Mechanism-based pharmacokinetic-pharmocodynamic modelling of opioids : role of biophase distribution and target interaction kinetics

Groenendaal, D.

Chapter 2

SCOPE AND OUTLINE OF INVESTIGATIONS

Ch2

1. GENERAL OBJECTIVES AND BACKGROUND

The objective of the research described in this thesis was the development of a mechanism-based pharmacokinetic-pharmacodynamic (PK-PD) model for the electro-encephalogram (EEG) effects of opioids. The central effects of opioids are determined by four main processes: (1) blood/plasma pharmacokinetics, (2) biophase distribution, which is mainly determined by blood-brain barrier (BBB) transport, (3) receptor interaction kinetics and (4) signal transduction. Under the assumption that within a single species the signal transduction mechanisms are equal for all opioids, the focus of this thesis was to characterise the role of biophase distribution and receptor interaction kinetics on the pharmacokinetic-pharmacodynamic (PK-PD) relationships of opioids.

In the investigations described in this thesis, a panel of opioids with a wide range of pharmacological and physicochemical properties was used. These drugs were specifically selected because of their (partial) agonistic properties at the μ-opioid receptor. The change in the delta frequency band (0.5-4.5 Hz) of the EEG was used as a pharmacodynamic endpoint because it is a sensitive and continuous measure of the central effects of opioids (Dingemanse *et al.* 1988). Previously, the EEG effects of the opioids alfentanil, fentanyl and sufentanil have been investigated. In these investigations, PK-PD analysis was performed with the effect compartment model, to account for hysteresis between the blood concentration and effect. This analysis showed that these opioids all behaved as high efficacy agonists albeit that differences in potency and hysteresis were observed (Cox *et al.* 1998).
To identify a mechanism-based PK-PD model, morphine, nalbuphine and butorphanol were included as model drugs. Morphine is the classical agonist of the μ-opioid receptor (Dhawan *et al.* 1996) and its biophase distribution is known to be complex, because of the interaction with the efflux transporters at the BBB, such as P-glycoprotein (Pgp) at the BBB (Henthorn *et al.* 1999; Letrent *et al.* 1998; 1999a;. 1999b; Mahar Doan *et al.* 2002; Schinkel *et al.* 1995; 1996). Nalbuphine and butorphanol are structurally related to morphine and have previously been identified as partial agonists *in vivo* (Emmerson *et al.* 1996). Nalbuphine was identified as a Pgp substrate *in vitro* (Mahar Doan *et al.*, 2002). So far little is known about the biophase distribution kinetics of nalbuphine and butorphanol.

2. CHARACTERISATION OF THE ROLE OF BIOPHASE DISTRIBUTION

The main objective of the research described in *section 2* was the characterisation of the role of biophase distribution in the PK-PD relationships of the selected opioids. Restricted transport across the BBB can be a critical factor in the PK-PD relationships of opioids. Apart from restricted paracellular diffusion by the presence of tight junctions between the endothelial cells of the BBB, active transport mechanisms may play an

important role, especially for Pgp-mediated efflux.

For the investigation of the PK-PD relationships of morphine, butorphanol and nalbuphine and the characterisation of BBB transport of morphine, first a sensitive HPLC method was developed to analyse blood and brain microdialysate concentrations. The method consisted of a liquid-liquid extraction with ethyl acetate of the blood samples followed by injection on an HPLC system coupled to electrochemical detection. The development and validation of this method is discussed in **chapter 3**. The microdialysate samples were injected without sample pre-treatment. The sample pre-treatment of blood samples was dependent on the compound. For morphine, an alkaline extraction was sufficient whereas for nalbuphine and butorphanol both and acidic and alkaline extraction were required. The mobile phase was a mixture of 0.1 M sodium phosphate buffer, methanol and octane-sulfonic acid with ratio and pH depending on compound and matrix. The limits of quantification in blood samples were 25, 50 and 25 ng/mL for nalbuphine, butorphanol and morphine, respectively and 0.5 ng/mL for morphine in microdialysate samples. Based on sample volume, sensitivity and reproducibility, these assays proved to be suitable for PK-PD studies.

Next, the membrane transport characteristics of opioids were investigated *in vitro*. The focus was on the relative contribution of passive permeability and Pgp-mediated efflux. The studies were conducted in an *in vitro* cell system using monolayers of either the MDCK:MDR1 cells, which were transfected with the MDR1 gene encoding for human P-glycoprotein (Pgp), or LLC-PK1:MDR1a cells, which were transfected with the MDR1a gene encoding for rodent Pgp (**chapter 4**). The interaction of the opioids with Pgp was determined a) indirectly by investigating the inhibition of Pgp-mediated efflux of ^{3}H-digoxin and b) directly by a substrate assessment study. In the latter study, the transport of opioids in the presence and absence of the potent and specific Pgp inhibitor GF120918 (Hyafil *et al.* 1993) was determined. The passive permeability, as reflected by the Papp, of the different opioids was determined in the presence of GF120918. In addition, regression analysis was performed to investigate the relationships between the physico-chemical properties and the P_{app} values. These studies have shown that the influence of Pgp on membrane transport is highly dependent on the passive permeability. Alfentanil, fentanyl and sufentanil have affinity for Pgp but the contribution of Pgp mediated transport to the overall transport rate is minimal because of the high passive permeability rates (>500 nm/sec). In contrast, Pgp has a significant influence on the transport of morphine, presumably because the passive permeability is very low (16 nm/sec).

For morphine, the role of biophase distribution kinetics in the PK-PD correlation was investigated using a novel combined EEG/microdialysis technique that allows simultaneous characterisation of both the brain extracellular fluid (ECF) concentration

and the EEG effect. In these investigations a wide dose range was investigated (4 to 40 mg/kg). The influence of Pgp was investigated by co-infusion of GF120918.

In **chapter 5**, the relative contributions of passive and active transport mechanisms of morphine across the BBB *in vivo* were determined in a quantitative manner upon intravenous administration of two distinct doses of morphine, 4 and 40 mg/kg. Complex brain distribution kinetics was observed in these investigations. Specifically, analysis of the brain microdialysate concentrations showed non-linear distribution kinetics in brain ECF with increasing dose and a distinct effect of Pgp inhibition by GF120918. To describe the complex brain distribution kinetics, a one compartment brain distribution model was developed, with separate expressions for passive diffusion, active saturable influx and active efflux. This active efflux component could be partly reduced by co-infusion with GF120918.

The focus of **chapter 6** was on the influence of biophase distribution and Pgp interaction on the EEG effects of morphine and to compare the biophase distribution kinetics for the EEG with the kinetics of morphine distribution to the brain ECF. Profound hysteresis was observed between blood concentrations and EEG effect. To describe the biophase distribution kinetics of morphine, an extended-catenary biophase distribution model was proposed. This model consists of two sequential compartments (a transfer and an effect compartment) and two rate constants; the k_{1e} which describes the transport through the transfer compartment and the keo, which describes the loss from the effect compartment. Co-infusion of GF120918 only influenced the k_{eo} and different values were found for k_{1e} and k_{eo}. The observation that GF120918 affects the efflux of morphine is consistent with the observations on the distribution in brain ECF. The predicted morphine biophase concentration-time profiles, however were distinctly different from the brain ECF concentration-time profiles as estimated by intracerebral microdialysis.

3. PHARMACOKINETIC-PHARMACODYNAMIC MODELLING OF THE EEG EFFECTS OF OPIOIDS

In *section 3* the PK-PD relationships of the whole set of opioids were evaluated on the basis of model discrimination. For the biophase distribution kinetics, two different models were investigated for each opioid (**chapter 7**): (1) the one-compartment biophase distribution model and (2) the extended-catenary biophase distribution model as proposed for morphine in chapter 6. Both symmetrical and a-symmetrical transport to the biophase was investigated. It was shown that only morphine displays complex biophase distribution kinetics, since the biophase distribution of the other opioids were best described with the one-compartment distribution model. Interestingly, a statistically significant correlation was observed between the values of k_{1e} and the *in vitro* passive permeability. It could be concluded that the relative contribution of passive

permeability and the interaction with transporters determines the complex biophase distribution of opioids.

In addition, the link between the *in vivo* concentration effect-relationships and the interaction at the μ-opioid receptor was investigated (**chapter 8**). The predicted biophase concentrations were used to analyse the concentration-effect relationships simultaneously with the empirical E_{max} model. Further analysis of the pharmacodynamic data was performed on the basis of the operational model of agonism as proposed by Black and Leff (1983) with the values of the system maximum E_m (123 μV) and n (1.44) constrained to the values of alfentanil which displayed the highest intrinsic activity. Between the opioids, wide differences in both *in vivo* affinity and intrinsic efficacy were observed. When the estimated *in vivo* pK_A values were correlated with the *in vitro* pK_i values, indications for two distinct subpopulations were obtained. In addition, a poor correlation was observed between the *in vitro* Na/GTP-shift and the *in vivo* log τ. These observations might be explained by 1) the involvement of active transport processes in distribution from blood to brain, 2) the existence of μ-opioid receptor subtypes and 3) the interaction with other types of opioid receptors.

4. SUMMARY, CONCLUSIONS AND PERSPECTIVES

To conclude this thesis, in *section 4* (**chapter 9**) the results of the separate studies are reviewed and discussed and perspectives for future research are presented.

5. REFERENCES

Black JW, Leff P (1983) Operational models of pharmacological agonism. *Proc.R.Soc.Lond B Biol.Sci.* **220**: 141-162

Cox EH, Kerbusch T, van der Graaf PH, Danhof M (1998) Pharmacokinetic-pharmacodynamic modeling of the electroencephalogram effect of synthetic opioids in the rat: correlation with the interaction at the mu-opioid receptor. *J Pharmacol.Exp.Ther.* **284**: 1095-1103

Dhawan BN, Cesselin F, Raghubir R, Reisine T, Bradley PB, Portoghese PS, Hamon M (1996) International Union of Pharmacology. XII. Classification of opioid receptors. *Pharmacol.Rev.* **48**: 567-592

Dingemanse J, Sollie FA, Breimer DD, Danhof M (1988) Pharmacokinetic modeling of the anticonvulsant response of oxazepam in rats using the pentylenetetrazol threshold concentration as pharmacodynamic measure. *J.Pharmacokinet.Biopharm.* **16**: 203-228

Emmerson PJ, Clark MJ, Mansour A, Akil H, Woods JH, Medzihradsky F (1996) Characterization of opioid agonist efficacy in a C6 glioma cell line expressing the mu opioid receptor. *J.Pharmacol.Exp.Ther.* **278**: 1121-1127

Henthorn TK, Liu Y, Mahapatro M, Ng KY (1999) Active transport of fentanyl by the blood-brain barrier. *J.Pharmacol. Exp.Ther.* **289**: 1084-1089

Hyafil F, Vergely C, Du VP, Grand-Perret T (1993) In vitro and in vivo reversal of multidrug resistance by GF120918, an acridonecarboxamide derivative. *Cancer Res.* **53**: 4595-4602

Letrent SP, Pollack GM, Brouwer KR, Brouwer KL (1998) Effect of GF120918, a potent P-glycoprotein inhibitor, on morphine pharmacokinetics and pharmacodynamics in the rat. *Pharm.Res.* **15**: 599-605

Letrent SP, Pollack GM, Brouwer KR, Brouwer KL (1999a) Effects of a potent and specific P-glycoprotein inhibitor on the blood- brain barrier distribution and antinociceptive effect of morphine in the rat. *Drug Metab Dispos.* **27**: 827-834

Letrent SP, Polli JW, Humphreys JE, Pollack GM, Brouwer KR, Brouwer KL (1999b) P-glycoprotein-mediated transport of morphine in brain capillary endothelial cells. *Biochem.Pharmacol.* **58**: 951-957

Mahar Doan KM, Humphreys JE, Webster LO, Wring SA, Shampine LJ, Serabjit-Singh CJ, Adkison KK, Polli JW (2002) Passive permeability and P-glycoprotein-mediated efflux differentiate central nervous system (CNS) and non-CNS marketed drugs. *J.Pharmacol.Exp.Ther.* **303**: 1029-1037

Schinkel AH, Wagenaar E, Mol CA, van Deemter L (1996) P-glycoprotein in the blood-brain barrier of mice influences the brain penetration and pharmacological activity of many drugs. *J.Clin.Invest* **97**: 2517-2524

Schinkel AH, Wagenaar E, van Deemter L, Mol CA, Borst P (1995) Absence of the mdr1a P-Glycoprotein in mice affects tissue distribution and pharmacokinetics of dexamethasone, digoxin, and cyclosporin *A. J.Clin.Invest* **96**: 1698-1705