

Supplemental e-Methods

This study part was performed in Milan, Italy, after having completed the analysis of the efficacy and safety data collected in Santa Cruz, Bolivia. All the inclusion and exclusion criteria were the same as in the main study protocol. We additionally included only subjects with fully preserved global cognitive functions and frontal lobe-related executive functions (MMSE > 26/30, FAB > 15/18). The study protocol was carried out in compliance with the Declaration of Helsinki and its further amendments. Written informed consent was obtained from all patients.

Patients were assessed in the morning on three consecutive days, after overnight withdrawal of levodopa (at least 12 hours) and fasting. Measured pharmacokinetic variables included time to peak (t_{max}), peak plasma levodopa concentration (C_{max}) and the area under the plasma concentration–time curve (AUC; Contin *et al.*, 2015). We measured plasma Levodopa concentrations at fixed time-points over a 3-hour period (baseline, 15', 30', 45', 60', 90, 120', 150', 180').

Considering the safety issues associated with the intake of the suprathreshold high dose of levodopa/benserazide (3.5mg/kg) (see **Table e-1**) and the better-than-expected results on efficacy measures of MP-based preparations, we decided to investigate the pharmacokinetics of MP after applying an overall reduction in levodopa dose.

In the main study, we found that the intake of levodopa alone (LD-DDCI) was associated with a considerably prolonged motor response (272 min), which was longer than with MP-Hd (p=0.042). In turn, MP+DDCI showed a 38 min shorter response (139 min) than LD+DDCI at the same dose (p=0.046; see **Table e-2**). When we calculated the ratio of the ON duration between MP+DDCI and LD+DDCI (0.79±0.32) and between MP-Hd and LD-DDCI (0.83±0.17), we found an approximately 20% shorter ON period (p<0.05 for both) with MP powder than pharmaceutical levodopa preparations with and without a DDCI, respectively. Therefore, we additionally explored a 1-to-0.8 ratio between MP and levodopa without DDCI adjusting for this putative discrepancy. In particular, (i) the dose of LD+DDCI (used as reference) was reduced from 3.5 mg/kg to patients' usual morning levodopa dose increased by 30%; (ii) the dose of levodopa alone without DDCI (LD-

DDCI) was reduced from 5-fold to 4-fold the dose of LD+DDCI in 4/4 patients; (iii) the ratio between MP and LD-DDCI was 1:1 in 2/4 patients (Supplementary Table 1, patients #1 and #4) and 0.8:1 in the other 2/4 patients (Supplementary Table 1, patients #2 and #3).

Data on plasma Levodopa curves after LD+DDCI and MP of one patient (patient#1 in the Supplementary Table e-1 and Supplementary Figure e-2) had been published in a previous paper (Cassani *et al.*, 2016; corresponding to patient#4 in Table 2).

The UPDRS motor score (part III) and the AIMS were used to assess motor response and dyskinesias, respectively. Additional response variables included the following: time to ON, time to dyskinesias (time from intake to the first onset of dyskinesias), time to OFF (time from intake to the OFF state), duration of ON time (time from the ON time to the OFF state).

Supplemental e-Results

Pharmacokinetics and pharmacodynamics

Pharmacokinetics and pharmacodynamics of MP in comparison to LD+DDCI and LD-DDCI in 4 Italian PD patients are provided in **Table e-4**. Plasma concentrations of levodopa are displayed in **Figure e-2**.

- *MP vs. LD+DDCI*. Consistently with the main study, MP was associated with a shorter latency to ON, longer ON duration and less dyskinesias than LD+DDCI. These clinical differences were associated with a shorter t_{max} after intake of MP than of LD+DDCI (median [25-75IQR], 15' [15-18.75] vs. 30' [15-45], respectively), along with an approximately 2-fold greater AUC (194.8 [118.5-269.5] vs. 98.4 [84.4-116.1]) and greater C_{max} (2.85 [2.5-3.5] vs. 1.17 [0.89-1.67]). We did not observe any relationship between pharmacokinetic measures (C_{max} and AUC) and either duration of the ON state or severity of dyskinesias.

- *MP vs. LD-DDCI*. Overall, UPDRS-III scores and dyskinesias were similar with the two treatments. In the two patients on 1:1 levodopa ratio, the AUCs with MP were smaller than with LD-DDCI (by 17% and 36%) and associated with a shorter ON duration by 30' and 65'. In the

other two patients whose LD-DDCI dose was reduced by 20% (because of the difference in the ON duration described above), we found a 165'-170' longer ON duration with MP than with LD-DDCI. The relative comparison of the duration of ON time between MP-Hd and LD-DDCI and between MP+DDCI and LD+DDCI showed that the duration of ON time was about 20% shorter with MP preparations (MP-Hd and MP+DDCI) than with similar doses of pharmaceutical preparations of levodopa (LD-DDCI and LD+DDCI, respectively). Considering these clinical data, one might hypothesize that MP powder is associated with lower levodopa bioavailability than LD-DDCI. However, when we adjusted levodopa doses according to this disparity (by reducing LD-DDCI to 80%), we found a large difference in clinical response, with a 165'-170' longer ON duration with MP than with LD-DDCI. These data suggest that the discrepancy in the ON duration between MP powder and standard levodopa preparations is unlikely to be merely due to major differences in levodopa bioavailability and that other mechanisms should be explored.

- *Safety.* No major adverse events occurred. One patient experienced somnolence after LD-DDCI. We found no differences in cardiovascular response among treatment arms (data not shown).

e-References

- Cassani E, Cilia R, Laguna J, Contin M, Cereda E, Isaias IU, et al. *Mucuna Pruriens* for Parkinson's Disease: Low-cost preparation method, Laboratory measures and Pharmacokinetics profile. *J Neurol Sci* 2016; 365:175-80.
- Contin M., Lopane G., Passini A., Poli F., Iannello C., Guarino M. *Mucuna pruriens* in Parkinson Disease: A Kinetic-Dynamic Comparison With Levodopa Standard Formulations. *Clin Neuropharmacol* 2015; 38:201-3.