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Systems pharmacology and blood-brain barrier functionality in Parkinson's disease

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Chapter 1

Scope and Outline of the Investigations

1. General objective

The objective of the research described in this thesis was to explore rotenone as a toxin for inducing Parkinson's disease in rats as a new rat model of this disease, and to use this rat model in pharmacokinetic(-pharmacodynamic) (PK-PD) studies on antiparkinson drugs with special reference to blood-brain barrier (BBB) functionality.

2. Scope and outline

Parkinson's disease is a progressive neurodegenerative disease, characterised by the loss of dopamine producing neurons in the striatum and substantia nigra pars compacta (SNc). Dopamine controls a variety of functions including locomotor activity, cognition, emotion, positive reinforcement, food intake, and endocrine regulation. Pathological conditions such as Parkinson's disease are linked to a dysregulation of dopaminergic transmission (Missale *et al*., 1998), resulting in clinical symptoms like bradykinesia, resting tremors, rigidity and postural instabilities (Thomas and Beal, 2007). Dopamine is formed from L-3,4-dihydroxyphenylalanine (L-DOPA) and the treatment of Parkinson's disease consists mainly of symptomatic treatment by replacing the lost dopamine with L-DOPA. L-DOPA is given in combination with a peripheral aromatic amino acid decarboxylase (AAADC) inhibitor, while at later stages other drugs (such as direct dopamine receptor agonists, monoamine-oxidase (MAO) inhibitors, catechol-O-methyltransferase (COMT) inhibitors) may be added. Unfortunately, treatment of Parkinson's disease is not without complications as typically the effect of symptomatic drug treatment wears off with progression of the disease and motor complications arise. At present it is poorly understood which mechanisms contribute to the loss of efficacy. Moreover there is an increasing interest in the development of treatments aimed at impairing disease progression. An important aspect in research on drug treatment of Parkinson's disease is its multifactorial nature. For example there may be changes in the brain distribution of drugs, the disposition of endogenous neurotransmitters, the expression and functionality of receptors etc. This underscores the importance of a system's approach towards the development of novel drug treatment paradigms. The investigations in this thesis focus on alterations on BBB transport in relation to Parkinson's disease progression.

Overview of the pathophysiology of Parkinson's disease and the current treatment modalities, emphasis on changes in the functionality of the BBB

In **Chapter 2**, a summary of Parkinson's disease characteristics, diagnosis and etiopathogenesis is given. In Parkinson's disease, the functional imbalance in dopaminergic transmission is thought to be responsible for the bradykinesia, which may be temporarily normalised by dopamine replacement therapy. In the treatment of Parkinson's disease, L-DOPA in combination with a peripheral AAADC inhibitor like Carbidopa or Benserazide, is the golden standard. In a later stage of the disease, L-DOPA is often administered in combination with a dopamine agonist, a COMT inhibitor, or a MAO-B inhibitor to overcome any motor complications associated with long-term L-DOPA treatment. However, the further the progression of Parkinson's disease, the more effective drug treatment fails and more complications occur.

In order to be able to answer the questions on the reasons behind this reduction in effectiveness, one should consider the mechanisms that may affect the target site distribution of the CNS drug and ultimately the drug response (e.g. plasma protein binding, BBB transport, within brain distribution, target interaction). To improve drug treatment (and drug development), detailed information on the interrelationship between disease, pharmacokinetics and drug response is needed as all individual mechanisms may vary among different physiological, pathological, and chronic drug treatment conditions, and therewith their relative importance in relation to the effect. **Chapter 2** presents an overview of the mechanisms involved in drug response and the factors which influence these mechanisms. Special emphasis is on the contribution of the BBB on the drug response, specifically under diseased conditions like Parkinson's disease. BBB transport of a particular CNS drug into and out of the brain is the sum of all actual BBB transport mechanisms applicable to that particular drug. Therefore, any changes in BBB transport mechanisms may affect actual BBB transport and therewith the effects of the drug. This may also apply for the drugs used in treatment of Parkinson's disease, like L-DOPA as well as the co-administered AAADC inhibitors. This may contribute to the motor complications that develop after long-term treatment with L-DOPA in the more advanced stages of the disease. This implies the need for research on the role of the BBB in the dose-response relationships of the Parkinson's disease related drugs along with the stage of the disease.

Overview of experimental animal models of Parkinson's disease

In **Chapter 3**, an overview of the most commonly used animal models in Parkinson's disease research together with their main disease characteristics is presented. Further information is provided on *in vivo* techniques to assess target site distribution as well as on behavioural tests for pharmacodynamic information.

As Parkinson's disease is composed of many components -each caused by the interplay of a number of genetic and nongenetic causes- a systems pharmacology approach to the improvement of present drug treatments and the development of novel drug treatments of Parkinson's disease is warranted. Animal-based models combined with integrated research approaches that address the individual mechanisms involved, including their time-dependencies, are needed to fully understand these aspects.

The currently applied animal models for Parkinson's disease are presented as toxin-induced and genetic animal models. The behavioural tests that can be used in animal models include drug-induced rotometry, the staircase- and the rotorod test, among others. Further emphasis has been put on *in vivo* techniques to assess the BBB functionality on drug transport into and out of the brain, and the distribution of drugs to the target site. To that end, intracerebral microdialysis is very useful as it is able to measure kinetics of exogenous compounds. Moreover, it also offers the possibility of monitoring endogenous compounds such as neurotransmitters (e.g. dopamine) and its metabolites and any changes in their kinetics as a consequence of disease and/or treatment.

Taken together, information obtained experimentally by applying animal models of Parkinson's disease in combination with intracerebral microdialysis and behavioural testing can be used for the development of advanced mechanism-based PK-PD models.

Experimental research on the 'rotenone model' of Parkinson's disease

Chapter 4 deals with experimental research on the 'rotenone model' of Parkinson's disease. The aim was to find a suitable and applicable animal model for Parkinson's disease resembling the characteristics of the disease, specifically displaying slow and selective lesioning of dopaminergic neurons over time in addition to the presence of Lewy Bodies (LB) in the remaining neurons and detectable motor deficits. To that end, the neurotoxin rotenone was used for inducing Parkinson's disease in rats, and a comparison was made between the systemic and the intracerebral route of administration.

Previously, the administration of low-dose intravenous or subcutaneous rotenone to rats has been shown to produce a slow, selective degeneration of nigrostriatal dopaminergic neurons accompanied by α-synuclein-positive LB inclusions as seen in Parkinson's disease (Betarbet *et al.*, 2000; Sherer *et al.*, 2003). Rotenone's advantages of being able to create an animal model exhibiting a slow progression of disease and the formation of LB-like structures outweighed the use of the well-documented but more acute neurotoxins 6-OHDA and MPTP which did not show any LB formation.

Ultimately, for application of an animal model as a tool for the development of systems PK-PD disease progression models, in which time-dependent changes in the biological system-specific parameters of diseased animals can be obtained (Post *et al.*, 2005), induction of a progressive form of the disease is important.

In the investigations described in **Chapter 4** the subcutaneous route of administration of rotenone was compared with intracerebral administration, directly into the median forebrain bundle (MFB), on parameters such as bodyweight and BBB permeability using sodium fluorescein as a marker and intracerebral microdialysis to quantify flurescein brain distribution. In addition, behavioural assessments were conducted using the rotorod for the subcutaneous model and amphetamine-induced rotometry for the intracerebral model. Post-mortem analysis consisted of assessing nigrostriatal damage based on immunohistological staining with TH and α-synuclein inclusions and peripheral organ pathology.

The results indicated that rotenone infused intracerebrally at the highest dose tested is able to create a progressive rat model for Parkinson's disease, which makes this model useful for research on PK-PD relationships at different stages of Parkinson's disease. The subcutaneous route of administration was found to be of limited value due to the occurrence of peripheral organ toxicity, which indirectly influenced the BBB permeability.

Research on the disposition and brain distribution of L-DOPA in the rotenone model of Parkinson's disease

Chapter 5 describes the research on the disposition and brain distribution of L-DOPA in the Rotenone model of Parkinson's disease.

Parkinson's disease treatment is still mainly focussed on symptomatic treatment by replacing the striatal dopamine by drugs like L-DOPA (Factor, 2008; Nyholm, 2006; Schapira, 2008). L-DOPA is actively transported across the BBB to the brain by the LAT-1 transporter and there are a number of indications on changes in BBB

transport of L-DOPA in animals (Carvey *et al.*, 2005) and in patients (Bartels *et al.*, 2008). In **Chapter 5**, we describe the investigation on potential changes in BBB transport of L-DOPA in conjunction with its brain conversion to the dopamine metabolites DOPAC and HVA, as a consequence of the disease using the intracerebral rotenone model as the rat model for Parkinson's disease. At 14 days after an unilateral injection of rotenone (5 μg) in Lewis rats, intracerebral microdialysis was used to measure endogenous and exogenous $brain_{ECF}$ concentrations of L-DOPA and $brain_{ECF}$ concentrations of DOPAC and HVA following different dosages of L-DOPA (10, 25 or 50 mg/kg). These measurements were used for the investigation of the relationship between plasma and $brain_{ECF}$ kinetics of L-DOPA, and the dopamine metabolites DOPAC and HVA in the untreated as well as in the treated brain side. Post-mortem analysis using striatal TH staining was used to determine "responders" to rotenone. Non-linear Mixed Effects Modeling (NONMEM) was used to develop a population based PK model of L-DOPA and metabolites. The results indicated that the disease conditions at 2 weeks post-rotenone-injection in the MFB did not result in any change in the kinetics (including the brain distribution) of L-DOPA. Merely, a clear effect of disease on the concentrations and elimination rates of DOPAC and HVA in brain was found, providing indirect information on decreased dopamine concentrations at the diseased brain side based on "formation-rate limited elimination" pharmacokinetic principles.

Summary, conclusions and perspectives

Finally, in **Chapter 6** the results presented in this thesis are discussed and perspectives for future research are presented.

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