TCH346 prevents motor symptoms and loss of striatal F-DOPA uptake in bilaterally MPTP-treated primates


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Abstract

The neuroprotective efficacy of the propargylamine TCH346 was studied in the primate model of Parkinson’s disease, the bilaterally MPTP-treated monkey.

Male rhesus monkeys received 2.5 mg MPTP into the left carotid artery and, 8 weeks later, 1.25 mg MPTP into the right carotid artery. Starting 2 h after the second MPTP infusion, either 0.014 mg/kg TCH346 or its solvent was subcutaneously injected twice per day for 14 days.

The first MPTP treatment induced mild Parkinson symptoms, reduced right limb movements, and reduced F-DOPA uptake in the left striatum. The second MPTP treatment made Parkinson symptoms worse, reduced left limb movements, and reduced F-DOPA uptake in the right striatum of solvent-treated monkeys. In contrast, the second MPTP treatment did not further worsen motor symptoms and did not decrease F-DOPA uptake in the right striatum of TCH346-treated monkeys. Although the effects of the second MPTP treatment were largely prevented, the effects of the first MPTP treatment were not reversed by TCH346. Immunohistochemical examination confirmed the dramatic loss of dopamine cells in vehicle-treated monkeys and the preservation of these neurons in the right brain side of the TCH346-treated animals.

In conclusion, systemic administration of TCH346 prevented motor symptoms and nigrostriatal degeneration induced by MPTP in primates.

Keywords: Parkinson’s disease; MPTP; F-DOPA; Propargylamine; TCH346; Primate; CGP 3466B; PET; Immunohistochemistry; Motor behavior
Introduction

Parkinson’s disease (PD) is a common neurodegenerative disorder affecting 1% of the population over age of 50. The disease is characterized by the death of dopaminergic neurons preferentially in the substantia nigra pars compacta. The disease is progressive and further loss of nigrostriatal function contributes to disability and development of levodopa-related motor response complications. Several pathogenic factors possibly contributing to the selective neuronal loss have been identified. Despite the fact that these factors offer clues to potential therapeutic strategies that could halt or slow disease progression, such strategies are not yet available in clinical practice. Indeed, developing and evaluating neuroprotective drugs is essential for improving the present day therapy of PD. The MAO-B inhibitor deprenyl is the most extensively explored drug in this respect. The compound exerts neuroprotective effects in a wide variety of cellular and rodent models for PD. Interestingly, an increasing number of studies reports neuroprotective effects of deprenyl in nondopaminergic cells, which indicates that MAO-B inhibition does not account for this. Clinical effects, however, are disappointing: a double-blinded, placebo-controlled, multicenter clinical trial, i.e., the DATATOP-study (Deprenyl and Tocopherol Antioxidative Therapy of Parkinsonism) showed that, although deprenyl significantly delayed the development of disability requiring levodopa therapy, a worsening of motor scores was observed after drug withdrawal. This indicates a symptomatic effect and a limited, if any, neuroprotective effect. Conversion of deprenyl into the toxic metabolites amphetamine and methamphetamine may explain the lack of neuroprotection and also its cardiovascular adverse effects. Indeed, propargylamines that lack these metabolic complications may be more effective in PD. The deprenyl analogues rasagiline and TCH346 (dibenzo[b,f]oxepin-10-yl-methyl-methyl-prop-2-ynylamine; labeled previously CGP 3466B) are excellent candidates in this respect. These drugs are not metabolized into (meth)amphetamine and provide neuroprotection in cellular and rodent models of PD. Rasagiline protects dopaminergic SH-SY5Y cells from 6-hydroxydopamine-induced apoptosis and is more effective than deprenyl in reducing oxygen/glucose deprivation damage in PC 12 cells. The siomer of rasagiline, TVP1022, is neuroprotective in mice with closed head injuries. Unlike rasagiline, TVP1022 does not inhibit MAO-B. Thus, similarly to deprenyl, MAO-B inhibition is not a prerequisite for neuroprotection by rasagiline. Further clinical studies are necessary to evaluate the neuroprotective efficacy of rasagiline in PD.

TCH346 has been tested in a wide variety of cellular and animal models of PD and exhibits neurorescuing properties qualitatively similar to, but about 100-fold more potent, than those of deprenyl. The compound is not only able to rescue dopamine neurons in vitro from death induced by apoptotic stimuli, but also has promising effects in rodent models of PD. Doses of 0.0014 – 1.4 mg/kg TCH346 given twice daily for...
18 days have neuroprotective effects in mice and, in addition, the compound prevents nigral degeneration and motor symptoms induced by low doses of 6-hydroxydopamine in the rat model of PD\textsuperscript{5, 52}. In our previous study\textsuperscript{3} and in the present study, we extend the evaluation of the neuroprotective effects of TCH346 to an animal more similar to the human disease in both brain pathology and symptomatology, namely the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-hydrochloride (MPTP)-treated rhesus monkey. Although MPTP primate models have allowed well-founded appraisal of a vast range of symptomatic treatments\textsuperscript{8, 9, 39}, not all MPTP primate models are suitable for assessment of the efficacy of neuroprotective drugs. Recovery from MPTP-induced deficits\textsuperscript{21, 34} and high interindividual variability in susceptibility to the neurotoxin\textsuperscript{20} are confounding factors that prevent adequate analysis of putative neuroprotective effects. In order to circumvent this, we used a two-phase intracarotid lesion approach\textsuperscript{4, 45}; not only does this approach induce a stable syndrome with little recovery over time\textsuperscript{11}, it offers the opportunity to estimate the sensitivity of each individual monkey to MPTP, prior to studying the effects of putative neuroprotective compounds such as of TCH346.

Our previous study has shown that TCH346 is able to prevent Parkinson symptoms in this primate model of PD, as assessed qualitatively with a nonhuman primate clinical rating scale\textsuperscript{3}. Here, we extended the behavioral evaluation of this compound with a quantitative analysis of motor symptoms by scoring goal-directed limb movements. Moreover, we investigated the in vivo efficacy of TCH346 to spare the integrity of nigro-striatal neurons using the 6-[\(^{18}\)F]fluoro-L-dopa (F-DOPA) PET scanning technique. Finally, the in vivo data were confirmed postmortem by light and confocal microscopical examination.

**Materials and methods**

**Subjects**

Preceding MPTP exposure, the dominant limb was selected as described earlier\textsuperscript{4}. Eight male Rhesus monkeys (Macaca mulatta) over the age of 7 years, weighing between 6 and 12 kg, were studied under an approved protocol that met all institutional guidelines and requirements stated in the *Principles of Laboratory Care* (NIH publication no. 85–23, revised 1985). The animals were individually housed in cages (90 x 180 x 200 cm) equipped with a climbing bar, toys, and a “squeeze cage” (60 x 60 x 70). Diet consisted of lab chow supplemented with fruit; water was available ad libitum. Monkeys were kept under stable room conditions (temperature = 22 ± 2°C) and exposed to a 12-h light–dark cycle (light on 7:00 a.m. to 7:00 p.m). This study was approved by the local ethics committee for animals of the University Medical Center Groningen, Groningen, The Netherlands.
MPTP treatment
A two-step bilateral MPTP lesion was created as first described by Smith et al.\(^4\), \(^45\); see Andringa et al., for details). In short, under total anesthesia a first dose of 2.5 mg MPTP was administered into the carotid artery contralateral to the dominant limb (being the right limb in all monkeys) and, 8 weeks later, a second dose of 1.25 mg MPTP was infused into the other carotid artery. The second dose was kept lower to reduce the risk of severe incapacitation or even death of the animals. This MPTP treatment procedure induces a stable model with little recovery, according to \([^{123}I]\)FP-CIT SPECT-experiments\(^1\).

Administration of TCH346
Monkeys received their first subcutaneous injection with TCH346 (0.014 mg/kg, generous gift of Novartis, Basel, Switzerland) or its solvent, saline, 2 h after the second MPTP infusion. During the consecutive 13 days, the monkeys were injected twice daily. The selected dose of 0.014 mg/kg was based on dose–range studies of TCH346 in rats\(^5\) and pilot studies in monkeys. These studies show bell-shaped dose–response curves, with an optimum around 0.014–0.14 mg/kg sc. The lowest effective dose (0.014 mg/kg) was chosen for evaluation in primates.

Assessment of motor symptoms
Monkeys were videotaped for 1 h each test day. Evaluation occurred 10 and 3 days prior to the first MPTP treatment (naive stage), 6 and 7 weeks after the first MPTP treatment (unilateral stage), and 3, 7, 14, 21, 28, and 35 days after the second MPTP treatment (bilateral stage). Given the lack of a significant difference, the two sets of data collected in the naive stage were averaged; the same held true for the two sets of data collected in the unilateral stage. The motor symptoms induced by the initial MPTP treatment were used to estimate the sensitivity of each monkey to MPTP: the unilaterally treated animals were then divided among the TCH346 and control group so that the mean Parkinson scores of the group was approximately equal, prior to infusing the second dose of MPTP in the opposite artery and starting the TCH346 or saline treatment. Motor symptoms were evaluated using a quantitative assessment of goal-directed limb movements\(^6\). Evaluation occurred in a blinded fashion and by a single trained investigator (GA). Animals were housed, treated with MPTP and TCH346, behaviorally assessed, and euthanized at the Central Animal Facility of the University of Nijmegen, The Netherlands.

F-DOPA PET scans
F-DOPA PET scans can be used to assess, in vivo, the status of the striatal dopaminergic system. Scans were conducted approximately 1 week prior to the first MPTP treatment.
(naive stage), approximately 7 weeks after the first MPTP treatment (unilateral stage), and approximately 5 weeks after the second MPTP treatment (bilateral stage).

Doudet and colleagues\(^\text{18}\) have shown that the interanimal variability of F-DOPA measures is small (COV~14\%) and thus appropriate to examine dopamine uptake repeatedly within the same animal. Animals were fasted overnight prior to each F-DOPA PET scan. Anesthesia of the monkeys was initiated with 7 mg/kg impentobarbital and 0.05 mg/kg imatropine. The animals were maintained under light anesthesia with isoflurane gas (1.5 \%) for the remainder of the scan. The peripheral decarboxylase inhibitor carbidopa was administered orally (3 mg/kg) approximately 30 min before F-DOPA administration. A venous access line was inserted and the monkeys were intubated. The monkey's head was always fixated in the same position with a modified stereotactic apparatus as head holder. Monkeys were placed in the camera in such a way that the center of the field of view was positioned just above the eyes. PET scans were performed on the Siemens ECAT Exact HR + scanner (Siemens, Munich/Erlangen, Germany). This tomograph has a resolution of 5 mm full width at half maximum in the center of the field of view and an axial field of view of 15.5 cm. Twenty minutes before the scanning, a transmission scan, using a $^{68}$Ge-ring, was performed for attenuation correction. F-DOPA was injected intravenously as a bolus in a volume of 6 ml saline over a period of 1 min. The injected dose ranged from 54 to 152 MBq, with a mean of 78.5 MBq and a standard deviation of 17.7 MBq. Scanning consisted of 21 frames starting directly after the tracer infusion and proceeding at gradual increasing intervals up to a total scan duration of 120 min.

Three regions of interest (left and right striatum and a reference region of nonspecific uptake) were defined. The size of the regions of interest was fixed in a standard template, which was used for all the scans. This approach avoids possible bias introduced by resizing regions to fit the apparent size of the striatum in an image, which may be affected by changes in function. The reference region included both cortical and white matter regions and its large size also reduced possible intersubject biases in placement. The estimate for each region was an average of separate measurements made on each of three planes. The center of each region was repositioned separately on each plane. Indices for comparing uptake in striatum with uptake in reference regions were calculated as uptake in striatum minus uptake in reference region, divided by uptake in reference region for both the left and right side, and referred to as Striatum reference index (SRI). Indices were calculated using the data between 75 and 105 min scanning time. F-DOPA PET scans were performed at the Groningen University Hospital PET Center, Groningen, The Netherlands.

**Histology and immunohistochemistry**

Seven weeks after the last TCH346 injection animals were euthanized with an overdose of sodium pentobarbital and transcardially perfused with saline, followed by
4% paraformaldehyde in 0.1 M phosphate buffer. Animal 4 did not wake up from the isofluorane anesthesia after the third PET scan. Pathological examination did not reveal any abnormalities and anesthetic oversensitivity was concluded to be the most probable cause of death. This animal was not included in the neuropathological examination. Histological and immunohistochemical examinations were performed at the Preclinical Safety Assessment Department, Novartis Pharma AG, Basel, Switzerland. Hemispheres of each brain were sectioned together into 5- to 8-mm-thick coronal slices; similar tissue slices were obtained by performing cross-sections through the brainstem and cerebellum. Specimens were embedded in paraffin wax and 5-µm-thick sections were obtained. Sections from three anteroposterior levels of the striatum and from the upper, mid, and lower levels of the midbrain were stained with hematoxylin and eosin or immunostained either for TH, the rate-limiting enzyme in catecholamine synthesis, or for GFAP, the major constituent of glial cytoplasmic filaments. Immunohistochemical procedures were performed as described previously. Dopaminergic integrity and astroglisisis, as assessed by TH- and GFAP-immunoreactive cells and processes, respectively, were evaluated in substantia nigra and striatum for each animal. The relative number of dopaminergic neurons was evaluated in three sections of three levels (upper, mid, and lower) in the left and right substantia nigra pars compacta. The number of nigral neurons present in a midpower field (20 x objective) was categorized as follows: low number (<15), moderate number (15–30), and high number (>30). TH immunoreactivity in the striatum, as well as GFAP immunoreactivity in the substantia nigra pars compacta and striatum, were semiquantitatively analyzed. For confocal microscopy analysis, paraffin sections from three anteroposterior levels of the left and right caudate and putamen were immunofluorescently labeled for TH (1:4000) followed by Alexa Fluor 488 goat anti-mouse IgG (1:250; Molecular Probes, The Netherlands) and counterstained with propidium iodide. Labeling was examined with a Leica TCS-NT (Leica Microsystems Ltd) confocal laserscanning microscope. Using a 63 x water immersion objective and 0.284-µm optical sections, the upper and lower limits of the TH-labeled fibers were imaged. Confocal settings were kept the same for all analyzed slides. Raw data were imported to a Silicon Graphics (SGI) workstation. The density of the TH-labeled fibers (green channel) was quantified using Voxelshop Pro software (Bitplane AG, Switzerland), by dividing the calculated volume of fibers by the total volume of the tissue section confocaled. The fiber density was pooled and averaged. Propidium iodide-labeled structures were excluded from the calculation.

Statistics

All data are expressed as means ± SEM. The effects of the first MPTP treatment were analyzed by comparing the scores of the solvent and TCH346-treated group in the naive stage with those in the unilateral stage, using the Wilcoxon signed rank test for
the Parkinson scores and the paired $t$-test for limb movements and PET scores. The effects of the second MPTP treatment were analyzed by comparing the scores of the solvent-treated group in the unilateral stage with those of the solvent-treated group in the bilateral stage, using the nonparametric Friedman test for Parkinson scores, a one-way ANOVA for limb movements followed by Dunnett’s posthoc tests where appropriate, and a paired $t$-test for the PET scores. The ability of TCH346 to prevent MPTP-induced behavioral and neurochemical deficits was analyzed by comparing Parkinson scores, limb movements, and F-DOPA uptake of the solvent-treated monkeys in the bilateral stage with those of the TCH346-treated monkeys in the bilateral stage using a twoway ANOVA with repeated measures. Posthoc $t$-tests were done where appropriate. A $p$ value $< 0.05$ was considered significant.

**Results**

**Naive stage**
In the naive stage animals spent an average time of 10.3 and 18.2 % on left and right limb movements, respectively (Fig. 1A B). Furthermore, all animals scored 0 on the Parkinson rating scale, which indicates that they all displayed normal motor behavior before MPTP administration (Table 1). Consistent with the behavioral data, F-DOPA uptake in the right and left striatum was within the normal range in all animals (Figs. 2A and 3 Table 2 ).
TCH346 prevents motor symptoms and loss of F-DOPA uptake in MPTP-treated primates

Figure 1: Percentage of time spent on left (A) and right (B) goal-directed limb movements in rhesus monkeys prior to MPTP treatment (naïve stage), after initial infusion of 2.5 mg MPTP into the left carotid artery (unilateral stage), and after a second infusion of 1.25 mg MPTP into the right carotid artery, 8 weeks later (bilateral stage). Monkeys received saline (n = 4) or TCH346 (n=4) twice per day for 2 weeks, starting 2 h after the second MPTP infusion. Data are expressed as mean ± SEM.

Table 1: SRI of F-DOPA uptake in the left and right striatum of naïve, unilaterally and bilaterally MPTP-treated rhesus monkeys.

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<th>Vehicle</th>
<th>Naïve</th>
<th>Unilateral</th>
<th>Bilateral</th>
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<tr>
<td></td>
<td>Day 3</td>
<td>Day 7</td>
<td>Day 14</td>
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<tr>
<td>TCH346</td>
<td>0</td>
<td>1.63±0.97</td>
<td>6.25±0.63</td>
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Note: F-DOPA uptake depicted as SRI in the left and right striatum in rhesus monkeys prior to MPTP treatment (naïve stage), after initial infusion of 2.5 mg MPTP into the left carotid artery (unilateral stage) and after a second infusion of 1.25 mg MPTP into the right carotid artery, 8 weeks later (bilateral stage). Monkeys received saline (n = 4) or TCH346 (n=4) twice per day for 2 weeks, starting 2 h after the second MPTP infusion. Data are expressed as means ± SEM.
Figure 2: (A) F-DOPA uptake in the left and right striatum in rhesus monkeys prior to MPTP treatment (naive stage) and after initial infusion of 2.5 mg MPTP into the left carotid artery (unilateral stage). SRI was calculated as F-DOPA uptake in striatum minus uptake in reference region, divided by uptake in reference region. Indices were calculated using the data between 75 and 105 min scanning time. Individual data and average SRI are depicted. Open circles, saline-treated primates; closed circles, TCH346-treated primates.

(B) F-DOPA uptake in the left and right striatum in rhesus monkeys after initial infusion of 2.5 mg MPTP into the left carotid artery (unilateral stage) and after a second infusion of 1.25 mg MPTP into the right carotid artery, 8 weeks later (bilateral stage). Monkeys received saline (n= 4) or TCH346 (n = 4) twice per day for 2 weeks, starting 2 h after the second MPTP infusion. SRI was calculated as F-DOPA uptake in striatum minus uptake in reference region, divided by uptake in reference region. Indices were calculated using the data between 75 and 105 min scanning time. Individual data and average SRI are depicted. Open circles, saline-treated primates; closed circles, TCH346-treated primates.
Table 2: SRI of F-DOPA uptake in the left and right striatum of naïve, unilaterally and bilaterally MPTP-treated rhesus monkeys.

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<th>Naïve</th>
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<th>Bilateral</th>
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<tbody>
<tr>
<td></td>
<td>Left</td>
<td>Right</td>
<td>Left</td>
</tr>
<tr>
<td>Saline</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
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<td>0.446</td>
<td>0.250</td>
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<tr>
<td>2</td>
<td>0.459</td>
<td>0.531</td>
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<tr>
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<tr>
<td>4</td>
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<td>0.498</td>
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<tr>
<td>Mean ± SEM</td>
<td>0.466±0.026</td>
<td>0.473±0.022</td>
<td>0.109±0.05</td>
</tr>
<tr>
<td>TCH346</td>
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<tr>
<td>5</td>
<td>0.512</td>
<td>0.463</td>
<td>0</td>
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<tr>
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<td>0.458</td>
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</tr>
<tr>
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<td>0.404</td>
<td>0.308</td>
</tr>
<tr>
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<td>0.414±0.027</td>
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<tr>
<td>Mean ± SEM</td>
<td>0.451±0.028</td>
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<td>0.161±0.056</td>
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<tr>
<td>Mean ± SEM</td>
<td>0.451±0.017</td>
<td>0.402±0.079</td>
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<tr>
<td>(n=16)</td>
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Note: F-DOPA uptake depicted as SRI in the left and right striatum in rhesus monkeys prior to MPTP treatment (naïve stage), after initial infusion of 2.5 mg MPTP into the left carotid artery (unilateral stage) and after a second infusion of 1.25 mg MPTP into the right carotid artery, 8 weeks later (bilateral stage). Monkeys received saline (n = 4) or TCH346 (n=4) twice per day for 2 weeks, starting 2 h after the second MPTP infusion. Data are expressed as means ± SEM.
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Figure 3: Representative images of F-DOPA labeling in coronal sections of a vehicle (A–C) and TCH346-treated (D–F) rhesus monkey brain prior to MPTP treatment (naive stage; A and D), after initial infusion of 2.5 mg MPTP into the left carotid artery (unilateral stage; B and E) and after a second infusion of 1.25 mg MPTP into the right carotid artery, 8 weeks later (bilateral stage; C and F). Monkeys received saline (n = 4) or TCH346 (n = 4) twice per day for 2 weeks, starting 2 h after the second MPTP infusion.

Figure 4: (A and B) TH immunoreactivity in transverse sections through the midbrain of a vehicle- (A) and a TCH346-treated (B) monkey. Five-micrometer sections were immunostained with TH using the streptavidin/peroxidase method and counterstained with hematoxylin. Note the absence of TH immunoreactivity in the left side of the substantia nigra in both animals. In the vehicle-treated monkey, a dramatic loss of TH immunoreactivity was also observed in the right substantia nigra contrasting with the intensely immunostained right substantia nigra of the TCH346-treated monkey. In the latter, only the ventrolateral zona compacta appears partly affected. Original magnification, 2.5x. Bar represents 0.4 mm. (C and D) Hematoxylin and eosin-stained sections through the left (C) and right (D) substantia nigra of a TCH346-treated monkey. Nigral neurons are virtually absent in the left substantia nigra and glial proliferation, characterized by an increase in small cell nuclei, is obvious. In the right substantia nigra, numerous dopaminergic neurons are present. Original magnification, 200x. Bar represents 50 μm. (E and F) TH immunoreactivity in semisuccessive sections to C and D, respectively, in a TCH346-treated monkey. Five-micrometer sections were immunostained with TH using the streptavidin/peroxidase method. Note the dramatic loss of TH-positive neurons and of their dopaminergic fiber network in the left midbrain (E). The neurons appear dystrophic with pyknotic perikarya and short interrupted processes (E). In the contralateral substantia nigra, the numerous neurons and dopaminergic fiber network are intensely immunostained for TH. Original magnification, 200x. Bar represents 50 μm. (G and H) TH immunoreactivity in 5-μm coronal sections through the paraventricular part of the left (G) and right (H) caudate nucleus of a TCH346-treated monkey; the ependymal layer of the left (G, right) and right (H, left) lateral ventricle is present. Sections were immunostained with TH using the streptavidin/peroxidase method. Scattered TH-positive dopaminergic fibers are present in the left nucleus caudate (G, arrows) and a dense dopaminergic fiber network is preserved in the right caudate nucleus (H). Original magnification, 200x. Bar represents 50 μm. (I and J) GFAP immunoreactivity in coronal 5-μm sections through the paraventricular part of the left (I) and right (J) caudate nucleus of a TCH346-treated monkey; same areas as in G and H. The left caudate nucleus displays astroglial proliferation (I), while in the right caudate nucleus, GFAP immunoreactivity is almost exclusively expressed in the ependymal/subependymal layer (J). Streptavidin/peroxidase method for GFAP, hematoxylin counterstaining. Original magnification, 200x. Bar represents 50 μm.
Fig. 5. Confocal photomicrographs showing TH immunofluorescence of dopaminergic fibers in the left (A) and right (B) caudate nucleus of a TCH346-treated animal. Five-micrometer sections were immunofluorescently labeled with TH followed by Alexa Fluor 488 goat anti-mouse IgG and counterstained with propidium iodide. Note the dramatic loss of TH-positive fibers in the left caudate nucleus (A), contrasting with the dense dopaminergic fiber network present in the right caudate nucleus (B). Original magnification, 630x. Bar represents 20 µm.

Unilateral stage: effects of unilateral MPTP administration

The initial MPTP infusion into the left carotid artery significantly reduced the percentage of time spent on goal directed right limb movements [naive vs unilateral stage (14) = 3.92, p < 0.001; paired t-test, Fig. 1B], but had no effect on the time spent on left limb movements [naive vs unilateral stage (14) = 0.92, p < 0.05; paired t-test; Fig. 1A]. Following unilateral MPTP administration, all monkeys displayed Parkinson symptoms that ranged from very mild (score = 1) to mild (score = 5) [naive stage vs unilateral stage: t(7) = 10.87, p < 0.01: Wilcoxon; Table 1]. Six of eight monkeys displayed symptoms that were restricted to the side contralateral to the MPTP infusion (in all monkeys being the right side of the body), while two monkeys exhibited bilateral symptoms. In line with the behavioral data, F-DOPA uptake was strongly reduced in the left striatum 7 weeks after MPTP injection into the left carotid artery [naive stage vs unilateral stage: t(6) = 3.65, p < 0.01; Figs. 2A and 3, paired t-test]. In contrast, F-DOPA uptake in the right striatum was increased [naive stage vs unilateral stage: t(6) = 2.62, p < 0.04; Figs. 2A and 3, Table 2, paired t-test].

Bilateral stage: protective effects of TCH346 on MPTP-induced deficits

Infusion of a second dose of MPTP into the right carotid artery strongly reduced the time spent on left limb movements in the saline-treated group [saline groups: unilateral stage vs bilateral stage: F(6,27) = 2.60, p < 0.05; Fig. 1A, one-way ANOVA] but did not further decrease the time spent on right limb movements [saline groups: unilateral stage vs bilateral stage: F(6,27) = 1.00, p < 0.05; Fig. 1B, one-way ANOVA]. In contrast to its effects in saline-treated monkeys, the second MPTP injection did not reduce the time spent on left limb movements in TCH346-treated animals [TCH346 groups: unilateral stage vs bilateral stage: F(6,27) = 0.19, p < 0.05; Fig. 1A]. Indeed, the percentage of time spent on left limb movements was significantly lower in the animals that had received saline than in the animals that had received TCH346 [saline; bilateral stage vs TCH346, bilateral stage: F(1,36) = 16.06, p < 0.001; Fig. 1A; two-way ANOVA]. In the TCH346-treated group, right limb movements were not affected by the second MPTP treatment [TCH346 unilateral stage vs TCH346 bilateral stage: F(26) = 0.52, p < 0.05; Fig. 1B; two-way ANOVA].
The second MPTP injection significantly worsened the Parkinson score in the saline-treated monkeys, including the monkey that exhibited bilateral symptoms after the initial MPTP treatment [saline group: unilateral stage vs bilateral stage: $F(6,27) = 78.45, p < 0.0007$; Friedman; Table 1]. Parkinson scores were very similar over all test days, which indicates that the MPTP-induced syndrome was stable in time. There was a positive correlation between the Parkinson score of the saline-treated animals in the unilateral and bilateral phase ($r = 0.95, p < 0.05$, data not shown), indicating that the severity of the parkinsonian symptoms after the first MPTP injection is a good predictor for the effects of the second MPTP treatment. The second MPTP injection only mildly affected the parkinsonian symptoms in the monkeys that had received TCH346, including the monkey that already exhibited bilateral symptoms after the initial MPTP treatment [total score, unilateral vs bilateral stage: $F(6,27) = 34.82, p < 0.001$; Friedman; Table 1]. The severity of Parkinsonian symptoms was much lower in the bilaterally MPTP-treated primates that had received TCH346 than in the animals that had received the solvent of TCH346 [$F(1,36) = 930.20, p < 0.0001$, two-way ANOVA, factor treatment]. The score between the saline and TCH346-treated group was significantly different on all test days.

In the saline group, the second MPTP treatment into the right carotid artery tended to decrease F-DOPA uptake in the right striatum. Compared to the first MPTP lesion on the left side, damage was less severe, in concordance with the reduced amount of MPTP given [unilateral stage vs bilateral stage: $t(3) = 1.85, p < 0.08$; Figs. 2B and 3, Table 2]. F-DOPA uptake in the left striatum was not altered by the second MPTP treatment (Table 2). In the group that had received TCH346, F-DOPA uptake in the right striatum remained unaffected after the second MPTP treatment. In line with this, F-DOPA uptake in the right striatum showed a tendency to be higher in the TCH346 group compared to the control group [$F(2,0.14) = 2.92, p < 0.067$, two-way ANOVA, factors treatment x stage; Figs. 2B and 3, Table 2], suggesting that TCH346 prevented the reduction in F-DOPA induced by the second MPTP administration. However, similarly to the saline group, F-DOPA uptake in the left striatum was unaltered, illustrating that TCH346 was not able to reverse the reduction in F-DOPA uptake that had been established by the first MPTP administration, 8 weeks before the start of the treatment with TCH346.

The main neuropathological findings are summarized in Table 3. All vehicle-treated monkeys showed a dramatic loss of the pigmented TH-positive neurons and of their dopaminergic network in the left substantia nigra (Fig. 4A). Two of these animals (1 and 2) exhibited a similar picture in the right side of the midbrain (Fig. 4A), while the third monkey (3) displayed a relatively preserved right substantia nigra, with moderate numbers of dopaminergic neurons. Increased astroglial proliferation was observed in the same areas. TH immunoreactivity was dramatically decreased in the caudate nucleus and the putamen, in both sides, in two of the vehicle-treated animals (1 and 2), while moderate
TH immunoreactivity was present in the right striatum of the third control monkey (3). Mild gliosis was observed in the striatum (bilaterally) of two control animals (1 and 2). In TCH346-treated monkeys, small (Fig. 4B, C, and E; monkeys 5 and 7) and moderate (monkeys 6 and 8) numbers of dopaminergic neurons were found in the left substantia nigra, where slight (monkey 6) to moderate (monkeys 5, 7, and 8) gliosis was present. All four animals exhibited moderate (monkey 8) and high (monkeys 5–7) numbers of neurons in the right substantia nigra (Fig. 4D and F), although the ventrolateral zona compacta appeared partly affected (Fig. 4B). Intense TH immunoreactivity (Fig. 4H), without gliosis (Fig. 4J), was observed in the right striatum of all four monkeys, while minimal (Fig. 4G; monkeys 5 and 7), slight to moderate (monkey 8), and moderate (monkey 6) TH immunoreactivity and slight astroglial proliferation (Fig. 4I) were found in the left striatum. Confocal microscopy analysis of TH labeling in the striatum (Figs. 5 and 6) enabled a direct comparison of dopaminergic fiber density between vehicle- and TCH346-treated groups and between individual animals. In all vehicle-treated animals, density of the TH-labeled fibers in the left striatum was very low (0.003–0.006). In three (monkeys 5, 7, and 8) of four animals treated with TCH346, fiber densities in the left striatum (Fig. 4I) corresponded to those recorded in the vehicle-treated monkeys (0.002–0.08), with one primate exhibiting higher fiber densities in the left striatum. In two of the vehicle-treated monkeys (1 and 2), there was a low density of TH-labeled fibers in the right striatum. In the third vehicle-treated animal (monkey 3), however, the right striatum showed higher fiber densities, averaging 0.32. In contrast, in the right striatum of the all TCH346-treated monkeys, TH-labeled fibers were much greater in density ranging from 0.34 to 0.41 (Figs. 5B and 6). Overall, different techniques used to analyze nigrostriatal integrity, i.e., F-DOPA PET scanning, TH light microscopy, and TH confocal microscopy rendered very similar outcomes in each individual animal.
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Table 3: Neuropathological examination of vehicle and TCH346-treated MPTP monkeys

<table>
<thead>
<tr>
<th>Treatment</th>
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<th>TCH346</th>
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<td>Animal no.</td>
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Note: overview of TH and GFAP immunohistochemistry in the left and right substantia nigra and striatum of vehicle and TCH346-treated primates. Monkeys were perfused 7 weeks after the last TCH346 or vehicle injection. Five-micrometer coronal sections were immunostained for TH or GFAP and analyzed using light microscopy. The terms gliosis refers to GFAP-positive cells and cell processes.

(-) almost absent
+ low/low number
++ moderate/moderate number
+++ high / high number

Fig. 6. Confocal microscope analysis of dopaminergic fiber density in the left and right striatum of monkeys treated with either TCH346 or vehicle. Density is calculated as the volume of fibers divided by the total volume of the examined tissue.
Discussion

The present data provide neurochemical, neuropathological, and behavioral evidence to demonstrate that TCH346 prevents the death of nigrostriatal dopaminergic neurons and concomitant motor symptoms in an animal model that most closely resembles the pathophysiology and symptomatology of PD, namely the bilaterally MPTP-treated monkey. The 14-day administration of TCH346 starting 2 h after the second 1.25 mg MPTP infusion was able to largely prevent motor deficits induced by such an MPTP treatment in saline-treated primates. Not only Parkinson scores were lower in the TCH346-treated group than in the solvent-treated group, TCH346-treated monkeys also did not show any reduction in the percentage of time spent on left limb movements. In accordance with this, the F-DOPA uptake in the right striatum appeared greater in the group that had received TCH346 than in the group that had received saline. Moreover, in three of four TCH346-treated monkeys, semiquantitative and quantitative analysis of TH and GFAP immunoreactivity in the substantia nigra and striatum confirmed the preservation of the nigrostriatal system in the right hemisphere. Our data are consistent with findings of Pate and colleagues, which show that a reduction in F-DOPA uptake correlates well with a reduction in the amount of dopamine nerve terminals in the striatum. Therefore, it is concluded that TCH346 was able to prevent the MPTP-induced degeneration of dopamine neurons in these primates.

Although TCH346 almost completely prevented the reduction in F-DOPA uptake in the right striatum induced by the administration MPTP 2 h earlier, as anticipated, it did not reverse the Parkinson symptoms or reduced F-DOPA uptake induced by the initial MPTP treatment 8 weeks earlier. The majority of neurodegeneration is established within a week after intracarotid administration of MPTP, thus indicating that the degenerative process had mostly ended by the time of TCH346 administration 8 weeks later.

Our data are in line with the accumulating evidence that TCH346 can interfere with the process of dopaminergic cell death whereas neurotrophic effects on surviving dopaminergic neurons have not been established.

A two-phase lesion approach was used to induce a bilateral Parkinsonian syndrome in rhesus monkeys. This MPTP treatment induced a significant reduction in limb movements and moderate to severe parkinsonian symptoms, as well as decreased striatal F-DOPA uptake. Our data confirm earlier reports that both motor symptoms and neurochemical effects are stable with little compensation in time: limb use and F-DOPA uptake remained constant from day 3 to day 35 in the bilateral phase. In the present model, the second lesion is produced with a lower concentration of MPTP, to more closely mimic the bilateral lesion progression seen in the disease. This allowed us to study the neuroprotective effects of TCH346 in slowing the disease process on the side, which is relatively spared, as might be the case in the clinical setting. Due to the lesser amount of MPTP, the difference between
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the saline-treated group and the TCH346-treated group of monkeys was small and less than the difference between the results of the naive group versus the unilateral group. This small difference lowered statistical power. Statistical power was also decreased by missing data in the TCH346-treated group of animals.

As anticipated, the interindividual susceptibility to the MPTP treatment varied markedly: whereas in six out of eight animals the initial MPTP treatment dramatically decreased F-DOPA uptake and striatal fiber density, the dopamine system remained partially intact on both sides in one TCH346-treated primate and on the right side in one saline-treated primate. Therefore dividing animals among the different treatment groups in order to obtain comparable groups appears essential for obtaining reliable data on putative neuroprotective compounds.

We observed a small but significant increase in the SRI between the naive and unilateral stages. Similar small increases in the contralateral striatal function after MPTP lesion have been observed\textsuperscript{24, 31}. The high capacity of dopaminergic neurons for functional compensation may underlie this effect\textsuperscript{46}. Indeed, the increased F-DOPA uptake is consistent with the reported upregulation of high-affinity dopamine uptake in the nonlesioned side of the MPTP-treated animals\textsuperscript{14}. In a further study, which showed evidence of early effects after MPTP lesions\textsuperscript{19}, the authors argue convincingly that these effects are consistent with the wider body of literature. The fact that two of the animals showed bilateral Parkinson symptoms at the unilateral stage supports the idea that the ipsilateral substantia nigra may have been affected to some small degree during the unilateral lesioning.

In contrast to many propargylamines, including rasagiline and deprenyl, TCH346 does not induce symptomatic effects by increasing synaptic dopamine through an inhibitory effect on MAO-B. Indeed, the indication that the effects of TCH346 were not symptomatic is underlined by the observation that the animals not only displayed low Parkinson scores during the 14-day TCH346 administration period, but also as long as 3 weeks after its last administration. Distribution studies have shown that midbrain levels of MPTP peak within 2 h of administration\textsuperscript{26}. Since TCH346 was administered 2 h after the MPTP infusion, it is unlikely that TCH346 interfered with the uptake of MPTP.

An increasing number of studies suggest that TCH346 can prevent dopamine cells from dying by blocking apoptotic pathways\textsuperscript{13, 33, 52}. Therefore it appears feasible that TCH346 prevented the Parkinson syndrome in our nonhuman primates by blocking MPTP-induced apoptosis of dopamine cells. A potential target mediating the antiapoptotic effects of TCH346 is glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Besides its role as enzyme in glycolysis, GAPDH has been found to be one of the key factors in apoptosis\textsuperscript{16}. TCH346 prevents neuronal apoptosis induced by GAPDH overexpression in vitro\textsuperscript{52}, possibly by preventing nuclear accumulation of GAPDH and/or by converting GAPDH from its usual tetrameric form to a dimeric structure\textsuperscript{13}. 

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There is no question that apoptotic cell death can occur in nigral dopamine cells: these neurons die from apoptosis in MPTP and 6-hydroxydopamine models of PD\(^ {10, 27, 54}\). Although not all postmortem studies report the morphological characteristics of apoptosis in PD\(^ {6, 12, 48}\), various executioners of apoptosis, including GAPDH, are activated in remaining dopaminergic substantia nigra neurons\(^ {15, 47, 51}\). The enzyme accumulates in the nucleus of the latter neurons, suggesting that the proapoptotic, nuclear translocation of GAPDH is not limited to cultured cells but actually plays a role in the pathogenesis of PD\(^ {47}\). Accordingly, it implicates that the neuroprotective actions of TCH346 through GAPDH could prove significant for protecting dopamine neurons in PD.

In conclusion, the data show that TCH346 prevented MPTP-induced Parkinson symptoms in the best animal model of PD, possibly through its inhibitory effect on GAPDH. This compound may prove useful for inhibiting the progression of dopaminergic degeneration in patients with PD.

**Acknowledgments**

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