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Research report

Anti-Parkinson effects of a selective alpha2C-adrenoceptor antagonist in the MPTP marmoset model

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HIGHLIGHTS

- Indirect targeting dopaminergic neurotransmission may provide innovative therapeutic potential in Parkinson's disease.
- The alpha2C-adrenoceptor antagonist JNJ27063699 mitigates motor deterioration in MPTP monkey model of Parkinson's disease.
- The anti-parkinsonian therapeutic dose of JNJ27063699 is devoid from side effects.
- Adrenergic intervention may be successful to improve motor control in Parkinson's disease.

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ABSTRACT

Current dopamine replacement therapies, in Parkinson's disease (PD), result in aversive side effects and rapid drug dose escalation over time. Therefore, a non-dopaminergic treatment would be an advantageous supplement to lower the dose of dopamine replacement treatment postponing the occurrence of side effects. The noradrenergic system plays an important role in the facilitation or maintenance of the activity of the nigrostriatal dopamine pathways. Here the putative anti-Parkinson effects of the oral selective alpha2C-adrenoceptor antagonist (JNJ27063699 0.1–10 mg/kg p.o.) and of vehicle (fruit syrup) were evaluated in the MPTP-marmoset model. Dose-related anti-Parkinson effects were assessed by means of a behavioural rating scale covering parkinsonian symptoms, body weight and body temperature, and two test systems assessing locomotor activity and complex motor skills of hand–eye coordination for controlled movements in MPTP- or saline-pretreated marmosets.

JNJ27063699, at the middle and higher doses, consistently improved locomotor activity and hand–eye coordination capabilities, which indicates an improvement in the coordination of motor control -or movements- in MPTP-pretreated monkeys. No additional effects on the parkinsonian symptoms or side effects were observed on other test systems. Overall, the findings link deficit in motor coordination with dysfunctional adrenergic signalling and it suggest that selective alpha2C adrenergic antagonism may contribute to behavioural improvement in the MPTP-monkey model of PD. In multi-drug medication JNJ27063699 might have potential in the treatment of motor deficit in PD.

1. Introduction

Parkinson's disease (PD) is a major progressive neurodegenerative disorder characterized by a loss of the dopamine (DA) neurons in the nigrostriatal circuitry governing the control of muscle movement [1]. The decrease in dopaminergic (DA-ergic) tone results in a disturbed balance in the neural circuitry of the basal ganglia [2] resulting in symptoms like akinesia, bradykinesia, rigidity, resting tremor and postural instability. Current pharmacological strategies are focussed on increasing DA-ergic neurotransmission using

Abbreviations: ANOVA, analysis of variance; CNS, central nerve system; DA, dopamine; DA-ergic, dopaminergic; GPi, globus pallidus interna; LC, locus coeruleus; L-DOPA, L-dihydroxy-phenylalanine; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NE, noradrenergic; PPN, pedunculopontine nucleus; SNR, substantia nigra reticula; UPDRS, Unified Parkinson's Disease Rating Scale.

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DA receptor agonists and L-dihydroxy-phenylalanine (L-DOPA). These compounds are still the most effective, although long term medication causes complications such as motor fluctuations and dyskinesia [3,4]. To overcome these side effects related to direct intervention in the DA system, Non-DA-ergic strategies using compounds that modulate indirectly the DA system would be a good alternative and/or supplement to the DA replacement therapies [5]. Indeed, the pathology is not only restricted to the *substantia nigra* of the basal ganglia and DA-ergic system, as the noradrenergic (NE), cholinergic and serotonergic systems are also affected [6]. Accordingly, the NE system, which finds its main origin in the *Locus Coeruleus* (LC) plays a pivotal role in the in the regulation of the nigrostriatal DA pathways [7]. Alpha2C adrenergic subtype receptors are of particular interest as it is part of a negative feedback loop to regulate NE release and therefore DA neurotransmission. Functionally, numerous selective alpha2-adrenoceptor modulators have been developed and proposed to provide an effective treatment in PD [8–10].

Recently we have developed a selective alpha2C adrenergic antagonist JNJ27063699 with high oral availability and metabolic stability. The half-life for rat is 3.7 h and for dogs 6.8 h. The oral absorption is very good: the bioavailability measured in three different species is 30–40%. JNJ27063699 displays a high in vitro potency and subnanomolar affinity (Ki = 0.28 nM) for the h-alpha2C adrenoreceptor with high selectivity over the human alpha2A- and alpha2B-adrenoceptor subtypes as well as over the alpha1A-receptor, while in vitro profiling across a wide range of other binding assays showed no activity (CEREP data on file). The maximum selected dose (10 mg/kg p.o.) resulted in >80% brain receptor binding in rats, measured 2 h after dosing. The present studies aimed at investigating the potency of JNJ27063699, in three consecutive sequential doses, to alleviate the parkinsonian symptoms and improve the motor deficits in the MPTP marmoset model of idiopathic PD. The MPTP challenge in non-human primates causes specific intoxication of DA-ergic structures and loss of nigrostriatal DA neurons leading to long-lasting motor and non-motor impairments characteristic and neuropathological features similar to PD in humans, which makes the MPTP marmoset model an essential tool suitable for translational studies in PD [11–15]. An advantage of the marmoset monkey is the availability of automated test systems for measuring motor deterioration in a quantitative manner. In human neurodegenerative diseases such as PD, spontaneous activity levels are greatly affected. In marmoset monkeys, a device/set up called the 'Bungalow' has been developed quantifying the spontaneous locomotor activity [16]. Furthermore, the fine motor skills of patients are reduced due to a combination of tremor, slowness of movement and disturbed motor planning. Indeed, assessment of these fine motor skills in MPTP non-human primates, e.g. hand–eye coordination is also affected [17]. The hand–eye coordination task according to Wolthuis et al. [18] proved to be very suitable for PD studies [19]. Both test systems were extensively validated in the MPTP marmoset model [15,19–22].

2. Methods

2.1. Animals

Twelve adult common marmoset monkeys (*Callithrix jacchus*) of both sexes between five and seven years of age and body weights between 300 and 450 g were obtained from the German Primate Centre (DPZ), Germany and the Biomedical Primate Research Centre (BPRC), The Netherlands. These animals were equally subdivided over two treatment groups ($n = 6$ per group). They were housed under controlled temperature ($25 \pm 2\,^{\circ}C$), humidity (>60%) and standard 12:12 light cycle regime (lights on from 7:00 a.m. to 7:00 p.m., illumination intensity: ~650 lx). The monkeys were daily fed with standard monkey-chow (SSNIFF and SDSS) enriched with fruit and vegetables of the season. Water was available at libitum. All animal procedures were approved by local laws and are in line with European Community guidelines. Protocols were reviewed by the local Ethical Review Committee on Experimental Animals, prior to the start of experiments.

2.2. Drug administration

The vehicle, for oral administration of the test compounds, consisted of water: strawberry syrup in a 2:1 proportion and was administered orally (po) in a dose of 1 ml/kg. The test compound JNJ27063699 obtained from Janssen Research and Development was dissolved in the vehicle, as mentioned above, in the following concentrations: 0.1 mg/kg for low dose, 1 mg/kg for middle dose and 10 mg/kg for high dose. MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine hydrochloride; Sigma Chemical Co., St. Louis, USA) was injected subcutaneous (sc) in the abdominal area for three consecutive days (respectively 2.0, 1.5, and 1.0 mg/kg), and continued after a 2-day rest period for another two consecutive days of MPTP injections (both at 1.5 mg/kg) to induce a moderate state of PD.

2.3. Experimental design

For the validation of the anti-Parkinson efficacy behavioural assays were used: Observational symptoms assessing parkinsonian features as immobility, apathy, rigidity, and tremors were analysed, while motor deterioration was assessed by analysis of the locomotor activity and complex motor behaviour.

Side effects of the treatments on the assays used were investigated in a separate group, in which the animals received saline-injections instead of MPTP during the disease induction.

All monkeys were trained on the hand–eye coordination task and baseline values of all test systems used were obtained before the start of the experiments. Based on hand–eye coordination performance, locomotor activity in the bungalow task, age and gender, the monkeys were stratified into two equal test groups. One group ($n = 6$) received five MPTP injections within a week time period till stable moderate parkinsonian symptoms were established (total MPTP dose: 7.5 mg/kg). The other group ($n = 6$) received time and volume-matched subcutaneous administration of saline.

After disease stabilization of two weeks, the effects of oral administration of vehicle and of JNJ27063699 (0.1, 1 and 10 mg/kg) were evaluated with a week time interval, respectively ($n = 6$ for each dose). A wash out period of one week was observed between each experiment. An observational scoring scale for clinical parkinsonian signs, behavioural assays (hand–eye coordination and locomotor activity), body weight and body temperature were used to test the efficacy of JNJ27063699. The observational parameters, body weight and body temperature were scored one day before administration, the morning before administration, 50 min after administration and one day after administration. The efficacy on both the hand–eye coordination and the locomotor activity were measured at 60 and 75 min after administration of the compounds, respectively.

2.4. Physical signs

Clinical assessment of PD symptoms in the clinic is mostly done with the Unified Parkinson's Disease Rating Scale (UPDRS). Here, we used a clinical rating scale to score cardinal parkinsonian symptoms in marmosets such as (a) inadequacy of grooming by inspection of the fur; (b) apathy by testing the responsiveness of the animal to its surrounding; (c) immobility; (d) rigidity; and (e) presence of