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In vivo blood-brain barrier transport of oxycodone in the rat – indications for active influx and implications for PK/PD

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D, Non standard abbreviations:

BBB – blood-brain barrier

LC-MS/MS – liquid chromatography tandem mass spectrometry

NONMEM – nonlinear mixed effects model

CNS – central nervous system

RBC – red blood cell

CV – coefficient of variation

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OFV – objective function value

RSE – relative standard error

ISF – interstitial fluid

Abstract

The blood-brain barrier (BBB) transport of oxycodone was studied in rats. Microdialysis probes were inserted into the striatum and vena jugularis. Ten animals were given a bolus dose followed by a 120 minute constant rate infusion to study the steady-state concepts of oxycodone BBB equilibration. Another ten animals were given a 60 minute constant rate infusion to study the rate of equilibration across the BBB. Oxycodone-D3 was used as a calibrator for the microdialysis experiments. The samples were analyzed with a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method and a population pharmacokinetic model was used to simultaneously fit all the data using NONMEM. A two-compartment model which allowed for a delay between the venous and arterial compartments best described the pharmacokinetics for oxycodone in blood and plasma, while a one-compartment model was sufficient to describe the pharmacokinetics in the brain. The BBB transport of oxycodone was parameterized as CL_{in} and $K_{p,uu}$. CL_{in} describes the clearance of oxycodone across the BBB into the brain, while $K_{p,uu}$ describes the extent of drug equilibration across the BBB. CL_{in} across the BBB was estimated to 1910 μL/min·g brain. $K_{p,uu}$ was estimated to 3.0, meaning that the unbound concentration of oxycodone in brain was 3 times higher than in blood, which is an indication of active influx of oxycodone at the BBB. This is the first evidence of an opioid having an unbound steady-state concentration in brain that is higher than unity, which can explain potency discrepancies between oxycodone and other opioids.

Introduction

The blood-brain barrier (BBB) is comprised of capillary endothelial cells connected by tight junctions. Its main function is to be a physical and active barrier to restrict and regulate the penetration of compounds into and out from the brain in order to maintain brain homeostasis. Transport into the brain across the BBB is essential for drugs which act within the central nervous system (CNS), while BBB penetration needs to be minimized for drugs with potential CNS side effects.

Brain distribution can be described with respect to the rate and extent of equilibration of a drug molecule across the BBB (Hammarlund-Udenaes, 2000). The rate of equilibrium can be expressed as clearances into and out of the brain, CL_{in} and CL_{out}, respectively. The extent of equilibration across the BBB can be expressed as the ratio of the steady-state concentration of unbound drug in brain over unbound drug in blood, $K_{p,uu}$ (Gupta et al., 2006). $K_{p,uu}$ is equivalent to the ratio of the area under the concentration versus time curve of unbound drug in brain and blood, $AUC_{u,brain}$/ $AUC_{u,blood}$, as well as to the ratio CL_{in} / $CL_{out.}$ This assumes that it is only unbound drug that crosses the BBB and that metabolism and interstitial bulk flow contribution to brain efflux is minor. The extent of equilibration equals the net flux of drug across the BBB.

Conclusions on the bidirectional transport properties of the BBB can be drawn based on the unbound concentrations in brain and blood (Hammarlund-Udenaes et al., 1997). If $K_{p,uu}$ is unity, the transport across the BBB is predominantly passive diffusion, or the impact of influx and efflux transport is equal. If $K_{p,uu}$ is below unity, a likely explanation is active efflux transport, metabolism at the BBB or substantial influence of bulk flow on drug brain elimination. A $K_{p,uu}$ above unity is an indication that active influx processes, such as influx

transporters are involved in the BBB transport. Using microdialysis, full time-profiles of unbound drug in both brain and blood can be obtained from each individual. Thus, microdialysis gives direct quantitative information about the *in vivo* net flux of possible influx and efflux mechanisms.

Oxycodone is an opioid receptor agonist (Monory et al., 1999) used to treat moderate to severe pain. The potency for oxycodone when used in postoperative pain treatment is similar to that of morphine (Silvasti et al., 1998), in spite of lower affinity to the opioid receptors (Peckham and Traynor, 2006). Given that the affinity to the opioid receptors is lower for oxycodone than for morphine, a higher concentration of oxycodone would be necessary to give rise to a similar effect. By observing only plasma concentrations it is therefore unclear how the two opioids can be equipotent after systemic administration. Oxycodone has been used clinically for the past 90 years, but little is known about its blood-brain barrier (BBB) transport properties. We recently showed that coadministration of the P-glycoprotein inhibitor PSC833 did not influence the antinociceptive effect nor the total brain concentrations of oxycodone (Bostrom et al., 2005). The objective of this study was to investigate the *in vivo* net flux of oxycodone BBB transport in rats.

Materials and methods

Animals

Male Sprague-Dawley rats (B&K, Sollentuna, Sweden) weighing 250-290 g were used. The animals were group housed at 22°C under a 12 h light-dark cycle for at least five days before the experiment. Food and water were available ad libitum. The study was approved by the Animal Ethics Committee of Tierp District Court, Tierp, Sweden (C 247/1).

Chemicals

Oxycodone hydrochloride and phenobarbital were obtained from Apoteket AB, Production & Laboratory, Sweden. Oxycodone-D3 and D6, noroxycodone, noroxycodone-D3, oxymorphone and oxymorphone-D3 were purchased from Cerilliant Corporation, Round Rock, USA. All chemicals were of analytical grade. All solvents were of HPLC grade.

In vitro blood/plasma partition of oxycodone

The partition of oxycodone between blood and plasma was examined *in vitro*. The experimental setting has been described previously (Kerbusch et al., 2001). Fresh rat blood spiked with two oxycodone concentrations (50 ng/mL and 500 ng/mL) were placed in a thermostatically controlled water bath at 37°C. At various time points (0, 15, and 60 min), samples were taken and centrifuged for 2 min at 10 000 rpm. After centrifugation, the plasma layer was separated from the red blood cells (RBC) layer and the RBCs were transferred to clean vials. All samples were stored at -20°C pending analysis. The experiment was performed in duplicates of each concentration.

Animal surgery

The animals were anaesthetized by inhalation of enfluran (Efrane®, Abbott Scandinavia AB, Kista, Sweden). A PE-50 cannula fused with silastic tubing was inserted into the left femoral vein for oxycodone administration. A PE-50 cannula fused with a PE-10 tubing was inserted into the femoral artery for blood sampling. In order to avoid clotting these catheters were filled with a heparinised saline solution (Heparin Leo®, 100 IE/mL, Leo Pharma AB, Malmö, Sweden). A CMA/20 blood probe (10 mm, CMA, Stockholm, Sweden) was inserted into the right jugular vein through a guide cannula and fixed to the pectoralis muscle with two sutures. The anaesthetized rat was placed into a stereotaxic instrument (David Kopf Instruments, Tujunga, USA) for the implantation of the brain probe. A midsaggital incision was made to expose the skull, and the CMA/12 guide cannula was implanted into the striatum with the coordinates 2.7 mm lateral and 0.8 mm anterior to the bregma and 3.8 mm ventral to the surface of the brain. After insertion the guide cannula was anchored to the scull with a screw and dental cement (Dentalon® Plus, Heraeus, Hanau, Germany). A CMA/12 probe (3 mm, CMA, Stockholm, Sweden) was inserted into the striatal guide. A 15 cm piece of PE-50 tubing was looped subcutaneously on the back of the rat to the surface of the neck in order to let the perfusion solution adjust to body temperature before entering the brain probe. All ends of the cannulae and catheters were passed subcutaneously to a plastic cup placed on the posterior surface of the neck out of reach from the rat. During the surgical procedure, the rat body temperature was maintained at 38°C by using a CMA/150 temperature controller (CMA, Stockholm, Sweden). The rats were placed in a CMA/120 system for freely moving animals (CMA, Stockholm, Sweden) with free access to water and food, and were allowed to recover for approximately 24 hours. All experiments were performed at the same time of the day.

Bolus dose plus constant rate infusion regimen

Ten animals were given a bolus dose followed by a 120 minute constant rate infusion. The blood and brain microdialysis probes were perfused with Ringer solution (147 mM NaCl, 2.7 mM KCl, 1.2 mM $CaCl_2$ and 0.85 mM $MgCl_2$) containing 45 ng/mL of the calibrator oxycodone-D3 at a flow rate of 1 µl/min by using a CMA/100 precision infusion pump (CMA, Stockholm, Sweden). Samples were collected in 15 minute intervals. After a 60 minute stabilization period, oxycodone was administered as an intravenous bolus dose of 0.277 mg/kg over 10 s, followed by a 120 min constant infusion of 0.533 mg/kg/h in the left femoral vein by using a Harvard 22 pump (Harvard Apparatus Inc., Holliston, USA). The animals were studied two at a time and the dose was calculated based on the average weight of the two animals. Brain and blood dialysates were collected in 10 min intervals during the first hour of the infusion and in 20 min intervals during the last hour of the infusion. Five rats were given an overdose phenobarbital right before the end of the infusion and were decapitated for determination of total brain oxycodone concentrations at steady-state. After the end of the infusion, brain and blood dialysates for the remaining five rats were collected in 10 min intervals during one hour and in 20 min intervals during the last hour of the experiment.

Constant rate infusion regimen

Another ten animals were given a constant rate infusion of oxycodone. Both microdialysis probes were perfused with Ringer solution (147 mM NaCl, 2.7 mM KCl, 1.2 mM $CaCl_2$ and 0.85 mM $MgCl_2$) containing 45 ng/mL of the calibrator oxycodone-D3 at a flow rate of 1 µl/min by using a CMA/100 precision infusion pump (CMA, Stockholm, Sweden). Samples were collected in 15 min intervals. After a 60 min stabilization period, oxycodone was administered as an intravenous constant rate infusion of 0.3 mg/kg over 60 min in the left

femoral vein by using a Harvard 22 pump (Harvard Apparatus Inc., Holliston, USA). Brain and blood dialysates were collected in 10 min intervals during the infusion and for the first hour after the stop of the infusion. Thereafter the dialysates were collected in 20 min intervals for the last two hours of the experiment.

One to eight blood samples from each rat were collected into heparinised Eppendorf vials (Brand, Wertheim, Germany) at pre-dose and 5, 10, 20, 30, 60, 75, 90, 105, 120, 130, 140, 180 or 240 min after the start of the oxycodone infusion. No more than 2 ml of blood was collected from each rat.

After the end of the collection interval, microdialysates were capped and stored at -20°C until analysis. The blood samples were centrifuged at 10 000 rpm for 7 min, and the plasma was transferred to Eppendorf vials (Brand, Wertheim, Germany). All brains were examined to ensure that there was no extensive bleeding around the brain probe. Plasma samples and brains were stored at -20°C until analysis.

Sample preparation

The Ringer samples were diluted with an equal volume of water spiked with the internal standard oxycodone-D6 at a concentration of 40 ng/mL, vortexed for 5 s and 16 µl was injected onto the column.

Fifty µl of plasma was precipitated with 100 µl acetonitrile spiked with the internal standard at a concentration of 30 ng/mL and vortexed for 5 s. After centrifugation at 10 000 rpm for 5 min, 30 µl of the supernatant was injected onto the column.

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Each individual brain tissue sample was pretreated as previously described (Bostrom et al., 2004). The sample was homogenized with a 5-fold volume of 0.1 M perchloric acid. The homogenates were centrifuged for 10 min at 3 000 rpm. One hundred µl of the supernatant was pretreated using a slightly modified solid phase extraction (SPE) method by Joel et al. (Joel et al., 1988). SPE columns were coupled to a vacuum manifold and conditioned. One hundred µL brain homogenate supernatant was vortexed with 20 µL water containing the internal standard and 3 mL 0.5 M ammonium sulphate (pH 9.5) for 5 seconds. Thereafter the samples were transferred to the column and drawn through the cartridge. The column was washed with 20 mL 5 mM ammonium sulphate (pH 9.5), 0.5 mL water and 100 µL methanol, before the analytes were eluated with 3 x 2 mL of methanol. A stream of nitrogen and a temperature of 45°C were used to evaporate the eluate and the residue was dissolved in 100 µL of the mobile phase. Forty µL of the reconstituted sample was transferred to a polyethylene autosampler vial and 30 µL was injected onto the column.

The RBCs were prepared according to a previously described method (Dumez et al., 2005). The RBCs were diluted with four times its volume with distilled water. After 5 min, the solution was vortexed and thereafter centrifuged at 10 000 g to precipitate the cell debris. Four hundred µl of the solution was conducted to the same solid phase extraction procedure as for the brain homogenate supernatant.

Drug quantification

Oxycodone and the microdialysis probe calibrator oxycodone-D3 were quantified using a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method (Bostrom et al., 2004). Oxycodone-D6 was used as internal standard. The LC/MS-MS system consisted of an

LC-10AD pump (Shimadzu, Kyoto, Japan) and a Triathlon 900 auto sampler (Spark Holland, The Netherlands) equipped with a 100 µL loop and a Zorbax SB-CN column (4.6 x 150 mm, Agilent Technologies, Wilmington, DE, USA). The mobile phase consisted of 45% acetonitrile in 5 mM ammonium acetate. The column was maintained at 50°C and a constant flow rate of 1.0 ml/min was employed. The flow was splitted allowing 0.2 ml/min to enter the MS (Quattro Ultima, Micromass Manchester, United Kingdom). The transition modes for oxycodone, oxycodone- D3 and oxycodone-D6 were set to m/z 316.1 → m/z 297.9, m/z 319.1 → m/z 301 and m/z 322.2 → m/z 304, respectively. MS control and spectral processing were carried out using MassLynx software, version 4.0 (Micromass, Manchester, United Kingdom).

All standard curves of oxycodone and oxycodone-D3 in different matrixes showed good linearity (coefficient of determination > 0.990). The limit of quantification of oxycodone and oxycodone-D3 was 0.5 ng/mL in Ringer and plasma. In RBCs and total brain, the limit of quantification was 5 ng/mL and 20 ng/mL, respectively. Runs were accepted if the precision and accuracy of the quality control samples had a coefficient of variation (CV) below 15%.

Data analysis

In order to estimate the CL_{in} of oxycodone across the BBB, unbound brain and blood concentrations as well as total plasma concentrations were modeled using population nonlinear mixed effects modeling by NONMEM (version VI) (Beal and Shiner, 1999). The method resolves both inter- and intra-animal variability in the parameters as well as the residual variability between observations and the model predictions The first-order conditional estimation with interaction method was used throughout the modeling procedure (Beal and Shiner, 1999). The subroutine ADVAN6 was used for the NONMEM analysis. The

model selection was based on the objective function value (OFV), pharmacokinetic parameter point estimates, standard errors, scientific plausibility, as well as graphical analysis using Xpose 3.104 (Jonsson and Karlsson, 1999) implemented into S-plus 6.1 (Insightful Corp., Seattle, WA, USA). To distinguish between two nested models a drop in the OFV of 6.63 was required. This value (χ^2 distributed) corresponds approximately to $p < 0.01$ for a one parameter difference. An exponential variance model was used to describe the inter-animal variability:

$$P_i = P_{pop} \cdot \exp(\eta_i) \qquad \text{(Eq. 1)}$$

where P_i and P_{pop} are the parameter in the i^{th} animal and the typical animal, respectively. η_i is the inter-animal variability, assumed to be normally distributed around zero and with a standard deviation ω to distinguish the i^{th} animal's parameter from the typical value as predicted from the regression model. The need for inter-animal variability was investigated in all model parameters.

The percentage relative standard error (RSE%) of the fixed effects parameter estimates was expressed as the standard error (SE) divided by the parameter estimate times 100. The RSE (%) of the random effects parameter estimates were expressed as the SE divided by the parameter estimate on the variance scale. Additive, proportional and slope-intercept models were considered for the residual variability. Different error models were explored for each of the five observation types. The possibility of joint residual errors for the different observation types was also investigated. The residual errors were assumed to be normally distributed around 0.

Initially, submodels were developed separately to speed up model development and facilitate location of model misspecification. The model developed was based on the integrated blood-

brain pharmacokinetic model for morphine with some modifications (Tunblad et al., 2004). The blood probe recovery and blood dialysate data were combined and a sub-model was developed. Thereafter the total plasma concentration data was included and thereafter also the brain probe recovery data and the brain dialysate concentrations. Each part of the model was re-evaluated after adding each observation type. Finally, all the data were simultaneously fitted. The final model is depicted in Fig. 1. One- and multi compartmental pharmacokinetic models were considered for both systemic and brain pharmacokinetics. To allow for a distribution delay between the arterial and venous compartments, the central unbound volume of distribution (V_c) was equally divided between central arterial and venous volumes, VA and VV. Elimination was assumed to occur only from the arterial compartment. The equations describing the model can be found in Tunblad et al (Tunblad et al., 2004), with the change that the BBB transport was parameterized in terms of CL_{in} and $K_{p,uu}$. $K_{p,uu}$ is equivalent to CL_{in}/CL_{out} (Hammarlund-Udenaes et al., 1997; Gupta et al., 2006). CL_{in} and $K_{p,uu}$ were estimated as:

$$CL_{in} = k_{in} \cdot VA \qquad \text{(Eq. 2)}$$

$$CL_{out} = k_{out} \cdot V_{u,brain} \qquad \text{(Eq. 3)}$$

$$K_{p,uu} = \frac{CL_{in}}{CL_{out}} \qquad \text{(Eq. 4)}$$

where $V_{u,brain}$ is the unbound volume of distribution in the brain.

This individual unbound volume of distribution in the brain was calculated using:

$$V_{u,brain} = (A_{brain} - V_{blood} \cdot C_{blood}) / C_{u,brain} \qquad \text{(Eq. 5)}$$

where A_{brain} is the total amount of oxycodone per gram of brain at steady-state. V_{blood} is the volume of blood per gram of brain. $C_{u,brain}$ is the unbound brain ISF concentration at steady-state. A_{brain}, C_{blood} and $C_{u,brain}$ were calculated using data obtained from rats decapitated at the

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end of the infusion (n = 5) in the bolus + constant rate infusion regimen. C_{blood} is the total concentration in blood which can be assessed by multiplying the plasma concentration with the partitioning between plasma and blood (C_{blood}/C_{plasma})) which was calculated according to Eq. 6 (Tozer, 1981):

$$\frac{C_{blood}}{C_{plasma}} = 1 - H + H \cdot \left(\frac{C_{RBC}}{C_{plasma}} \right) \qquad \text{(Eq. 6)}$$

where C_{blood}, C_{plasma}, and C_{RBC} are the oxycodone concentration in blood, plasma and red blood cells, respectively. *H* is the hematocrit, estimated to 42 % in the rat (Leonard and Ruben, 1986). The volume of blood in the rat brain was fixed to 14 µL/g brain (Bickel et al., 1996). The average value of $V_{u,brain}$ from five animals was used as a fixed parameter in the model. $V_{u,brain}$ and C_{blood}/C_{plasma} are presented as averages with standard deviations (SD).

The final model was validated by running a bootstrap validation procedure. Two hundred datasets were generated, randomly sampled from the original dataset with replacement. Then each of the datasets were fit to the final pharmacokinetic model and the mean, SE and 95% confidence intervals of the model parameters were calculated and compared to the final parameters estimates and SE generated by NONMEM.

In order to be able to make comparisons with blood flow, blood clearance across the BBB ($CL_{in,blood}$) was calculated using Eq. 7:

$$CL_{in,blood} = \frac{CL_{in} \cdot f_u}{\left[\frac{C_{blood}}{C_{plasma}} \right]} \qquad \text{(Eq. 7)}$$

where CL_{in} and f_u are the final model estimates of the fixed effects and C_{blood}/C_{plasma} are calculated from Eq. 6.

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CL_{out} was calculated according to Eq. 8:

$$CL_{out} = \frac{CL_{in}}{K_{p,uu}} \qquad \text{(Eq. 8)}$$

where CL_{in} and $K_{p,uu}$ are the final model estimates of the fixed effects.

The partitioning of drug between brain and blood at steady-state can be calculated as:

$$K_p = \frac{C_{tot,brain}}{C_{plasma}} \qquad \text{(Eq. 9)}$$

$$K_{p,u} = \frac{C_{tot,brain}}{C_u} \qquad \text{(Eq.10)}$$

$$K_{p,uu} = \frac{C_{u,brainISF}}{C_u} \qquad \text{(Eq.11)}$$

where K_p is the partition coefficient between total brain concentrations ($C_{tot,brain}$) and total plasma concentrations (C_{plasma}), $K_{p,u}$ is the partition coefficient between total brain concentrations ($C_{tot,brain}$) and unbound blood concentrations (C_u) and $K_{p,uu}$ is the partition coefficient between unbound drug in interstitial fluid (ISF) and unbound drug in blood. K_p, $K_{p,u}$ and $K_{p,uu}$ are presented as averages with standard deviations (SD).

Results

After the bolus dose plus constant rate infusion regimen, oxycodone steady-state concentrations in plasma and blood dialysate were reached rapidly. The brain dialysate concentrations also rapidly reached steady-state, where the unbound oxycodone concentrations in brain ISF were higher than those in blood (Fig. 2a). To achieve stable estimates of CL_{in}, there was a need for information on the time aspects of oxycodone equilibration across the BBB. Therefore a constant rate infusion was given. Higher unbound brain concentrations compared to blood was also seen after the constant rate infusion regimen (Fig. 2b).

$K_{p,uu}$ was estimated to 3.03 (0.038). After the initially even higher brain ISF concentrations, the ratio decreased and remained at 3 throughout the experiment, i.e. over a wide range of concentrations (Fig. 3). No difference was found between the two dosage regimens during or after the end of the infusion. After the end of the infusions, the unbound concentrations in brain and blood declined with the same half-life. The partition coefficients K_p and $K_{p,u}$ were 4.5 ± 2.8 and 7.8 ± 1.2, respectively. The total brain, plasma and unbound blood oxycodone concentration at steady-state was 484 ± 69 ng/g brain, 84 ± 16 ng/mL and 62 ± 9 ng/mL, respectively. This resulted in an average $V_{u,brain}$ of 2.20 ± 0.53 ml/g brain.

The partitioning of oxycodone between blood and plasma resulted in a C_{blood}/C_{plasma} ratio of 1.3 ± 0.3. The binding was not concentration dependent in the range 50-500 ng/mL. Equilibrium was achieved instantaneously and was stable during the study period of 60 minutes.

A two-compartment model which allowed for a delay between the venous and arterial compartments best described the pharmacokinetics for oxycodone in blood and plasma, while a one-compartment model was sufficient to describe the pharmacokinetics in the brain (Fig 1). The influx clearance across the BBB, CL_{in}, was estimated to 1910 (0.20) μL/min·g brain. Using Eq. 7, this corresponds to a $CL_{in,blood}$ of 1092 μL/min·g brain. CL_{out} was calculated to 630 μL/min·g brain. The final model parameters are given in Table 1 and were all estimated with high precision. The data supported inter animal variability in blood and brain recovery, CL, V1 and f_u.

A slope intercept model with variances $\sigma^2_{prop,brain}$ and $\sigma^2_{add,brain}$ was used to describe the brain dialysate data. Proportional error models with variances $\sigma^2_{prop,blood}$, and $\sigma^2_{prop,plasma}$ were used to describe the residual variability in the blood dialysate and plasma data, respectively. Additive error models with variances of $\sigma^2_{add,RECblood}$ and $\sigma^2_{add,RECbrain}$ were used to describe the residual variability in the blood and brain recovery data, respectively.

The final parameter estimates for the bootstrap validation were in good agreement with the estimates of the final model (Table 1). The goodness of fit plots for the different observation types are shown in Fig. 4. A good, medium and poor fit for the blood dialysate concentrations, brain dialysate concentrations and plasma concentrations are shown in Fig. 5.

Discussion

In this study, oxycodone showed a $K_{p,uu}$ of 3 suggesting that the influx clearance is 3-fold greater than the efflux clearance. This is the first evidence of an opioid having an unbound steady-state concentration in brain that is higher than in blood. The finding indicates that oxycodone is actively influxed at the BBB. Other opioids have much lower ratios: morphine 0.29 (Tunblad et al., 2003), morphine-6-glucorunide 0.27 (Tunblad et al., 2005), morphine-3-glucorunide 0.10 (Xie et al., 2000) and codeine 1.0 (Xie and Hammarlund-Udenaes, 1998). The much higher ratio of oxycodone is in spite of similar lipophilic properties of oxycodone and morphine (Peckham and Traynor, 2006). The very lipophilic drug diazepam, which is generally thought to pass membranes only by passive diffusion, showed an unbound concentration ratio between brain and blood of 1 (Dubey et al., 1989).

Reports comparing the pain relieving effects of morphine and oxycodone conclude that the two opioids are equipotent (Silvasti et al., 1998). This is in contrast to the lower affinity for the μ-opioid receptor for oxycodone than morphine (Peckham and Traynor, 2006). As the opioids are active in the CNS, the equipotency of the two drugs may be explained by the oxycodone BBB net flux of 3, as compared with the morphine BBB net flux of 0.29. This results in a higher brain concentration for oxycodone than what could be anticipated from plasma concentrations. The knowledge of the net flux across the BBB may therefore be of importance when interpreting PK/PD relationships for CNS drugs, especially when comparing different routes of administration.

It is difficult to speculate about the reason for the rapid and very high brain uptake of oxycodone. Mechanistic information about the transporter responsible for the extensive uptake of oxycodone into the brain would give strong incentives to drug development, since it

could provide knowledge about strategies of transporting drugs into the brain. In the present study we have quantified the transport. Other experiments are needed to understand more about the mechanisms with which oxycodone is transported. The initially even higher ratio might suggest that the transport mechanism is saturable. If this was true, one would expect the ratio to increase after the end of infusion. This was not the case for oxycodone.

There are a variety of capacity limited ATP dependent transport proteins at the BBB. These include the well known P-glycoprotein and multidrug resistance proteins which actively efflux drugs at the BBB (Loscher and Potschka, 2005). Several CNS drugs such as morphine and methadone are substrates for such efflux transporters at the BBB (Letrent et al., 1998; Dagenais et al., 2004; Wang et al., 2004). There is also evidence of transport proteins that are involved in the influx of nutrients to the brain and that drugs such as L-dopa and M6G use influx transporters to enter the brain (Kageyama et al., 2000; Bourasset et al., 2003). There are reports describing peptides (Thomas et al., 1997) gabapentin (Luer et al., 1999) fentanyl (Henthorn et al., 1999) and pentazocine (Suzuki et al., 2002) using saturable mechanisms to enter the brain. However, a net steady state flux between brain and blood above unity, indicating that active influx is dominating over active efflux has only been reported in very few investigations. The ratio of unbound AUC for the two enantiomers of gacyclidine in spinal cord compared to unbound AUC in plasma ranged between 3 – 7 (Hoizey et al., 2000). Sam et al. reported an unbound concentration ratio for the R(-) and S(+) enantiomer of apomorphine between brain ISF and blood of 12 and 5, respectively (Sam et al., 1997). This would indicate that also the net flux of apomorphine across the BBB is dominated by influx transport. As only a few drugs have been investigated with methods that are able to discriminate between BBB transport and brain tissue binding *in vivo*, there are likely more examples of drugs that have a net influx across the BBB greater than one. Genetic

polymorphism has been described for P-glycoprotein (Kerb, 2006). Similar polymorphism in the transporter responsible for oxycodone BBB influx might have implications for individualized dosing, effective analgesia and perhaps abuse.

The unbound CL_{in} for oxycodone was 1910 μL/min·g brain, resulting in a rapid rate of equilibration across the BBB. This is even higher than the cerebral blood flow of 1440 μL/min·g brain (Shockley and LaManna, 1988). Recalculating CL_{in} to the blood clearance of oxycodone across the BBB, $CL_{in,blood}$, it was 1092 μL/min·g brain. This makes oxycodone a drug of high extraction into the brain. The CL_{in} for oxycodone is much greater than for morphine, M6G and M3G of 11.4, 1.66 and 0.11 μL/min·g brain, respectively (Xie et al., 2000; Tunblad et al., 2003; Tunblad et al., 2005). However, it is in the same range as for fentanyl with a value of 1840 μL/min·g brain from in situ brain perfusion studies (Dagenais et al., 2004). The CL_{out} for oxycodone was 630 μL/min·g brain. The interstitial bulk flow in rat brain has been reported to be 0.18-0.29 μL/min·g brain (Szentistvanyi et al., 1984), which makes the contribution of bulk flow to the total elimination of oxycodone from the rat brain negligible.

The terminology of BBB transport differs in the literature. The influx clearance across the BBB, CL_{in} in this study, can be compared to the initial brain uptake clearance in brain perfusion studies, remembering that the artificial fluid flow and composition in *in situ* brain perfusion differ from physiological blood flow. This will matter if the drug has a perfusion limited transport across the BBB, which seems to be the case for oxycodone. To directly describe the equilibration across the BBB and thereby quantify the *in vivo* net result of active processes in the BBB, the extent of transport across the BBB is in this study described by $K_{p,uu}$ (Gupta et al., 2006). The term K_p includes both binding in brain tissue as well as protein

binding in blood, and can therefore not be used to draw direct conclusions on BBB transport itself. $K_{p,u}$ relates the unbound drug concentration in blood to the total concentration in brain. This gives information on the combined impact of transport across the BBB and distribution within the brain. The average $K_{p,u}$ in this study was 7.9 ± 1.2 indicating that oxycodone is highly distributed into the brain. As this can be the result of either extensive binding to brain tissue and/or influx or efflux mechanism at the BBB it cannot be interpreted further without the use of unbound brain ISF concentrations. The $V_{u, brain}$ of 2.20 ml/g brain was larger than the brain interstitial space of 0.15 ml/g brain (Goodman et al., 1973). This shows that oxycodone is likely distributed intracellularly and/or binds to tissue components in the brain. Together with the $K_{p,uu}$ ratio of 3, this shows that both binding in the brain tissue and influx mechanisms contribute to the high $K_{p,u}$ value.

Population modeling was applied to the data to obtain the typical values and RSEs of the pharmacokinetic parameters including the BBB parameters CL_{in} and $K_{p,uu}$. The same type of integrated model has previously been used to describe morphine pharmacokinetics in the rat (Tunblad et al., 2004). The advantage of this integrated model is the possibility to estimate the pharmacokinetics, the BBB transport and the blood- and brain microdialysis probe recoveries simultaneously. The precision of the estimates was validated with the bootstrap resampling procedure, which showed a high degree of stability in the parameter estimates.

The insertion of a microdialysis probe into the brain region of interest is necessary to study the brain ISF pharmacokinetics of a drug. In the present study the experiments were performed 24 hours after surgery and probe implantation. The tissue trauma and the possible loss of BBB integrity associated with probe insertion are often discussed and could if present lead to errors in the interpretations of microdialysis data. Benveniste and coauthors showed

that the local cerebral blood flow and the local cerebral glucose metabolism were nearly normalized 24 hours after probe implantation (Benveniste et al., 1987). In contrast, Groothius et al. showed that the BBB permeability was affected 24 hours after probe insertion and that microdialysis may overestimate the rate of transfer into and out of the brain (Groothuis et al., 1998). However, the many microdialysis studies in our and other's hands showing an unbound brain to blood ratio far below unity are a good evidence for a working experimental setup (Xie et al., 2000). With a very slow implantation of the microdialysis probes we are confident that the results are describing an *in vivo* situation of a $K_{p,uu}$ above unity.

In conclusion, oxycodone was found to have 3 times higher unbound concentrations in the brain than in blood. The BBB influx clearance based on blood concentrations was close to brain blood flow, in spite of rather low lipophilicity. This indicates that oxycodone is actively influxed across the BBB in rats and might be an important keystone when interpreting pharmacodynamic data from different routes of administration.

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Legends for Figures:

Fig. 1.

A schematic view of the model describing the systemic pharmacokinetics and the blood-brain barrier transport of oxycodone. The circles represent the observed data. The volume term associated with each compartment is noted in the right bottom corner of the respective compartment. Dotted arrows represent the transformations made from the observed data to obtain the unbound concentrations in brain and blood. Thin arrows represent mass transport. The equations used for estimation of parameters have previously been described (Tunblad et al., 2004), unless stated in the Materials and Methods section. The conversions from rate constants to the estimated unbound parameters are shown in the legend.

Fig. 2.

Observed concentration-time profiles of unbound oxycodone concentrations (mean ± SD) a) in brain (◊) and blood (♦) of rats receiving a bolus dose followed by a 120 min constant rate infusion of oxycodone, and b) in brain (□) and blood (■) of rats receiving a 60 minute constant rate infusion of oxycodone.

Fig. 3.

The unbound concentration ratio of oxycodone between brain and blood in rats (mean ± SD). Filled circles (●) represent the rats from the bolus + constant rate regimen, and the open circles (o) represent the rats from the constant rate regimen.

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Fig. 4.

Goodness of fit plots for the oxycodone concentrations in blood dialysate, brain dialysate and plasma, and the oxycodone-D3 concentrations in the blood and brain dialysates. The left panels show the observations vs. the population predictions and the right panels show the observations vs. the individual predictions.

Fig. 5.

Individual plots of the observations (●), predictions (---) and individual predictions (—) for a good, medium and poor fit of the model to the data. The top panel shows the blood dialysate concentrations, the middle panel shows the brain dialysate concentrations and the bottom panel shows the plasma concentrations.

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Tables

Table 1. Unbound parameter estimates of the final population pharmacokinetic model for oxycodone and the stability of the parameters using bootstrap resampling validation.

	Original Data Set		Bootstrap Resampling	
	Estimate	RSE [a] (%)	Mean	RSE [a] (%)
CL (mL/min)	37.4	3.4	37.6	3.1
Vc (mL)	1010	5	1016	4.8
Q (mL/min)	4.37	19	4.40	19
Vper (mL)	230	13	234	13
Q_{AV} (mL/min)	45.1	7.9	45.2	8.1
f_u (%)	74.3	7.1	74.3	6.7
REC_{blood} (%)	65.8	2.3	65.8	2.4
REC_{brain} (%)	16.4	6	16.5	6.1
CL_{in} (mL/min)	1.91	20	1.95	20
$K_{p,uu}$	3.03	3.8	3.04	3.7
Inter animal variability				
ω_{CL}	0.14	33	0.14	34
ω_{Vc}	0.16	36	0.16	39
ω_{fu}	0.28	51	0.26	52
$\omega_{RECblood}$	0.10	28	0.097	30
$\omega_{RECbrain}$	0.22	31	0.21	32
Residual variability				
$\sigma_{prop,plasma}$ [b] (%)	0.195	9.3	0.189	9.6
$\sigma_{prop,blood}$ [b] (%)	0.171	8.1	0.169	8.5
$\sigma_{prop,brain}$ [b] (%)	0.152	18	0.143	20
$\sigma_{add,\ brain}$ [b] (mg/mL)	0.226	42	0.224	43
$\sigma_{add,RECblood}$ [c] (mg/mL)	2.44	12	2.43	11
$\sigma_{add,RECbrain}$ [c] (mg/mL)	2.07	12	2.03	12

a, RSE = relative standard error
b, oxycodone
c, oxycodone-D3

Figure 1

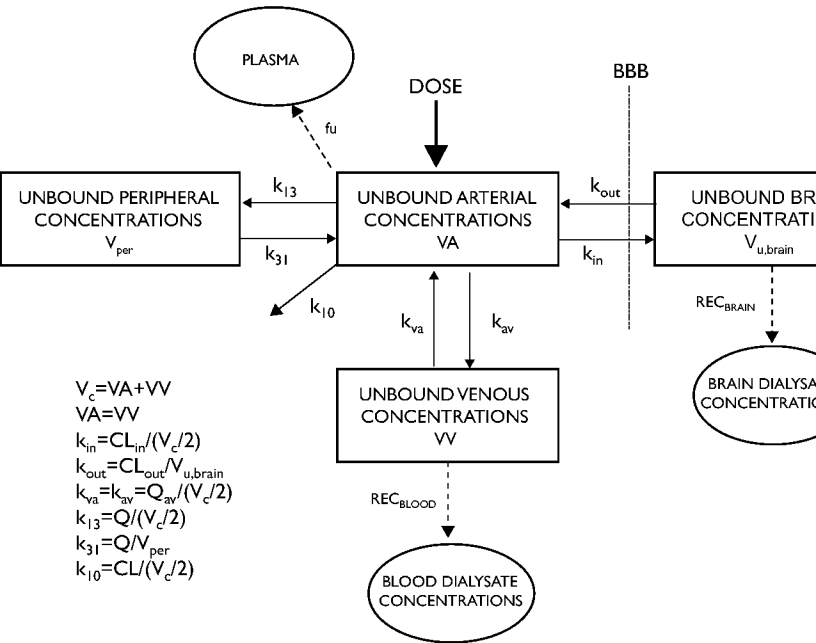

PLASMA
DOSE
BBB
fu
UNBOUND PERIPHERAL CONCENTRATIONS
V_{per}
k_{13}
k_{31}
UNBOUND ARTERIAL CONCENTRATIONS
VA
k_{out}
k_{in}
UNBOUND BR
CONCENTRATI
$V_{u,brain}$
k_{10}
k_{va}
k_{av}
REC_{BRAIN}
BRAIN DIALYSA
CONCENTRATIO
UNBOUND VENOUS CONCENTRATIONS
VV
V_c=VA+VV
VA=VV
$k_{in}=CL_{in}/(V_c/2)$
$k_{out}=CL_{out}/V_{u,brain}$
$k_{va}=k_{av}=Q_{av}/(V_c/2)$
$k_{13}=Q/(V_c/2)$
$k_{31}=Q/V_{per}$
$k_{10}=CL/(V_c/2)$
REC_{BLOOD}
BLOOD DIALYSATE CONCENTRATIONS

Figure 2a

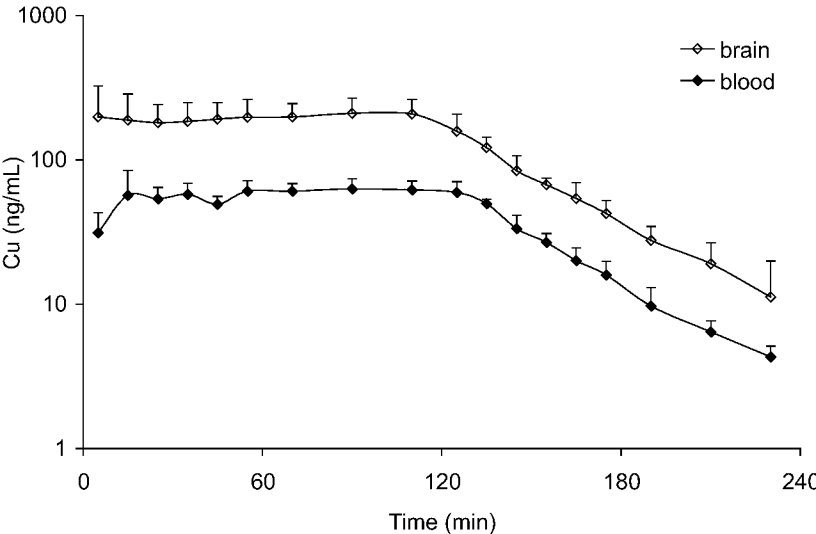


Figure 2b

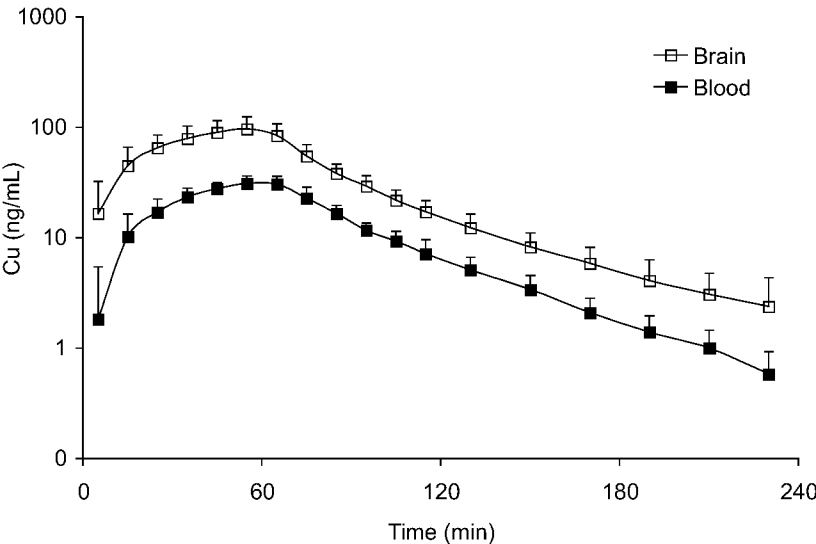


Figure 3

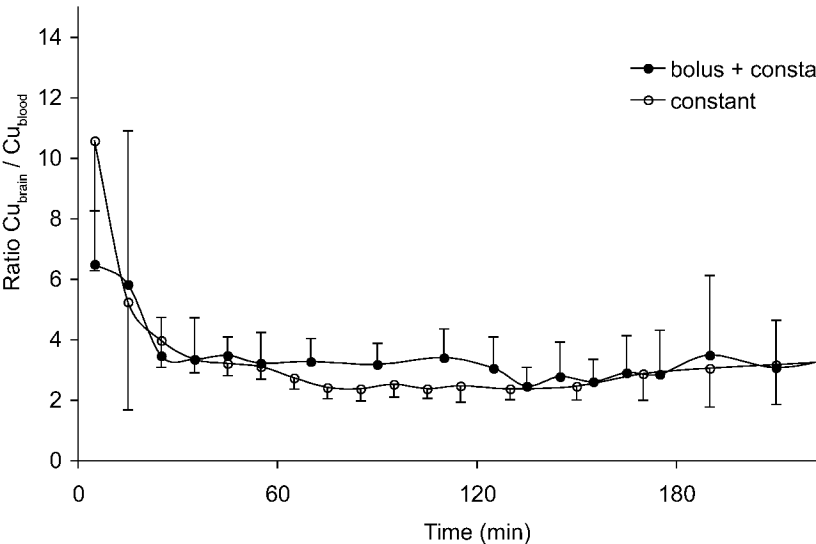

Ratio Cu_{brain} / Cu_{blood}
14
12
10
8
6
4
2
0
0
60
120
180
Time (min)
bolus + consta
constant

Figure 4

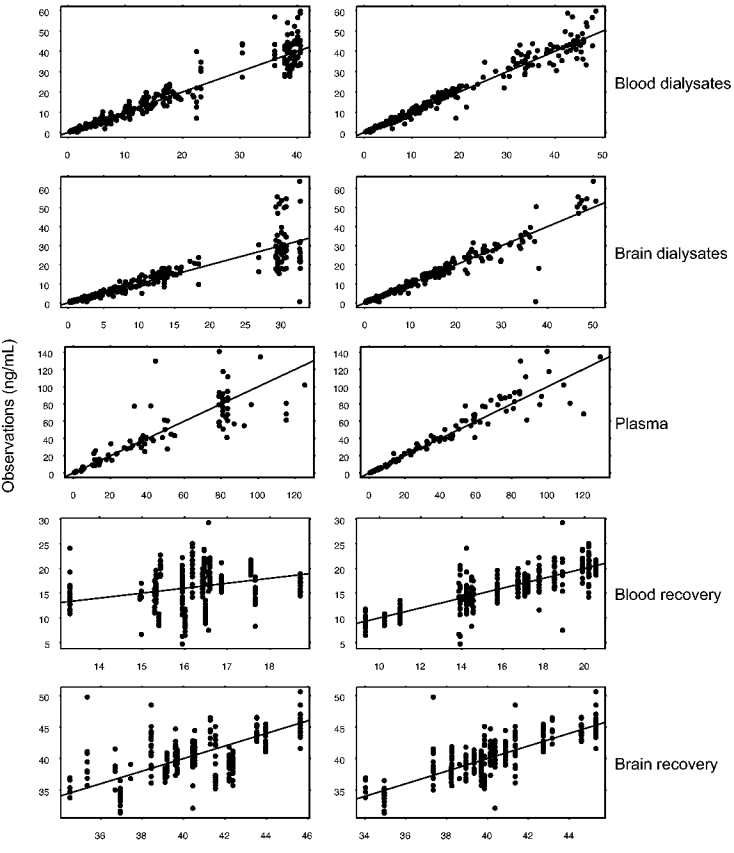
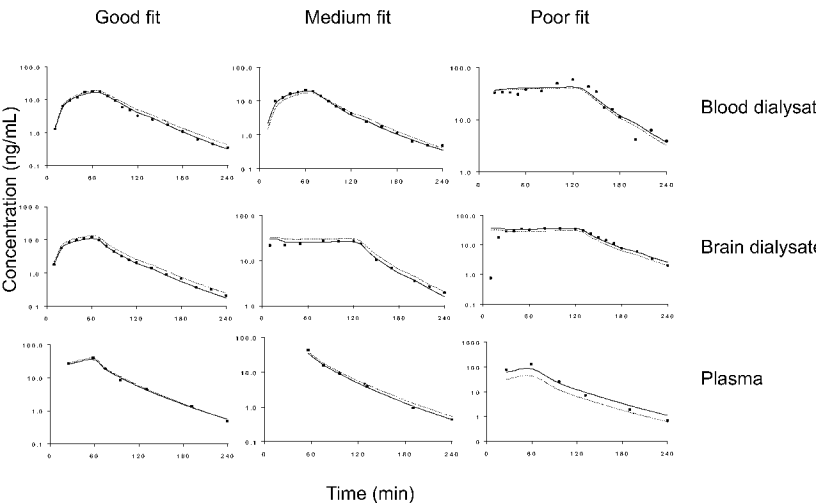

Blood dialysates
Brain dialysates
Plasma
Blood recovery
Brain recovery
Observations (ng/mL)
Population predictions (ng/mL)
Individual predictions (ng/mL)

Figure 5


Good fit
Medium fit
Poor fit
Blood dialysat
Brain dialysate
Plasma
Concentration (ng/mL)
Time (min)