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Simvastatin decreases levodopa-induced dyskinesia in monkeys, but not in a randomized, placebo-controlled, multiple cross-over ("n-of-1") exploratory trial of simvastatin against levodopa-induced dyskinesia in Parkinson's disease patients

François Tison [a,b,c,*], Laurence Nègre-Pagès [d], Wassilios G. Meissner [a,b,c], Sandrine Dupouy [a,b,c], Qin Li [e,f], Marie-Laure Thiolat [a,b], Thibaud Thiollier [a,b,g], Monique Galitzky [h], Fabienne Ory-Magne [h], Agathe Milhet [d], Laurent Marquine [h], Umberto Spampinato [c,i], Olivier Rascol [h,j,k], Erwan Bezard [a,b,c,e,f]

[a] Université de Bordeaux, Institut des Maladies Neurodégénératives, UMR 5293, Bordeaux, France
[b] CNRS, Institut des Maladies Neurodégénératives, UMR 5293, Bordeaux, France
[c] Service de Neurologie, CHU de Bordeaux, Pessac, France
[d] LN Pharma, Toulouse, France
[e] Institute of Laboratory Animal Sciences, China Academy of Medical Sciences, Beijing, China
[f] Motac neuroscience, Manchester, UK
[g] Cynbiose, Marcy l'étoile, France
[h] CIC Toulouse, France
[i] Université de Bordeaux-INSERM U862, Neurocentre Magendie, Bordeaux, France
[j] Départements de Pharmacologie Clinique et Neurosciences, INSERM CIC9302, CHU de Toulouse, France
[k] Service de Pharmacologie, Faculté de Médecine, CHU de Toulouse, Université de Toulouse, France

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ABSTRACT

Background: Simvastatin may improve levodopa-induced dyskinesia through striatal Ras-extracellular signal-regulated kinase pathway modulation.
Methods: (1) Six 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-treated macaques were assessed for parkinsonism and dyskinesia severity following acute co-administration of levodopa and simvastatin (0, 1.5, 3 and 6 mg/kg). (2) A "n-of-1" design randomized, placebo-controlled, 3 cross-over trial was then conducted in 10 Parkinson's disease patients with troublesome dyskinesia. The primary endpoint was a 7-point scale rating subjective discomfort caused by troublesome dyskinesia. Secondary endpoints related to dyskinesia severity and duration and functional impairment, severity and duration of OFF periods, motor scores and investigator- and patient-rated global impressions. (3) The pharmacodynamic variable for both studies consisted in a multiplex analysis of kinase-induced phosphorylation in T and B-lymphocytes by flow cytometry.
Results: (1) In the macaque, simvastatin reduced dyskinesia scores (45%), at the dose of 3 mg/kg (2) In the "n-of-1" trial no significant response was observed in the primary end point and all secondary endpoints. No serious adverse events were reported. (3) Simvastatin 3 mg/kg significantly reduce kinase-induced phosphorylation in monkeys but not simvastatin 40 mg in patients.
Conclusions: Simvastatin reduced dyskinesia in primates using high doses over 3 mg/kg but the exploratory trial in patients revealed no effect at 40 mg/d suggesting that higher doses, not compatible with a safe prolonged administration, are necessary.

Complications of long-term levodopa therapy, mainly motor fluctuations and levodopa-induced dyskinesias (LID), constitute a major shortcoming of Parkinson's disease (PD) management [1]. Reducing frequency and intensity of troublesome LID while preserving patient's motor autonomy is a yet unmet challenge [2], despite continuous efforts towards identification of non-dopaminergic strategies [3].

Regulation of striatal gene expression is a likely mechanism underlying neuronal plasticity in LID [4]. The extracellular signal-

* Corresponding author. Service de Neurologie, Hôpital du Haut-Lévêque, CHU de Bordeaux, Avenue Magellan, 33604 Pessac cedex, France. Tel.: +33 5 57 65 64 61; fax: +33 5 57 65 66 97.
E-mail address: francois.tison@chu-bordeaux.fr (F. Tison).

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regulated kinase (ERK) cascade is a key regulator of striatal plasticity and an interesting candidate for drug targeting [5,6]. In animal models of PD, the supersensitivity of striatal D1 receptors leads to ERK hyperactivation in response to levodopa, which correlates positively with LID severity [7-11]. Moreover, inhibition of this pathway with systemically active drugs [8,12], or viral vectors has been recently proposed as a treatment for LID [10].

Interestingly, statins, besides being specific inhibitors of the rate-limiting enzyme in cholesterol biosynthesis, can also interfere with the Ras-ERK pathway, by inhibiting Ras isoprenylation and activity and subsequent phosphorylation (activation) of Ras-ERK1/2 [13]. Accordingly, we demonstrated that statins reduce both LID and ERK1/2 phosphorylation in a 6-hydroxy-dopamine lesion rat model of PD [12]. Among statins, simvastatin exhibits interesting pharmacokinetic properties such as blood brain barrier crossing [14,15].

We herein describe the further translation of this hypothesis to the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-intoxicated monkey model of PD, and the subsequent completion of a randomized, placebo-controlled, multiple cross-over ("n-of-1") trial in PD patients suffering from troublesome LID. Such exploratory trial design is highly suitable for rapid proof-of-concept challenging of a new indication of available drugs for LID management [16].

1. Material and methods

1.1. Preclinical study in the MPTP monkey model

Animals. Non-human primate models of PD and LID were produced in male rhesus monkeys (Macaca mulatta, Xierxin, Beijing, PR of China; mean weight = 5.3 ± 0.8 kg; mean age = 5 ± 1 years) as previously described in details [10,17,18]. They were housed in individual cages under controlled conditions of humidity, temperature, and light (12-h light/12-h dark cycle, lights on at 8.00 am); food and water were available ad libitum in an AAALAC-accredited facility. Animal care was supervised by veterinarians skilled in the healthcare and maintenance of non-human primates. Experiments were carried out in accordance with European Communities Council Directive of 24 November 1986 (86/609/EEC) for care of laboratory animals and all efforts were made to minimize animal suffering, to reduce the number of animals used and to utilize alternatives to in vivo techniques, if available.

Induction of parkinsonism and dyskinesia. Six monkeys were intoxicated with MPTP hydrochloride (0.2 mg/kg i.v. for 15 days), according to our standard method [19]. Such regimen of intoxication leads to near complete nigrostriatal denervation and stable parkinsonism [19,20]. After inducing a stable, bilateral parkinsonism (constant disability scores over two consecutive weeks after at least 8 weeks post-MPTP), the animals received daily oral administration of L-DOPA (Modopar®, L-DOPA/benserazide, ratio 4:1) for 12 weeks at a tailored dose producing full reversal of parkinsonian symptoms (ranging from 15 to 20 mg/kg/day) [10,17,18,21] and developed stable and moderate-severe LID (dyskinesia severity grade 2–4, according to the NHP Dyskinesia Disability Scale.)

Behavioural assessment procedures. Assessments of parkinsonism (PD scores), locomotor activity and dyskinesia (LID scores) were carried out in an observation cage for 350 min following oral administration of L-DOPA alone (individual dose ranging from 15 to 20 mg/kg) or co-administration of the same dose of L-DOPA with simvastatin. The battery of behavioural tests was performed as previously described [10,17,18,21] and in keeping with the recommended practice [22]. This study assessed drug effects in duplicate according to a within-subject design with treatments randomized in a latin square design. In the PD motor scale, a PD score of 0 corresponds to a normal monkey and a score above 6 indicates severe parkinsonism. The severity of dyskinesia was rated using the NHP Dyskinesia Disability Scale (0, dyskinesia absent to 4, severe, almost continuous dyskinetic activity, disabling to the animal and replacing normal behaviour) discriminating both choreic-like and dystonic-like movements (max score 4 × 2 = 8) [22]. Locomotor activity was concomitantly measured every 5 min with infrared activity monitors.

The duration of anti-parkinsonian action, ON-time, was defined as the number of minutes for which the bradykinesia score is zero (PD motor scale). In addition, the duration of ON-time associated with dyskinesia of varying severity was calculated [22]: "GOOD ON-time" represents the number of minutes for which the bradykinesia score is zero while dyskinesia is absent, mild or moderate, (total score < 4). "BAD ON-time" represents the number of minutes for which the bradykinesia score is zero while the dyskinesia score is marked or severe (total > 4).

Time course scale-based experiments were analysed using the Wilcoxon matched-pairs signed rank test. Area under curve (AUC) were analysed using non-parametric one-factor analysis of variance (ANOVA) with repeated measures (Friedman test), followed by Dunn's multiple comparison test. AUCs of locomotor counts were analysed using one-way ANOVA with repeated measure followed by Bonferroni multiple comparison test.

1.2. Clinical trial

"n-of-1" trial design. The randomized, double blind, placebo-controlled, multiple cross-over, proof-of-concept pilot study was performed as previously described in details (Fig. 1) [16]. Each patient received simvastatin and placebo in 3 consecutive cross-overs in a random order. Each cross-over lasted 28 days and was composed of 2 treatment periods of 10 days each of simvastatin (A) or placebo (B), separated by 4 days of placebo wash-out. Two tertiary movement disorders centers (Bordeaux and Toulouse, France) participated. All procedures were carried out with the adequate understanding and written consent of the subjects involved and with the ethical approval of the authors' institutional review board (CPP Sud-Ouest et Outre-Mer 3); The study was registered in EudraCT database under the number 2009-011736-35.

Participants. Non demented PD patients suffering from troublesome LID were selected to participate in the study [16]. Briefly, eligible patients were aged ≥ 30 and <80 years, had a diagnosis of idiopathic PD and should have LID during more than 25% of the waking day and should be at least moderately disabling (item IV UPDRS 32 >1; item 33>1). A stable dose of anti-parkinsonian drug treatment was required for at least 1 month prior to inclusion. Patients were excluded if they underwent PD brain surgery or currently used (or within 3 months), amantadine, statins and dopamine receptor blocking agents (besides domperidone) or budipine, riluzole, dextromethorphan and memantine.

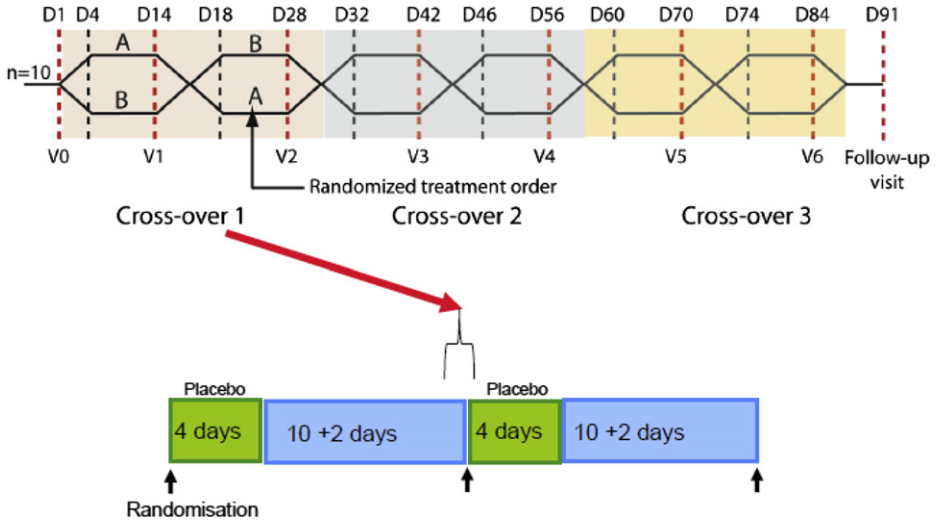

Fig. 1. N-of-1 clinical trial design. Each patient was evaluated during 3 randomized cross-over periods lasting 28 days (D) and was composed of 2 treatments periods of 10 days ± 2 each of simvastatin (A) or placebo (B), separated by 4 days of placebo wash-out.