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

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Chapter 6

Summary, Conclusions and Perspectives

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. Understanding drug response in Parkinson's disease: the role of the BBB

In most neuropharmacological studies the effect of drugs in the CNS are still related to the dose and the mechanisms which may affect the disposition (e.g. absorption, distribution, metabolism and excretion) and thereby ultimately the drug response is not considered. Different factors like genetics, species, gender, age, environmental and pathological conditions can influence the drug response. Parkinson's disease is a progressive neurodegerative disease that lacks good treatment, especially at later stages. Apart from plasma pharmacokinetics, mechanisms that govern CNS drug distribution and response include the rate and extent of BBB transport and the kinetics of distribution within the brain including the brain target distribution. For the development of new drugs as well as for the optimisation of therapy with the current drugs, the variability of these individual mechanisms and contribution in terms of rate and extent should be investigated. **Chapter 2** summarised the mechanisms and sources of variation in the response to drugs used in the treatment of Parkinson's disease. As the BBB is a key player in the relationship between plasma and brain pharmacokinetics, the influences of disease states on BBB functionality in the various stages of the disease is important in order to judge on drug effects. To that end, more integrative research approaches are needed. This warrants the application of a systems pharmacology approach in investigations on variability in drug response in Parkinson's disease.

3. Animal models as a tool in systems pharmacology research on Parkinson's disease treatment

For the development of mechanism-based PK-PD models, animal models are essential. An animal model for Parkinson's disease displaying the slow progressive nature of the disease would provide the biological system-specific parameters needed to determine the neuroprotective properties of new drugs, preclinically. In **Chapter 3**, we presented an overview of the currently available

animal models of Parkinson's disease, with their main characteristics, followed by a summary of available behavioural tests and a discussion on the microdialysis technique for the assessment of BBB functionality. The use of a chronic animal model for Parkinson's disease is a first step in the characterisation of drug effects on disease processes and disease progression. A technique such as intracerebral microdialysis may be applied to determine extracellular, unbound concentrations of endogenous compounds (e.g. biomarkers for Parkinson's disease or BBB functionality) as well as of exogenous compounds (e.g. antiparkinson drugs or marker compounds for specific transport mechanisms of the BBB). Furthermore, behavioural tests may also be applied as a pharmacodynamic read-out. The data obtained from these experiments give information on processes on the causal path between drug administration and response (e.g. target site distribution, target binding and activation, transduction/homeostatic feedback and diseases processes/progression). With this, mechanism-based PK-PD models can be developed which can have properties for extrapolation and prediction in systems pharmacology research (Danhof *et al.*, 2007).

4. The exploration of rotenone as a toxin for inducing Parkinson's disease in rats

In **Chapter 4**, two methods of inducing Parkinson's disease in rats are introduced and compared using the neurotoxin rotenone. The administration of low-dose intravenous or subcutaneous rotenone to rats had previously been shown to produce a slow, selective degeneration of nigrostriatal dopaminergic neurons accompanied by the formation of α-synuclein-positive LB-like 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. Ultimately, this animal model would be applied as a tool for mechanism-based PK-PD disease progression models, in which time-dependent changes in the biological system of diseased animals are taken into account (Post *et al.*, 2005).

The subcutaneous route was chosen as this was less labor intensive and produced the same results as administration via jugular vein cannulation (Sherer *et al.*, 2003). Studies were performed in which we investigated the effect of subcutaneous rotenone on bodyweight, behaviour and BBB permeability with sodium fluorescein as a marker and intracerebral microdialysis as a tool to assess

intracerebral fluorescein concentrations. The intracerebral microdialysis technique offers the advantage of allowing repeated or continuous sampling in freely moving animals. Herewith, it provides important information on the BBB transport and brain distribution. Post-mortem analysis consisted of assessing nigrostriatal damage based on immunohistological staining with TH as well as peripheral organ pathology. The results indicated that subcutaneously administered rotenone failed to produce dopaminergic lesions in the SNc and striatum and, moreover, led to extensive peripheral organ toxicity. BBB permeability for fluorescein following subcutaneously administered rotenone was changed, however due to peripheral toxicity. Other labs have shown similar results on systemically administered rotenone (Fleming *et al.*, 2004; Hoglinger *et al.*, 2003; Lapointe *et al.*, 2004; Zhu *et al.*, 2004).

These results led to the development of the method in which rotenone was administered directly into the median forebrain bundle (MFB) in the brain to be able to overcome any peripheral toxicity. Intracerebrally-administered rotenone (MFB or SNc) is able to decrease striatal and nigral dopamine and its metabolites (Antkiewicz-Michaluk *et al.*, 2004; Saravanan *et al.*, 2005) and to induce behavioural changes similar to Parkinson's disease (Sindhu *et al.*, 2006). An infusion into the MFB was chosen as this might develop a slower, more progressive degeneration of dopaminergic cells as compared to an intranigral infusion (Sindhu *et al.*, 2005). To determine the "optimal" dose for inducing Parkinson's disease in rats, three different doses of rotenone were tested (0.5, 2.0 and 5.0 µg). Here, we monitored bodyweight and performed post-mortem peripheral organ toxicology to confirm the absence of any influence of the rotenone peripherally. Additionally, BBB permeability was assessed with sodium fluorscein as a marker and intracerebral microdialysis. In a separate experiment rotational behaviour was assessed using amphetamine in animals receiving the highest rotenone dose (5.0 µg). Post-mortem analysis using TH on the striatum and SNc was performed. The SNc were analysed for α-synuclein inclusions. Results showed a progressive lesion of the nigrostrial dopaminergic pathway with no associated peripheral toxicity. Furthermore, a large increase in amphetamine induced rotational behaviour was seen and a few rats showed α-synuclein immunoreactivity and aggregation. However, no changes in passive BBB permeability were detected. The results indicated that rotenone infused intracerebrally (specifically the 5.0 µg-dose) is able to create a progressive rat model for Parkinson's disease, which could then be used in PK-PD and other types of experiments.

5. The intracerebral rotenone model of Parkinson's disease in rats: altered conversion of L-DOPA into DOPAC and HVA without changes in BBB transport

In **Chapter 5**, the relationship between plasma and brain$_{ECF}$ kinetics of L-DOPA and its effect on dopamine metabolites DOPAC and HVA was measured in the untreated as well as in the brain side 14 days after an injection of rotenone. For this purpose rotenone (5 µg) was infused unilaterally into the MFB to induce Parkinson's disease in Lewis rats as presented in **chapter 4**. The contralateral, non-infused brain side was used as control. Three groups were used in the experiment, each receiving a different dose of L-DOPA (10, 25 or 50 mg/kg). Plasma samples were collected to determine plasma PK of L-DOPA and (dual-probe) intracerebral microsialysis was used as a tool to measure extracellular concentrations of L-DOPA, DOPAC and HVA in the striatum of both the lesioned and control/untreated brain sides. This enabled us to compare the diseased brain concentrations to the untreated side. Dopamine could not be detected as the concentrations were below the limit of quantification. Post-mortem analysis using TH immunostaining on the striatum was performed. These data were used to determine "responders" to rotenone, which had a TH staining percentage of 40% or lower and were considered to be diseased. NONMEM was used to develop a population based PK model. The results described in this chapter are the first in which both plasma and brain$_{ECF}$ PK of L-DOPA under untreated and diseased conditions are described by one population PK model. 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 of L-DOPA. Merely, a clear effect of disease on the levels and elimination rates of DOPAC and HVA in brain were found, providing indirect information on decreased dopamine concentrations at the diseased brain side based on flip-flop kinetic principles.

6. Discussion and future perspectives

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 PK(-PD) studies on antiparkinson drugs with special reference to BBB functionality in the context of disease.

The results indicated that subcutaneously administered rotenone failed to produce dopaminergic lesions and led to extensive peripheral organ toxicity. BBB permeability for fluorescein following subcutaneously administered rotenone

was changed, however due to peripheral toxicity. Other labs have shown similar results on systemically administered rotenone (Fleming *et al.*, 2004; Hoglinger *et al.*, 2003; Lapointe *et al.*, 2004; Zhu *et al.*, 2004). Rotenone infused intracerebrally (specifically the 5.0 µg-dose into the MFB) is, however, able to create a progressive rat model for Parkinson's disease, which could be used in PKPD and other types of experiments. We showed in a few cases α-synuclein immunoreactivity and aggregation. These have not been observed in previous experiments using an intracerebral infusion of rotenone (Alam *et al.*, 2004; Antkiewicz-Michaluk *et al.*, 2004; Saravanan *et al.*, 2005; Sindhu *et al.*, 2005). Furthermore, the decrease in striatal TH staining seems to reach a minimal plateau at 28 days post-injection. Further experiments to follow the progression at longer post-injection intervals would be required to evaluate if there is disease progression and further α-synuclein development beyond 28 days. Many studies have demonstrated substantial and important age-related changes in neurochemistry and neurobiology. Data on the decreases and alterations in the dopaminergic neurotransmitter pathway have been relatively consistent in both animal and human studies (Adolfsson *et al.*, 1979; Bucht *et al.*, 1981; Wenk *et al.*, 1989). Also, age seems to increase the sensitivity of dopaminergic neurons to rotenone toxicity in rats (Phinney *et al.*, 2006). These findings indicate to further investigate this for the intracerbrally infused rotenone rats and to study the influence that age might have on the development of α-synuclein inclusions.

In our intracerebral microdialysis studies using sodium fluorescein as a marker for BBB permeability, we did not demonstrate any changes in striatal BBB permeability. We performed our microdialysis study with sodium fluorescein at one particular time point at a specific location in the brain (striatum), and it therefore cannot be concluded that no changes in BBB permeability would occur in other stages of the disease, or at other places within the brain such as the SNc. Carvey and collegues (Carvey *et al.*, 2005) have found leakage of FITC in the SNc and striatum which was always patchy in appearance. This suggests that the 6-OHDA lesion which they applied in their studies, leads to multiple focal breakdowns in the BBB function. It is worthwhile investigating this phenomenon in our animal model at different timepoints after the injection of rotenone into the MFB. The evidence in literature for alterations in BBB functionality in Parkinson's disease is still growing and shows that BBB research in this disease needs more future attention. Parkinson's disease patients have shown to have an increase in vascular density in the SNc, but not the ventral tegmental area (Faucheux *et al.*,

1999), and a reduced P-gp function was found using PET to measure brain uptake of [^{11}C]-verapamil (Bartels *et al.*, 2008; Kortekaas *et al.*, 2005). A rat model using 6-OHDA has demonstrated that dyskinesias in animals were associated with increased entry of L-DOPA into the striatum (Carta *et al.*, 2006; Westin *et al.*, 2006) which was also seen using PET imaging in patients with peak-dose dyskinesias at 1 hour after L-DOPA administration (Fuente-Fernandez *et al.*, 2004). The dyskinetic 6-OHDA rats also exhibited a significant increase in total blood vessel length and a visible extravasation of serum albumin in the SNc (Westin *et al.*, 2006), indicating a role for the BBB in the altered transport of L-DOPA to the brain.

In our studies using the intracerebral rotenone model no change in the plasma kinetics, nor in the brain distribution kinetics of L-DOPA was seen at 2 weeks post-rotenone-injection. However, a clear effect of disease on the levels and elimination rates of DOPAC and HVA in brain were found, providing indirect information on decreased dopamine concentrations at the diseased brain side based on flip-flop kinetic principles. The percentage of intact TH staining found in the rotenone-treated hemisphere compared to the untreated hemisphere was below 40% in 12 out of 17 rats used in this experiment, and higher than 90% in the remaining 5 rats. Further experiments might be directed towards investigating the kinetics of L-DOPA in rats with more advanced lesions. Bromocriptine, a D2 antagonist used in the treatment of Parkinson's disease, has shown to be a substrate for P-gp (Vautier *et al.*, 2006). Given the evidence that P-gp function might be altered in Parkinson's disease (Bartels *et al.*, 2008; Kortekaas *et al.*, 2005), it would be worthwhile to investigate possible changes in the pharmacokinetics (and pharmacodynamics) of bromocriptine in the intracerebral rotenone rat model. Another D2 receptor agonist used in the treatment of Parkinson's disease is pramipexole which is a cationic drug which not only crosses the BBB by diffusion but also via organic cation-sensitive transporter (Okura *et al.*, 2007). This drug might be an interesting model drug to investigate Parkinson's disease related changes in this BBB transporter.

7. Conclusion

In the intracerebral rotenone model of Parkinson's disease, studies were performed at day 14 after injection of rotenone. At this time-point no changes were found in passive BBB permeability, nor in BBB transport modes of L-DOPA (LAT transporter & passive permeability). However, a diseased condition was

present as indicated by the clear effect of rotenone on the levels and elimination rates of DOPAC and HVA in brain that provided information on decreased dopamine concentrations at the diseased brain side.
Altogether it was concluded that the intracerebral infusion of rotenone into the MFB is able to create a chronic and progressive rat model for Parkinson's disease, which is suitable as a tool in systems pharmacology research on Parkinson's disease.

8. Reference list

Adolfsson, R., Gottfries, C.G., Roos, B.E., Winblad, B., 1979. Post-mortem distribution of dopamine and homovanillic acid in human brain, variations related to age, and a review of the literature. *J. Neural Transm.* 45, 81-105.

Alam, M., Mayerhofer, A., Schmidt, W.J., 2004. The neurobehavioral changes induced by bilateral rotenone lesion in medial forebrain bundle of rats are reversed by L-DOPA. *Behav. Brain Res.* 151, 117-124.

Alexander, G.M., Schwartzman, R.J., Grothusen, J.R., Gordon, S.W., 1994. Effect of plasma levels of large neutral amino acids and degree of parkinsonism on the blood-to-brain transport of levodopa in naive and MPTP parkinsonian monkeys. *Neurology* 44, 1491-1499.

Antkiewicz-Michaluk, L., Wardas, J., Michaluk, J., Romaska, I., Bojarski, A., Vetulani, J., 2004. Protective effect of 1-methyl-1,2,3,4-tetrahydroisoquinoline against dopaminergic neurodegeneration in the extrapyramidal structures produced by intracerebral injection of rotenone. *Int. J. Neuropsychopharmacol.* 7, 155-163.

Bartels, A.L., Willemsen, A.T., Kortekaas, R., de Jong, B.M., de Vries, R., de Klerk, O., van Oostrom, J.C., Portman, A., Leenders, K.L., 2008. Decreased blood-brain barrier P-glycoprotein function in the progression of Parkinson's disease, PSP and MSA. *J. Neural Transm.* 115, 1001-1009.

Betarbet, R., Sherer, T.B., MacKenzie, G., Garcia-Osuna, M., Panov, A.V., Greenamyre, J.T., 2000. Chronic systemic pesticide exposure reproduces features of Parkinson's disease. *Nat. Neurosci.* 3, 1301-1306.

Bhidayasiri, R. and Truong, D.D., 2008. Motor complications in Parkinson disease: clinical manifestations and management. *J. Neurol. Sci.* 266, 204-215.

Bucht, G., Adolfsson, R., Gottfries, C.G., Roos, B.E., Winblad, B., 1981. Distribution of 5-hydroxytryptamine and 5-hydroxyindoleacetic acid in human brain in relation to age, drug influence, agonal status and circadian variation. *J. Neural Transm.* 51, 185-203.

Carta, M., Lindgren, H.S., Lundblad, M., Stancampiano, R., Fadda, F., Cenci, M.A., 2006. Role of striatal L-DOPA in the production of dyskinesia in 6-hydroxydopamine lesioned rats. *J. Neurochem.* 96, 1718-1727.

Carvey, P.M., Zhao, C.H., Hendey, B., Lum, H., Trachtenberg, J., Desai, B.S., Snyder, J., Zhu, Y.G., Ling, Z.D., 2005. 6-Hydroxydopamine-induced alterations in blood-brain barrier permeability. Eur. *J. Neurosci.* 22, 1158-1168.

del Amo, E.M., Urtti, A., Yliperttula, M., 2008. Pharmacokinetic role of L-type amino acid transporters LAT1 and LAT2. *Eur. J. Pharm. Sci.* 35, 161-174.

Factor, S.A., 2008. Current status of symptomatic medical therapy in Parkinson's disease. *Neurotherapeutics.* 5, 164-180.

Faucheux, B.A., Bonnet, A.M., Agid, Y., Hirsch, E.C., 1999. Blood vessels change in the mesencephalon of patients with Parkinson's disease. *Lancet* 353, 981-982.

Fleming, S.M., Zhu, C., Fernagut, P.O., Mehta, A., DiCarlo, C.D., Seaman, R.L., Chesselet, M.F., 2004. Behavioral and immunohistochemical effects of chronic intravenous and subcutaneous infusions of varying doses of rotenone. *Exp. Neurol.* 187, 418-429.

Fuente-Fernandez, R., Sossi, V., Huang, Z., Furtado, S., Lu, J.Q., Calne, D.B., Ruth, T.J., Stoessl, A.J., 2004. Levodopa-induced changes in synaptic dopamine levels increase with progression of Parkinson's disease: implications for dyskinesias. *Brain* 127, 2747-2754.

Hoglinger, G.U., Feger, J., Prigent, A., Michel, P.P., Parain, K., Champy, P., Ruberg, M., Oertel, W.H., Hirsch, E.C., 2003. Chronic systemic complex I inhibition induces a hypokinetic multisystem degeneration in rats. *J. Neurochem.* 84, 491-502.

Kortekaas, R., Leenders, K.L., van Oostrom, J.C.H., Vaalburg, W., Bart, J., Willemsen, A.T.M., Hendrikse, N.H., 2005. Blood-Brain Barrier dysfunction in Parkinsonian midbrain in vivo. *Ann. Neurol.* 57, 176-179.

Lapointe, N., St Hilaire, M., Martinoli, M.G., Blanchet, J., Gould, P., Rouillard, C., Cicchetti, F., 2004. Rotenone induces non-specific central nervous system and systemic toxicity. *FASEB* J. 18, 717-719.

Nyholm, D., 2006. Pharmacokinetic optimisation in the treatment of Parkinson's disease : an update. *Clin. Pharmacokinet.* 45, 109-136.

Phinney, A.L., Andringa, G., Bol, J.G., Wolters, E.C., van Muiswinkel, F.L., van Dam, A.M., Drukarch, B., 2006. Enhanced sensitivity of dopaminergic neurons to rotenone-induced toxicity with aging. Parkinsonism. *Relat Disord.* 12, 228-238.

Post, T.M., Freijer, J.I., DeJongh, J., Danhof, M., 2005. Disease system analysis: basic disease progression models in degenerative disease. *Pharm. Res.* 22, 1038-1049.

Saravanan, K.S., Sindhu, K.M., Mohanakumar, K.P., 2005. Acute intranigral infusion of rotenone in rats causes progressive biochemical lesions in the striatum similar to Parkinson's disease. *Brain Res.* 1049, 147-155.

Schapira, A.H., 2008. Progress in neuroprotection in Parkinson's disease. *Eur. J. Neurol.* 15 Suppl 1, 5-13.

Sherer, T.B., Kim, J.H., Betarbet, R., Greenamyre, J.T., 2003. Subcutaneous rotenone exposure causes highly selective dopaminergic degeneration and alpha-synuclein aggregation. *Exp. Neurol.* 179, 9-16.

Sindhu, K.M., Banerjee, R., Senthilkumar, K.S., Saravanan, K.S., Raju, B.C., Rao, J.M., Mohanakumar, K.P., 2006. Rats with unilateral median forebrain bundle, but not striatal or nigral, lesions by the neurotoxins MPP+ or rotenone display differential sensitivity to amphetamine and apomorphine. *Pharmacol. Biochem. Behav.* 84, 321-329.

Sindhu, K.M., Saravanan, K.S., Mohanakumar, K.P., 2005. Behavioral differences in a rotenone-induced hemiparkinsonian rat model developed following intranigral or median forebrain bundle infusion. *Brain Res.* 1051, 25-34.

Vautier, S., Lacomblez, L., Chacun, H., Picard, V., Gimenez, F., Farinotti, R., Fernandez, C., 2006. Interactions between the dopamine agonist, bromocriptine and the efflux protein, P-glycoprotein at the blood-brain barrier in the mouse. *Eur. J. Pharm. Sci.* 27, 167-174.

Wenk, G.L., Pierce, D.J., Struble, R.G., Price, D.L., Cork, L.C., 1989. Age-related changes in multiple neurotransmitter systems in the monkey brain. *Neurobiol.* Aging 10, 11-19.

Westin, J.E., Lindgren, H.S., Gardi, J., Nyengaard, J.R., Brundin, P., Mohapel, P., Cenci, M.A., 2006. Endothelial proliferation and increased blood-brain barrier permeability in the basal ganglia in a rat model of 3,4-dihydroxyphenyl-L-alanine-induced dyskinesia. *J. Neurosci.* 26, 9448-9461.

Zhu, C., Vourc'h, P., Fernagut, P.O., Fleming, S.M., Lacan, S., DiCarlo, C.D., Seaman, R.L., Chesselet, M.F., 2004. Variable effects of chronic subcutaneous administration of rotenone on striatal histology. *J. Comp Neurol.* 478, 418-426.