A Slow Progressive MPTP Marmoset Model for Idiopathic Parkinson's Disease

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ENGLISH SUMMARY

The work in this thesis is concerned with behavioural, molecular and neurochemical alterations elicited by the administration of low doses MPTP in the common marmoset in order to achieve progressive and chronic parkinsonian symptoms and mimic early staged Parkinson's disease (PD). The aim of the reported studies was to gain insight into the molecular, cellular and behavioural processes underlying the clinical expression of sporadic PD. In addition, inter- and intra-familiar differences in susceptibility to MPTP itself were investigated, as well as compensatory molecular and cellular mechanisms associated with behavioural recovery after discontinuation of MPTP injection. Taken together, the thesis describes observations at multiple levels that support the use of chronic MPTP exposure in marmosets as a clinically relevant model for PD in humans.

Chapter 1 describes the basic terminology and principles used in PD research. PD is a movement disorder characterized by tremor, cognitive impairment, gastro-intestinal deregulations and sleep disturbances. Neuropathological hallmarks involve degeneration of dopaminergic neurons of the substantia nigra and dopaminergic projections in the striatum. In addition, protein aggregates (Lewy bodies) are detected in surviving neurons in post mortem brain tissue of PD patients. Although the exact cause of PD is still unknown, various disturbances of cellular processes are observed, including protein aggregation, oxidative stress, proteasomal dysfunction, mitochondrial dysfunction, inflammatory responses and excitotoxicity. Available treatments nowadays only alleviate symptoms but cannot stop, delay or reverse neurodegeneration. Therefore more research is necessary. Several animal models exist that replicate parkinsonian symptoms, among which MPTP treatment is the most used and best resembling idiopathic PD. Non-human primates in general, and the necessity and value of marmosets in PD research is also discussed.

Chapter 2 is concerned with a clinical and behavioural assessment of marmosets after chronic exposure to low dose MPTP. Several motor and non-motor behaviours were found to be affected due to MPTP exposure and these clinical parkinsonian symptoms increased in severity during exposure. In particular, we observed disturbances in locomotor activity, sleep patterns and jumping behaviour, but not in hand-eye coordination. Discontinuation of the neurotoxin injections reversed the MPTP-induced behavioural phenotype to some extent, as was evident from home cage activity measurements, the bungalow and tower test. Post-mortem analyses after the recovery period revealed no alterations in the number and size of tyrosine hydroxylase positive dopamine neurons in the substantia nigra. Also the levels of tyrosine hydroxylase in putamen and caudate nucleus were unaltered, and no differences were observed in the levels of several neurotransmitters in the caudate nucleus. Finally, proteomics analysis revealed no global changes in protein expression in substantia nigra and putamen between treatment groups. These findings indicate that parkinsonian symptoms can occur without detectable damage at the cellular or molecular level. Moreover, it seems that parkinsonian symptoms may be reversible when diagnosed and treated early.

Chapter 3 addresses differences in individual and familiar susceptibility to MPTP in marmosets. Monkeys from different breeding families were selected to investigate inter- and intra-family differences in behavioural and neurochemical parameters associated with MPTP exposure. Individual differences were observed in the response to similar doses of MPTP at the level of clinical signs, in particular non-motor PD related behaviours, while motoric behaviours such as locomotor activity, jumping behaviour and fine motor skills were affected equally in all animals. Individual variability in disease manifestation could be traced back through genealogy to siblings of same families. Subsequent neurochemical analysis showed that susceptibility to MPTP correlates with different neurotransmitter levels in the caudate nucleus, in particular noradrenalin. These differences may be caused by MPTP treatment, and may reflect individual differences in disease progression. On the other hand, familial differences in basal noradrenalin levels, either by nature or by nurture, may also explain the observed differences in MPTP susceptibility.

Chapter 4 describes the proteomics analysis of brain tissue from marmosets at different time points after MPTP induction. The substantia nigra and putamen were analysed after acute MPTP exposure, during chronic MPTP exposure, and 10 weeks after discontinuation of MPTP treatment when symptoms had subsided, allowing distinction between acute toxicity, disease progression and recovery from disease symptoms. Untreated animals served as controls. In these experiments, in-gel digestion was used in combination with data-dependent acquisition to reveal changes in protein expression by mass spectrometry. Stringent statistical analyses including correction for multiple testing did not identify differentially regulated proteins between groups due to high variance in the dataset. Less stringent analysis was performed to explore possible trends in the data, resulting in 281 candidate proteins with differential expression patterns in one or more contrasts. However, among these proteins, no enrichment was observed for particular cellular functions, and when three random proteins (MARCKS, GAD67 and SPTN-4) were selected for validation by immunoblotting, correlation between protein expression values obtained by mass spectrometry and immunoblotting was observed for only one of them (SPTN-4). Taken together, variability in protein expression measures reported in this Chapter, either induced by biological differences or by technical variation, preclude robust conclusions on the molecular changes that correlate with MPTP-induced disease progression or recovery.

In Chapter 5, we tested whether reducing technical variation in mass spectrometry-based proteomics analysis might improve data quality when applied to marmoset or human brain tissue. In particular, synaptic protein fractions from healthy human and marmoset hippocampal tissue were analysed using a filter-aided separation method in combination with SWATH analysis (FASP/SWATH). We then compared FASP/SWATH-derived protein expression data to the IGD/DDA protein analysis of the marmoset putamen and substantia nigra as presented in Chapter 4. FASP/SWATH analysis of hippocampal synaptosomes resulted in less variability compared to IGD/DDA of the substantia nigra and putamen, and thus offers possibilities to re-investigate differentially expressed proteins in the latter. However, it should be noted that synaptosomes were compared here with whole tissue extracts, and

that differences in sample complexity may have contributed to differences in variability. Also, tissue preparation was also different between the two experiments, which may have resulted in less homogeneous samples in case of the substantia nigra and the putamen, which are more difficult to dissect that the hippocampus. Finally, comparison of FAPS/SWATH-derived hippocampal tissue of humans and marmoset showed 857 differently regulated proteins between the two species, suggesting that despite their similarities they may differ considerably at the level of synaptic protein expression.

Chapter 6 discusses the clinical relevance of behavioural, cellular and molecular research of the marmoset MPTP model. The possibilities and challenges of working with an outbred animal strain are addressed with regard to behaviour and proteomics analysis. Proteomics as a discovery tool to identify new drug targets is discussed. Finally, implications and future perspectives are considered, including the importance of the research presented in this thesis in the broader scope of clinical PD research.