Discussion and future perspectives
Correlation between clinical parameters and striatal dopaminergic tracer uptake

Animal studies

The studies described in this thesis investigate the relation between clinical parameters and striatal uptake measures of dopaminergic tracers in patients with PD and parkinsonian non-human primates. Also the relation between these parameters and pathological findings in monkeys is described.

In monkeys we found a significant negative correlation between striatal F-DOPA uptake and parkinsonian symptom scores and a positive correlation between striatal F-DOPA uptake and the number of limb movements. This is in concordance with other studies. Uptake of β-CIT and fluoro-methyltyrosine (FMT) both correlate well with motor symptoms in MPTP-lesioned monkeys\(^{11-13, 26}\) and F-DOPA uptake is altered in MPTP-treated monkeys\(^{8-10}\).

In our study striatal F-DOPA uptake and motor symptoms correlated with immunohistochemical findings. In monkeys with high parkinsonian scores and decreased striatal F-DOPA uptake, more gliosis in substantia nigra and striatum was observed compared to monkeys in which striatal F-DOPA uptake remained intact and only moderate parkinsonian scores were present. On the other hand, monkeys with normal F-DOPA uptake and moderate parkinsonian scores displayed more TH-positive neurons and fibres in substantia nigra and striatum than monkeys with a decrease in striatal F-DOPA uptake and high parkinsonian scores. These data are consistent with the results of Pate et al\(^{29}\) who show that a reduction of F-DOPA uptake is correlated with a reduced amount of dopamine nerve terminals in the striatum. Oiwa et al\(^{26}\) showed that in MPTP-treated monkeys different stages of parkinsonism correlated with different amounts of DA concentrations in the caudate nucleus and putamen of the contralateral hemisphere. FMT-PET uptake correlated well with these biochemical data and proved to be a good predictor of DA levels in the contralateral striatal regions. Despite those positive correlations between striatal F-DOPA uptake, motor behaviour and immunohistochemical findings, the exact quantitative relation between those parameters has not been explored in a systematic manner yet.

Human studies

According to the studies described in this thesis, a significant correlation was found between clinical examination in the ‘off’-state (UPDRS-III and H&Y) and striatal uptake of F-DOPA and FP-CIT. This is in agreement with other authors. Many studies have described a significant correlation between striatal uptake by means of F-DOPA PET or FP-CIT SPECT and clinical parameters. Correlation coefficients between striatal uptake and UPDRS-III scores ranged from 0.51 to 0.81\(^{2, 4, 17, 31, 35, 39, 42}\), while the correlation coefficients in our studies are lower (0.38 and 0.45 for striatal uptake of respectively F-DOPA and FP-CIT). This discrepancy
may be explained by methodological differences in data processing. Although patients were examined in the clinical ‘off’-state, long-lasting effects of antiparkinsonian therapy on motor signs cannot be excluded. Pirker et al showed that striatal uptake of \[^{123}\text{I}]\beta\text{-CIT SPECT}\) was significantly correlated with UPDRS-II and UPDRS-III, disease duration and Hoehn & Yahr stage. UPDRS motor sub scores for bradykinesia showed a good correlation, while rigidity showed a moderate, but significant correlation with striatal uptake. No significant correlation could be found between tremor scores and striatal uptake. Bradykinesia appears to be directly related to the degree of nigral cell loss, striatal dopamine deficiency and to the decrease of homovanillic acid in cerebrospinal fluid. However, no such correlation could be found for parkinsonian tremor.

In patients with early PD striatal uptake values of FP-CIT are reduced to 70.9% (caudate), 46.8% (anterior putamen) and 24.0% (posterior putamen) of normal values. This is in agreement with Morrish et al who found that symptom onset in Parkinson’s disease was estimated at a mean putaminal F-DOPA uptake of 75% of normal and a mean caudate F-DOPA uptake of 91% of normal. They concluded that the mean preclinical period is less than seven years. Other studies estimated a preclinical period of 6.5 years and 3.1 years. Estimation of the preclinical period may vary depending on the method of analysis and extrapolation. It is estimated that at least 30-50% of nigral cell loss has occurred at the onset of symptoms of PD. At that time patients have lost approximately 80% of striatal dopamine content. Striatal reduction of F-DOPA uptake correlates with subsequent post-mortem dopaminergic cell density in the substantia nigra of post-mortem-confirmed patients with PD.

Nigral cell loss is just one of the processes involved in the pathogenetic mechanism of PD. Many mechanisms have been proposed to be involved in the initiation of the neurodegenerative process, like oxidative stress, glutamate excitotoxicity, free radical damage, mitochondrial (complex I) dysfunction and inflammatory processes or proteasomal dysfunction. These processes will eventually result in nigrostriatal dysfunction and cell death. Braak et al have proposed an ascending pattern for the development of PD. The olfactory system and brainstem nuclei are involved in the early stages, i.e. stage 1 and 2. Substantia nigra is involved in stage 3, while involvement of anteromedial temporal mesocortex is characteristic to stage 4. At stage 5 and 6 the cortical areas become involved.

F-DOPA PET and FP-CIT SPECT measure only a small piece of this complex process. Each of these imaging techniques measures an aspect of dopaminergic function. However, they do not measure the number of nigral dopaminergic neurons and results derived from these studies do not directly assess the causes of the measured biochemical abnormalities.
Although in many cross-sectional studies in patients with PD reduction in striatal uptake correlated significantly with increasing severity of PD measured by UPDRS, this relationship remains unclear in longitudinal studies. In the CALM-PD and the REAL-PET study the possibility of dopamine agonists to modify disease progression in PD were tested. In both studies percentage loss of uptake from baseline correlated with change in UPDRS score from baseline. Both studies demonstrated a significantly lower loss in the rate of striatal tracer uptake in patients treated with dopamine agonists compared with L-DOPA. However, the REAL-PET study showed that after 2 years of treatment motor function was superior in the patients treated with L-dopa compared with ropinirole. In the CALM-PD study no significant differences in UPDRS scores between pramipexole treated patients and L-dopa treated patients could be detected after 22 months of treatment. The discrepancy between a slower loss of striatal uptake and a worse clinical condition in dopamine-agonist treated patients compared to L-dopa may be explained by several factors. First, in the REAL-PET study patients were scored in the clinical ‘on’-condition, because of the inherent problems associated with true ‘off’- treatment assessments. L-dopa has a greater symptomatic efficacy compared to ropinirole, which may explain the lower UPDRS-scores in the L-dopa treated group. Although patients in the CALM-PD group were clinically examined 12h after study drug and antiparkinsonian medications, long-lasting symptomatic effects confounding the UPDRS-scores cannot be ruled out. Second, neuroimaging tracer dynamics may be influenced by the tested drugs. In the CALM-PD study, patients were scanned without stopping study drugs in advance. Third, in early PD the temporal patterns for rate of loss of dopamine transporter and change in UPDRS-scores may not be congruent. It cannot be excluded that in early phases of PD striatal uptake decreases to a large degree, while the change in UPDRS is only minimal. This problem can be overcome by a long-term follow up. Due to the mentioned problems faced with the interpretation of imaging studies for evaluation of therapies, some assume that imaging studies should not be used as surrogate endpoints in clinical trials of PD.

Neuroprotective studies

According to our study described in this thesis, the compound TCH346 is able to prevent motor symptoms and nigrostriatal deficits induced by MPTP in primates. Therefore TCH346 seemed a promising agent for neuroprotective therapy in Parkinson’s disease. The animal study we performed was followed by a double-blind, randomised, controlled trial in humans with Parkinson’s disease. In that study, no significant differences between any of the TCH346 doses administered and placebo were observed regarding time to the development of disability requiring dopaminergic treatment, and regarding changes in...
clinical rating scores. This is not the first time that a promising preclinical neuroprotective agent fails to show any neuroprotective effect in the clinical human situation. In 2003, the National Institutes of Health (NIH) committee proposed 59 possible agents to be tested as potential neuroprotective compounds. However, until now no therapy has been proven to be able to stop or even slow the progression of Parkinson’s disease. This discrepancy between preclinical animal studies and clinical studies with parkinsonian patients may be explained by several factors, like inappropriate animal model or inappropriate study design. These factors have probably both played a role in the disagreement found between our preclinical and the clinical TCH346-studies. It is unlikely that the discrepancy in results can be explained by the use of a wrong tracer in our animal study: our animal study showed that administration of TCH346 to MPTP-treated primates prevents striatal dopaminergic defects as well as parkinsonian behaviour.

Inappropriate animal model
Many animal models use MPTP which induces acute damage, limited to dopaminergic neurons. This may not correctly reflect the underlying pathogenetic mechanisms of cell death in Parkinson’s disease. Neuroprotective effects of a compound observed in a MPTP-treated animal model may therefore not be relevant for the human situation. However the exact mechanism of cell death in Parkinson’s disease is not known yet, making it difficult to design a perfect animal model.

Inappropriate study design
Developing an appropriate study design to evaluate neuroprotective effects of a compound in Parkinson’s disease is difficult. While the evaluation of symptomatic treatment has obvious endpoints, like change in signs and clinical ratings scales, the measure of effects in neuroprotective treatment is less clear. An increase of symptoms may not be a direct reflection of the changes at a cellular or biochemical level. The Unified Parkinson Disease Rating Scale (UPDRS), which is often used in clinical trials as a measure for the severity of parkinsonian symptoms is relatively insensitive to small deteriorations in clinical state. This forms especially a problem for patients, enrolled in neuroprotective studies, as they are in the beginning of the disease when signs and symptoms may be mild and subtle. More specific and sensitive parameters need to be designed for accurate assessment of neuroprotective effects. Only patients with very short duration of signs and symptoms should be enrolled in neuroprotective studies. In the beginning of the disorder, progression is fastest and neuroprotection is most effective. However, it is not easy to enrol patients in the beginning of the disease, as signs and symptoms are subtle and easily misinterpreted both by patients and by physicians. This may lead to a delay in diagnosis. In this respect
the possibility of performing PET or SPECT scans may be helpful in diagnosing Parkinson’s
disease in an early phase of the disease.
Many neuroprotective trials include an observation time of 1 to 2 years. This may be a too
short period of time to evaluate a (small) long-term neuroprotective effect. Longer periods
of follow-up and observation time may be needed to detect neuroprotective outcome.
However, studies with a longer period of follow-up will be difficult to perform and the
clinical relevance of small neuroprotective effects is doubtful.

FP-CIT SPECT and F-DOPA PET

In this thesis we evaluated the usefulness of both F-DOPA PET and FP-CIT SPECT in clinical
practice and of F-DOPA PET in research settings (using animals).

The biological processes
Both tracers assess one or more aspects of the dopaminergic nerve terminal system.
F-DOPA PET reflects the uptake and conversion of fluorodopa to fluorodopamine. This
process contains several steps. After i.v. injection, F-DOPA crosses the blood-brain barrier
and is taken up into cells. It is converted by aromatic acid decarboxylase (AADC) to
fluorodopamine. PET scans with F-DOPA allow quantification of striatal dopa decarboxylase
activity and storage capacity of F-dopamine.
FP-CIT belongs to a group of compounds derived from cocaine, which has a high affinity
for the dopamine transporter (DAT). Removal of dopamine from the synaptic cleft is mainly
performed by reuptake through the DAT. DAT levels correlate with the density of DA nerve
terminals, but are also regulated by striatal DA concentration and is regulated at several
other levels.

Both imaging techniques do not calculate the number or density of nigral dopaminergic
neurons. Results from tracer uptake studies do not directly quantify the biologic processes
as in vitro studies. Therefore, the correspondence between tracer uptake studies and
biological parameters will always be indirect and will always need to be validated, to
obtain a measure of specific uptake.
The pattern of F-DOPA or FP-CIT uptake in patients with PD is practically identical. Uptake
in caudate remains normal in the early phases, while putaminal uptake is reduced, mainly
the dorsal part of the putamen. Although both putamina often show reduced uptake of
tracer, the putamen contralateral to the clinical signs and symptoms will be most affected.
Comparison FP-CIT and F-DOPA

From the studies described in this thesis it can be concluded that using either FP-CIT SPECT scans or F-DOPA PET scans patients with de novo – clinically typical- Parkinson’s disease can be separated completely from healthy volunteers. This is in agreement with Ishikawa et al. F-DOPA PET and FP-CIT SPECT significantly discriminated patients with PD and healthy volunteers with comparable accuracy\(^\text{17}\). Lee et al examined the striatal uptake of different dopaminergic tracers in patients with PD\(^\text{20}\). DAT labelling by means of methylphenidate (MP) was compared with F-DOPA uptake in patients with PD at an early stage of the disease. In mildly affected parkinsonian patients, striatal F-DOPA uptake was relatively higher than the uptake of MP in caudate and striatum. This pattern suggests up regulation of AADC activity and down regulation of DAT. Although no direct proof has been given of this assumption in human studies, it is in line with experimental animal studies, which suggests a compensation of the loss of dopaminergic neurones by increasing the relative synthesis and release of dopamine from the remaining dopaminergic neurons\(^\text{46, 47}\).

According to the studies in this thesis, no significant difference could be detected between FP-CIT SPECT and F-DOPA PET in the detection of presynaptic dopaminergic deficits. This may possibly be due to the small sample size. Until so far, there is no convincing evidence for superiority of one of those tracers in assessment of presynaptic dopaminergic deficits\(^\text{33}\).

F-DOPA PET and FP-CIT SPECT in clinical setting

We have compared both techniques in patients with clinically strong evidence of Parkinson’s disease, which are not difficult to diagnose correctly for neurologists. It is more tempting and clinically useful to compare both scanning methods in patients with only subtle parkinsonian signs and symptoms, which cause problems to be diagnosed correctly in clinical practice. It remains to be investigated, whether those patients with recent or subtle parkinsonism can also be discriminated from patients with suspicious symptoms but without Parkinson’s disease by means of these imaging techniques.

Which tracer should be used in clinical practice depends on several factors. FP-CIT SPECT scans are widely available, but have a lower spatial resolution. F-DOPA PET scans have a better spatial resolution, but restricted availability of PET instruments and the difficult production of F-DOPA limit its use.

Patient preparation necessary for F-DOPA PET and FP-CIT SPECT scans is different. For F-DOPA PET it is recommended that patients do not consume large amounts of protein before the start of the scan\(^\text{21}\). Patients are allowed to take their (antiparkinsonian) medication. Only COMT-inhibitors should be taken into account before performing a F-DOPA PET scan\(^\text{32}\). In contrast, drugs interfering with the dopamine receptor like cocaine, amphetamine-like drugs (including methylphenidate), benzatropine, bupropion, mazindol, phenteramine, phentany and sertraline, have to be stopped 5 half-times before undergoing a FP-CIT
SPECT scan\textsuperscript{38}. Also other selective serotonin reuptake inhibitors (SSRI’s), like citalopram, escitalopram and paroxetine should be stopped\textsuperscript{6, 30}. Antiparkinsonian medication is allowed to be continued for FP-CIT SPECT scans.

Acquisition time of F-DOPA PET differs from FP-CIT SPECT. Acquisition time for FP-CIT SPECT scans is about 45 minutes. For F-DOPA PET scans one static 3D acquisition of 6 minutes is performed. This short scanning time may be advantageous for patients with Parkinson’s disease. All the above mentioned factors may influence the final decision which scanning technique to prefer as sensitivity and specificity of FP-CIT SPECT and F-DOPA PET are almost equal.

**Future perspectives**

In the recent past, many centers acquired a PET camera. About 10 years ago, only 3 centers in the Netherlands possessed one or more PET cameras, while nowadays circa 30 – 40 PET cameras are available in the Netherlands. PET cameras are mostly used as a diagnostic tool in oncology using FDG as radiotracer. FDG can be transported easily and is commercially available. This makes it unnecessary for a hospital to produce FDG by itself and therefore to possess an on-site cyclotron. Until now, F-DOPA is not commercially available. However, if neurologists discover the possibility of performing PET scans in their own center, it may be expected that the demand for F-DOPA PET scans will increase and that F-DOPA will become commercially available.

SPECT is often used to study the dopamine transporter (DAT) system. As PET provides higher resolution than SPECT, new dopamine transporter radiotracers for PET, labelled with $^{11}$C or $^{18}$F, have been developed\textsuperscript{14, 15, 44, 45}. It may be expected that one or more of the $^{18}$F-labeled DAT radiotracers will become commercially available in the future.

The development of new PET cameras with higher spatial resolution results in higher quality of PET images and the ability to visualize small cerebral regions.

The combination of the development of commercially available presynaptic dopaminergic radiotracers for PET and the higher resolution of PET cameras will probably result in high-quality diagnostic tools for detecting dopaminergic striatal deficits in daily clinical practice as well as in experimental settings for the assessment of possible neuroprotective effects of new therapies.
Reference List

1. Dopamine transporter brain imaging to assess the effects of pramipexole vs levodopa on Parkinson disease progression. *JAMA* **287**:1653-1661, 2002
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