonoko Nozaki, Mitsutoshi Yamamoto, Hiroaki Miyaoka, Masahiro Nagai, Noriko Nishikawa, Tatsuo Yamada, Naokazu Sasagasako, Takayuki Kondo, Shigehiro Imamura, Yoshito Sonoda, Satoshi Takahashi, and Hitoshi Yamada.

References

1. Cotzias GC, Van Woert MH, Schiffer LM (1967) Aromatic amino acids and modification of parkinsonism. New Engl J Med 276:374–379
2. Lang AE, Lozano AM (1998) Parkinson's disease. First of two parts. New Engl J Med 339:1044–1053
3. Marsden CD, Parkes JD (1976) "On-off" effects in patients with Parkinson's disease on chronic levodopa therapy. Lancet 1:292–296
4. Ahlskog JE, Muenter MD (2001) Frequency of levodopa-related dyskinesias and motor fluctuations as estimated from the cumulative literature. Mov Disord 16:448–458
5. Rascol O, Brooks DJ, Korczyn AD, De Deyn PP, Clarke CE, Lang AE (2000) A five-year study of the incidence of dyskinesia in patients with early Parkinson's disease who were treated with ropinirole or levodopa. N Engl J Med 342:1484–1491
6. Parkinson Study Group (2000) Pramipexole vs levodopa as initial treatment for Parkinson disease: a randomized controlled trial. JAMA 284:1931–1938
7. Parkinson Study Group (2004) Pramipexole vs levodopa as initial treatment for Parkinson disease: a 4-year randomized controlled trial. Arch Neurol 61:1044–1053
8. Oertel WH, Wolters E, Sampaio C, Gimenez-Roldan S, Bergamasco B, Dujardin M et al (2006) Pergolide versus levodopa monotherapy in early Parkinson's disease patients: the PELMO-PET study. Mov Disord 21:343–353
9. Baronti F, Mouradian MM, Davis TL, Giuffra M, Brughitta G, Conant KE et al (1992) Continuous lisuride effects on central dopaminergic mechanisms in Parkinson's disease. Ann Neurol 32:776–781
10. Engber TM, Susel Z, Juncos JL, Chase TN (1989) Continuous and intermittent levodopa differentially affect rotation induced by D-1 and D-2 dopamine agonists. Eur J Pharmacol 168:291–298
11. Nutt JG, Obeso JA, Stocchi F (2000) Continuous dopamine-receptor stimulation in advanced Parkinson's disease. Trends Neurosci 23(Suppl):109–115
12. Olanow CW, Obeso JA, Stocchi F (2006) Continuous dopamine-receptor treatment of Parkinson's disease: scientific rationale and clinical implications. Lancet Neurol 5:677–687
13. Scheller D, Ullmer C, Berkels R, Gwarek M, Lübbert H (2009) The in vitro receptor profile of rotigotine: a new agent for the treatment of Parkinson's disease. Naunyn-Schmiedebergs Arch Pharmacol 379:73–86
14. Boroojerdi B, Wolff HM, Braun M, Scheller DKA (2010) Rotigotine transdermal patch for the treatment of Parkinson's disease and restless legs syndrome. Drugs Today 46:483–505
15. Pfeiffer RF (2002) Potential of transdermal drug delivery in Parkinson's disease. Drugs Aging 19:561–570
16. Cawello W, Braun M, Boekens H (2009) Absorption, disposition, metabolic fate, and elimination of the dopamine agonist rotigotine in man: administration by intravenous infusion or transdermal delivery. Drug Metab Dispos 37:2055–2060
17. Malik M, Andreas JO, Hnatkova K, Hoeckendorff J, Cawello W, Middle M et al (2008) Thorough QT/QTc study in patients with advanced Parkinson's disease: cardiac safety of rotigotine. Clin Pharmacol Ther 84:595–603
18. Elshoff JP, Braun M, Andreas JO, Middle M, Cawello W (2012) Steady-state plasma concentration profile of transdermal rotigotine: an integrated analysis of three, open-label, randomized, phase I multiple dose studies. Clin Ther 34:966–978
19. Poewe WH, Rascol O, Quinn N, Tolosa E, Oertel WH, Martignoni E et al (2007) Efficacy of pramipexole and transdermal rotigotine in advanced Parkinson's disease: a double-blind, double-dummy, randomised controlled trial. Lancet Neurol 6:513–520
20. LeWitt PA, Lyons KE, Pahwa R (2007) Advanced Parkinson disease treated with rotigotine transdermal system: PREFER Study. Neurology 68:1262–1267
21. Pagano M, Gauvreau K (2000) Principles of biostatistics, 2nd edn. Duxbury Press, Belmont
22. Food and Drug Administration, HHS (2005) International conference on harmonisation; guidance on E14 clinical evaluation of QT/QTc interval prolongation and proarrhythmic potential for non-antiarrhythmic drugs; availability. Notice. Fed Regist 70:61134–61135

Journal of Human Genetics (2014) 59, 163–172

www.nature.com/jhg

ORIGINAL ARTICLE

Molecular epidemiology and clinical spectrum of hereditary spastic paraplegia in the Japanese population based on comprehensive mutational analyses

Hiroyuki Ishiura[1], Yuji Takahashi[1], Toshihiro Hayashi[1], Kayoko Saito[2], Hirokazu Furuya[3], Mitsunori Watanabe[4], Miho Murata[5], Mikiya Suzuki[6], Akira Sugiura[7], Setsu Sawai[8,9], Kazumoto Shibuya[10], Naohisa Ueda[11,12], Yaeko Ichikawa[1], Ichiro Kanazawa[13], Jun Goto[1] and Shoji Tsuji[1]

Hereditary spastic paraplegia (HSP) is one of the most genetically heterogeneous neurodegenerative disorders characterized by progressive spasticity and pyramidal weakness of lower limbs. Because >30 causative genes have been identified, screening of multiple genes is required for establishing molecular diagnosis of individual patients with HSP. To elucidate molecular epidemiology of HSP in the Japanese population, we have conducted mutational analyses of 16 causative genes of HSP (*L1CAM*, *PLP1*, *ATL1*, *SPAST*, *CYP7B1*, *NIPA1*, *SPG7*, *KIAA0196*, *KIF5A*, *HSPD1*, *BSCL2*, *SPG11*, *SPG20*, *SPG21*, *REEP1* and *ZFYVE27*) using resequencing microarrays, array-based comparative genomic hybridization and Sanger sequencing. The mutational analysis of 129 Japanese patients revealed 49 mutations in 46 patients, 32 of which were novel. Molecular diagnosis was accomplished for 67.3% (33/49) of autosomal dominant HSP patients. Even among sporadic HSP patients, mutations were identified in 11.1% (7/63) of them. The present study elucidated the molecular epidemiology of HSP in the Japanese population and further broadened the mutational and clinical spectra of HSP.
Journal of Human Genetics (2014) **59**, 163–172; doi:10.1038/jhg.2013.139; published online 23 January 2014

Keywords: array-based comparative genomic hybridization; hereditary spastic paraplegia; resequencing microarray

INTRODUCTION

Hereditary spastic paraplegia (HSP) is a neurodegenerative disorder characterized by progressive lower limb spasticity and pyramidal weakness, which is one of the most genetically and clinically heterogeneous disorders.[1,2] HSP is clinically divided into two forms, pure and complicated forms, depending on whether the neurological symptoms are basically confined to spasticity and pyramidal weakness of the lower limbs or accompanied by additional neurological symptoms such as cognitive dysfunction, cerebellar signs, optic atrophy, retinitis pigmentosa, amyotrophy and peripheral neuropathy. HSP is characterized by enormous genetic heterogeneity; to date, more than 50 genetic loci (SPG1-57) and 37 causative genes have been identified: *L1CAM* (SPG1), *PLP1* (SPG2), *ATL1* (SPG3A), *SPAST* (SPG4), *CYP7B1* (SPG5A), *NIPA1* (SPG6), *SPG7* (SPG7), *KIAA0196* (SPG8), *KIF5A* (SPG10), *SPG11* (SPG11), *RTN2* (SPG12), *HSPD1* (SPG13), *SPG15/ZFYVE26* (SPG15), *BSCL2* (SPG17), *ERLIN2* (SPG18), *SPG20* (SPG20), *SPG21* (SPG21), *DDHD1* (SPG28), *KIF1A* (SPG30), *REEP1* (SPG31), *ZFYVE27* (SPG33), *FA2H* (SPG35), *PNPLA6* (SPG39), *SLC33A1* (SPG42), *GJC2* (SPG44), *GBA2* (SPG46), *AP4B1* (SPG47), *KIAA0415* (SPG48), *TECPR2* (SPG49), *AP4M1* (SPG50), *AP4E1* (SPG51), *AP4S1* (SPG52), *VPS37A* (SPG53), *DDHD2* (SPG54), *C12ORF65* (SPG55), *CYP2U1* (SPG56), and *TFG* (SPG57).

Because of the limited availability of information on genotype–phenotype correlations and locus heterogeneity, it is often difficult to prioritize genes for the mutational analysis of HSP. Therefore, it is essential to incorporate knowledge of the molecular epidemiology of HSP and relative frequencies of the types of mutations (substitution, insertion/deletion or rearrangement) in each gene into the algorithm of molecular diagnosis of HSP. We also need to be aware that different

[1]Department of Neurology, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan; [2]Institute of Medical Genetics, Tokyo Women's Medical University, Tokyo, Japan; [3]Department of Neurology, Neuro-Muscular Center, National Omuta Hospital, Fukuoka, Japan; [4]Department of Neurology, Institute of Brain Science, Hirosaki University Graduate School of Medicine, Aomori, Japan; [5]Department of Neurology, National Center of Neurology and Psychiatry, Tokyo, Japan; [6]Department of Neurology, Higashisaitama Hospital, National Hospital Organization, Saitama, Japan; [7]Department of Neurology, Shizuoka Institute of Epilepsy and Neurological Disorders, Shizuoka, Japan; [8]Department of Molecular Diagnosis, Graduate School of Medicine, Chiba University, Chiba, Japan; [9]Division of Laboratory Medicine and Clinical Genetics, Chiba University Hospital, Chiba, Japan; [10]Department of Neurology, Graduate School of Medicine, Chiba University, Chiba, Japan; [11]Department of Neurology, Chigasaki Municipal Hospital, Kanagawa, Japan; [12]Department of Neurology, Yokohama City University School of Medicine, Kanagawa, Japan and [13]Graduate School, International University of Health and Welfare, Tokyo, Japan
Correspondence: Dr S Tsuji, Department of Neurology, Graduate School of Medicine, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan.
E-mail: tsuji@m.u-tokyo.ac.jp
Received 13 September 2013; revised 16 November 2013; accepted 29 November 2013; published online 23 January 2014