Effects of Talipexole on Contraversive Rotation and Functional Impairment in MPTP-Induced Chronic Hemiparkinsonian Monkeys

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ABSTRACT—The effects of talipexole on functional motor improvement in comparison with contraversive circling were studied in five chronic (5–7 years post MPTP-lesioned) hemiparkinsonian *Macaca nemestrina* monkeys. Talipexole induced contraversive rotations in a dose of 32 $\mu\text{g}/\text{kg}$ for about 1 hr after i.m. injection. Larger doses (56 and 100 $\mu\text{g}/\text{kg}$, i.m.) produced less effect due to sedation. Three different rating scales were used to assess functional improvement, including a clinical parkinsonism rating scale, volitional responses to fruit presentations, and number of significant hand movements. The optimal dose of talipexole was 32 $\mu\text{g}/\text{kg}$, i.m. Functional improvement by talipexole, including clinical parkinsonian rating scales and significant hand movements, as well as contraversive circling in hemiparkinsonian monkeys, confirm that this chronic animal model is useful in preclinical testing of drugs for the treatment of human parkinsonism.

Keywords: Talipexole, MPTP, Chronic hemiparkinsonism (monkey), Functional improvement, Dopamine D₂-like receptor agonist

Parkinson's disease results primarily from degeneration of dopamine (DA) containing neurons in the pars compacta of the substantia nigra. In addition to DA supplementation by administration of the DA precursor levodopa, DA-receptor agonists are also effective in the treatment of Parkinson's disease via activation of postsynaptic striatal-receptors. Contraversive circling induced by DA-receptor agonists in hemiparkinsonian monkeys, as in rats with a lesion of the nigrostrial pathway, is one of the useful indicators for the evaluation of the effectiveness of DA-receptor agonists in Parkinson's disease (1, 2). However, the relationship between DA agonist induced contraversive circling and functional improvement of the parkinsonian syndrome is not clearly understood and, up to now, there have been no reports regarding this issue in nonhuman primates.

The present study was undertaken to clarify the anti-parkinsonian effects of talipexole (6-allyl-2-amino-5,6,7,8-tetrahydro-4*H*-thiazolo-[4,5-*d*]-azepine dihydrochloride; B-HT 920 CL2; Domin[®]) by examining the ability of this drug to induce contraversive circling and functional motor improvement in MPTP-lesioned monkeys. Talipexole is a potent non-ergot D₂-like agonist with α_2 agonist and 5-HT₃ antagonist activity (3–9). It is effective

in the treatment of Parkinson's disease in controlled clinical studies (10).

MATERIALS AND METHODS

Some of the methods used in this study have been described previously (1–2, 11).

Animals

Five adult female *Macaca nemestrina* (pig-tailed macaque) monkeys, ranging in weight from 5.4–7.6 kg at the time of this study, were purchased from the Charles River Co., Port Washington, NY, USA. The animal care and use program conforms to the standards in The Guide for the Care and Use of Laboratory Animals (12) and B virus guidelines for monkey handlers (13). Each animal was given an enriched environment consisting of ease of visualization of their colleagues, Kong toys, TV entertainment, etc.

The monkeys were placed in a sound-quiet, air conditioned certified animal room (31.5 × 11.3 × 8.0 feet) on a light/dark cycle with lights on from 7:00 A.M. to 7:00 P.M. Food and water were given in the mornings except when a drug or its vehicle was tested, in which case the

animals were fed in the afternoon. Monkey chow and water were available ad lib until consumed. The animals' diet was supplemented with fruit and vegetables as appropriate. Each adult female monkey was housed in a standard individual cage, was not pregnant, and menstruated monthly. At the time of the present experiments, the animals had symptoms of hemiparkinsonism for 5–7 years, which did not show any gross change over this period of time. During the intervening years, the animals were given various selected D₁-like and D₂-like agonists i.m., as well as levodopa/carbidopa orally in their fruit. Thus, the animals in this study represent a chronic animal model.

MPTP-induced hemiparkinsonism

The method of Bankiewicz et al. (14), with modifications, was used 5–7 years previously to slowly infuse MPTP unilaterally into one common carotid artery in a total dose of 2.5 to 3.5 mg, depending upon the size of the monkey, for a total dose of approximately 0.6 mg/kg. Briefly, each animal in her own squeeze cage was anesthetized with ketamine hydrochloride (5–10 mg/kg, base dose) given i.m. Subsequently, each animal was given 30 mg/kg, i.v. pentobarbital to maintain deep anesthesia and secured on an operating table in the prone position for exposure of the right or left common carotid artery at its bifurcation. The common artery supplying blood to the dominant cerebral hemisphere was selected. The major vessels of the external carotid were isolated and fine arterial clips were applied temporarily. Surgery lasted approximately 30 min. The wound was then irrigated with H₂O₂ solution and closed with a continuous nylon suture and skin clips. Each animal was given 300,000 units of sterile penicillin G benzathine and penicillin G procaine in an aqueous suspension.

Drug administration

Talipexole (B-HT 920 CL₂) was obtained from the Boehringer Ingelheim KG, Ingelheim, Germany. Five percent dextrose in sterile water (D5W) was purchased from Abbott Laboratories, North Chicago, IL, USA. Talipexole was given i.m. in logarithmic doses to all five monkeys. The drug was given on the same day each week, usually Thursday A.M. Therefore, intervals between drug administration were at least 1 week apart. If the drug was given more than once in the same dose, mean data were used for analysis. The same volume of vehicle (D5W) was injected i.m. as the control.

Observations of circling behavior

Each monkey was placed in a standard primate cage modified with a clear Plexiglas front for viewing and recording free moving behavior. The upper cages were

illuminated with 34 watt fluorescent ceiling lights. The lower cages were illuminated with 20 watt fluorescent lights. Gross animal behaviors were observed via three separate video color cameras. The behaviors of two monkeys in separate cages were recorded with one camera. Thus, a total of three video cameras with zoom autofocus were used. All three cameras were connected electrically to their own videocassette recorders. The time and date of each camera were synchronized. The animal was video recorded in its own home cage without human presence for 30 min after vehicle injection and then for an additional 2 hr or more, as necessary, following drug or vehicle injection. The videotapes were scored by persons blind to the purpose of the study for ipsiversive and contraversive circling to the side of the brain lesion. The number and direction of complete 360° turns during each consecutive 5-min period were counted and recorded. The total number of complete contraversive turns in 2 hr after drug administration was computed. This time period was used since all of the drugs studied were relatively short acting. The coefficient of reliability for two interraters was >0.9.

Clinical monkey rating of parkinsonism

A modified parkinsonian rating scale (PD scale) was used to quantify the clinical status of lesioned monkeys before, 15, 30, 60 and 120 min after talipexole administration in doses of 10–100 µg/kg, i.m. This scale includes drug side effects. Use of such a scale allows comparability of data from different centers using similar lesion paradigms. Each monkey was videotaped in its own cage and rated every 15 min from the videotape recordings by a trained researcher blind to the drug dose. The scale consisted of the sum of eight parkinsonian features (tremor, posture, gait, bradykinesia, balance, gross motor skills, defense reaction and freezing; 1 point each), three drug-related side-effects (dyskinesia/dystonia, psychological disturbance and vomiting; 1 point each), gross motor activity (1–4 points each) for a total of 25 points. Thus, 0 = normal and 25 = the worst.

Volitional responses to fruit presentation

Volitional responses to presentation of pieces of fruit were scored in overnight fasted monkeys. Two pieces of food were offered sequentially; the second piece was presented when the first piece had been taken by one hand and brought to the animal's mouth. In normal monkeys, the second piece of food was always taken with the other hand. In hemiparkinsonian monkeys, however, the first piece was held in the mouth while the same hand that was used initially was again used to obtain the second piece of food; the rigid and tremorous limb contralateral to the MPTP infusion side remained unused. The ability to use

the "parkinsonian arm" to obtain a second piece of food was used as an index of improvement in volitional motor function. Monkeys were scored by a trained researcher according to the following rating scale: 0: normal use; 1: can grasp small objects, independent use; 2: can grasp only big objects without assistance of intact arm, tremor present; 3: can grasp large objects but only with assistance of the intact limb; 4: arm use only when intact arm used; 5: unable to use affected limb. A score of less than 3 indicates effective arm use.

Scoring each significant hand and arm movement

The entire experiment was videotaped without the presence of the investigators. All hand and arm movements counted the same, 1 point each. The total score for 5 min was the sum of the points for each hand's movements as described below: 1) Grasps the cage with the hand; 2) Supports the body with the hand; 3) Grooms with the hand; 4) Grasps and holds food with the hand; 5) When the monkey circles it will use the hand to assist itself. Sometimes the monkey will use the hand several times during a single circling movement; 6) The hand will be moved in what appears to be voluntary motion even in the air.

The following hand movements were very difficult to score: 1) Grooming incessantly with the same hand, 2) Holding food to eat and moving the hand incessantly, 3) Grasping and shaking the cage incessantly, 4) Playing with and touching an object incessantly.

All hand movements were scored in real time from the videotapes. When the observed hand was transiently behind the monkey and could not be seen, hand movements were not scored. If the last four hand movements continued for more than 2 sec, they were scored as 1 movement. The sum of hand movements per each 5-min period began at -30 min with the drug vehicle injection, and at 0 min when the actual drug was administered. Hand movements were scored for a minimum of 120 min, even though the duration of action of the drug was shorter.

Statistical analyses

Correlation coefficients, Dunnett multiple comparison tests, and Student's *t*-tests were run using the Statview program (Abacus Concepts Inc., Berkeley, CA, USA) on a MacIntosh SE30 computer (Apple Computer Inc., Cupertino, CA, USA). Two way ANOVA were run using the BMDP program (BMDP Statistical Software, Inc., Los Angeles, CA, USA).

RESULTS

Behavior of MPTP-induced hemiparkinsonian monkeys

Within a week or so after MPTP, each monkey showed

evidence of hemiparkinsonism on the side contralateral to the brain lesion. This consisted of a characteristic "extrapyramidal" hand in a flexed position at the wrist. The lesioned animal would not use the contralateral arm and leg for eating, scratching, etc. Generally, the animal would circle ipsiversively, although after many months to years, some spontaneous contraversive circling was observed, especially when it was excited. Rarely, resting tremor was seen, especially of the contralateral arm and hand. All animals showed completely normal motor activity on the side ipsiversive to the injection of MPTP. Therefore, they could easily feed and groom themselves. On casual observation, personnel who were unaware of the fact the animals were hemiparkinsonian thought the monkeys were normal. All of the animals were healthy, gained weight and menstruated regularly. They were housed in individual cages and had no opportunity to become pregnant or to fight amongst themselves.

Effects of talipexole on significant hand movements

Talipexole significantly increased contraversive hand movements when a two way ANOVA test was done for doses of 10, 32, 56 and 100 $\mu\text{g/kg}$ ($F(12,60)=2.856$, $P=0.003$). As illustrated in Fig. 1A, talipexole in a dose of 32 $\mu\text{g/kg}$, i.m. produced the greatest increase in mean contraversive (rigid) hand movements. An increase in ipsiversive (normal) hand movements was caused by talipexole (Fig. 1B). This was also significant by two way ANOVA ($F(12,60)=2.588$, $P=0.0077$). In both cases, the duration of action was less than 1 hr. There was a 6.88-fold improvement in significant movements of the affected hand when the data were normalized to the D5W vehicle control (not shown, but note findings in Figs. 2 and 3). Larger and smaller doses of talipexole were less effective.

Effects of talipexole on clinical parkinsonism

The mean effects of increasing doses of talipexole on a clinical rating scale of parkinsonism in monkeys are shown in Fig. 2. Talipexole improved the clinical parkinsonism rating score. Two way ANOVA for doses (10, 32 and 56 $\mu\text{g/kg}$) and time showed significant reduction of the score ($F(12,60)=8.677$, $P<0.01$). One way ANOVA with repeated measures was done for all 5 animals for the talipexole doses of 0 (control D5W), 10, 32 and 56 $\mu\text{g/kg}$ before ($F(3,19)=2.54$, $P<0.106$), which was not significant, although at 10 min ($F(3,19)=3.99$, $P<0.035$), 60 min ($F(3,10)=10.36$, $P<0.0001$) and 120 min ($F(3,10)=5.56$, $P<0.013$) after talipexole, there were statistically significant reductions of parkinsonian signs. Thus, the vehicle alone produced no significant change, but there was a highly significant improvement with a dose of 32 $\mu\text{g/kg}$ (paired *t*-test, $P<0.05$). Doses of 100

Fig. 1. Effects of talipexole on hand movements. The mean $\pm$ S.E.M. data of all 5 monkeys are plotted every 30 min before and after various doses of talipexole (10 μ g/kg, 32 μ g/kg, 56 μ g/kg, 100 μ g/kg) as shown. In the upper panel A, the contraversive and in the lower panel B, the ipsiversive hand movements are illustrated. Talipexole increased both hand movements, but the percentage increase above the control number was greater in the rigid affected hand contraversive to the side of the brain lesion. Note that the scale for mean contraversive hand movements is different from that for mean ipsiversive hand movements. Statistical significance: * $P < 0.05$, *** $P < 0.001$.

Fig. 2. Talipexole dose-dependent modification of parkinsonism in MPTP-lesioned monkeys. The mean $\pm$ S.E.M. data of 5 monkeys are plotted. The 5% dextrose in water vehicle control (dose 0, ■) had no effect. A dose of 10 μ g/kg, i.m. (▨) talipexole had a tendency to reduce parkinsonian signs, but these effects were not statistically significant. Doses of 32 (▤) and 56 (▥) μ g/kg of talipexole significantly improved the clinical signs of parkinsonism, but 100 μ g/kg (▧) made them worse. The latter dose produced significant sedation and, hence, a marked decrease in body movements. Note the duration of effects of talipexole on this rating scale was at least 120 min. Statistical significance: * $P < 0.05$.

Fig. 3. Relationship between the effects of talipexole on hand movements and circling behavior in MPTP-induced hemiparkinsonian monkeys. The mean $\pm$ S.E.M. motor activity data of 5 monkeys are plotted every 5 min from -30 min when the control vehicle D5W is given i.m. to 0 when talipexole is given i.m. in different doses as shown for the next 120 min. Ordinate values greater than 0 represent circling or hand movements contralateral to the side of the brain lesion. $\square$: ipsiversive rotation, $\circ$: contraversive rotation, $\blacksquare$: ipsiversive hand movements, $\bullet$: contraversive hand movements. Note the most pronounced improvements occur with 32 µg/kg, i.m. talipexole.

$\mu\text{g/kg}$ produced significant sedation and, hence, appeared to make the parkinsonian symptoms worse due to motor inactivity.

Effects of talipexole on volitional responses to fruit

Talipexole did not significantly improve volitional responses of the affected hand to fruit presentations. The animals were scored before, 10, 60 and 120 min after the D5W vehicle control or 10, 32, 56 or 100 $\mu\text{g/kg}$, i.m. talipexole given at weekly intervals. The rating scores were all about 5.1 and were not statistically significantly different. Although clinically, talipexole seemed to improve affected hand performance, the volitional response test was not sensitive enough to determine any effects.

Correlation of significant hand movements and circling behavior

Talipexole in doses of 10, 32 and 56 (not shown) $\mu\text{g/kg}$ increased contraversive rotation: two way ANOVA for doses and time course showed a statistical significance ($F(12, 60)=2.155$, $P=0.026$) (Fig. 3). The most prominent effects were seen with 32 $\mu\text{g/kg}$ (Student's *t*-test, $P<0.05$). However, an increase in contraversive rotation was not observed with the higher doses that induced sedation. Ipsiversive rotation was not significantly affected with talipexole of 10, 32 and 56 $\mu\text{g/kg}$ (Fig. 1B) (two way ANOVA: $F(12,60)=1.18$, $P=0.315$).

As described above, the dose-effect relationships between mean significant hand movements of both the normal and affected hand and the relationship to circling behavior over a 120-min period are illustrated in Fig. 3. Note that Fig. 3 includes the control vehicle D5W and not the 56 $\mu\text{g/kg}$ talipexole mean data. Both normal and affected hand movements increased. The time course of these effects were very similar to the effects on contraversive circling. The optimal dose of talipexole was 32 $\mu\text{g/kg}$, i.m. Both 10 and 56 $\mu\text{g/kg}$ of talipexole were less effective. A dose of 100 $\mu\text{g/kg}$, i.m. produced significant sedation and, thus, any beneficial effects of talipexole could not be observed.

DISCUSSION

The present research showed that talipexole induced contraversive circling and an increase in functional hand movements concomitantly with a reduction of clinical features of parkinsonism in MPTP-lesioned chronic monkeys. There was a positive correlation between contraversive rotation and functional hand movements. These findings indicate that talipexole reduced parkinsonian symptoms and improved functional hand movements in these chronic hemiparkinsonian monkeys. Similar antiparkinsonian effects of talipexole have been

reported in other MPTP-lesioned subhuman primates such as the rhesus monkey (7), marmoset (15, 16) and cynomolgus monkey (17).

In agreement with our previous findings (11), talipexole induced an inverted "U" dose-effect relationship with an optimal dose of 32 $\mu\text{g/kg}$, i.m. The observation that the effects of larger doses were less effective appear to be due to a sedative action. The clinical parkinsonism rating scale observing a variety of symptoms was less sensitive than the number of significant hand responses. The volitional responses to fruit presentations unexpectedly were not altered. The lack of use of the affected hand to take fruit may have been due to the fact that it is customary to use the normal hand in such a chronic hemiparkinsonian model.

The duration of action of i.m. talipexole was about 1 hr in hand movements and circling behavior and 2 hr in clinical ratings. This means that talipexole would be more therapeutically useful in human parkinsonian patients if given multiple times per day.

The major side effect of talipexole in these animals is sedation due to its α_2 -agonist property. Sedation was very pronounced in a dose of 100 $\mu\text{g/kg}$, i.m. This means that clinically talipexole must be given slowly in small incrementing amounts to achieve an optimal dose and maintained at that level. Larger doses would be desirable only if sedation is decreased during repeated administration of talipexole.

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