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Neuroprotective effects of riluzole in early phase Parkinson's disease on clinically relevant parameters in the marmoset MPTP model

Peternella S. Verhave [a,b,*], Marjan J. Jongsma [a], Roland M. Van Den Berg [a], Raymond A.P. Vanwersch [a], August B. Smit [b], Ingrid H.C.H.M. Philippens [c]

[a] BU CBRN Protection, TNO Defence, Security and Safety, Lange Kleiweg 137, PO Box 45, 2280 AA Rijswijk, The Netherlands
[b] Department of Molecular and Cellular Neurobiology, Center for Neurogenomics and Cognitive Research, Neuroscience Campus Amsterdam, VU University, De Boelelaan 1085, 1081 HV Amsterdam, The Netherlands
[c] Department of Immunobiology, Division Neuropathology, Biomedical Primate Research Centre, Lange Kleiweg 161, 2288 GJ Rijswijk, The Netherlands

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ABSTRACT

The present study evaluates neuroprotection in a marmoset MPTP (1-methyl-1,2,3,6-tetrahydropyridine) model representing early Parkinson's disease (PD). The anti-glutamatergic compound riluzole is used as a model compound for neuroprotection. The compound is one of the few protective compounds used in the clinic for a neurodegenerative disorder.

Marmoset monkeys were randomized into three groups of six: 1) an MPTP group receiving a total MPTP dose of 7 mg/kg (4 injections over two weeks, s.c.) 2) a riluzole group receiving besides MPTP, a twice daily dose of riluzole (10 mg/kg, p.o.), starting one week before MPTP and continuing for one week after the final MPTP injection and 3) a control group receiving saline instead of MPTP and riluzole. The marmosets' Parkinsonian symptoms were scored daily and their activity level, hand-eye coordination, jumping behavior, axial turning and night sleep parameters were tested and recorded weekly. At three weeks following the last MPTP challenge, brains were dissected and dopamine levels in the striatum and the tyrosine hydroxylase (TH) expressing dopamine (DA) neurons in the substantia nigra (SN) were compared. MPTP affected all behavioral parameters and sleep architecture and induced a relatively mild (50%) decline of DA neurons in the substantia nigra (SN). Riluzole relieved the Parkinsonian signs, and improved the hand-eye coordination as well as turning ability. Moreover, riluzole prevented the impact of MPTP on sleep architecture and rapid eye movement behavioral disorder (RBD). Riluzole also increased the number of surviving DA neurons in MPTP-treated marmosets to 75%. However, riluzole did not prevent the MPTP-induced impairments on locomotor activity and jumping activity.

In conclusion, reduction of excitotoxicity by riluzole appeared to be effective in reducing progressive neurodegeneration and relieved several clinically relevant PD symptoms in an animal model representing the early phase of PD.

1. Introduction

Parkinson's disease (PD) is one of the most common progressive neurodegenerative disorders worldwide especially in aging societies. The challenge for today's scientific community is to discover new treatment strategies. A current priority in PD research is to move beyond symptom control to neuroprotective therapy (Jenner, 2008) in order to prevent the neurons from dying so that PD progression is slowed. Crucial for this is the ability to evaluate neuroprotection at an early stage of the disease. Here, we aim to show the neuroprotective effects on behavior and pathology in a model for early PD.

One of the features involved in neurodegeneration is mitochondrial dysfunction (Philippens et al., 2010) that affects cellular function via the accumulation of intracellular calcium levels (Greenamyre et al., 1999). In PD the mitochondrial impairment is of crucial importance. Dopamine (DA) neurons in the substantia nigra (SN), the main target cells in PD, are also generating action potentials even in the absence of synaptic input. This is called the autonomous firing capacity and involves ion channels that enable

* Corresponding author. BU CBRN protection, TNO Defence, Security and Safety, Lange Kleiweg 137, PO Box 45, 2280 AA Rijswijk, The Netherlands. Tel.: +31 15 284 3047; fax: +31 15 284 3963.
E-mail address: nellekeverhave@gmail.com (P.S. Verhave).

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the entry of calcium into the cytosol (Chan et al., 2009). This calcium is removed by active pumping that requires energy in the form of ATP. Therefore, loss of mitochondrial function in PD may lead to premature dopaminergic cell death in the SN owing to a failure to control calcium homeostasis. Under these conditions, free oxygen and nitrogen radicals (Fiskum et al., 2003; Nicholls, 2008) are produced which puts neurons under stress and also activates pathways leading to neuronal degeneration. Excitotoxicity has been implicated in relatively slowly progressing neurodegenerative disorders such as PD (Mandel et al., 2003) in which vulnerable neurons may not survive elevated glutamate concentrations that would not normally be harmful. Therefore, reducing the effects of glutamate and calcium influx may prevent further progression of neuron deterioration. Indeed, counteracting the actions of glutamate, directly or through sodium or calcium channel manipulation (Song et al., 1997), should have neuroprotective effects in neurodegenerative disorders (LeWitt and Taylor, 2008). To this end, the anti-glutamatergic compound riluzole may be expected to influence the calcium influx through various pathways, including the inhibition of glutamate activity in the synapse by the blockade of NMDA receptors (Doble, 1996) and blocking voltage-dependent sodium channels (Hubert et al., 1994) on nerve endings and cell bodies. However, a direct interaction between riluzole and glutamate receptors has never been observed. Nevertheless, riluzole appears to prevent neuronal damage at the start of neurodegeneration in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-challenged mice, rhesus monkeys and marmosets (Araki et al., 2001; Benazzouz et al., 1995; Obinu et al., 2002) and also in other PD models (Barneoud et al., 1996; Fumagalli et al., 2006). Riluzole is an FDA-approved drug treatment because of its life-prolonging effects in amyotrophic lateral sclerosis (ALS) (Bensimon et al., 1994). In this study we use riluzole to model neuroprotection and show the effects of the reduced neurodegeneration in a model for early PD.

Riluzole appears to be less effective in clinical studies if treatment (50 mg, twice daily) is delayed until the motor phase of PD becomes evident, by which time many DA neurons will already have degenerated (Jankovic and Hunter, 2002). Complete prevention of neuronal cell loss may only be possible if the treatment is initiated before or simultaneously with the actual start of the apoptotic cell death process. Patients would then benefit from premotor phase diagnosis of PD and start treatment at a stage while there are still neurons left to protect. Thus, in order to find new targets for neuroprotective therapies, animal models need to be employed that enable investigation of treatment options early in the pathogenesis of the neurodegenerative process.

In this study, we investigated the neuroprotective effects of riluzole in the marmoset model on several clinically relevant behavioral and sleep parameters which may be used as biomarkers for neuropathology. This translational, integrative approach may contribute to the understanding of the mechanisms during PD progression. Additionally, this approach offers a model for the early phase of PD to test new neuroprotective treatment strategies.

2. Materials and methods

2.1. Experimental design

Eighteen common marmoset monkeys (*Callithrix jacchus*) of both sexes, between 2 and 3 years of age, were purchased from the Biomedical Primate Research Centre in the Netherlands. The monkeys were housed in individual primate cages (61 × 61 × 41 cm) under controlled conditions of humidity (60%), temperature (23–25 °C) and lighting (12 h light/dark cycles; lights on at 7:00 h). Marmosets were fed daily with pellet chow. Diet was enriched with peanuts, fruit and vegetables, raisins, sunflower seeds and an occasional grasshopper. Water was available ad libitum. Toys and wood were available to all marmosets to provide variety in their cage environment. Experiments were conducted after approval by the Ethical Review Committee of the institute.

The marmosets were divided into three groups semi-randomly based on their baseline activity level in the bungalow test. Two groups ($n = 6$) received 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine hydrochloride (MPTP, Sigma Aldrich, St. Louis, USA) by subcutaneous (sc) injections in the abdominal area. MPTP was administered in a schedule of four injections (2, 2, 1.5, and 1.5 mg/kg) over a two-week interval on Mondays and Thursdays. Moderate Parkinsonian symptoms were established following this treatment. A third (control) group ($n = 6$), received sc injections of 0.9% saline which were match for time and volume with the treatment groups. One of the two MPTP-treated groups ($n = 6$) received riluzole orally (riluzole group). Treatment started one week before MPTP treatment and lasted until one week after the final MPTP injection. Riluzole was suspended in 2:1 (vol:vol) water:syrup (Karvan Cevitam®) containing 0.5% methylcellulose (Sigma Aldrich, St Louis, USA). Dosage was 10 mg/kg given twice daily at 7 am and 5 pm. The two other groups received the diluent vehicle alone at the same times in equivalent volumes.

All marmosets were observed each morning and evening on every day of the study for signs of PD. Symptoms were scored on two observation scales. Other tests were performed intermittently. The functionality and activity tests were performed on Wednesdays or Thursdays between 1 pm and 5 pm, while sleep was recorded weekly during the night. To ensure that direct effects of MPTP did not influence the analysis, only the last three weeks after the MPTP challenge (i.e. including the two weeks without riluzole treatment) were used for behavioral analysis. At the end of the experiments the brains were collected for post-mortem analysis.

2.2. Observational scores for Parkinson's disease symptoms

The observational tests were scored daily before treatment. Items were rated from 0 (= normal/healthy) to 4 (= severely affected). Clinical scores include measures for apathy (no interest in their surrounding), immobility, muscle rigidity (as measured by the stiffness of the legs and tail) and tremors at rest. Immobility was also quantified separately to determine home cage activity at rest and serve as a comparison for the out of cage activity tests. In order to record activities that were not covered by this clinical scoring scale, we additionally used the Abnormal Involuntary Movements Scale (AIMS). This scale included scores for facial behavior items (jaw, facial muscles, tongue and lips) and full body behavior items (upper, lower and trunk). It is normally used for observing L-dopa induced dyskinesia.

2.3. Functionality tests

2.3.1. Hand-eye coordination

The marmosets reward-related hand-eye coordination was tested with an automated test setup (van Vliet et al., 2006). During a session of 42 trials, small pieces of marshmallow were presented behind a window (5 × 8 cm) at three different speeds: (stationary) non-moving (0.0 m/s for a maximum of 30 s); slow (0.04 m/s) and fast (0.08 m/s) moving. All trials started with a brief sound signal to alert the animal. Before the start of the study, all animals were trained until they grabbed 75% or more of the presented rewards. The percentage of correct hits was the measure of an animal's performance.

2.3.2. Tower

In the Tower (Verhave et al., 2009) not only a marmoset's jumping behavior but also its movement between different levels (= measure of activity) were evaluated. The trespa Tower (35 × 35 × 250 cm) contains 7 levels of horizontal crossbars. Their distances apart increase the higher up the cage they are so that marmosets have to jump to reach the highest bars. During each test the marmoset could move around freely between the 7 levels for 5 min. To motivate the marmoset to visit each level, a small piece of marshmallow was available at each level. All marmosets were habituated to the Tower before testing. The marmoset's location (level) was recorded by non-automated video analysis. The highest bar reached was a measure of functionality.

2.3.3. Hourglass

In the Hourglass test a marmoset's axial turning ability was evaluated in a Plexiglas cylinder (11 × 27 cm) (Verhave et al., 2009). A trial consisted of turning the cylinder vertically through 180°. A test consisted of five consecutive trials at intervals of 30 s. The time the marmoset needed to return to an upright position, after inversion of the cylinder, was measured by means of non-automated video analysis by an observer unaware of the treatment. The maximum time allowed was 30 s (this value was also given to marmosets which did not turn upright at all). Only the three fastest turns were included in the statistical analyses.

2.3.4. Bungalow

Activity of the marmoset was quantified in the so-called Bungalow test system. This is an automated system to record the number of times the marmoset moves from one compartment to another (van Vliet et al., 2006). The Bungalow consisted of four equal compartments (23 × 23 × 23 cm) connected to each other so that the marmoset could move freely between them. A video tracking system registered the movement pattern and the position of the monkey in the apparatus. The number of compartment changes over a 20-min. period was used as a measure for activity.