Dose Response to Intraventricular Glial Cell Line-Derived Neurotrophic Factor Administration in Parkinsonian Monkeys

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ABSTRACT
A double-blinded study was conducted to evaluate the dose response of hemiparkinsonian rhesus monkeys to intracerebroventricular (ICV) injections of recombinant methionine human glial cell line-derived neurotrophic factor (GDNF). Thirty rhesus monkeys with stable hemiparkinsonian features were divided into six treatment groups (vehicle, 10, 30, 100, 300 and 1000 μg GDNF; n = 5/group). Each animal received 4 ICV administrations spaced at four week intervals. In addition, the animals were followed for 4 mo after the last injection. Standardized video taped behavioral tests were used to rate parkinsonian features using a nonhuman primate rating scale and assess side effects from treatment. Significant behavioral improvements were measured in animals receiving 100 to 1000 μg GDNF. One month after the last GDNF administration, parkinsonian features in animals receiving 100 and 1000 μg GDNF began to return to baseline levels. However, 300 μg GDNF recipients continued to display behavioral improvements. Parkinsonian features significantly improved were: bradykinesia, rigidity, posture and balance. The most common side effect was a transient weight loss after GDNF administration. Only one other side effect was observed, one animal receiving 1000 μg GDNF displayed dyskinetic movements. The results provide additional information for evaluating the possible clinical application of GDNF for treating Parkinson’s disease.

GDNF, as a mature protein, is a glycosylated, disulfide-bonded, 133 amino acid member of the transforming growth factor-β superfamily which exerts potent effects on rodent midbrain dopamine neurons in vitro and in vivo (Lin et al., 1993; Hoffer et al., 1994; Tomac et al., 1995; Lapchak et al., 1996). Our group has found that intracerbral GDNF injections into adult rhesus monkeys promotes significant increases in midbrain dopamine neuron perikaryal size along with an increased number of neurites and higher dopamine levels in both normal and parkinsonian animals (Gash et al., 1995, 1996). Although behavioral changes following GDNF treatment are small in normal monkeys, parkinsonian rhesus monkeys demonstrate significant improvements in movement functions including bradykinesia, rigidity, posture and balance (Gash et al., 1996). GDNF administered via ICV injections every four weeks maintains the functional improvements (Gash et al., 1996).

Because of its strong dopaminergic trophic actions, GDNF may prove useful in treating Parkinson’s disease which results from the progressive degeneration of midbrain dopamine neurons (Hornykiewicz, 1993). Because GDNF does not pass through the blood brain barrier and must be administered intracerebrally, our studies have focused on the ICV injections that inherently produce less tissue damage than injections directly into brain parenchyma. At both 1 and 24 hr after intraventricular administration, strong 125I-GDNF labeling is found bilaterally in the rat substantia nigra (Lapchak et al., 1997a). In addition to dopamine neurons, GDNF has also been found to exert trophic effects on other central nervous system neurons including motor neurons (Henderson et al., 1994; Oppenheim et al., 1995), forebrain cholinergic neurons (Williams et al., 1996; Lapchak et al., 1997b) and locus ceruleus noradrenergic neurons (Arenas et al., 1995). The antiparkinsonian actions of GDNF could be at least partially mediated by these or other neurotransmitter systems affected by GDNF.

Our study was conducted to determine the dose response in parkinsonian rhesus monkeys to GDNF injected into the right lateral ventricle. In our original studies, the GDNF...
doses used were based on effective levels in rodents (Hudson et al., 1995), scaling up for the larger size of the rhesus monkey brain. Both 100 and 450 μg ICV doses were effective in the initial study in parkinsonian monkeys (Gash et al., 1996). The improvements from a single injection lasted for at least 4 wk and then, as indicated by pilot data, began to return to preinjection levels. In our study, the effects of four ICV injections spaced 4 wk apart of vehicle or GDNF at five different dose levels (10, 30, 100, 300 and 1000 μg) were analyzed. After the fourth ICV administration, dosing was stopped and behavioral changes monitored for another 4 mo. Throughout the study, movement functions were evaluated using standardized video taped tests. In addition to analyzing changes in parkinsonian features using a nonhuman primate rating scale (Ovadia et al., 1995), the animals were evaluated for possible side effects from the surgical procedures and drug treatment.

Materials and Methods

Animals. Thirty adult female rhesus monkeys (Macaca mulatta) obtained from a commercial supplier (HRP, Alice, TX) were used in our study. The animals ranged in age from 7 to 22 yr old and in weight from 4 to 9 kg. The animals were housed in individual primate cages with a Lexan panel on the right side to permit videotaping and maintained on a 12-hr light/12-hr dark cycle with food and water provided ad libitum. In addition to standard primate biscuits, the diet was supplemented daily with fresh fruits and vegetables. There was a small Lexan panel on the left side of the cage and an automated monitor was mounted on the panel to record home cage activity (Smith et al., 1993). All testing and surgery was conducted in the Laboratory Animal Facilities of the University of Kentucky, which are fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care. Animal care was supervised by veterinarians skilled in the health care and maintenance of nonhuman primates. All protocols used were approved by the Animal Use Committee of the University of Kentucky.

MPTP administration. All animals received right carotid artery infusions of MPTP to induce continuously expressed parkinsonian features (Bankiewicz et al., 1986; Smith et al., 1993; Ovadia et al., 1995). The doses used were age specific as described in detail elsewhere (Ovadia et al., 1995). Our published procedures were modified for animals older than 16 yr, in which the MPTP dose was 0.4 mg/kg for animals up to 6 kg in weight. Animals in this age range over 6 kg received a total dose of 2.4 mg as brain size was not significantly larger than in lower weight monkeys. After MPTP treatment, the animals were evaluated for 6 wk to assess their parkinsonian features. Animals not meeting the drug treatment entry level criterion of consistently expressing parkinsonian features ≥ 5 on our nonhuman primate rating scale (Ovadia et al., 1995) received another MPTP infusion and were restested for another 6 wk. As the expression of parkinsonian features stabilizes by 6 wk post-MPTP treatment (Smith et al., 1993), active testing and treatment of the animals began at that time point. The animals were divided into six groups (n = 5 in each group) balanced for age and severity of parkinsonian features (see table 1).

Drug administration. A double-blinded experimental design was followed until the data were reviewed. Each test group of five animals was randomly assigned to one of six GDNF dose levels: 0, 10, 30, 100, 300 and 1000 μg. Each animal in the group then received four ICV injections of that dose spaced at 4-wk intervals. Behavioral changes were followed for 4 wk after each injection and for an additional 3 mo after the last ICV injection. During the third ICV injection, one of the 30-μg GDNF recipients experienced a surgical complication (hematoma) and was removed from the study. All other animals successfully completed the study.

The ICV injections of GDNF (recombinant methionine human GDNF, Amgen, Thousand Oaks, CA) or vehicle (10 mM citrate, 150 mM NaCl, pH 4.96) into the right lateral ventricle were conducted using MRI-guided stereotaxic procedures (Gash et al., 1995, 1996). Before the ICV injection, the animals were anesthetized with isoflurane gas (1-2%) following a preoperative dose of ketamine hydrochloride (30 mg/kg i.m.) and atropine (0.04 mg/kg, i.m.). Animals were then placed in a MRI-compatible stereotaxic frame. Using the stereotaxic coordinates obtained from the MRI scans of each animal and after sterile operating procedures, 80 μl of vehicle and GDNF or vehicle alone were delivered by a 22-gauge spinal needle attached to a 100-μl Hamilton syringe at the rate of 10 μl/min into the right lateral ventricle. The needle was left in place for 3 min before being withdrawn at the rate of 1 mm/min.

Behavioral testing. Body weight was recorded weekly while the animals were in the ICV treatment phase of the study and biweekly during the washout period. Animal behavior was assessed by continuously recording home cage activity levels and weekly video taping home cage behavior under standardized conditions (Smith et al., 1993; Ovadia et al., 1995). Home cage behavior was video taped once a week for 2 consecutive hr starting at 1:00 P.M. At the beginning of each hour, a technician entered the room and placed a small piece of food on a ledge above the cage door to elicit standing and reaching movements. The animal was then videotaped for 15 min with no one in the room. At the end of the second 15-min session, a larger piece of fruit that required both hands to manipulate was placed in the cage to elicit left and right arm/hand movements. Each tape was coded with four digit number and evaluated blindly by two raters using our nonhuman primate parkinsonian rating scale (Ovadia et al., 1995). As previous studies (Gash et al., 1996; Miyoshi et al., in press) had shown that tremor was not improved by GDNF treatment, it was not included in the rating scale. Although there had not been problems in using the food retrieval task to assess fine motor movement in shorter term studies, more than 30% of the animals learned to use their nonimpaired right hand to retrieve food from the left food receptacle during the present study, negating its usefulness in the rating scale. The parkinsonian features rated were: bradykinesia, rigidity of both upper and lower limbs, posture and balance.

In addition to rating parkinsonian features, the tapes along with activity and weight data were analyzed to determine if the animals displayed side effects from the treatments. Changes in weight of more than 10% at any point during the course of the study were considered significant. Changes in home cage activity levels ≥ 15% during the treatment phase of the study were considered as indicators of hypoactivity if the activity level decreased or hyperactivity if the activity level increased. Other adverse effects rated in the tapes were the number of incidents of dyskinetic and dystonic movements, vomiting and stereotypic behavior. The video taped behavior was also evaluated for incidents of abnormal visual tracking, that is staring off into space or following movements on the ceiling or blank walls, which was considered abnormal behavior.

Statistical analysis. Changes in parkinsonian features were analyzed by ANOVA procedures for repeated measures in which the between animal factor was always taken to be the dose while the within subjects factor were the monthly average parkinsonian rating scores. In the presence of significance dose by repeated measures

<table>
<thead>
<tr>
<th>No. of Animals</th>
<th>GDNF Dose (μg)</th>
<th>Age (± S.E.M.)</th>
<th>Baseline Parkinsonian Score (± S.E.M.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>0</td>
<td>14.0 ± 2.3</td>
<td>7.4 ± 0.6</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>14.2 ± 2.5</td>
<td>7.0 ± 0.5</td>
</tr>
<tr>
<td>30</td>
<td>30</td>
<td>11.2 ± 1.5</td>
<td>7.0 ± 0.4</td>
</tr>
<tr>
<td>100</td>
<td>100</td>
<td>12.6 ± 2.2</td>
<td>7.2 ± 0.6</td>
</tr>
<tr>
<td>300</td>
<td>300</td>
<td>12.8 ± 2.7</td>
<td>7.0 ± 0.5</td>
</tr>
<tr>
<td>1000</td>
<td>1000</td>
<td>14.4 ± 2.3</td>
<td>7.4 ± 0.7</td>
</tr>
</tbody>
</table>
interaction effects, post hoc comparisons of means depended on Fisher’s protected least significant differences procedures facilitated by the use of least square mean’s due to a missing response created by the loss of one animal from the 30-μg dose group halfway through the treatment period.

As a repeated measures ANOVA showed a significant dose by month interaction (see “Results”), the following post hoc analysis was conducted. For the 4 ICV dosing months, emphasis was placed on comparing the change in monthly average scores between each dose and vehicle. Change was defined to be the difference in the mean response for an animal between a given treatment month and the baseline month. The analysis was carried out by using a series of one-way ANOVAs for each ICV month. For the 3-mo washout period, the emphasis was placed on determining whether the mean score in a given washout month was significantly different than the mean score in the last ICV treatment month. This analysis was conducted using a series of repeated measures ANOVAs for each dose group.

Further post hoc ANOVAs were conducted to evaluate specific parkinsonian features at the peak dose response (3rd ICV treatment of 1000 μg GDNF) and how these features changed in the wash-out period. A similar analysis was also conducted comparing the response following the fourth 300 μg ICV injection to the washout period to determine if there was any change in the maintenance of specific parkinsonian features. Linear regression analysis was used to 1) determine the correlation between GDNF dose levels and improvements in parkinsonian features and 2) assess correlations at the peak dose response between age, severity of parkinsonian features and improvements in parkinsonian features. Changes in weight were analyzed using ANOVA procedures. Statistical significance was determined at the 0.05 level throughout the analysis.

**Results**

A strong correlation ($r^2 = 0.55, P \leq .01$) was found between the GDNF dose level and improvements in parkinsonian features in rhesus monkeys receiving monthly ICV injections into the right lateral ventricle (fig. 1). In addition, a repeated measures ANOVA revealed a highly significant dose by month interaction ($P \leq .0001$) during the four month treatment period. In comparing improvements after each GDNF ICV administration to the vehicle response, significant decreases in overall parkinsonian scores were seen at the 100-, 300- and 1000-μg dose levels (fig. 2). Although significant improvements were not measured in the other three treatment groups, parkinsonian features tended to improve over baseline scores.

For the washout period, the issue addressed was whether the parkinsonian features changed over the 3-mo interval after the fourth treatment month. In the 30-, 100- and 1000-μg GDNF treatment groups, parkinsonian scores were significantly higher in the three month follow-up period (fig. 3). In contrast, no significant changes in the overall parkinsonian score occurred during the washout period in the 0-, 10- and 300-μg treatment groups. A post hoc analysis of the 300 μg recipients revealed that this was also the case for individual parkinsonian features as well as the composite score (data not shown).

The peak dose response to GDNF administration was seen at the 1000 μg level in the 3rd mo. At this time point (fig. 4), all four parkinsonian features evaluated (bradykinesia, rigidity, balance and posture) were improved. Three of these features (bradykinesia, rigidity and posture) significantly

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**Fig. 1.** The correlation between the GDNF dose and the average improvement in parkinsonian score (4 mo average ± S.E.M.) on the nonhuman primate rating scale over the 4 mo of dosing was highly significant ($R^2 = 0.55, P \leq .01$).

**Fig. 2.** The percent change in the parkinsonian score over the 4-wk test period after each ICV injection is shown for each test group. The baseline for each test group is its pre-ICV parkinsonian score. Significant changes from the vehicle recipients were seen in the 100-, 300- and 1000-μg GDNF treatment groups (*$P \leq .05$; **$P \leq .01$).

**Fig. 3.** The parkinsonian score during the 4-wk period after the fourth ICV injection is compared to the subsequent 3 washout months. The baseline for each test group is its pre-ICV parkinsonian score. Significant changes in the washout months from the fourth ICV treatment period were found in the 30-, 100- and 1000-μg treatment groups (*$P \leq .05$; **$P \leq .01$).
worsened during the washout period. In the five 1000 μg GDNF recipients, functional recovery at the peak response correlated strongly to age ($r^2 = 0.81$, $P \leq .05$) with the youngest animals demonstrating the greatest improvements (fig. 5). In this group, there was not a significant correlation between the severity of the parkinsonian features and the response to GDNF treatment ($r^2 = 0.22$, NS).

The most commonly observed side effect from GDNF treatment was weight loss (table 2). Eleven of the 15 animals in the three highest dose groups experienced a $\geq 10$% weight loss at some point over the 4-mo GDNF treatment period. Weight loss was generally most pronounced in the week after an ICV injection and then increased toward pretreatment levels. By the 4th wk after the fourth ICV injection, weight levels were not significantly different from pre-ICV levels in any test group. All animals tended to gain weight throughout the drug wash out period. By the end of this 4-mo period, the group of 29 animals that completed the entire study was significantly heavier ($P \leq .0001$) compared to pre-ICV baseline weights. In addition, there were no significant weight differences between the test groups at this time point. The only other side effect seen in this study was dyskinetic movement by one of the 1000 μg GDNF recipients. The animal tended to hold the left upper limb in an extended rather than flexed position when at rest and display choreiform left arm and head movements in moving around the cage.

**Discussion**

The results demonstrate that GDNF promotes dose-dependent improvements in movement functions in rhesus monkeys with MPTP-induced parkinsonism. As seen in our previous nonhuman primate studies (Gash et al., 1995, 1996; Miyoshi et al., in press), the response to a single ICV injection lasts for at least 4 wk and is maintained by repeated injections. The most consistent GDNF-induced improvements were seen at two highest dose level tested, 300 and 1000 μg. This suggests that the ceiling for GDNF effects may not have been reached in our study.

In animals receiving 1000 μg of GDNF, improvements were seen in all parkinsonian features evaluated: bradykinesia, rigidity and postural stability. These results confirm our earlier study (Gash et al., 1996) in which intracerebral injections of GDNF into any of three sites—substantia nigra, caudate nucleus or lateral ventricle—promoted functional improvements in the same features. The effective dose levels, from 100 to 1000 μg GDNF, identified in our study are consistent with those seen previously to be efficacious in nonhuman primates (Gash et al., 1996; Miyoshi et al., in press) in which intracerebral administrations ranged from 100 to 450 μg GDNF. The GDNF levels used in the earlier studies were based on scaling up from effective doses in rat studies where consistent effects on dopamine neurons were seen with nigral injections of 10 μg GDNF (Hoffer et al., 1994; Hudson et al., 1995).

The magnitude of improvement in the 100 to 1000 μg GDNF dose range of 15 to 22% over preinjection levels is comparable to the best response we have found in a levodopa dose-response study in this animal model of Parkinson’s disease (Miyoshi et al., in press). In evaluating treatment efficacy, it’s important to keep in mind that lesion severity as measured by the reduction of dopamine levels in the right striatum of hemiparkinsonian monkeys is similar to or exceeds that of end stage Parkinson’s patients. Dopamine levels on the right side in hemiparkinsonian monkeys, which is most severely affected by MPTP infusion, are less than 1% of normal levels in the putamen and caudate nucleus and around 7% of normal substantia nigra levels (Gash et al., 1995, 1996). In comparison, putamen, caudate and substantia nigra dopamine tissue levels measured at autopsy in patients with severe Parkinson’s disease are, respectively, around 2, 18.5 and 5 to 22% of control levels (Bokobza et al., 1984; Hornykiewicz and Kish, 1986: Kish et al., 1988). In parkinsonian rhesus monkeys demonstrating functional improvements after GDNF administration, dopamine levels were increased twofold in the substantia nigra, but not significantly changed in the caudate or putamen (Gash et al., 1996).

Based on the maintenance of functional improvements over the 3-mo washout period, the 300 μg GDNF dose level...
appears to be the most effective. The response in the washout period followed a U-shaped curve with no significant change seen the beneficial reduction in parkinsonian features in animals at the 300-μg dose level, but at higher and lower dose levels the symptoms increased in severity. The reasons why the functional improvements were retained in the 300-μg treatment group are not clear, but may indicate that the effects of GDNF at some dose levels last for months. Obviously, additional studies are needed to clarify the long term effects of GDNF administration on the central pathways regulating movement functions.

Two observations emerging from this study which are important in considering potential clinical applications of GDNF in treating Parkinson’s disease. Age was an important variable in the 1000-μg treatment group response with the youngest animals displaying the greatest functional improvements. The incidence of Parkinson’s disease increases rapidly from age 40 through 75 with a median age of onset of around 54 years (Schoenberg, 1986; Bergmann et al., 1986). This suggests that the younger Parkinson’s patients might receive more benefits from GDNF treatment. The degree of recovery did not correlate with the severity of Parkinson’s features which would suggest that GDNF could benefit patients even in the advanced stages of parkinsonism. This possibility is also suggested by the fact that the right striatum in the hemiparkinsonian model used demonstrates a more severe depletion of dopamine then is typical of the late stages of Parkinson’s disease.

Although the magnitude of functional improvement from the higher doses of GDNF was similar to that of conventional levodopa treatment, there were significant differences. The effects from levodopa wear off with 4 to 6 hr of administration (Miyoshi et al., in press), whereas the functional improvements from a single GDNF administration were maintained for weeks. In addition as the disease progresses and higher doses of levodopa are required for treatment, patients experience increasing side effects including dyskinetic movements, dystonias and psychotic episodes (Yahr, 1989). To date, the most commonly observed side effect from GDNF administration has been fluctuations in weight levels. Although this is a potential concern, it should be noted that the animals in the study were examined daily by both Laboratory Animal Resources staff and our research group throughout the study and at no time manifest signs of clinical distress or malnourishment. Post hoc review of the video taped behavioral test revealed that the GDNF recipients experiencing the weight fluctuations appeared healthy, alert and active. However, as the overall activity level was not higher in the GDNF recipients, it is likely the weight loss reflected appetite reduction resulting from the stimulation of central dopaminergic pathways. Although weight fluctuations have been observed in our previous nonhuman primate studies, the dyskinesias displayed by in one of the 1000 μg GDNF recipients has not been seen previously. Whether the response in this one animal represents an anomaly or suggests that increasing side effects will be seen above the 1000 μg dose level can only be determined by additional studies.

In summary, the results from this study confirm and extend previous observations that intracerebrally administered GDNF significantly improves parkinsonian features in monkeys with MPTP-induced movement dysfunctions. Significant functional improvements were seen from 100 μg to the highest dose tested, 1000 μg. The animal model used mimics the late stages of human Parkinson’s disease in lesion severity where conventional levodopa therapy is less effective (Yahr, 1993) and new therapeutic approaches are especially needed. Side effects common to levodopa treatment seen in only one animal at the highest dose level in response to GDNF treatment. However, trophic factor recipients did experience significant fluctuations in weight levels. The results add to the growing body of evidence that GDNF could be of significant therapeutic benefit in treating Parkinson’s disease.

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References


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