It is standard practice to co-administer levodopa with the dopa-decarboxylase (DDC) inhibitor carbidopa to inhibit its peripheral metabolism and maximize bioavailability. Preclinical studies have shown that continuous subcutaneous delivery of solubilized carbidopa, not only improves the bioavailability of carbidopa, but also broadens the concentration range of levodopa available for therapeutic effect. The aim of these two open-label studies was to establish the concentration of subcutaneously administered carbidopa that provides optimal bioavailability of a constant fixed concentration of a novel proprietary liquid formulation of levodopa (ND0612) when given continuously via subcutaneous infusion.

**Pharmacokinetic analyses**

Blood samples were taken for pharmacokinetic analysis from pre-dose until 52 hours post-dose in healthy volunteers and until 8 hours in PD patients.

Standard PK parameters (Cmax and AUC) were estimated for plasma levodopa and carbidopa plasma concentrations. In Study 1, the relative levodopa bioavailabilities (assessed by geometric mean ratios [GMRs] of C0-8 and AUC0-8 of ND0612 and oral levodopa) and at each carbidopa concentration treatment were compared using mixed modeling techniques. Study 1 also included exploratory trend analyses to investigate if there was an identifiable trend between the increasing dose of carbidopa for levodopa exposure levels for both ND0612 and ND0612H.

In Study 2, the Fluctuation Index of levodopa, was assessed using a mixed model repeated measures analysis. Using data from ND0612L, the levodopa fluctuation index was calculated and compared versus the oral levodopa/carbidopa regimen, for each carbidopa dose level.

**Results**

- **Study 1: ND0612-004**
  - This study characterized the impact of two doses of carbidopa (7.5 mg/mL and 14 mg/mL) on levodopa bioavailability.
  - The study was conducted in clinic, and included adult patients (32 males and 4 females; mean ±SD age: 44 ± 7 years) experiencing well-defined morning OFF periods (≥80% of days) and a good levodopa response.
  - Patients were randomized to either ND0612L (total levodopa dose of 15 mg/day) or ND0612H (total levodopa dose of 30 mg/day).
  - The concentration of levodopa was 60 mg/mL in all treatment regimens throughout the study (figure 2).

- **Study 2: ND0612-005a**
  - This study characterized the impact of three descending doses of carbidopa (7.5, 6 and 4 mg/mL) on levodopa bioavailability.
  - The study was conducted in clinic, and included 21 male or female healthy volunteers (60-65 yrs).
  - Subjects were allocated to 3 consecutive infusions of either ND0612H for 60 hours to a total levodopa dose of 720 mg) or ND0612L (over 24 hours to a total levodopa dose of 270 mg) in a randomized double-blind manner (figure 1).
  - Each consecutive infusion was given with decreasing concentrations of carbidopa and there was a washout of 12 h between infusions.

- **Study 1: ND0612 Pharmacokinetics in patients with PD**
  - Fluctuations in levodopa plasma levels were significantly reduced (14% difference for the ND0612L regimen versus oral levodopa/carbidopa).
  - Both ND0612 regimens maintained steady therapeutic levodopa plasma concentrations. Levodopa plasma concentrations were dose proportional with ND0612H achieving around 3-fold higher plasma levels than ND0612L in accordance with the approximately 3-fold increase in daily levodopa dose infused in the ND0612H versus ND0612L treatment groups.
  - No relevant differences in levodopa bioavailability were observed with the two carbidopa concentrations (figure 1).
  - Levodopa exposure levels increased proportionally with carbidopa dose level (both ND0612 regimens).

- **Study 2: ND0612 Pharmacokinetics in healthy volunteers**
  - Comparisons of GMRs indicated, that over the whole infusion period, observed levodopa exposure levels were similar (≤10% difference for the ND0612L regimen versus oral levodopa/carbidopa) for the three carbidopa doses.
  - However, exploratory analysis indicated a positive trend between carbidopa dose levels in the ND0612L regimen with respect to levodopa exposure, with higher AUCs observed at a carbidopa dose of 7.5 mg/mL. This trend was not apparent for the entire range of carbidopa doses when given with the ND0612H regimen (figure 2).
  - As expected, carbidopa exposure levels increased proportionally with carbidopa dose level (both ND0612 regimens). The 7.5 mg/mL dose of carbidopa resulted in an AUC over 2000 pg/mL, which is considered to provide maximal inhibition of DDC.

**Conclusions:**

- Taken together, these two studies confirmed that the concentration of subcutaneously administered levodopa/carbidopa that provides optimal levodopa bioavailability in ND0612 is 7.5 mg/mL.
- Increasing the carbidopa concentration above this level did not improve the levodopa bioavailability. However, lower concentrations could potentially compromise levodopa bioavailability for the lower carbidopa dose level (ND0612L).
- This dosing ratio equates to a carbidopa: levodopa dose ratio of 1:4. The lower dose of carbidopa required versus current oral carbidopa/levodopa formulations reflects the significantly higher bioavailability of subcutaneously administered carbidopa.
- Based on these results the final formulation of ND0612 was defined as carbidopa 7.5 mg/mL/levodopa 60 mg/mL. With this formulation, steady levodopa levels were consistently maintained in PD patients within the desired levodopa range.