Continuous dopaminergic stimulation reduces risk of motor complications in parkinsonian primates

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Abstract
Levodopa or short-acting dopamine (DA) agonist treatment of advanced parkinsonian patients exposes striatal DA receptors to non-physiologic intermittent stimulation that contributes to the development of dyskinesias and other motor complications. To determine whether continuous dopaminergic stimulation can delay or prevent onset of motor complications, four MPTP-lesioned, levodopa-naive cynomolgus monkeys were implanted subcutaneously with apomorphine containing ethylene vinyl acetate rods. Three other MPTP-lesioned monkeys received daily injections of apomorphine. Animals receiving apomorphine rods showed improved motor function (ON state) within 1 day of implantation, and remained continually ‘ON’ for the duration of treatment (up to 6 months) without developing dyskinesias. Injected animals also showed similar improvement in motor function after each apomorphine injection. However, these primates remained ‘ON’ for only 90 min and within 7–10 days all developed severe dyskinesias. Implanted monkeys evidenced local irritation, which was alleviated by steroid co-therapy.

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Introduction
Alterations in the motor response to standard dopaminergic therapy constitute a major source of disability in patients with later stage Parkinson’s disease (PD) (Ahlskog and Muentter, 2001; Martignoni et al., 2003; Miyawaki et al., 1997). Increasing evidence suggests that the intermittent stimulation of striatal dopamine (DA) receptors contributes to the pathogenesis of these progressive complications (Bezard et al., 2001; Chase, 2004; Chase and Oh, 2000a,b; Olanow et al., 2000). In parkinsonian rats, 3 weeks of twice-daily levodopa alters motor responses in ways that mimic human motor fluctuations; these responses do not occur if levodopa is administered by round-the-clock infusion (Juncos et al., 1989; Papa et al., 1994). In parkinsonian monkeys, dyskinesias begin within 1–2 weeks of daily dopaminomimetic therapy (Smith et al., 2003), but do not appear when treated via continuous infusion for 27 days (Morissette et al., 1997). Dyskinesias develop sooner in parkinsonian primates given levodopa (90 min half-life) than in those treated with DA agonists (with half-lives exceeding 4 h) that provide relatively more constant dopaminergic stimulation (Jenner, 2000). Similarly, motor complications tend to subside in PD patients when intermittent dopaminergic therapy is replaced by more continuous modes of administration (Baronti et al., 1992; Hadj Tahar et al., 2000; Mouradian et al., 1990). These observations suggest that the risk of motor response complications may be reduced by therapeutic regimes that provide steady and thus more physiologic DA replacement for this normally tonically operating system (Chase et al., 1989; Skirboll et al., 1990).

The only currently available means to constantly administer dopaminergic agents, by continuous infusion
has limited practicality. Recently, an ethylene vinyl acetate (EVA) copolymer system has been developed that enables the continuous release of drugs at therapeutic levels over extended periods (Lesser et al., 1996; White, 2003). When loaded with apomorphine (Raasch et al., 2000), a highly effective DA agonist for the relief of parkinsonian symptoms (Bravi et al., 1994; Manson et al., 2002; Poe and Wenning, 2000; Wenning et al., 1999), these implants have performed safely and reliably upon subcutaneous placement in pigs (IDB, Titan Pharmaceuticals). Here, we evaluated the hypothesis that the long-term continuous administration of apomorphine will maintain antiparkinsonian efficacy but not induce dyskinesias in previously untreated MPTP lesioned primates. In addition, the safety and tolerability of these apomorphine-containing EVA rods administered by themselves and with steroid co-therapy was assessed in this primate PD model.

Subjects and methods

Animals

Studies were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals in 7 adult male cynomolgus (Macaca fascicularis) primates weighing 6.5–7.3 kg. All were housed individually, under stable room conditions, with a 12-h light/dark cycle. Each received a standard biscuit diet twice daily supplemented with fruit and had free access to water. All primates were rendered parkinsonian by the subcutaneous administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) HCl (Research Biochemicals Intl., Natick, MA) once per week at 0.5–1 mg/kg until definite parkinsonian features, including tremor, appeared (scores of 4–5 points on the Laval Disability Scale, where the normal state extends from 0 to 2 points and maximum disability is 10 points) (Bibbiani et al., 2001). The average cumulative MPTP dose was 4.3 mg/kg (range 2.5–7.5). Following observation for two additional months to ensure stable disability, animals were selected for equivalent parkinsonian severity (disability score of 4.5–5 points). These animals, not previously exposed to dopaminergic agents, were randomly divided into two groups: the first (n = 3) received daily subcutaneous injections of apomorphine (2 mg/kg), the minimally effective dose to turn animals ‘ON’, that is, reverse all parkinsonian signs at rotating sites for 14 days (Bibbiani et al., 2003); each primate in the second group (n = 4) was treated by implantation of three apomorphine-containing EVA rods (see below for procedure).

Response assessments

All behavioral ratings were performed by the same blinded investigator. Evaluations in animals receiving daily injections (apomorphine or placebo) occurred every 30 min over a period of 3 h and were performed separately from those in the EVA implanted animals. The continuously infused monkeys were scored once daily. Parkinsonian severity was scored on the Laval Disability Scale (Bibbiani et al., 2001). Dyskinesias were assessed on an Abnormal Involuntary Movement Scale (Bibbiani et al., 2001) which measures the severity of choreiform and dystonic dyskinesias independently: occasional or mild = 1; intermittent or moderate = 2; continuous or severe = 3. Safety monitoring (for skin irritation) was performed by a different rater who was not blind to treatment.

Drug treatments

Apomorphine hydrochloride (Sigma, St Louis, MO) was used for both injection and implantation therapy. Dexamethasone (Sigma) (0.5 mg/kg i.m.) and Triamcinolone acetonide (Kenacort A, Bristol-Myers Squibb, Anagni, Italy) (0.5 mg s.c. per injection site) were used for the prophylaxis of cutaneous inflammation.

Materials

Non-erodible EVA rods capable of prolonged zero order delivery of drugs (Langer and Folkman, 1976) (Titan Pharmaceuticals, Inc., South San Francisco, CA) were 26 mm long and 2.4 mm in diameter; each contained approximately 98 mg of apomorphine. Animals were sedated with 0.3 ml ketamine and a small incision (0.5 cm) was made between the shoulder blades. Implants were positioned between the scapulae by means of a trocar, and a stylet was used to slide the rod from the trocar into the subcutaneous tissue. This technique allows rod placement through a minimal incision. The first two implanted animals received rods that were pre-washed for 8 h with saline prior to sterilization, while the third and fourth implanted animals received rods that had been pre-washed for 30 min with ethanol prior to sterilization to minimize any initial burst drug release. The implantation procedure took approximately 15 min and produced no complications.

Plasma analysis

Post-implant plasma apomorphine concentrations were measured at hours 6, 12, 24, 36, 48, then daily until day 7, weekly until week 4, and monthly for the remainder of study (6 months). Plasma apomorphine levels were determined by extraction into an organic solvent (liquid–liquid extraction) followed by HPLC separation and MS/MS identification and quantification. Plasma samples were diluted with acetonitrile containing a known amount of an internal standard (nor-apomorphine), extracted with hexane, and the solvent evaporated. The resulting residue was reconstituted in 100 µL water/acetonitrile (90:10) and injected onto an HPLC column. The individual components were separated and analyzed by MS/MS both for identity and amount by comparison to the internal standard. Upon explantation, the
residual apomorphine content in each rod was determined by HPLC analysis.

**Histology**

Sections of skin surrounding the implant site were fixed in 10% formalin, embedded in paraffin, sectioned at 5-µm intervals, and stained with hematoxylin and eosin.

**Statistical analysis**

The primary outcome measure of this study was the appearance of dyskinesias. This essentially “all-or-nothing” phenomenon, taken together with the limited sample size precluded formal statistical analysis.

**Results**

All MPTP-lesioned monkeys given apomorphine by subcutaneously implanted rods showed essentially total remission of parkinsonian signs (fully ‘ON’) within 6–12 h after implantation, and continued to evidence stable motor benefit until explantation after 11 (first animal), 22 (second animal) and 24 weeks (third and fourth animal) with no fluctuations in response (constant Disability Scale scores of 0–1). Animals treated once daily by apomorphine injection (2.0 mg/kg) also turned fully ‘ON’ beginning immediately after injection and lasting up to about 90 min. The ‘ON’ state induced by apomorphine administered by rod or by injection was clinically similar (Laval scores: 0–1). However, within 7–10 days (mean 8.3 days), all animals treated by daily apomorphine injection developed dyskinesias (range 6–10 on the Involuntary Movement Scale). In contrast, animals receiving apomorphine implants remained ‘ON’ for up to 6 months and never developed dyskinesias (Fig. 1). Once the EVA rods were removed, all animals were ‘OFF’ and oral levodopa therapy was initiated.

Plasma apomorphine concentrations were measured periodically in all primates (Fig. 2). Apomorphine levels immediately after implantation in animals receiving saline-washed rods were initially high (Cmax of 10 and 27 ng/ml), but declined to their ultimate steady state levels within a week. One of the monkeys receiving saline-washed implants showed signs of excessive dopaminergic stimulation (vomiting, prostration) for 2 days following rod insertion, which resolved as drug levels reached steady state. Use of ethanol-washed rods in the remaining two monkeys, eliminated this initial burst release. Except as noted, apomorphine levels remained stable in all rod-implanted animals throughout the entire period with very little variability (0.66 ± 0.08 ng/ml at steady state). During this time, the release rate of apomorphine from the implanted rods, calculated from the drug remaining in the rod upon explantation, was approximately 0.25 mg/day/rod. In animals receiving apomorphine injections, plasma drug levels rose after 20 min to approximately 27 ng/ml, and declined to about 5 ng/ml by 80–90 min post-injection (at which point the animals had returned to the ‘OFF’ state). Plasma levels required to maintain the ‘ON’ state in implanted animals (0.19–1.1 ng/
ml) were substantially lower than those required in injected animals (>5.0 ng/ml).

Local irritation was observed clinically in the first 2 implanted primates. The first implanted monkey manifested signs of irritation within 2 days that continued until explantation at 11 weeks. Histological examination of the implant sites revealed a dermal zone of necrosis surrounded by viable and degenerated neutrophils, macrophages, multinucleate giant cells, and plasma cells. The second implanted animal was pretreated systemically with dexamethasone (0.5 mg/kg i.m.) 1 day before implantation, on the day of implantation, and 2, 4, and 6 days thereafter. This primate showed no irritation until 22 weeks when the implants were removed. The third and fourth implanted animals were also pretreated systemically with dexamethasone and, in addition, received local triamcinolone acetonide (0.5 mg injection s.c. per implant site) at 2, 4, and 6 months after insertion. No clinical signs of irritation were evident in these animals that were explanted at 6 months, as per protocol. Microscopic examination, however, revealed a mild inflammatory response immediately surrounding each implant. There were no systemic adverse reactions to the implants or to the anti-inflammatory treatments.

Discussion

Results from this study indicate that subcutaneous implanted EVA rods can stably release apomorphine for up to 6 months, allowing continuous ‘ON’ time in MPTP-lesioned primates without the appearance of dyskinesias. Apomorphine injections produced equivalent, although transient improvement in parkinsonian signs, but lead to the development of dyskinesias within 7–10 days. Plasma apomorphine concentrations required for optimal motor improvement in the injected animals were an order of magnitude higher than those required in the rod implanted primates. Conceivably, higher agonist levels may be required with intermittent stimulation to prolong the time DA receptors are exposed to suprathreshold stimulation. It is also possible that that postsynaptic DA receptors in a system that normally functions tonically, respond more efficiently to stable physiologic-range stimulation than to intermittent pulsatile activation, possibly owing to restore changes occurring within or downstream from striatal dopaminergic spiny neurons (Chase, 2004).

The present results further suggest that the continuous administration of dopaminomimetics, at doses sufficient to ameliorate parkinsonian signs can substantially reduce the risk of inducing dyskinesias. All apomorphine-injected primates developed dyskinesias within 1–2 weeks of treatment initiation, consistent with the findings of earlier investigations in which DA agonists were intermittently administered to MPTP lesioned primates (Bibbiani et al., 2003; Smith et al., 2003). In contrast, no apomorphine implanted animal developed dyskinesias over treatment intervals lasting up to 6 months. Previous studies of continuous dopaminergic administration in rodent and primate models of PD (Morissette et al., 1997; Papa et al., 1994) showed protective results for up to 4 weeks. Since no other therapeutic regimen has been reported capable of maintaining a similar degree of dyskinesia-free antiparkinsonian efficacy over such an extended period, findings from this study provide additional evidence in support of the hypothesis that the intermittent and thus, non-physiologic stimulation of striatal dopaminergic receptors contributes to the onset of dyskinesias and related motor response complications (Chase, 2004). The nonphysiologic stimul-
tion of dopamine receptors on medium-sized spiny neurons, first due to the progressive denervation caused by nigrostriatal system degeneration and subsequently due to the intermittent high-intensity stimulation associated with dopaminomimetic treatment, has been shown to activate signaling cascades that regulate the phosphorylation state of coexpressed ionotropic glutamatergic receptor subunits (Chase and Oh, 2000a,b). Resultant NMDA and AMPA receptor sensitization modifies cortical excitatory input to these spiny efferent neurons, thus altering striatal output in ways that compromise motor function (Chase and Oh, 2000a,b; Olanow et al., 2000).

EVA rods of the type used here produced no clinical or histological evidence of tissue reaction in animals or in patients when loaded with various other drugs (White, 2003). The adverse tissue response observed in two of the apomorphine implanted animals thus appears attributable to apomorphine itself rather than to the polymeric matrix. Apomorphine is known to induce cutaneous nodules when injected subcutaneously (Manson et al., 2001; Muguet et al., 1995; Østergaard et al., 1995). On the other hand, steroidal co-therapy at doses low enough to preclude systemic effects appeared to largely suppress this reaction. In the two primates receiving both local and systemically administered steroid co-therapy, the apomorphine was well tolerated for up to 5–6 months. Histological examination of tissues from both these animals showed inflammation similar in character but considerably diminished in severity compared to that observed in the two animals that received no or only systemic steroids and manifested signs of local irritation. Thus, the combination of systemically administered dexamethasone at the time of implantation and locally injected triamcinolone every 2 months thereafter appears capable of preventing a clinically significant tissue reaction.

The continuous delivery of dopaminergic agents to the human striatum remains a major challenge for the treatment of patients with PD. None of the previously utilized cutaneous, intravenous, intranasal, sublingual, duodenal, or rectal routes of administration have documented long-term success due to inconvenience, adverse effects, or limited efficacy (Dewey et al., 1996; Montastruc et al., 1995; Neef and van Laar, 1999; Pollak et al., 1993). Apomorphine is an attractive candidate for use in continuous administration paradigms to parkinsonian patients due to its proven efficacy, high potency, solubility, and comparatively well-balanced D1 and D2 DA receptor agonist properties (LeWitt, 2004; Muguet et al., 1995). Delivery systems like the one used in this study may constitute an important advance in our ability to constantly deliver dopaminergic agents to PD patients and thus warrant further evaluation.

References


Morissette, M., Goulet, M., Soghomonian, J.J., Blanchet, P.J., Calon, F., Bedard, P.J., Di Paolo, T., 1997. Preproenkephalin mRNA expression in the caudate-putamen of MPTP primates after chronic treatment with the...
D2 agonist U91356A in continuous or intermittent mode of administration: comparison with L-DOPA therapy. Brain Res. Mol. Brain Res. 49, 55–62.


