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Riluzole delayed appearance of parkinsonian motor abnormalities in a chronic MPTP monkey model

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Abstract

Preliminary studies have shown that riluzole, a Na^+ channel blocker with antiglutamatergic activity, has neuroprotective efficacy in several models of acute dopaminergic neurodegeneration. A chronic 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) monkey model which comes closer to the slow evolution of Parkinson's disease has recently been developed in order to allow dynamic studies. The present results show that riluzole delayed the appearance of parkinsonian motor abnormalities in this dynamic model, using from 10.2 ± 1.6 daily injections for the MPTP-treated monkeys ($n = 4$) to 16.5 ± 2.0 daily injections for the MPTP + riluzole-treated monkeys ($n = 4$). These results strongly suggest that riluzole may be beneficial to slow down the rate of progression of Parkinson's disease.
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1. Introduction

Parkinson's disease is due to the progressive death of pigmented nigrostriatal dopaminergic neurones (Ehringer and Hornykiewicz, 1960) in the substantia nigra pars compacta. Palliative treatment of Parkinson's disease with levodopa (Birkmayer and Hornykiewicz, 1961) although efficacious over a variable period of time, inevitably result in drug-related side-effects (e.g., Nutt et al., 1992).

Thus, to develop a neuroprotective drug able to reduce or block nigral neurodegeneration has become a priority. Results of recent studies in rodents (e.g., Turski et al., 1991; Sonsalla et al., 1992; Tabatabaei et al., 1992) and in primates (Zuddas et al., 1992; Lange et al., 1993) suggest that excitatory amino acid antagonists have a neuroprotective effect against pro-parkinsonian neurotoxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), toxicity.

Riluzole (2-amino-6-trifluoromethoxy-benzothiazole), a Na^+ channel blocker with antiglutamatergic activity, has been shown to display protective activity in various models, using pro-parkinsonian neurotoxins in rodents (Boireau et al., 1994a,b; Barnéoud et al., 1996). Riluzole has been reported to protect against the MPTP-induced drop in dopamine levels in mice (Boireau et al., 1994a). Clear protection was also found in a model of 1-methyl-4-phenylpyridinium toxicity (Boireau et al., 1994b). In addition, riluzole was shown to alleviate the circling behaviour in 6-hydroxydopamine-treated rats and to reduce the suppression of dopamine metabolism, at both striatal and nigral levels (Barnéoud et al., 1996). Both neuroprotective and palliative effects have also been obtained in an acute model of MPTP intoxication in the monkey (Benazzouz et al., 1995).

We have recently developed a chronic MPTP monkey model that reflects the slow evolution of Parkinson's disease (Bezard et al., 1997c,d). We now aimed to test the hypothesis that riluzole is able to delay the appearance of parkinsonian motor abnormalities in this dynamic MPTP monkey model.

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2. Materials and methods

2.1. Animals

Experiments were carried out with nine male cynomolgus monkeys (*Macaca fascicularis*) weighing 3–4 kg. The monkeys were housed in individual primate cages and their care was supervised by veterinarians skilled in the healthcare and maintenance of primates. Our laboratory operates under the guidelines laid down by the National Institute of Health and is authorized by the French Ministry of the Environment.

2.2. Behavioural assessment

The animals' behaviour was evaluated on a parkinsonian monkey rating scale (Kurlan et al., 1991; Benazzouz et al., 1995; Bezard et al., 1997a,b) using videotape recordings of monkeys in their cages as well as blind clinical neurological evaluation. During each 30-min session, two observers evaluated the animals' levels of motor performance, coaxing them to effect various tasks by rewarding them with appetizing fruits. A simultaneous independent and blind assessment was made by a third observer watching a video recording. The three observers eventually converged to a coefficient (Kendall's) greater than 0.95 in their rating of all behaviours, before starting actual data collection. Any differences in rating were discussed regularly to eliminate observer idiosyncracy (Taylor et al., 1994).

We assessed the following symptoms: tremor (0–3), variation in the general level of activity (0–3), body posture (flexion of spine; 0–3), vocalization (0–2), freezing (0–2), rigidity of each arm (0–3 for each upper limb), and arm movements (reaching for food with each arm; 0–3 for each upper limb). The minimum score was 0 and the maximum disability score was 25. A score ≥ 8 corresponds to recognizable but moderate parkinsonism and a score ≥ 15 to full parkinsonism, similarly to stage IV on Hoen and Yahr's scale (Hoehn and Yahr, 1967).

2.3. Experimental protocol

A five-day assessment of normal behaviour for each monkey was carried out before starting the MPTP protocol.

One monkey was treated with vehicle daily, and the other eight animals were injected with MPTP (MPTP hydrochloride, Sigma, St. Louis, USA) daily at the dose of 0.2 mg/kg i.v., as previously described to reflect as much as possible the slow evolution of Parkinson's disease (Bezard et al., 1997d). The animals were treated daily with MPTP until they reached a score of over eight on the clinical rating scale. Parkinsonian clinical symptoms then continued to develop for several days after the treatment was stopped, due to the long-term degenerative process (Jackson-Lewis et al., 1995).

Four animals (Group I) received daily MPTP treatment (10 AM) and twice daily vehicle (10 ml of water p.o. at 11 AM and at 4:30 PM). Four animals (Group II) received daily MPTP treatment (10 AM) and twice daily riluzole (Rhone-Poulenc Rorer, France; 4 mg/kg p.o. in 10 ml of water at 11 AM and at 4:30 PM). When the animals reached a clinical score of eight, MPTP injections were stopped in accordance with the design of the model (Bezard et al., 1997d), whereas riluzole or vehicle administrations continued until the animals reached a state of stable parkinsonism, attested to by a maximum score persisting over a period of seven consecutive days.

Clinical evaluation was carried out daily throughout the protocol at 9 AM (before MPTP injection and riluzole or vehicle administration). The X of Kendall test was used to compare the number of MPTP doses necessary to develop parkinsonism in Group I and in Group II. Analyses of variance (ANOVA) were used to compare the time course of the clinical scores between the two groups, then a *t*-test was used to compare daily the clinical evaluations after parkinsonism was established.

3. Results

The vehicle-treated monkey's motor behaviour remained normal throughout the protocol.

There was a significant difference in the number of MPTP doses necessary to develop parkinsonism between Group I (MPTP) and Group II (MPTP + riluzole) (10.2 ± 1.6 injections and 16.5 ± 2.0 injections, respectively; mean $\pm$ S.D.) ($P < 0.05$). Monkeys from Group II (Riluzole) thus developed parkinsonian motor abnormalities later than did monkeys of Group I (Fig. 1).

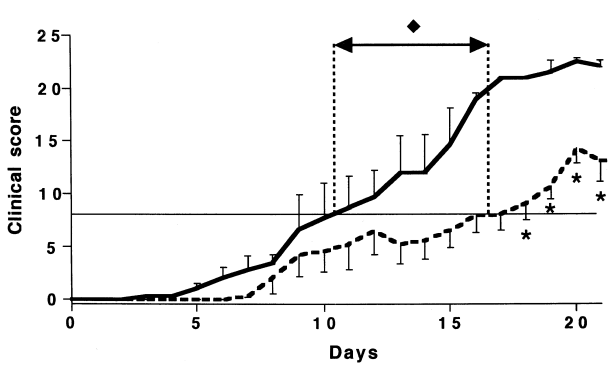

Fig. 1. Evolution of daily clinical scores (clinical score $\pm$ S.D.). Continuous line: MPTP-treated group. Dotted line: MPTP + riluzole-treated group. ◆: Comparison of the latency of appearance of the score 8 (or more) on the clinical rating scale (i.e., the time when MPTP injections were stopped) in the MPTP-treated group and in the MPTP + riluzole-treated group, $P < 0.05$, X of Kendall test. * Comparison of clinical scores from D18 to D21, $P < 0.05$, *t*-test.

ANOVA comparing the time courses showed a significant difference in the evolution of clinical scores between the two groups ($P < 0.01$). When parkinsonism was well-established in both groups, the clinical scores of Group I (21.5 ± 0.9; mean $\pm$ S.D.) were significantly higher than those of Group II (11.7 ± 4.0; mean $\pm$ S.D.) ($P < 0.05$) from D18 to D21 (Fig. 1). The same items of the clinical rating scale were increased for both groups since all monkeys were fully parkinsonian even if monkeys of Group II exhibited less serious symptoms.

4. Discussion

The results showed that riluzole delayed the appearance of parkinsonian motor abnormalities in a chronic monkey model of MPTP toxicity, designed to come closer to Parkinson's disease (Bezard et al., 1997d). This added new insight to a previous report of a neuroprotective effect observed in an acute model of MPTP intoxication in monkeys (Benazzouz et al., 1995).

Immunohistochemical determination of tyrosine hydroxylase in surviving dopaminergic neurones (data not shown) showed a sharp cell depletion in cells of the substantia nigra pars compacta in both groups of monkeys without a difference between the two groups. This absence of difference was due to the experimental design which was aimed to allow observation of a delay in the appearance of clinical signs and not of a neuroprotective effect at the cellular level at the end of such a protocol. Monkeys of both groups, however, were treated with MPTP until they were parkinsonian. Since it is generally accepted that parkinsonian motor abnormalities appear only when loss of dopamine neurones exceeds a critical threshold and that full parkinsonism corresponds to an extreme loss of these dopamine neurones (Bezard and Gross, 1998), it is not surprising that no neuroprotection was observed at the cellular level at the end of the protocol.

At the end of the experiment, monkeys of Group II (MPTP + riluzole) showed lower scores for clinical signs than did those in Group I (MPTP). The fact that monkeys of Group II (MPTP + riluzole) exhibited less pronounced parkinsonian motor abnormalities may have been due to a compensatory hyperactivity of surviving dopaminergic neurones, leading to a significant difference in striatal dopamine concentration as previously reported to be due to the administration of riluzole (Benazzouz et al., 1995).

The main finding of this study was that riluzole clearly delayed the onset of clinical signs. Thus, based on our behavioural data, riluzole provokes a clear slowing of the degenerative process induced by chronic MPTP intoxication. Since this dynamic model has been validated by direct comparison with the evolution of Parkinson's disease (Bezard et al., 1997d, 1998), one can postulate that the delay in appearance of clinical signs, which was 6 days in our study, would correspond to a proportional period in patients. The recent report of the possibility of a presymptomatic diagnosis of Parkinson's disease (Bezard et al., 1997b) and the improvement of neuroimaging as a tool for early diagnosis (Kraus, 1996) should permit an early start of treatment with a neuroprotective compound, thus slowing the rate of progression of Parkinson's disease.

In conclusion, the results now obtained with a chronic model of intoxication in the monkey are well consistent with the results obtained in the pilot study with riluzole (Benazzouz et al., 1995). As far as we are aware, riluzole is the only compound with Na^+ channel blocking properties in glutamatergic neurones which exerts behavioural neuroprotective activity in primate models of Parkinson's disease. Further studies are needed to investigate the neuroprotection at the cellular level by treating groups of animals with the same cumulative dose of MPTP. A protocol aimed to test the neuroprotective effect should help us to demonstrate more precisely to what extent riluzole is able to protect dopamine neurones from the action of MPTP.

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