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Intraventricular dopamine infusion alleviates motor symptoms in a primate model of Parkinson's disease

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ABSTRACT

BACKGROUND

Continuous compensation of dopamine represents an ideal symptomatic treatment for Parkinson's disease (PD). The feasibility in intracerebroventricular administration (i.c.v.) of dopamine previously failed because of unresolved dopamine oxidation.

OBJECTIVES

We aim to test the feasibility, safety margins and efficacy of continuous i.c.v. of anaerobic-dopamine (A-dopamine) with a pilot translational study in a non-human primate model of PD.

METHODS

Continuous and circadian i.c.v. of A-dopamine was administered through a micro-pump connected to a subcutaneous catheter implanted into the right frontal horn of 8 non-human primates treated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). A-dopamine was assessed at acute doses previously reported for dopamine as well as evaluating the long term therapeutic index of A-dopamine in comparison to anaerobically prepared L-dopa or methyl ester L-dopa.

RESULTS

Over 60 days of a continuous circadian i.c.v. of A-dopamine improved motor symptoms (therapeutic index from 30-90 mg/day) without tachyphylaxia. No dyskinesia was observed even with very high doses. Death after 1 to 10 days (without neuronal alteration) was only observed with doses in excess of 160mg whereas L-dopa i.c.v. was not effective at any dose. The technical feasibility of the administration regimen was confirmed for an anaerobic preparation of dopamine and for administration of a minimal infusion volume by micro-pump at a constant flow that prevented obstruction.

CONCLUSION

Continuous circadian i.c.v. of A-dopamine appears to be feasible and shows efficacy without dyskinesia with a safe therapeutic index.

INTRODUCTION

A loss of dopamine, as a result of progressive neuronal degeneration in the substantia nigra pars compacta (SNpc), is the primary neurotransmitter marker of Parkinson's disease (PD).¹ Since dopamine does not cross the digestive mucosa or the blood brain barrier, the oral administration of its lipophilic precursor L-dopa remains pivotal as a therapy.² However there are many pharmacokinetic drawbacks with L-dopa that contribute to the occurrence of motor fluctuations and dyskinesia. These include a short half-life, limited and variable reabsorption through cellular barriers, the requirement of aromatic L-amino acid decarboxylase that declines in the striatum with disease progression and potentially harmful peripheral distribution.^{3,4} Continuous dopamine administration is considered more physiologically appropriate by preventing swings in neurotransmitter concentration between doses.^{5,6,7,8,9} Indeed, under normal conditions, the dopaminergic neurons in the SNc fire in a continuous manner (tonic low-frequency firing), so that the DA concentrations in the striatum are maintained at a relatively constant level. However, in a DA-depleted state, intermittent oral doses of L-dopa induce discontinuous stimulation of striatal DA receptors, and upon long-term treatment this can contribute to dysfunctional dopaminergic pathways that lead to L-dopa related complications development.^{5,6} Clinical observations with the treatments of long-acting dopamine agonists, dopamine metabolism inhibitors, direct delivery of a gel form of levodopa to the duodenum and subcutaneous infusions of apomorphine have reinforced this concept of continuous dopaminergic stimulation.^{7,8,9}

In a pilot study, De Yebenes *et al.*¹⁰ suggested that intracerebroventricular (i.c.v.) administration of a very high dose of dopamine (~300 mg/day) in the presence of a conservator (sodium metabisulfite) transiently improved motor handicap and increased dopamine in rat brains with unilateral neurotoxin 6-hydroxydopamine (6-OHDA)-induced damage as well as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) intoxicated

monkeys. The clinical feasibility of this administrative route has been supported by two independent PD patient case reports in which a low infusion dose of dopamine (16- 45 mg/day) into the frontal ventricle reduced motor handicap.^{11,12} However, concerns regarding dopamine oxidation inducing toxicity and tachyphylaxia further prevented therapeutic development of i.c.v dopamine.

By reducing dopamine oxidation, dopamine in anaerobia (A-dopamine) administered by continuous circadian i.c.v. close to the striatum has been shown to be efficient and safe in two rodent models of PD (acute intoxicated MPTP mouse and chronic unilateral 6-OHDA rat).¹³ Here, we aim to further test the feasibility, margins of safety and efficacy of continuous i.c.v. of A-dopamine administered by a clinic-approved programmable pump in a translational study on a non-human primate PD model that uses chronic MPTP .

METHODS

A-dopamine preparation

A-dopamine was formulated as a solution of dopamine hydrochloride (2, 30 and 100 mg/mL) solubilized with sodium chloride (0.9%) in a sterile anaerobic nitrogen isolator (oxygen < 0.1% ideally at < 0.01%) without any other excipient (Patent #WO2015173258 A1) and manufactured by the Central Pharmacy of the University Hospital of Lille. Maintaining the pH at 4 (sodium hydroxide addition) and oxygen level of A-dopamine < 1% allowed storage at 4 ° C in amber glass vials for 3 months and in a pump at 37°C for 1 month. Oxidation of the compound was regularly tested in accordance with the European Pharmacopoeia.

Continuous infusion of A-dopamine through a subcutaneous silicone catheter into the right frontal horn of the brain was maintained by either a Siromedes™ (Tricumed) or Prometra™ (Flowonix) pump and refilled transcutaneously. A reduction in dose by 25-50% during the night mirrored circadian rhythm. Batches of L-dopa (2 mg/mL) or L-dopa methyl ester (2 and

30 mg/mL) were also prepared anaerobically, since L-Dopa and ME-L-dopa are also known to rapidly oxidized within 1 hour in aerobic conditions. L-dopa and ME-L-dopa were unable to be prepared in saline at concentrations greater than 3 or 100 mg/ml respectively without compound precipitation.

Animals

Experiments were performed in accordance with the European Union directive on the protection of animal use for scientific purposes in an AAALAC-accredited facility (2010/63/EU) following study design acceptance by the Institute of Lab Animal Science (Chinese Academy of Science, Beijing, China). 8 male macaques (*Macaca mulatta*, Xieerxin, Beijing, PR of China) were housed in individual primate cages under controlled conditions that allowed visual contact and interaction with monkeys housed in adjacent cages. Food and water were available *ad libitum*. Animal care was supervised daily by veterinarians skilled in the healthcare and maintenance of non-human primates (NHPs).

Model preparation for MPTP and orally administered L-DOPA

The MPTP intoxication protocol, chronic L-DOPA treatment, clinical assessment and terminal procedure were conducted as previously published.¹⁴ Animals were rendered parkinsonian with MPTP-hydrochloride (0.2mg/kg, i.v., Sigma) dissolved in saline. A 30 min daily (9 a.m.) assessment of parkinsonism was performed within home cages by two blinded observers using a validated rating scale assessing tremor, general level of activity, body posture (flexion of spine), vocalization as well as rigidity, freezing and frequency of upper limbs (**Supplemental Table 1, Table 1**).¹⁵ On average, MPTP treatment duration is 21-27 days. Monkeys were left untreated until their parkinsonian disabilities stabilised, which was defined as a consistent PD score over a 3-week period. A tailored doses of Madopar was

selected to maximally reverse parkinsonian motor symptoms and cause hyperactivity in animals, which has been associated with the development of dyskinesia (over 3 week period). It takes around 3 months to induce a stable PD and dyskinetic syndrome. Animals are then followed for additional weeks for monitoring the stability of symptoms.¹⁴

Surgery

In the same surgical procedure, pumps were placed in the abdominal cavity whilst the catheter was stereotaxically inserted into the brain for i.c.v administration. Pre- and post-surgical animal care was under the supervision of a certified veterinarian. All surgeries were done under aseptic conditions with animals generally anaesthetised using ketamine (10 mg/kg, intramuscular) and isoflurane inhalation. To prevent infections, amoxicillin (Duphamox, 15 mg/kg; subcutaneous) was given one day before surgery, and again one day post-surgery. Ketoprofen (Ketophen, 2 mg/kg; subcutaneous) was administered over the first 3 days post-surgery to reduce pain.

The standard Horsley-Clarke stereotaxic technique has been improved by using sagittal and frontal ventriculography to accurately locate the borders of the third ventricles as well as the anterior and posterior commissures.¹⁶ After craniotomy without damaging the dura matter, a ventriculographic cannula mounted on a glass syringe was introduced into the anterior horn of the lateral ventricle and a contrast medium (Omnipaque, Nycomed, Norway) injected. A stereotaxic atlas was used for precise adjustment before insertion of the infusion cannula into the brain.¹⁷ Placement of the infusion cannula (PlasticOne, Roanoke, VA, USA) in the lateral ventricle was then performed using a dedicated PlasticOne Kopf-compatible cannula holder at the positions defined from the ventriculography following previously described procedures.¹⁸ Positioning was confirmed with a further sagittal x-ray. The plastic pedestals of the cannula were secured to the skull with stainless steel self-tapping slot pin screws (Stryker, France). Once the infusion cannula had been fixed to the skull, the wounds were closed with layers of

absorbable suture material. The pump was then connected to the cannula via a dedicated catheter and inserted subcutaneously in the abdomen. The macaques were then allowed to recover for at least 96 hours.

Behavioural assessment

After surgery, general health state, body temperature and any adverse events were monitored daily by an observer blinded to treatment. Each morning parkinsonism, anti-parkinsonian response and dyskinesia were characterized according to standard guidelines (**Supplemental Table 1**).^{15,19} Acute L-dopa challenge was performed post-surgery but before A-dopamine administration to check the model, according to standard guidelines.¹⁹ We determined in all NHPs, the minimum efficient dose of L-dopa which allowed the disappearance of the PD symptoms (PD score of 0). In all NHP, the disappearance of the PD symptoms was at the expense of dyskinesia (**Table 1**). No animal displayed an “On state” (PD score of 0) without dyskinesia, this pathological state of dyskinesia prevented from assessing the remaining akinesia and posture abnormality (PD score rated at 0 because of hypotonia, chorea). An exploratory cognitive/behaviour assessment was performed to detect any bad outcome on behaviour (i.e. rating on an observational 4 points scale that considered PD symptoms, cognition and mood: “individual classical parkinsonian state”, “slightly more aggressive”, “slightly more quiet”, “definitely more interactive and alert than in individual's parkinsonian state”).

Post-mortem tissue processing

The brain was rapidly removed and bisected along the sagittal midline. Brains were post-fixed in buffered paraformaldehyde (4%) for 2 months and then in PBS supplemented with sodium azide until required. The left hemisphere was used for anatomopathological analysis and processed accordingly. Briefly, four coronal sections were taken at the level of the frontal

pole, occipital pole, basal ganglia/SN and one horizontal section through the pons and cerebellum. These samples were embedded in paraffin, cut into 6 μ slides, and then either stained with haematoxylin-eosin or immunostained for microglia (CD68-KP1, antibody MA5-13324, Invitrogen).

The right hemispheres were used for magnetic resonance (MR) analyses. After being degassed with a vacuum pump for 5h, they were fully immersed into a dedicated vial filled with fomblin (perfluoropolyether, Sigma-Aldrich) at room temperature (18-22°C). MR acquisitions were performed on a 7T micro-MR system (BioSpec Brucker, Billerica, Massachusetts, US). A 3D multi-echo gradient-echo sequence was performed (FOV=60×60×76mm; matrix=240×240×304, voxel=0.250×0.250×0.250mm; TEs=4, 10, 16, 22, 28, 34 ms; TR=50ms; flip angle=15°) to evaluate the canula path and the presence of intracranial haemorrhage.

Plans of analysis

- Phase N°1: Low doses of A-dopamine

A-dopamine doses were assessed within a range previously reported in two human cases reports as efficient for aerobic dopamine (i.e. 8 to 16 mg)^{11,12}. A-dopamine (2 mg/mL) was primarily titrated at 1 mg/day up to 6.5 mg to monitor safety. Efficacy in each monkey was then tested with a faster titration of 1 mg/h within a day at a range of doses (0-30 mg).

- Phase N°2: High doses of A-dopamine

At a comparable dose in which aerobic dopamine has been previously reported to efficiently rescue MPTP intoxicated in NHPs (25mg/hour thus 300 mg/day)¹⁰ A-dopamine (100 mg/mL) was rapidly titrated at 25 mg/h until a change in behaviour (improvement or worsening) was observed.

- Phase N°3: Therapeutic index of A-dopamine at 60 days

Long-term safety and efficacy of A-dopamine (30 mg/mL) was assessed upon a slow titration (5 mg/ day) until 30-90 mg maximum was reached.

- Phase N°4 A-L-dopa and A-ME-L-dopa

To assess whether A-L-dopa (2 mg/mL) or A-ME-L-dopa (30 mg/mL) could induce a better outcome than A-dopamine in terms of efficacy, a slow titration of 10 mg/day was set each day until 40 mg where upon 5 mg/day increments were used until a 95 mg maximum was reached.

RESULTS

Phase N°1: Low doses of A-dopamine based upon human case reports

The **Table 1** presents the 8 monkeys with their weight, baseline PD score and dyskinesia score under an acute challenge of L-dopa. One NHP died 14 days after the surgical procedure due to an infection and before raising the dose above 6 mg/day. In 7 NHPs, the safety profile was good with no adverse events for dosing A-dopamine (2 mg/mL) with titration of 1mg/day. The lack of effect on motor symptoms with low doses of 6-10 mg, triggered an acute test with titration of 1 mg every hour up to 30 mg. At a dose of 30 mg, body temperature increased up to 39°C and there was an observed behavioural change (e.g. lying down on the cage floor). The same adverse events occurred in two NHPs (Y11 and CC16) treated with the saline control injected at the same volume (i.e. 15 ml), defining the volume safety limit to be below 15 ml (~20% of the CSF volume). The safety was good with a volume administrated below 15 ml. The low concentration and the high volume prevented from assessing higher doses and required a higher concentration in the phase N°2.

Monkey ID/pump	Weight (kg)	Baseline PD score	Dysk Score/ L-dopa dose	Dose range tested (mg/d) / number of days	Motor improvement	Adverse events	Interpretation
Y11/F	6.6	5	2/120	0.25-25 /13	No	-No until 25 mg - Transient fever with 15ml (i.e. 30 mg)	Well tolerated with volume below 15 ml Dose too low

CC16/F	6.8	5-6	3/80	0-30 /13	No	-No until 25 mg - Transient fever with 15ml (i.e. 30 mg)	Well tolerated with volume below 15 ml Dose too low
CC07/F	6.5	3	1/120	0-30 /30	No	- Transient fever and lying down with 15ml (i.e. 30 mg)	Well tolerated with volume below 15 ml Dose too low
CC20/F	6.7	5-6	3/140	0-6 /13	No	No	Well tolerated Dose too low
S2/T	6.6	3	1/120	0-3 /43	No	No	Well tolerated Dose too low
S26/T	6.3	5-6	3/140	0-3 /43	No	No	Well tolerated Dose too low
Y30/T	6.2	5	1/120	0-3 /38	No	No	Well tolerated Dose too low
S25/T	5.8	5	1/120	0-6/13	No	Died 14 days after surgery due to infection	NA

Table 1: Phase N°1: Low doses of A-dopamine

Phase N°2: High doses of A-dopamine based upon prior monkey case report

All Five NHPs that received A-dopamine >30 mg (concentration of 100 mg/mL and titration of 25 mg/h over 12h until a change in behaviour) showed an improvement of the motor handicap and cognitive state (**Table 2a**). The 3 remaining NHPs were not submitted to Phase N°2 analysis due to welfare concerns at a lower dose or equipment malfunction. Y11 presented with transient vomiting at the dose of 40 mg, leading to the monkey being maintained at the same dose without any further adverse events. CC20's catheter was partially obstructed and only 75% of the predicted dose could be delivered. CC16's catheter was completely obstructed.

Monkey	Daytime dose range / number of days	Baseline score (PD scale)	Motor improvement (PD scale)	Cognitive/ behaviour state (4- points scale)	Dysk Score under L-dopa	Dysk Score Under A-dopamine	Interpretation
Y11	0-200 / 1 then 40 /10	5	40% (score of 3) at 30 mg/day	Improvement: more interactive and alert at 30 mg/day time	2	0	Efficient No dyskinesia
CC20	0-100 / 20	5-6	40% (score of 3)	Improvement: more interactive and alert	3	0	Efficient No dyskinesia
S2	0-162 / 10	3	40% (score of 3)	Improvement: more interactive	1	0	Efficient No dyskinesia

				and alert			
S26	0-162 / 8	5-6	40% (score of 3)	Improvement: more interactive and alert	3	0	Efficient No dyskinesia
Y30	0-100 / 9	5	20% (score of 4)	Improvement: more interactive and alert	1	0	Efficient No dyskinesia
CC07	0-500 / 1	3	NA	NA	1	NA	Over dosage leading to death at 24 h

Table 2a: Phase N°2: Efficacy of high doses of A-dopamine

Three NHPs were able to tolerate very high doses between 100 to 300 mg without any obvious adverse event (including no dyskinesia) during 8-10 days and in 1 NHP an acute dose of 500 mg was tolerated for 24h before dying of a suspected sudden cardiovascular dysfunction. Taking these observations into consideration, the No Observed Adverse Effect Level (NOAEL) was defined at 90 mg over a day. As an additional observation, urine from monkeys whilst on a very high dose of A-dopamine (> 100 mg) was clear upon emission but turned black after storage in air for a few hours. This was likely to be due to oxidation of the excess dopamine excreted in the urine.

Monkey	Daytime dose range / number of days	Adverse events	Interpretation
Y11	0-200 / 1 then 40 /10	- Vomiting with rapid titration of doses at 100 mg and above - Abnormal behaviour with dose above 90 mg/day time	Dose too high
CC20	0-100 / 20	Transient pump dysfunction which explained that the high dose of 300 mg was well tolerated (real calculated dose of 70-100 mg)	Dose too high
S2	0-162 / 10	Death 10 days after an overdose of 162 mg/day	Overdose during 10 days leading to death
S26	0-162 / 8	Death 8 days after an overdose of 162 mg/day	Overdose during 8 days leading to death
Y30	0-100 / 9	Death 9 days after an overdose of 100 mg/day	Overdose during 9 days leading to death
CC07	0-500 / 1	Death 1 day after an overdose of 500mg/day	Huge over dosage leading to death at 24 h

Table 2b: Phase N°2: Safety of high doses of A-dopamine

- Phase N°3: Therapeutic index of A-dopamine over 60 days

Within the 2 remaining monkeys with a functional catheter (CC20 and Y11), motor and cognition improved with a dose reduction to 30 mg/day and no dyskinesia was observed. The

dose was slowly re-increased by 5 mg/daytime until a maximal of 90 mg was reached and maintained for up to 2 months. Efficacy during this time was maintained without an adverse event and there was no evidence of tachyphylaxia (**Table 3**) over chronic dosing. This defined the therapeutic index over 60 days to be 30-90 mg/day.

Similar to an acute administration of L-dopa (80-140 mg), fully dopa-responsive segmental motor symptoms were improved (**Table 2**) but axial symptoms (i.e. reduced vocalization and flexed posture) were not, conferring a general improvement of ~40%. The systematic dyskinesia with axial and segmental chorea and hypotonia (dyskinesia score from 1 to 3, **Table 2**) that was evident with acute administration of L-dopa was not apparent with A-dopamine.

The urine from monkeys whilst on minimum efficient dose of A-dopamine at 30 mg/day remained clear upon emission and storage.

Monkey	Dose range tested (real doses checked)	Period of treatment	Motor improvement (PD scale)	Cognitive/ behaviour state (4- points scale)	Adverse events	Therapeutic index: maximum efficacy and safety
Y11	Daytime: 0-70 mg Night: 15 mg/12h	6 weeks	40% (score from 5 to 3)	Improvement: more interactive and alert than PD state	No	Doses from 30 and 70 mg No dyskinesia
CC20	Daytime: 0-90 mg Night: 35 mg/12h	8 weeks	40% (score from 5 to 3)	Improvement: more interactive and alert than PD state	No	Doses from 30 and 90 mg No dyskinesia

Table 3: Phase N°3: Therapeutic index of A-dopamine at long term

- Phase N°4 A-L-dopa and A-L-dopa methyl ester assessment

Administration of A-L-dopa (2 mg/ml) induced no benefit in Y11 and CC20. Similarly, no motor improvement was observed in Y11 and CC20 at doses of A-ME-L-dopa up to 65 mg (**Table 4**). At 50 mg A-ME-L-dopa, a small level of dyskinesia was observed but not enough for the speed in amplitude score to reach 1; associated with nausea and anorexia. The urine in both animals remained clear at emission but again turned black within an hour with high doses (65-150 mg).

Monkey	Dose range tested (real doses checked)	Period of treatment	Motor improvement	Cognitive/behaviour state improvement	Adverse events	Interpretation
Y11	Daytime: 0-65 mg Night: 15 mg	4 weeks	No	No	- 60 mg: nausea - 55 mg: transient temperature increase - 50 mg anorexia	No improvement despite dose in the therapeutic index of A-dopamine
CC20	Daytime: 0-150 mg Night: 15 mg	4 weeks (150 mg during one day)	No	No	No	No improvement despite dose in the therapeutic index of A-dopamine

Table 4: Phase N°4: A-L-dopa and A-L-dopa methyl ester assessment

Post-mortem histological and MRI assessment

No abnormalities in organ (heart, lungs, liver, kidney) necropsy or weight was observed in all animals irrespective of the A-dopamine dose given. Gross morphology and MRI also showed no abnormalities except along the canula path and a small subacute hematoma in the thalamus caused by the canula placement. In the brains from monkeys that received a very high dose of A-dopamine (>100mg), a brown coloration of the ependymal surface of the third ventricle was observed. This was likely to be due to dopamine deposition that remained several hours post-mortem and oxidized upon contact with the air. This theory was support by the brown/black staining of the ventricle wall correlating with time after death and not the administered dose of A-dopamine. Microscopic analysis of the whole brain from all 6 monkeys processed for post-mortem histology was normal, without any signs of cortical or subcortical neuronal loss, gliosis or inflammatory changes.

DISCUSSION

An improvement of motor segmental symptoms, known to be dopa-responsive in human PD and a NHP model, was achieved in all monkeys that received A-dopamine at >30 mg for several days. Remarkably, irrespective of the A-dopamine dose no dyskinesia was observed and this allowed for much higher doses to be administered. However, the axial symptoms such as posture, that are typically not dopa-responsive in PD patient, were also not improved

by A-dopamine in NHPs. Whilst oral L-dopa appeared to improve both axial and segmental symptoms it also systematically induced concomitant dyskinesia, with segmental and axial chorea associated with hypotonia. Using a symptomatic PD assessment this inferred that the monkeys transitioned from a PD pathological state to a dyskinetic pathological state. This dyskinetic pathological state prevented from assessing akinesia, rigidity or posture. This is typically what is observed in PD patients. During the first years of disease progression, oral L-dopa treatment is improving the motor symptoms by about 30 to 50% without dyskinesia. Then after several years of disease progression and L-dopa treatment (and other dopaminergic treatments to a lesser extent), a pathological state of dyskinesia occur during which the patients wrongly seemed to have a full recovery of PD symptoms during an acute L-dopa challenge however the motor handicap remains with a bad functional outcome during dyskinesia.²⁰ This indicates that A-dopamine administered by i.c.v. does not induce the same effect as L-dopa administered orally. Furthermore, A-ME-L-dopa delivered by i.c.v. at the same level as A-dopamine had no clear behavioural benefit but maintained the adverse events known to occur with oral delivery of L-dopa. Despite this outcome, oral administration of ME-L-dopa (melevodopa with carbidopa) in PD patients has been shown to have a quicker efficacy than L-dopa (with carbidopa) and less pharmacokinetic variability.²¹ It should therefore be concluded that L-dopa is still an effective treatment for PD but i.c.v. delivery of L-dopa is not a viable therapeutic strategy.

The present results in NHP support the clinical feasibility of this administrative route previously reported by two independent PD patient case reports in which a low infusion dose of dopamine (16- 45 mg/day) into the frontal ventricle reduced motor handicap.^{11,12} However, a very advanced stage of dementia in the case studies prevented high motor benefits from being observed without deterioration of the psychiatric symptoms (i.e hallucinations). Moreover, concerns regarding dopamine oxidation inducing toxicity and tachyphylaxia

further prevented therapeutic development of i.c.v dopamine. The respect of a circadian administration (i.e. less than 50 % of the diurnal dose during the night) and importantly, the preparation and administration under anaerobia prevented from the tachyphylaxia.

A safe therapeutic index for A-dopamine was demonstrated, with a minimum efficient dose at 30 mg and a 3-fold increase in tolerability whereby A-dopamine remained efficient at 90 mg.

The lack of tachyphylaxia observed with A-dopamine administered up to 60 days in two monkeys was in contrast to aerobically prepared dopamine that has been repeatedly demonstrated to require regular dose increments in order to maintain efficacy.^{11,12} The NOAEL in NHPs was established at 90 mg, steadily titrated over a day, as minimal side effects (i.e. vomiting and low temperature) were only observed above this dose or with a more rapid titration. A slow titration of 5 mg/ day was found to be completely safe and avoided any adverse events. In humans, the safe titration was previously reported at 1-2 mg/day.^{11,12}

Uncharacteristic of many biologics including insulin, overdosing of A-dopamine required a 10-15-fold increase over the “human” dose (i.e. 160-500 mg) before death. This is likely to be related to the nature of A-dopamine and the highly efficient physiological mechanisms in place to capture and detoxify dopamine.

This is reflected by the high levels of excreted dopamine (that subsequently turned black upon exposure to air) associated with high doses of A-dopamine or A-ME-L-dopa. Presumably dopamine passes from cerebrospinal fluid into the venous system and then eliminated via the kidney. A similar observation did not occur with <120mg L-dopa BID or with A-dopamine within or below the index therapeutic dose. Lastly, even with A-dopamine dosing outside the index therapeutic range and when dopamine deposition was evidence on the ventricle walls, no neuropathology was detected. The lack of peripheral dopamine exposure with minimal efficient dose will have to be further demonstrated and carefully monitored in the next coming clinical trials notably for kidney function.²²

The technical feasibility of this therapeutic regimen was confirmed with the requirement of a highly technical pump for continual circadian administration and A-dopamine preparation to prevent deleterious oxidation and avoid the use of a potential deleterious conservator (e.g. sodium metabisulfite).²³ Further improvements are required in the preclinical progression of these studies in NHPs as the pumps, originally designed for larger sized humans, caused swelling for up to 1 month in the monkeys and increased a propensity for infection (e.g. S25) after surgical implantation. Also, a major technical issue identified in this study was the obstruction at the tip of the catheter by dopamine accumulation and oxidation after stopping the pump for more than 2 weeks. This can be avoided by maintaining a constant flow through the catheter, even when not administering the drug. However, vital information gained from this study demonstrates that the total volume administered by i.c.v. must be <20% of CSF at all time. Whilst in NHPs this was below 15 ml, in humans it would equate to 30mls in the 150ml total CSF volume. Lastly, small hematomas are a classic complication in neurosurgery and need to be minimized. While no clinical effect related to hematomas were observed in this NHP study, the potential for surgical haemorrhaging in patients can be dramatically reduced by per-operative MRI targeting; a technique typically used by neurosurgeons for deep brain stimulation targeting.

To conclude, the study was set to test the safety limits of a new therapeutic strategy of A-dopamine in order to anticipate the constraints of feasibility and safety in humans. The efficacy on dopa-responsive symptoms within a safe therapeutic index and without dyskinesia or tachyphylaxia is promising. Several important aspects have been defined by the study in preparation for a future proof of concept clinical trial. These include; slow titration, dose limits (NOAEL) that do not result in dyskinesia, volume of infusion limits that require a constant flow but respect a circadian rhythm and the use of MRI targeting of the ventricle for catheter placement.

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DD, CM, J-CD & Matthieu Fisichella (MF) have equity stake in InBrain Pharma.

CM has received grants from the France Parkinson charity and received various honoraria from pharmaceutical companies (such as Orkyn, Apopharma, and Boston Scientific) for consultancy and lectures on Parkinson's disease at symposia.

Alexandre Demailly and MF are employees of InBrain Pharma.

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Erwan Bezard has equity stake in Motac holding Ltd and receives consultancy payments from Motac Neuroscience Ltd.

Pascal Odou (PO) is the director of the pharmaceutical department of the University Hospital of Lille and leads the research group on injectable forms and associated technologies. As such, PO has signed contracts with many pharmaceutical companies. All contracts are signed by delegations from the University of Lille or CHU Lille. No personal contract.

DD also served on several Scientific Advisory Boards for Orkyn, Air Liquide, Lundbeck, Ever Pharma and Boston Scientific and has received PHRC grants from the French Ministry of Health and research funding from the France Parkinson and ARSLA charities. DD has received various honoraria from pharmaceutical companies for consultancy and lectures on Parkinson's disease at symposia.

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Legends

Table 1: Phase N°1: Low doses of A-dopamine

F= flowonix, T=tricumed, NA: not applicable; PD and dyskinesia scores according to the NHP scale¹⁵. The estimated dose delivered was checked by measuring the remaining volume in the pump.

Table 2: Phase N°2: Efficacy (Table 2a) and safety (Table 2b) of high doses of A-dopamine

Dysk Score: dyskinesia score at the acute challenge of oral administration of L-dopa; NA: not applicable; Max: maximum. PD and dyskinesia scores are according to NHP scale.¹⁵

Cognitive/behaviour state was rated on an exploratory daily observational 4 points scale that considered PD symptoms, cognition and mood: “individual classical parkinsonian state”, “slightly more aggressive”, “slightly more quiet”, “definitely more interactive and alert than in individual's parkinsonian state”. The estimated dose delivered was checked by measuring the remaining volume in the pump.

Table 3: Phase N°3: Therapeutic index of A-dopamine at long term

Dysk: dyskinesia. PD and dyskinesia scores are according to NHP scale.¹⁵

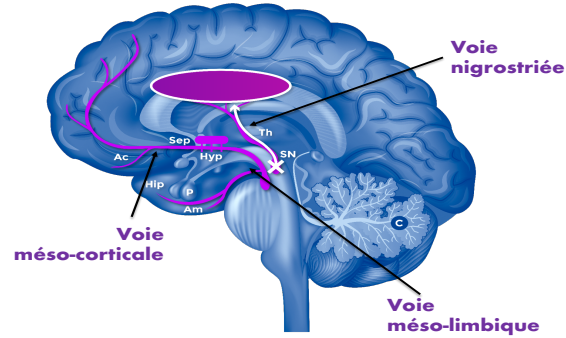
Cognitive/behaviour state was rated on an exploratory daily observational 4 points scale that considered PD symptoms, cognition and mood: “individual classical parkinsonian state”, “slightly more aggressive”, “slightly more quiet”, “definitely more interactive and alert than in individual's parkinsonian state”. The doses were estimated by measuring the remaining volume in the pump.

Table 4: Phase N°4: A-L-dopa and A-L-dopa methyl ester assessment

Dysk: dyskinesia. PD and dyskinesia scores are according to NHP scale.¹⁵

Cognitive/behaviour state was rated on an exploratory daily observational 4 points scale that considered PD symptoms, cognition and mood: “individual classical parkinsonian state”, “slightly more aggressive”, “slightly more quiet”, “definitely more interactive and alert than in individual's parkinsonian state”. The doses were estimated by measuring the remaining volume in the pump.

Central depletion of dopamine in Parkinson's disease



→ Need: central, continuous circadian compensation by controlled dose of dopamine

Continuous intracerebroventricular administration of L- dopamine

