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Levodopa Pharmacokinetics in Brain after Both Oral and Intravenous Levodopa in One Patient with Advanced Parkinson's Disease

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Abstract

Objective: One patient received oral levodopa during a study aiming for better understanding of the basal ganglia and of the mechanisms of deep brain stimulation of the subthalamic nucleus (STN DBS) with and without intravenous (IV) levodopa infusion in patients with Parkinson's disease (PD). The results from oral and IV levodopa treatment are presented. **Methods:** Five patients with advanced PD were included in the original study. During planned STN DBS surgery microdialysis probes were implanted in the right putamen and in the right and left globus pallidus interna (Gpi). During the study, microdialysis was performed continuously and STN DBS, with and without IV levodopa infusion, was performed according to a specific protocol. After DBS surgery, but before STN DBS was started, one patient received oral levodopa/benserazide and entacapone tablets out of protocol due to distressing parkinsonism. **Results:** The levodopa levels increased prompt in the central nervous system after the first PD medication intakes but declined after the last. Immediately the levodopa seemed to be metabolized to dopamine (DA) since the levels of DA correlated well with levodopa concentrations. Left STN DBS seemed to increase DA levels in left Gpi and right STN DBS seemed to increase DA levels in right Gpi while all STN stimulation seemed to increase the DA levels in right putamen. There was no obvious effect on levodopa levels. **Conclusions:** The results indicate that PD patients still have capacity to metabolize levodopa to DA despite advanced disease with on-off symptoms and probably pronounced nigral degeneration. STN DBS seems to increase DA levels with a more pronounced effect on ipsilateral structures in striatum.

Keywords

Parkinson's Disease, Levodopa, Dopamine, Brain, Microdialysis, Deep Brain Stimulation

1. Introduction

Parkinson's disease (PD) is a neurological condition with loss of dopamine (DA) producing neurons in the substantia nigra (SN) of the basal ganglia (BG). Levodopa, the precursor of DA, has long been gold standard in the treatment of PD and gives good symptom relief the first years of the disease. Levodopa, unlike DA, crosses the blood-brain-barrier (BBB) and is given together with a dopa decarboxylase inhibitor (DDI), to avoid high peripheral levodopa metabolism and elevated DA concentrations causing side effects such as nausea, orthostatic hypotension and vomiting. Adding a DDI results in higher levels of levodopa available to cross the BBB and further addition of a catechol-O-methyltransferase (COMT) inhibitor results in even higher levels of levodopa [1]-[11].

In most patients, oral treatment with levodopa eventually induces dyskinesia (LID) [12] [13] [14] [15]. The mechanisms of LID are not completely understood but in rat models it has been shown that intermittent levodopa treatment combined with degeneration of tyrosin hydroxylase containing neurons induces dyskinesia while continuously given levodopa (continuous dopaminergic stimulation = CDS) reduces LID [12] [16] [17] [18]. Deep brain stimulation (DBS) has also been shown effective in reducing motor symptoms and also in decreasing the daily doses of dopaminergic drugs in PD patients [19]-[25] with maintained effect several years after the surgical intervention [25] [26]. However, the mechanisms of DBS are still unclear [27]. In a previous study our group investigated the effect of DBS of the subthalamic nucleus (STN) on different neurotransmitters in the BG in PD patients to learn more about the mechanisms of DBS [28] using perioperative stereotactic microdialysis, which is a method for measuring concentrations of neurotransmitters in the human brain [29]-[35].

During the study one patient received oral levodopa on several occasions before the start of STN DBS due to distressing parkinsonism. We decided to report our results from this oral intake of levodopa since similar data are sparse in literature from human BG *in vivo*. From this patient, we present all data from the levodopa given orally or as intravenous (IV) infusion and compare the levodopa levels with the DA levels in BG. Since STN DBS was performed we also report the effects on levodopa and DA during these periods.

2. Materials and Methods

2.1. Study Design

STN DBS surgery is an established treatment available for patients with advanced PD. Patients with disabling motor function in spite of optimal oral medication are considered for this treatment. Before surgery all patients are tested for cognitive impairment, speech disturbances, and balance disabilities. STN DBS surgery is only performed on patients that fulfill the clinical criteria for surgery during this testing. From this group patients were asked to participate in the study and five consecutive patients were included in the original study. The PD diagnosis was made according to UK Parkinson's disease society brain bank

criteria. The study was performed with approval by the Regional Ethical Review Board in Linköping (No. 51-04) and written informed consent was obtained from all participating patients. The study design was previously described [28] and in short as follows:

Day 1: The patients were admitted to the ward and their antiparkinsonian medication was discontinued the night before DBS surgery.

Day 2: The DBS electrodes were implanted bilaterally in STN. During the surgery three microdialysis probes were implanted, one each in the right putamen and bilaterally into the globus pallidus interna (Gpi). The probes were connected to the pumps immediately after implantation and flow rate was set at 0.5 μL/min. Microdialysate sampling started at 6 pm, app. 3 hours after DBS surgery and continued during the entire study period, until Day 5 at 8 am. Probe and tubing delayed the fraction samples with 6 min. Fractions were collected every hour during daytime and every two hours during night (9 pm - 8 am).

Day 3 - 4: STN DBS was performed according to a specific protocol.

Day 4: A microdialysis probe was placed in a brachial vein and samples were collected every hour. IV levodopa infusion (75 mg/h) was given in the contralateral arm during 3 h. After one hour baseline recording, levodopa infusion combined with bilateral STN DBS was performed for another 6 h. The IV microdialysis was then discontinued.

Day 5: The last microdialysis samples from brain were taken at 8 am and all the probes were then removed.

Due to distressing parkinsonism one of the patients received oral levodopa/benserazide and entacapone on 3 occasions before DBS surgery and on 5 occasions after surgery but before the start of STN DBS, Figure 1. The oral medication was not discovered until after the dialysates were analyzed and the patient then approved to publish the data. On day 4 the patient was given levodopa infu-

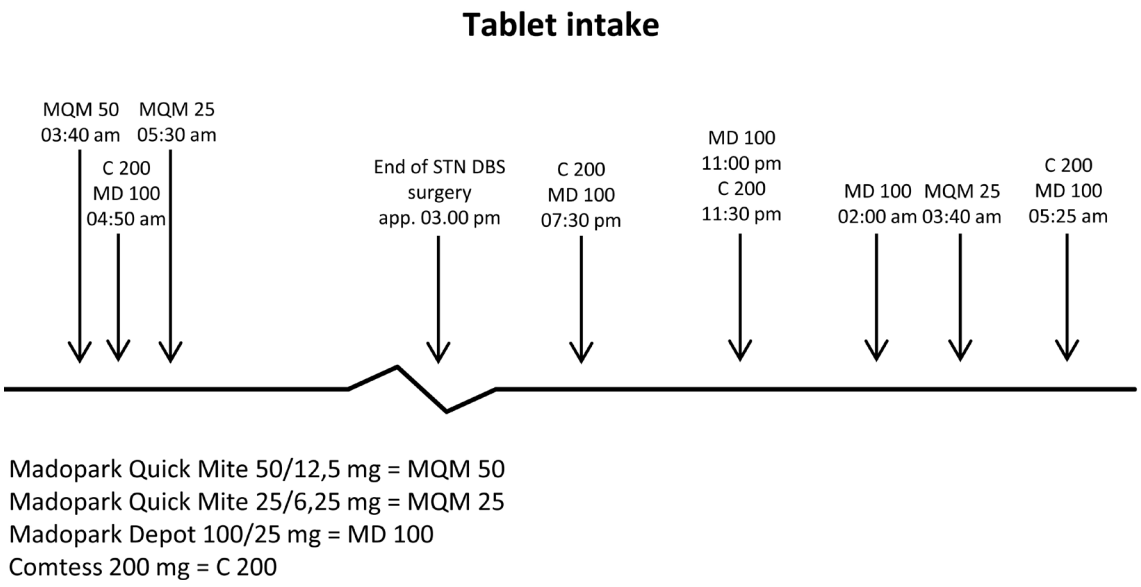

Figure 1. Time table for levodopa intake and STN DBS surgery.

sion for 1 + 7 h (instead of 3 + 6 h) due to technical failure in the beginning of the infusion period and prolonging of the second infusion period. The patient was a 57-years-old man with PD since 8 years and with symptoms starting in the left side of the body. The patient had oral levodopa treatment for 7 years and the daily levodopa dose was 850 mg in addition to rasagiline, entacapone and pramipexole. The patient suffered from disabling dyskinesia and bradykinesia and was considered a suitable candidate for STN DBS after completing the DBS pre-testing.

Levodopa from recovery and IV samples was analyzed with a high-performance liquid chromatography (HPLC) system previously described [28]. The HPLC analyses of the dialysates from central nervous system (CNS) were purchased from Pronexus Analytical AB, Stockholm, Sweden.

2.2. Materials and Chemicals

The microdialysis set was from CMA Microdialysis AB (Stockholm, Sweden) and consisted of CMA/107 Microinjection Pumps with 2-mL syringes connected to both the brain (CMA 65) and the IV probes, respectively. Both microdialysis probes were manufactured by CMA Microdialysis AB. The length of the brain probe shaft was 190 mm and the length of the dialysis membrane was 10 mm, with a golden tip for radiologic confirmation of position. The membrane cut-off of the brain probe was 20 kDa because of better recovery for cathecholamines (64%) compared to 100 kDa (13%) [36]. Chemicals used were Carbocain® adrenalin, 5 mg/mL + 5 µg/mL, Astra Zeneca (Sweden), Madopark® (100 mg levodopa + 25 mg benserazide), Roche AB, Sweden, Madopark® Quick mite (50 mg levodopa + 12.5 mg benserazide), Roche AB, Sweden and Comtess® (200 mg entacapone), Orion Pharma Espoo, Finland.and Levodopa IV infusion 5 mg/mL (Levodopa, Fresnius Kabi AB, Uppsala, Sweden).

2.3. Statistics

Mean value, maximal concentration (C_{max}) and minimum concentration (C_{min}) are used. The changes in levodopa and DA concentrations are presented in percent because of large differences in concentrations in right putamen, right Gpi and left Gpi and these values are standardized towards baseline value in fraction 1. Because of data from only one patient it is not possible to calculate significance levels between the concentrations.

3. Results

The first two baseline fractions contained low levels of levodopa indicating that all intake of PD medication before DBS surgery had been fully metabolized, Figure 2. After DBS surgery oral medication was given on 5 occasions, during fractions 2, 4, 6 and 7. After the first medication at 7.30 pm (levodopa/benserazide 100/25 mg and entacapone 200 mg, fraction 2; sampling time 7 - 8 pm) an increase of levodopa was seen in fraction 3 (sampling time 8 - 10 pm), Figure 2 and Figure 3. The mean value of the levodopa concentration in the three brain structures increased from 37 to 320 nmol/L (an increase of 761%). The levels

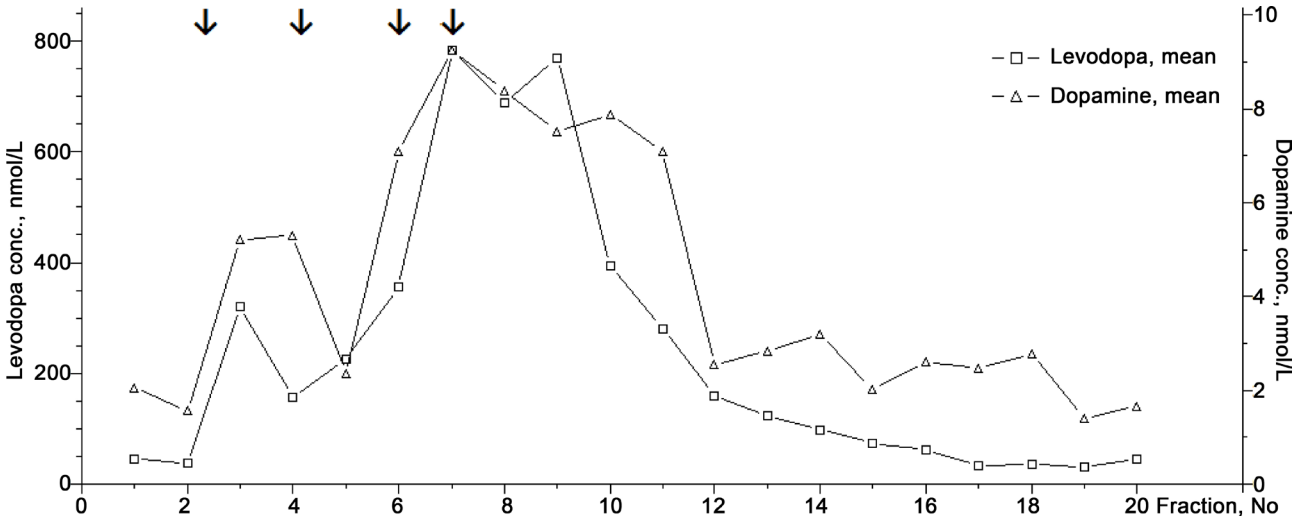

Figure 2. The mean concentrations of levodopa and DA in the BG during PD medication intake. Arrow = fraction with PD medication intake.

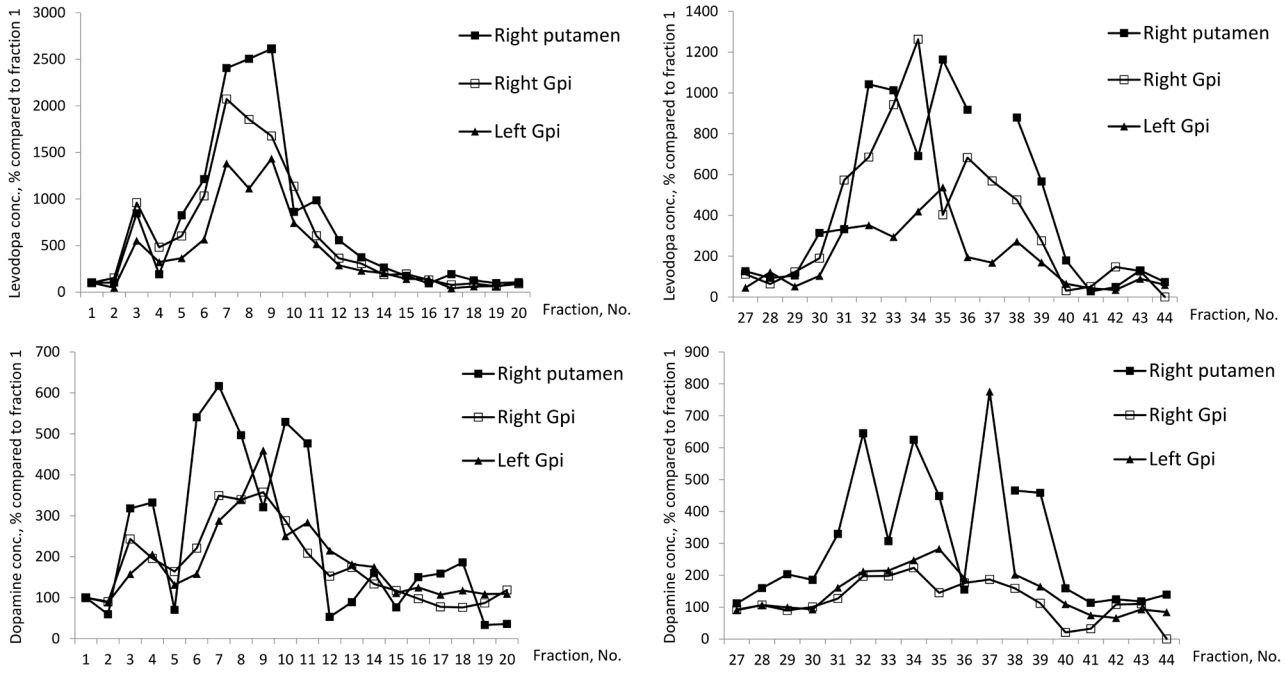

Figure 3. The changes in levodopa and dopamine concentrations in % during oral levodopa treatment (left) and levodopa infusion (right). The values are standardized towards the baseline value in fraction 1.

decreased again in fraction 4 (sampling time 10 - 12 pm). In this fraction oral medication was given again at 11 and 11.30 pm (levodopa/benserazide 100/25 mg and entacapone 100 mg respectively). In fraction 6 levodopa/benserazide 100/25 mg was given again at 2 am and 25/6.5 mg was given at 3.40 am. The levodopa concentration reached mean C_{max} 783 nmol/L in fraction 7 (sampling time 4 - 6 am), an increase of 397% from fraction 4. The last oral medication (levodopa/benserazide 100/25 mg and entacapone 100 mg) was given at 5.25 am (fraction 7). The mean values of levodopa concentration were mainly unchanged during fraction 7 - 9 (mean 747 nmol/L). After the last oral medication C_{max} for

levodopa differed between the three structures. The C_{max} was reached earlier in right Gpi (fraction 7; 677 nmol/L) than in both putamen and left Gpi (fraction 9; 533 nmol/L and 1227 nmol/L respectively). The differences between C_{max} and C_{min} of levodopa during the period with oral levodopa (fraction 2 - 7) were 2260%, 1258% and 2804% in right putamen, right Gpi and left Gpi respectively. Left STN DBS was performed during fraction 9 - 11 and the levodopa levels decreased during these fractions (from 769 to 280 nmol/L). Levodopa also decreased during the right STN DBS but remained unchanged during bilateral stimulation.

After DBS surgery a total amount of levodopa/benserazide 425/106.25 mg and entacapone 600 mg was given orally. The levels of levodopa in brain tissue returned to baseline levels again 10 - 12 hours after the last oral medication intake and the elimination followed the earlier described first order kinetics.

The DA curve had a different outline compared to the levodopa curve, **Figure 2** and **Figure 3**. DA increased with a small delay compared to levodopa and reached mean value 5.30 nmol/L in fraction 4 (an increase of 239% from fraction 2). In fraction 5, when levodopa started to increase again, DA decreased to lower levels to mean 2.35 nmol/L (a decrease of 56%). DA then started to increase again in fraction 6 and reached mean C_{max} 9.25 nmol/L in fraction 7, an increase of 294% from fraction 5. The highest levels of DA were seen in the right putamen with C_{max} 16.7 nmol/L in fraction 7 compared to C_{max} 6.8 and 7.1 nmol/L in fraction 9 in right and left Gpi respectively. The differences between C_{max} and C_{min} of DA during fraction 2 - 7 were 940%, 287% and 226% in right putamen, right Gpi and left Gpi respectively.

During left STN DBS (fraction 9 - 11) the DA level increased in left Gpi and in right putamen while it decreased in right Gpi. Right STN DBS was performed during fractions 13 - 15. DA increased in right putamen and in right Gpi from fraction 12 to 14 while there was a decrease in left Gpi during the stimulation period. Bilateral STN DBS was performed during fractions 17 - 19 and this resulted in increased DA in putamen and decreased or unchanged DA levels in right and left Gpi.

In the study protocol levodopa was given IV with and without concomitant bilateral DBS stimulation. A total amount of 600 mg levodopa was given during 1 + 7 hours (75 mg/h during fraction 30 + 32 - 38). For comparison we also report the findings from this treatment. During infusion treatment we observed that the levodopa levels in brain followed the IV levels well, but with a slight delay in right putamen and right Gpi during the first infusion period. The levels of levodopa in brain reached mean C_{max} 304 nmol/L in fraction 34 when 300 mg levodopa had been given. C_{max} for levodopa was 237 nmol/L in fraction 35 in right putamen, 412 nmol/L in fraction 34 and 459 nmol/L in fraction 35 in right and left Gpi respectively. The DA levels reached mean C_{max} 8.3 nmol/L in fraction 34 with C_{max} 17.4 nmol/L in fraction 32 in right putamen, 4.2 nmol/L in fraction 34 and 12 nmol/L in fraction 37 in right and left Gpi. The differences between C_{max} and C_{min} during the period with continuous levodopa IV (fraction 32 - 38) were 68%, 214% and 220% for levodopa and 317%, 54% and 307% for

DA in right putamen, right Gpi and left Gpi respectively.

4. Discussion

In this paper, we report how oral intake of PD medication influences the concentrations of levodopa and DA in human brain. We could show that the levodopa levels increased prompt in CNS after the medication and the levodopa seemed to be quickly metabolized to DA since the levels of DA correlated well with the levodopa concentrations, but with a slight delay.

The patient received oral levodopa on five occasions during a 10-h period. After the first medication, where both levodopa/benserazide and entacapone were given, the levodopa concentration reached higher levels in the following fraction and then decreased to a low level in the next fraction. However, the levodopa concentration did not return to the same low levels as before the first medication and it seemed as if levodopa was accumulated extracellularly in brain. This might indicate a higher half-life (T1/2) of levodopa in brain compared to peripheral blood. Previous studies have shown that the metabolism of levodopa in blood is decreased when adding a DDI [1]-[7] and COMT-inhibitors have also been shown to increase levodopa levels and T1/2 of levodopa in blood [1] [8] [9] [10] [11] [37]. Adding DDI and/or COMT-inhibitor to levodopa also result in higher levels of levodopa in brain [1] [38]. Levodopa was given together with the DDI benserazide at all five occasions with medication and the COMT-inhibitor entacapone was given during the first, second and last medications. After the second intake of PD medication the levodopa did not decrease as after the first also indicating an extracellular accumulation of levodopa in brain after repeated levodopa doses. The possible extracellular accumulation of levodopa could indicate difficulties for the DA producing neurons to metabolize all levodopa available due to a state of saturation. After the last levodopa intake in fraction 7, the levodopa levels did not increase further indicating increased levodopa uptake.

One question is if other neurons than the dopaminergic also may be involved in the metabolism of levodopa to DA. For example, the serotonergic neurons have been shown to convert exogenous levodopa to DA and release it as a "false transmittor" giving symptom relief in PD patients [39] [40] [41] [42] [43]. Even though the serotoninergic innervation in the striatum also is affected in PD, it is not degenerated to the same extent as for the dopaminergic neurons [44] and the serotonin neurons may therefore play a role in the converting process of exogenous levodopa to DA. Our results with an initial increase of the levodopa levels in brain and with a declined increase after repeated levodopa doses could be explained by a levodopa storing capacity in serotonergic neurons in our patient. One theory is that the auto-regulating function of the DA release is lacking in serotonergic neurons resulting in an un-controlled DA release after levodopa administration and thus causing LID [45] [46] [47]. It seems to be of importance to avoid high levodopa peaks in brain in PD patients and CDS has been shown effective in reducing LID. The serotonergic hyperinnervation and the dysregu-

lated DA release in different areas of the brain could be possible factors in the origination of several non-motor symptoms (NMS), for example impaired cognition, depression and anxiety [48]-[56].

Levodopa given IV allowed us to study the pharmacokinetics of levodopa without the high first-passage metabolism in the gastrointestinal system seen during oral treatment with levodopa. The bioavailability for levodopa IV is 100% compared to app. 30% for oral levodopa [6] [57]. Avoiding high levodopa peaks in CNS has been considered of importance in delaying the development of dyskinesia in PD patients. The differences between C_{max} and C_{min} of levodopa during the levodopa infusion seemed more discrete than during oral medication indicating less pronounced levodopa peaks in brain during levodopa infusion. During treatment with IV levodopa the concentrations of levodopa in CNS did not reach the same high levels as during the oral medication intake (mean C_{max} 304 nmol/L in fraction 34 when 300 mg levodopa had been given IV during 4 h compared to mean C_{max} 783 nmol/L in fraction 7 when 425 mg levodopa had been given orally during 10 h). The explanation could be the absence of enzyme inhibitors during IV treatment resulting in a higher metabolism of levodopa in blood and also to a higher amount of levodopa metabolites that could compete with levodopa across the BBB. However, despite the lower levodopa levels in the BG during the period of levodopa given IV, the levels of DA seemed to reach comparable levels (mean C_{max} 8.3 nmol/L in fraction 34 during IV levodopa compared to 9.3 nmol/L in fraction 7 during oral levodopa). This could be explained by storage of levodopa in dopaminergic neurons during the period of oral levodopa intake. However, the levodopa possibly stored in these neurons should have been used during the period of time between fraction 7 and 30 when the patient did not obtain any levodopa treatment. It is also possible that the optimal DA level for this patient was around 8 - 9 nmol/L and that the remaining DA producing neurons were able to auto-regulate the DA levels. However, this opposes the theory presented above about serotonergic neurons taking over the converting process of exogenous levodopa since the serotonergic neurons lack the auto-regulating function of DA release. It is possible that this PD patient, despite having the disease for many years, had enough dopaminergic neurons to both accumulate levodopa and to regulate the DA released. Another possibility is that astrocytes or other neurons than dopaminergic or serotonergic are involved in the converting process of levodopa to DA in PD patients. It has also been suggested that many of the large neutral amino acids that are actively transported over the BBB from blood to brain also can be actively transported in the opposite direction by a Na^+-dependent transport system. Levodopa is one of these substances and if actively transported out from the brain the availability of levodopa would decrease [58] explaining the observed decreased levels in brain despite levodopa treatment. However, this should not have led to lower levels of levodopa during IV levodopa treatment compared to oral levodopa treatment and it could not explain the DA levels. Another explanation for the lower levodopa levels in the BG during the IV treatment could be technical failure of the

microdialysis probes. However, it seems unlikely that all three microdialysis probes in the BG started to fail at the same time.

In our previous paper, we could see that STN DBS increased DA levels in putamen indicating a direct action of STN DBS on substantia nigra pars compacta (SNc) resulting in increased release of putaminal DA [28]. In the actual report we could confirm these observations and we could also see an increase of DA in the left-sided Gpi structures during left-sided stimulation and an increase in the right-sided Gpi structures during right-sided stimulation also indicating a direct action on SNc. There was no obvious pattern for levodopa during these stimulation periods.

One shortcoming in this report is that we only have data from one patient. The results are therefore presented in a descriptive way and it is difficult to apply the results generally. To take in consideration is that failure of individual microdialysis samples has a greater impact of the results when few patients are included. However, this period of oral levodopa medication gave us the opportunity to compare it with levodopa given IV and to study the levodopa and DA levels in brain from the same patient. Another shortcoming is that we did not analyze levodopa and DA from the left putamen which would have been preferable. Bilateral microdialysis in putamen was considered to be precarious in case of complications. In this paper we could study the effects of oral levodopa on levodopa and DA concentrations in brain during oral medication that was given according to the needs of the patient. A more strategic and planned dosing of levodopa at different doses combined with and without specific enzyme inhibitors would probably have resulted in better data. However, to perform a study according to that design is difficult from ethical point of view.

In this paper, we could conclude that levodopa, both given orally and IV in PD patient, resulted in increased levels of levodopa and DA in the BG. During oral medication levodopa and DA followed each other well showing that a PD patient with severe disease and probably pronounced nigral degeneration, still can metabolize levodopa to DA. This is an evidence of that treatment with levodopa still is beneficial, even in advanced PD. This could also be one explanation to why levodopa still is the golden standard treatment in PD. The conversion to DA gives good symptom relief and few side effects in contrast to DA agonists and enzyme inhibitors that often cause psychiatric side effects in late stages of the disease. We could also see that STN DBS seems to increase DA levels with a more pronounced effect on ipsilateral structures in striatum.

To investigate the metabolism of levodopa to DA *in vivo* in human brain is difficult both due to ethical considerations and because of the delicate and complex structures involved. However, more studies are of importance to get better knowledge about the mechanisms of the disease and the mechanisms of the treatments, both medication and DBS.

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Conflicts of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Abbrevations

BBB	Blood-brain-barrier
BG	Basal ganglia
CDS	Continuous dopaminergic stimulation
C_{max}	Maximal concentration
C_{min}	Minimum concentration
CNS	Central nervous system
COMT	Catechol-O-methyltransferase
DA	Dopamine
DBS	Deep brain stimulation
DDI	Dopa decarboxylase inhibitor
Gpi	Globus pallidus interna
HPLC	High-performance liquid chromatography
IV	Intravenous
LID	Levodopa-induced dyskinesia
NMS	Non-motor symptoms
PD	Parkinson's disease
SN	Substantia nigra
SNc	Substantia nigra pars compacta
STN	Subthalamic nucleus
T1/2	Half-life