Cerebral and lung kinetics of morphine in conscious sheep after short intravenous infusions

R. N. Upton\textsuperscript{1*}, G. L. Ludbrook\textsuperscript{1}, A. M. Martinez\textsuperscript{1}, C. Grant\textsuperscript{1} and R. W. Milne\textsuperscript{2}

\textsuperscript{1}Department of Anaesthesia and Intensive Care, Royal Adelaide Hospital, University of Adelaide, SA 5005 Adelaide, Australia. \textsuperscript{2}Centre for Pharmaceutical Research, School of Pharmacy and Medical Sciences, University of South Australia, Adelaide, Australia

*Corresponding author. E-mail: richard.upton@adelaide.edu.au

Background. The analgesic effects of morphine are delayed relative to its concentration in blood. The rate of equilibration of morphine between blood and brain may contribute to this delay, but the kinetics of this process have not been modelled. This was determined in conscious instrumented sheep. The lung kinetics of morphine were also determined given their importance in defining systemic kinetics after i.v. bolus administration.

Methods. Sheep were given short i.v. infusions of morphine (30 mg over 4 min). Cerebral kinetics were inferred from arterio–sagittal sinus concentration gradients and cerebral blood flow, and lung kinetics from the pulmonary artery–aortic gradient and cardiac output. These data were fitted to flow- and membrane-limited models of the kinetics in each organ.

Results. Morphine had minimal cardiovascular effects, did not alter cerebral blood flow and caused insignificant respiratory depression. Lung kinetics were best described by a single distribution volume (2036 ml) with a first-order loss (1370 ml min\textsuperscript{-1}), which was attributed to deep distribution. The cerebral kinetics of morphine were characterized by a significant permeability barrier. Permeability across the barrier (7.44 ml min\textsuperscript{-1}) was estimated with good precision, and was approximately one-fifth of the nominal cerebral blood flow. The distribution volume of morphine in the brain was estimated with less precision, but was described by a brain:blood partition coefficient of approximately 1.4. The time required for 50% equilibration between brain and blood concentrations was approximately 10.3 min.

Conclusion. The cerebral equilibration of morphine was relatively slow, and was characterized by significant membrane limitation.

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The time course of the cerebral effects (e.g. analgesia, changes in the EEG) of many clinically used opioids lag behind their concentration in blood.\textsuperscript{1} As this lag is similar in magnitude to the time required for equilibration of the central nervous system (CNS) concentrations with blood concentrations, it has been proposed that CNS equilibration is a major component of the lag.\textsuperscript{2} Furthermore, differences in the rate of CNS equilibration between opioids appear to correspond to their clinically observed duration of action after i.v. bolus administration.\textsuperscript{2} This delayed equilibration becomes of particular clinical significance in postoperative patients in whom multiple doses of parenteral opioids are titrated against pain levels, such as when patients are ‘loaded’ with opioid in the recovery ward, or during subsequent patient-controlled analgesia. Simulations of CNS opioid concentrations in these settings were found to be more consistent with clinical experience than simulations of blood concentrations.\textsuperscript{2} While many aspects of this type of...
Cerebral kinetics of morphine in sheep

Methods

Animal preparation

All experimental procedures were approved by the Animal Ethics Committee of the University of Adelaide. Female Merino sheep of similar ages and body mass (approximately 50 kg) were used, and were instrumented under general anaesthesia as reported previously.3 Catheters were chronically implanted via the femoral vessels in the abdominal aorta (for sampling of arterial blood), in the right atrium (for drug administration), in the pulmonary artery (for blood sampling and thermodilution measurement of cardiac output), and in the dorsal sagittal sinus (the appropriate site for sampling cerebral venous blood in sheep.13) A Doppler transducer was placed over the sagittal sinus using a previously validated method to provide an index of cerebral blood flow.13,14 After recovery from anaesthesia, the sheep were housed in metabolic crates and their catheters maintained with a saline/heparin lock (0.9%/50 i.u. ml−1).

Study design

Six sheep were studied. For each study, instrumented sheep were placed in non-weight-bearing slings inside metabolic crates and were prepared for physiological measurements and blood sampling. After a period of baseline measurements, an i.v. morphine infusion (morphine sulphate injection, David Bull Laboratories, Mulgrave, Victoria, Australia) was begun at time zero at a rate of 7.5 mg min−1 for 4 min. The sheep were not intubated, and breathed room air spontaneously throughout. For each study, the following data were collected.

Pharmacokinetic measurements

Cerebral pharmacokinetics were determined from simultaneous measurements of arterial and sagittal-sinus drug concentrations and cerebral blood flow, as performed previously in this preparation.3 Arterial and sagittal-sinus blood samples (0.5 ml) were taken at 0, 0.5, 1, 1.5, 2, 3, 4, 4.5, 5, 5.5, 6, 8, 10, 15, 20, 30, 45, 60 and 75 min after the start of the infusion.

Lung pharmacokinetics were determined in an analogous manner, from pulmonary arterial samples taken at the same times as the arterial samples.

Whole-blood samples were stored frozen (−20 °C) before assay. Morphine is stable for up to 2 years at this temperature.15 Although some studies of drug uptake into organs have used concurrent intravascular markers, no such marker was used in the present study as previous experience and computer simulations suggested that intravascular transit times for both organs were too rapid to be resolved using the proposed blood sampling schedules.
Cardiovascular and blood gas measurements

Immediately before morphine infusion, cardiac output was measured three times using a thermodilution method. The values were averaged to yield baseline cardiac output. Arterial pressure was recorded continuously via a pressure transducer on one of the arterial catheters. Cerebral blood flow was measured using the Doppler flow probe and a flow meter (Bioengineering, University of Iowa, USA). Both were recorded for 5 min before the start of drug infusion (baseline), and throughout the infusion, using an analog-to-digital card (Metabyte DAS 16-G2) and a personal computer (486 based IBM compatible).

Additional arterial blood samples for blood gas analysis were taken immediately before the infusion and at 4, 10 and 30 min after the start of the infusion (ABL System 625, Radiometer, Sweden). Arterial oxygen tension (\(P_{aO_2}\)), carbon dioxide tension (\(P_{aCO_2}\)), and arterial oxygen saturation (\(S_{aO_2}\)) were also recorded.

Morphine analysis

Morphine was assayed using high-pressure liquid chromatography. A method for liquid–liquid extraction from whole blood was modified from that of McLean and colleagues. Whole-blood samples (with hydromorphone as an internal standard) were extracted into dichloromethane at pH 9 (0.2 M bicarbonate buffer) followed by evaporation to dryness and reconstitution in mobile phase. The use of an organic solvent (dichloromethane) ensured red cell lysis. The chromatographic conditions were based on those reported by Evans and Shanahan with UV detection (210 nm). The mobile phase consisted of acetonitrile 7.5%, methanol 2.5% in 70 mM phosphate buffer (adjusted to pH 3) and was pumped through a C18 column (Alphabond, Alltech, Illinois, USA) at a flow rate of 1 ml min\(^{-1}\). All assays were calibrated using six-point standard curves prepared in blood taken from the same animal before drug administration. The average \(r^2\) value of these standard curves was 0.998 (SD 0.002). The limit of quantitation of the assays was 0.04 \(\mu\)g ml\(^{-1}\). The coefficient of variation of the assay was 5.5% at 0.25 \(\mu\)g ml\(^{-1}\), and 3.3% at 2 \(\mu\)g ml\(^{-1}\). Thus, five replicates would have reduced the contribution of assay variability of the mean data to less than 2.5%. It would be expected that the extraction phase would exclude the glucuronide metabolites of morphine, and normorphine was shown not to co-chromatograph with morphine.

Pharmacokinetic analysis

All kinetic analyses were based on the time course of the mean concentrations. Model parameters therefore represent estimates of the behaviour of the average sheep, which is optimal for discriminating between various models of organ kinetics. Four different kinetic models of the brain were fitted to the data: (i) a null model that tested the hypothesis that there was no concentration gradient across the organ; (ii) a single flow-limited compartment defined by a single distribution volume and cerebral blood flow; (iii) a single flow-limited compartment with an apparent first-order loss representing either deep distribution or metabolism; (iv) a two-compartment membrane-limited model with a permeability term representing distribution into a deep compartment.

The basic forms of the equations describing these models have been published previously, and are given below in a general form where \(C_{in}\) and \(C_{out}\) are the afferent and efferent drug concentrations of an organ, respectively, and \(Q\) is organ blood flow.

\[
C_{out} = C_{in} \quad (1)
\]

\[
V_1 \frac{dC_{out}}{dt} = Q.(C_{in} - C_{out}) \quad (2)
\]

\[
V_1 \frac{dC_{out}}{dt} = Q.(C_{in} - C_{out}) - PS_{loss}.C_{out} \quad (3)
\]

\[
V_1 \frac{dC_{out}}{dt} = Q.(C_{in} - C_{out}) + PS.(C_2 - C_{out})
\]

\[
V_2 \frac{dC_2}{dt} = PS.(C_{out} - C_2) \quad (4)
\]

\(V_1\) is the volume of the first compartment of the organs, and \(V_2\) and \(C_2\) are the volume of, and concentration in, the second compartment of the organ (if appropriate). \(PS\) is the permeability term for loss or exchange from the first compartment.

For the cerebral kinetic models, these parameters were appended with the subscript ‘\(brn\)’, \(C_{in}\) was the arterial concentration, \(C_{out}\) the sagittal-sinus concentration and \(Q\) was cerebral blood flow. For the lung kinetic models, these parameters were appended with the subscript ‘\(lng\)’, \(C_{in}\) was the pulmonary arterial concentration, \(C_{out}\) the arterial concentration and \(Q\) was cardiac output.

A hybrid modelling approach was used (i.e. where one part of the model is physiologically realistic, and the remainder is empirical descriptions of available data). To illustrate the principle, note that Equation 2 can be used to predict \(C_{out}\) as a function of time only if \(C_{in}\) and \(Q\) are known. As both \(C_{in}\) and \(Q\) are time-dependent variables, an algebraic solution for the equation is virtually impossible. However, Eqn 2 can be solved using a differential equation solving program (e.g. via the Runge–Kutta algorithm) if continuous mathematical functions describing \(C_{in}\) and \(Q\) with time are included in the system of equations to be solved. In hybrid modelling, these functions are derived by curve fitting the available experimental data, which consist of measurements of \(C_{in}\) and \(Q\) at discrete time points. The form of these interpolation or ‘forcing’ functions (e.g. sums
of exponentials or polynomials) is not important, provided the resultant curve closely matches the experimental data for \( C_{in} \) and \( Q \). In essence, these functions can be used to ‘force’ \( C_{in} \) and \( Q \) in Eqn 2 to follow the time course of the observed data.\(^{20}\) In practical terms, this means that the time course of \( C_{out} \) predicted by the equation now depends only on the one remaining unknown of the equation (\( V_1 \)), which can be estimated from \( C_{out} \) by curve fitting. The advantage of this approach is that organ kinetics (e.g. \( V_1 \)) can be estimated without having to develop models for the many factors that affect \( C_{in} \) and \( Q \) \textit{in vivo}.

In the present study, the input concentrations were interpolated using exponential forcing functions. The measured changes in blood flow were interpolated using polynomial forcing functions, but with the baseline value based on previous measurements.\(^{13}\) The output concentrations were curve fitted to determine model parameters using modelling software (Scientist for Windows, version 2, Micromath, Utah, USA). The best fit was determined by maximising the model selection criterion (MSC) of this software, which has been described in detail previously.\(^{19}\) The MSC is essentially the Akaike information criterion scaled to compensate for data sets of different magnitudes, and is calculated from the following formula:

\[
MSC = \ln \left( \frac{\sum_{i=1}^{n} w_i (Y_{obs_i} - Y_{cal_i})^2}{\sum_{i=1}^{n} w_i (Y_{obs_i} - Y_{obs})^2} \right) - \frac{2p}{n} \tag{5}
\]

where \( w_i \) is a weighting term and \( p \) is the number of parameters. No weighting scheme was used.

\textit{Calculated variables}

Model parameters were used to calculate secondary variables to facilitate comparison with other opioids and literature values. Cerebral equilibration times for morphine (and other opioids) were calculated by using the final cerebral kinetic model to simulate the time course of the brain concentrations for a step increase in the arterial concentration from 0 to 1. The times required for the cerebral concentration to reach 50% and 95% of the arterial concentration were recorded. The former is equivalent to the half-time of cerebral equilibration only for a flow-limited (single-compartment) model.

The apparent permeability of the BBB (\( P_S \)) was compared with a nominal cerebral blood flow of 40 ml min\(^{-1}\).\(^{13}\) The apparent brain:blood partition coefficient was calculated from \( V_b \) and a nominal real volume of 65 ml for the region of the brain drained by the sagittal-sinus catheter.\(^{13}\)

Total arterial (\( AUC_{art} \)) and sagittal-sinus (\( AUC_{ss} \)) area under the blood morphine concentration–time curves to 30 min were calculated using the trapezoidal rule. Drug retention (\( R_e \)) in the brain was calculated as follows:

\[
R_e = \left(1 - \frac{AUC_{art}}{AUC_{ss}}\right) \cdot 100 \tag{6}
\]

This indicates the amount of morphine that had entered the brain via the arterial blood but had not left the brain via the blood flow.
sagittal sinus by the end of the study period. Retention in the brain could be the result of metabolism or deep distribution. An analogous calculation was also performed for the lung using arterial and pulmonary artery AUC values.

**Results**

Deviations from the protocol were as follows. In one study, the measured arterial concentrations were found to be approximately half of the concurrent pulmonary artery and sagittal-sinus concentrations. A partially extravascular catheter that re-sampled catheter flush solution was postulated to account for this apparent dilution, and the data were excluded. In another study, it was not possible to sample pulmonary artery blood because of catheter failure. The total number of studies of arterial–sagittal sinus gradients across the brain was therefore five, and of pulmonary artery–arterial gradients across the lungs was four.

*Cardiovascular and blood gas data*

Morphine had minimal effects on cerebral blood flow, arterial carbon dioxide tension and mean arterial pressure (Fig. 1). Arterial oxygen tension and haemoglobin saturation were unchanged from baseline. Thus, although the dose of morphine used was relatively high, it was not associated with significant respiratory or cardiovascular side-effects.

A fixed baseline cerebral blood flow of 40 ml min⁻¹ was assumed in subsequent modelling of cerebral kinetics, in keeping with previous measurements in this preparation. Mean baseline cardiac output was 6210 ml min⁻¹ (95% confidence interval [CI] 4740–7680 ml min⁻¹). This baseline value was used in subsequent modelling of lung kinetics.

In all sheep, morphine was noted to produce a mild degree of dysphoria (agitation, mouthing of crate, nystagmus).

*Pharmacokinetic data*

Mean observed blood morphine concentrations are shown in Figure 2. Peak arterial morphine concentration was 2.11 µg ml⁻¹ (95% CI 0.91–3.30 µg ml⁻¹), and this occurred for the last sample taken during the infusion. The concentration differences of morphine across the lungs and brain were small, and the differences were most obvious in the intra-infusion period (Fig. 2). Concentration gradients were therefore only analysed until 30 min after the infusion. If the arterio–venous concentration difference is small, a large number of replicates may be required to resolve the contribution of organ kinetics from that of assay variability. However, for the present data, the time course of the arterio–venous difference showed a consistent non-random pattern, with uptake occurring into both organs during the infusion, and with limited elution from the organ in the post-infusion period (Fig. 3), suggesting that the number of replicates was sufficient for this purpose. As a further precaution against this phenomenon, the null model (C_out=C_in) was included for all modelling analysis. For both the lungs and brain, the null model was a poor alternative to structural models of organ kinetics (Tables 1 and 2).

*Fig 2* (A) Measured pulmonary artery (triangle) and arterial concentrations (circle) of morphine in four sheep. (B) Measured arterial (circle) and sagittal-sinus (inverted triangle) concentrations of morphine in five sheep. Data are shown as the mean at each time point. The variability of the data can be deduced from Figure 3. Solid lines are the best fit of the kinetic model with the highest model selection criteria. These were the ‘flow limited with loss’ model for the lungs (Table 1), and the membrane-limited model with V₁ fixed for the brain (Table 2).
compartment in the lung, for which uptake was essentially irreversible on the time scale of the study. The loss to this compartment was better described as a unidirectional loss term ($PS_{loss}$), as the volume of the deep compartment of the membrane-limited model tended to very small values during fitting, essentially defaulting to the ‘flow with loss’ model. There was good agreement that the effective clearance of this loss was approximately 1.4 litre min$^{-1}$. For a cardiac output of 6 litre min$^{-1}$, this amounts to an effective extraction of 23% across the lung during the study. The retention of morphine in the lungs after 30 min was 12.3% (95% CI ±30.8% to 55.4%).

Cerebral kinetics
Parameter estimates for the various models of cerebral kinetics are summarized in Table 2. The flow-limited and null model were equally poor descriptions of the data, suggesting that the cerebral kinetics of morphine were not compatible with flow-limited uptake. Data were better described by models where there was a permeability term describing loss from the first volume of the brain. In contrast to the lungs, this permeability term was better described by transfer of morphine into a deeper compartment (membrane-limited model) rather than a first-order loss term (‘flow with loss’ model). When the data were used to estimate both volumes of the membrane-limited model, the first volume tended to a small number and was therefore constrained to be greater than $10^{-3}$ ml. As an alternative, this volume was fixed at the nominal volume of the vascular compartment of the brain in sheep (5% of 90 ml, or 4.5 ml). This volume is remarkably similar to the volume of the first compartment estimated by fitting the other models (Table 2). This model was felt to be consistent with the notion of rate limitation for the movement of morphine across the BBB, and produced good parameter estimates. These were used for subsequent calculations. The permeability value of morphine was 7.4 ml min$^{-1}$, and approximately one-fifth of the nominal blood flow (40 ml min$^{-1}$). The final volume of the deep compartment was 92 ml. Given the nominal real volume of the region of the brain drained by the sagittal-sinus catheter (70% of 90 ml$^{13}$), this equates to a brain:blood partition coefficient of approximately 1.4 (Table 3). Retention of morphine in the brain after 30 min was 8.4% (95% CI −17.5% to 34.4%).

Discussion

Cardiovascular and blood gas changes

These data (Fig. 1) are in general agreement with the literature in that morphine had minimal effects on the cardiovascular system$^{4}$ and cerebral blood flow.$^{22}$ Slight and transient respiratory depression was evident, as suggested by the small rise in carbon dioxide partial pressure but this was not statistically significant. There was no associated hypoxia.

Lung kinetics

A number of in vitro and in vivo studies have confirmed that morphine is not metabolized by the lung.$^{7}$ However, in agreement with the present data, a number of studies suggest retention of morphine in the lung, which can manifest as extraction or clearance across the lung in short-term studies. In rabbits, the lung retention of morphine was reported to be 33% following bolus administration.$^{23}$ The value of 23%
calculated from the present data in sheep is comparable. Importantly, the gradient of morphine across the lungs of sheep was reported to be minimal after 5 h of a constant-rate infusion. Therefore, it can be assumed that deep distribution was complete by 5 h. Assuming the process is first order, a half-life of less than 1 h can be calculated. In man, first-pass retention of morphine in the lungs has been reported as 7% and 4%. Low first-pass uptake is consistent with membrane-limited uptake into the lungs, as suggested by the present data.

Cerebral kinetics

The parameters of the model estimated from the present morphine data are shown in Table 3, together with parameters estimated for other opioids in the same experimental preparation. Alfentanil and pethidine were shown to have no rate limitation across the BBB, and cerebral kinetics were therefore flow limited and dictated by their cerebral distribution volumes. As these differed markedly, their cerebral equilibration half-times also differed.

In keeping with the literature, note that the permeability of morphine across the BBB was low compared with these other opioids, as was its cerebral distribution volume. The data unequivocally show that the cerebral kinetics of morphine could not be described by a flow-limited (or venous-equilibrium) model. The permeability of morphine across the BBB was estimated relatively precisely, and was approximately one-fifth of cerebral blood flow. While the calculated brain:blood partition coefficient was relatively uncertain, it was evident that the time required for 50% equilibration was longer than for the other opioids (10.3 min, Table 3). However, this was shorter than the values for the half-time of the delay between morphine effects and blood concentrations reported previously for opioids of approximately 13 min, 17 min and 34 min. Thus, while acknowledging potential differences in species and methodology, it may be that there is a component to this pharmacodynamic delay in addition to the time required for cerebral equilibration. Potential factors contributing to this delay were given in the introduction.

Concentration–effect relationships and active metabolites

Morphine-6-glucuronide is active at the μ-opioid receptor, while morphine-3-glucuronide may antagonize analgesia. While it is clear that a kinetic–dynamic analysis of morphine must account for the systemic and cerebral kinetics of parent morphine, and also the systemic and cerebral kinetics of its glucuronide metabolites, the latter was not performed in the present study. Some understanding of kinetic–dynamic relationships for morphine alone can be inferred from studies in the rat, which produces no morphine-6-glucuronide. In this species, the time course of parent morphine concentrations in the whole brain and brain extracellular fluid showed a good relationship with the time course of analgesia. In contrast, earlier work using vocalization as an analgesic end-point suggested that analgesia lagged behind brain morphine concentrations.

Global vs regional brain concentrations

The model presented here can be used to predict the global brain concentration of morphine. This will be useful if the global concentration is representative of the concentration surrounding opioid receptors. This is not to imply that opioid concentrations in all regions of the brain and spinal cord are equal, but rather that they change in parallel and this is consistent with the literature on morphine distribution throughout the brain. Positron emission tomography studies of morphine distribution in the brain of monkeys showed homogenous distribution. Direct measurements of the concentrations of morphine in different brain regions (including the spinal cord) showed a significant difference in absolute concentration between regions, but are superficially consistent with the notion that regional concentrations changed in parallel.
Contribution of P-glycoprotein

An analysis of the cerebral kinetics of morphine is complicated by the fact that morphine is a P-glycoprotein substrate, and is actively pumped out of the BBB. Abolishing this transport produces brain concentrations and analgesia 3–4 times higher than normal, in the face of minimal changes in systemic kinetics. As sheep also express the gene for P-glycoprotein, the permeability term estimated for morphine in the brain in the present model is therefore a composite of the permeability resulting from diffusion and the effective efflux from the membrane by P-glycoprotein. This should be accounted for when correlating permeability with physicochemical properties.

Conclusion

The present data suggest that the delay in cerebral equilibration of morphine contributes to the slow onset and long duration of action for morphine. However, the quantitative role of other contributing factors to this clinical behaviour has yet to be determined. Nevertheless, the present model is a building block for these more complicated models of cerebral kinetic–dynamic relationships for morphine.

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