Translational pharmacology of dopamine receptor agonists and antagonists : prolactin and oxytocin as biomarkers

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Conclusions and general discussion

For dopaminergic agents, oral dosing is the most common route of administration. Absorption via this route is largely influenced by gastrointestinal- and first-pass metabolic processes. Medicinal Chemistry programs in this field have typically faced significant challenges to overcome high first-pass clearance and deliver clinical candidates which could provide sufficient systemic exposure following oral administration (Attkins, et al., 2009). Moreover, for compounds active in the central nervous system (CNS), transport across the blood-brain barrier (BBB) may restrict the distribution to the target site in the brain (El Ela, et al., 2004). As a consequence, relatively high doses are required to obtain efficacious concentrations in the brain (Deleu, et al., 2002), which increases the risk of adverse events driven by peripheral mechanisms, such as cardiac valve fibrosis in the treatment of Parkinson's disease. Another complicating factor may be the relatively slow onset of the effect in e.g. the treatment of sexual dysfunction (Attkins, et al., 2009). Therefore investigations have focused on alternative routes of administration.

The existence of a direct nose–to–brain transport route has been reported in numerous studies, although very few studies allow quantification of drug transport by this route. Consequently, although intranasal administration is generally accepted as an alternative route for drug administration, controversy remains on direct absorption from the nasal cavity into the brain. This controversy is fueled by limitations of currently used animal models in terms of attainable data and sampling techniques. In general, there is a strong demand for less invasive animal models, that allow measurement of drug and biomarker concentrations at the target site, which is the brain extracellular fluid (ECF) for most drugs acting in the CNS (Jansson and Bjork, 2002; De Lange and Danhof, 2002; Illum, 2004; Graff and Pollack, 2005a; Dhuria, et al., 2009; Watson, et al., 2009).

The objective of the investigations described in this thesis was to develop a scientific basis for the translational pharmacology of dopaminergic drugs following systemic and intranasal administration, focusing on the brain distribution kinetics and biomarker responses. After recognizing the main scientific challenges and opportunities to achieve this goal (chapter 2), we first developed a new, minimal stress rat model, in which the pharmacokinetics and pharmacodynamics (PK–PD) following intranasal administration of compounds can be compared to intravenous control administration

in freely moving rats (chapter 3, (Stevens, et al., 2009)). These experimental techniques allowed serial blood- and brain ECF sampling, by using intracerebral microdialysis (De Lange, et al., 1999; Chaurasia, et al., 2007). To measure the concentrations of the dopamine D2-receptor antagonist remoxipride as a paradigm compound in mechanism-based PK–PD studies, new analytical methods were developed for remoxipride measurements in small plasma, brain ECF and brain homogenate samples (chapter 4, (Stevens, et al., 2010)). Subsequently, nonlinear mixed effect modeling (Beal and Sheiner, 1992) was applied to develop the PK model of remoxipride in plasma and brain ECF (target site for remoxipride (Nadal, 2001)) following intranasal and intravenous administration. This advanced mathematical modeling approach proved, for the first time in a strictly quantitative manner, the existence of a direct nose–to–brain transport route for remoxipride (chapter 5). Next, the effects of remoxipride on prolactin as translational biomarker for dopaminergic system activity were assessed. Based on intravenous remoxipride administration studies in the rat, a novel mechanism-based PK–PD model was developed. With use of translational modeling approaches, the model allowed good prediction of human remoxipride PK–PD (chapter 6).

In addition, we provide evidence that, following intranasal administration of remoxipride, the time to maximal drug concentrations in plasma and brain ECF is almost equal to that following intravenous administration (chapters 3 and 5). This shows that for remoxipride the absorption from the nasal cavity into the brain is fast. Therefore, intranasal administration can be of added value in diseases for which a fast time to onset of action is required and intravenous administration is highly impractical (e.g. off-phase symptoms of Parkinson's disease, menopausal hot flashes, migraine, epilepsy). Also, for remoxipride, the direct nose–to–brain transport route appears to be responsible for prolonged brain ECF exposure. As a consequence, enhanced brain distribution following intranasal administration may indeed result in smaller efficacious doses (compared to e.g. oral dosage), and has therefore the potential to reduce (peripheral) side effects.

In this thesis, in further studies, the effects of remoxipride were assessed using prolactin as a translational biomarker for dopaminergic system activity. Based on intravenous remoxipride administration studies in the rat, a novel mechanism-based PK–PD model was developed. This model is based on the concept of a physiological indirect response model with a pool depletion component (Movin-Osswald and Hammarlund-Udenaes, 1995). Detailed analysis of the prolactin profiles following single and repeated intravenous administrations of remoxipride revealed that a more elaborate

model was needed to describe the data. The new aspects in this model consisted of; i) a biological system response model, describing homeostatic feedback on the synthesis of prolactin and; ii) a dopamine antagonism component based on target site- (brain ECF) rather than plasma concentrations. This model could predict the prolactin response of remoxipride following intranasal administration, thereby confirming the validity of the model. Moreover, the model allowed good prediction of the prolactin response in humans on basis of scaling of the model in rats, indicating good translational properties (chapter 6).

Below, the main findings of the investigations are summarized. In addition, the implications and future prospects are discussed.

MAIN FINDINGS

- a novel chronically instrumented animal model allows quantification of the PK, brain distribution and PD of dopaminergic drugs following intranasal administration under minimal stress conditions
- a novel semi-physiological compartmental PK model has been developed which allows quantification of the direct nose–to–brain transport of dopaminergic drugs following intranasal administration
- a novel mechanism-based PK–PD model has been developed to describe the prolactin response following remoxipride administration. Special features of the model are; i) remoxipride target site concentrations (brain ECF) that drive the effect on prolactin release and ii) prolactin plasma concentrations that drive prolactin synthesis to allow description of the tachyphylaxis component of prolactin release in rats
- the novel mechanism-based PK–PD model was successfully applied in animal to human extrapolation. Special features of the translational approach are; i) use of simulated human target site (brain ECF) concentrations; ii) literature values of drug-specific- and biological system specific parameters, and iii) allometric scaling of prolactin turnover. This resulted in a reasonable prediction of human plasma prolactin response profiles.

IMPLICATIONS AND FUTURE RESEARCH

■ Rate and extent of brain distribution following intranasal administration

This thesis provides, for the first time, quantitative insight in the rate and extent of transport of a drug after intranasal administration. This was achieved by using a novel chronically instrumented rat model for stress free intranasal administration, using intracerebral microdialysis to measure brain

target site drug concentrations combined with advanced mathematical modeling. This approach opens the possibility of systematic investigations on the CNS distribution of drugs following intranasal administration.
In terms of nasal morphology and physiology, potential routes of transport into the brain include the respiratory- and olfactory epithelium pathway, as described in chapter 2. Following absorption across the respiratory epithelium, compounds diffuse into trigeminal nerves which may allow compounds to further enter the brain. Compounds that enter the brain by the olfactory epithelial pathway may diffuse into the perineural spaces that cross the cribriform plate. This ends up in the cerebrospinal fluid of the subarachnoid space, and compounds may further diffuse throughout the brain. The olfactory epithelial pathway also allows intracellular transport through the sensory neurons, into the olfactory bulb. On the cell surface of sensory neurons, transporter proteins (glycoproteins) are present allowing compounds to bind and to be absorbed via endocytosis. After intracellular transport, the compound is exocytosed in the olfactory bulb. Which specific pathway is responsible for direct nose–to–brain transport is therefore likely to depend on the physico-chemical properties of drugs (e.g. lipophylicity, ionization, osmotic properties). Furthermore, it has been suggested that certain intranasal administration devices may favor specific pathways (Dhuria, et al., 2009).

The nose has its barriers to prevent entrance of compounds or particles into the body. The secreted nasal mucus helps to identify and destroy viruses, prevents bacteria from entering the nasal epithelium, and plays an eminent role in immunology (Jones, 2001). For drugs, the presence of nasal mucus may counteract nose–to–brain transport. Then, also nose–to–brain barriers exist, as nasal tissues express specific metabolic enzymes, as well as specific influx- and efflux transporters and mechanisms that may differ between the respi-ratory- and olfactory epithelium. As an example, both the olfactory epithelium and the endothelial cells surrounding the olfactory bulb express the efflux transporter P-glycoprotein (P-gp) (Graff and Pollack, 2003; Graff and Pollack, 2005b). This will obviously have an impact on nose–to–brain transport of P-gp substrates.

Specific knowledge on all these factors is important to predict target site exposure for CNS drugs following intranasal versus systemic administration. Then, to specifically predict nose–to–brain transport following intranasal administration in humans, several additional challenges must be overcome, like e.g. correcting for difference in olfactory surface area (absorption) and characterizing the impact of disease on the physiological state of the nasal epithelium and mucocilliary clearance, as has been reported for Alzheimer's

disease (Talamo, et al., 1989). For that, simultaneous modeling of data obtained in preclinical- and small scale human studies is useful to validate nose–to–brain distribution in man (chapter 6).

■ Mechanism-based PK–PD model

Information on target site exposure is important as it drives the PD. However, measurement of CNS concentrations in humans is highly restricted. In animals, intracerebral microdialysis can be applied, providing data on the free drug in the brain ECF that is close to or even indistinguishable from target site concentrations, which may be distinctively different from plasma concentrations because of the influence of e.g. BBB transport, intra-brain distribution and brain elimination of the drug (De Lange, et al., 2005).

This thesis describes the development of a mechanism-based PK–PD model for the prolactin response in plasma following remoxipride administration. This population-based model consists of; i) semi-physiological PK model for remoxipride (chapter 5); ii) a pool model incorporating the synthesis-, lactotroph storage-, plasma release-, and central elimination of prolactin; iii) an innovative biological system response model, describing homeostatic feedback by interconnecting prolactin plasma concentrations and the synthesis of prolactin, and; iv) a dopamine antagonism component interconnecting free remoxipride brain ECF concentrations and prolactin release. The structure of the model is adequate in describing prolactin response profiles in rats and humans alike. Consequently, mechanism-based PK–PD modeling of dopaminergic agents in preclinical studies *in vivo* can help to predict efficacious doses and the time-course of dopaminergic drug effects in man.

Dopaminergic modulation is a combination of complex processes, and theoretically there is no limit to the number of biological system responses (feedback) that can/should be included. However, without some way of quantifying functional biomarkers, they remain hypothetical. To this end, animal experimental methods are critical as these allow serial measurement of functional PK–PD biomarkers in both plasma and brain ECF. This is important as the BBB and blood-cerebrospinal-fluid-barrier may prevent free distribution of CNS biomarkers into plasma. E.g. it would be of much interest to obtain dopamine concentrations in brain ECF, to directly monitor dopaminergic modulation over time.
The pool- and biological system response structures of the preclinical (rat) PK–PD model are based on physiological processes and can therefore be considered mechanistic (Ben Jonathan, et al., 2008). In the mechanism-based PK–PD model this is parameterized in terms of positive feedback of prolactin on its own synthesis. Using *in vitro* bioassays and lactotroph cell cultures,

it would be of considerable interest to obtain detailed information on the kinetics of the prolactinergic feedback in both rat and man. In such an approach, following prolactin exposure, target occupancy and signal transduction should be monitored in a quantitative manner, in terms of prolactin transcription and –storage. This improves the mechanistic properties of the model, while simultaneously providing a comparative tool for the positive feedback parameter estimates found in these studies.

The preclinical PK–PD model for dopaminergic inhibition by remoxipride administration as developed in this thesis can be seen as an essential first step. An important feature is the validation of this proposed model. A crucial step in this respect is to demonstrate that the system specific parameters are independent from the drug parameters. In this respect it is important to administer different dopaminergic partial agonists and antagonists. While for dopaminergic antagonist only affinity (receptor occupancy) plays a role, for agonists also intrinsic efficacy should be taken into account. Such can be done in a similar preclinical setting. Subsequent modeling using the proposed mechanism-based PK–PD model should result in changes in the estimation of drug–effect parameters (absorption rate constant, maximal effect, concentration of drug that induces 50% of the maximal effect and the Hill-factor), but not in the pool- and biological system parameters. Hence, the pool- and system feedback models can be validated. Such an approach has been successfully applied in the development of mechanistic PK–PD models for adenosine A1-receptor agonists (Van der Graaf, et al., 1997), GABA-ergic compounds (Visser, et al., 2002), and 5-HT1a-receptor agonists (Zuideveld, et al., 2004).

While prolactin concentrations in plasma are considered an adequate biomarker in plasma that reflects inhibition of the dopaminergic system, it is not useful as biomarker for full dopaminergic stimulation. This is because baseline prolactin concentrations in rats are low and dopamine stimulation would cause prolactin concentrations to quickly drop below the limit of quantification. As a result, it will be extremely difficult to describe a drug–effect relationship. Therefore, inclusion of other dopaminergic system activity biomarkers should be considered, to expand the mechanism-based PK–PD model and allow description of stimulatory dopaminergic system functionality. Specifically the measurement of oxytocin in plasma should be considered, as its release into plasma is increased by dopamine receptor activation. Moreover, it would be of much interest to include this mechanism in the currently proposed PK–PD model, as interaction between oxytocin and prolactin is well documented (Freeman, et al., 2000). In the early stage of the experimental studies for this thesis, we considered measuring oxytocin con-

centrations. However, at the time of the experiments, no analytical methods were yet available to quantitatively measure oxytocin in small plasma samples. Next to these biomarkers that refer to physiological measurement in the integral biological system (type 4), measurement of functional biomarkers that refer to the degree of dopaminergic target occupancy (type 2, e.g. Positron Emission Tomography) and target site activation (type 3, electroencephalograms and functional Magnetic Resonance Imaging) should be pursued (chapter 2). These non-invasive techniques provide additional information on the causal chain between drug PK and effect and can be performed easily in both rats and humans.

In general, inclusion of multiple biomarkers for mechanisms on the causal chain between dose administration and effect will increase the mechanistic properties of the PK–PD model. In such approaches, information on target occupancy and transduction should be included, both in rat and human, as previously proposed (Danhof, et al., 2007; Ploeger, et al., 2009). Consequently, obtainment of multiple functional biomarkers (biomarker "fingerprint"), to reflect dopaminergic modulation in the brain, will increase the power of the model to predict the PK–PD relationships for other dopaminergic compounds, but also for the translation to the human situation. Ultimately, mechanistic understanding of dopaminergic modulation can be applied in models for disease progression in diseases related to dopaminergic dysfunction like e.g. Parkinson's disease, schizophrenia and depression.

SUMMARIZING

For mechanism-based investigations on PK–PD relationships following intranasal administration, the use of advanced animal models and analytical techniques are crucial. As described in this thesis, quantitative information on distinction between extent as well as rate of absorption between nose-to-systemic and nose–to–brain distribution can now be obtained. Using plasma prolactin concentrations as a biomarker for dopamine D2 inhibition, a mechanism-based PK–PD model was developed. Most important aspects in this approach were incorporation of target site exposure (brain ECF) of remoxipride and a biological system response (positive feedback) mechanism on the synthesis of prolactin, thereby increasing the mechanistic insight in modulation of the dopaminergic system in rats. Simulating remoxipride brain ECF concentrations in humans, allometric scaling and use of independent information on interspecies differences proved that the structural model is applicable in both rats and man.

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