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RESEARCH PAPER

Blood–brain distribution of morphine-6-glucuronide in sheep

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Background and purpose: At present there are few data regarding the rate and extent of brain–blood partitioning of the opioid active metabolite of morphine, morphine-6-glucuronide (M6G). In this study the cerebral kinetics of M6G were determined, after a short-term intravenous infusion, in chronically instrumented conscious sheep.
Experimental approach: Five sheep received an intravenous infusion of M6G 2.2 mg kg^{-1} over a four-minute period. Non-linear mixed-effects analysis, with hybrid physiologically based kinetic models, was used to estimate cerebral kinetics from the arterio-sagittal sinus concentration gradients and cerebral blood flow measurements.
Key results: A membrane limited model was selected as the final model. The blood-brain equilibration of M6G was relatively slow (time to reach 50% equilibration of the deep compartment 5.8 min), with low membrane permeability (PS, population mean, 2.5 ml min^{-1}) from the initial compartment (V_1, 13.7 ml) to a small deep distribution volume (V_2) of 18.4 ml. There was some between-animal variability (%CV) in the initial distribution volume (29%), but this was not identified for PS or V_2.
Conclusion and Implications: Pharmacokinetic modelling of M6G showed a delayed equilibration between brain and blood of a nature that is primarily limited by permeability across the blood-brain-barrier, in accordance with its physico-chemical properties.
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Keywords: M6G (morphine-6-glucuronide); pharmacokinetics; drug distribution; blood–brain barrier; cerebral blood flow; sheep physiological model

Abbreviations: AUC_{art}, Total arterial area under the blood concentration-time curves; AUC_{sag}, total sagittal sinus area under the blood concentration-time curves; BBB, blood–brain barrier; C_2, concentration of the second compartment of the brain; C_{in}, afferent arterial drug concentrations of the brain; C_{out}, efferent sagittal sinus drug concentrations of the brain; CBF, cerebral blood flow; CI, confidence intervals; CNS, central nervous system; CO, cardiac output; CSF, cerebrospinal fluid; ECF, extracellular fluid; HPLC, high-performance liquid chromatography; M6G, morphine-6-glucuronide; PS, a term describing loss or exchange of drug from the first compartment; QC, quality controls; Q_{CBF}, cerebral blood flow; $R_{\%}$, drug retention; V_1, volume of the first compartment of the brain (which includes blood); V_2, volume of the second compartment of the brain

Introduction

Morphine is still, 200 years after its first isolation from opium, considered the gold standard in pain treatment. Morphine is characterized by its long onset of effect (30 min) and moderate duration of action (4–6 h). The cerebral kinetics of morphine have recently been investigated in sheep and found to be characterized by a significant membrane limitation and slow cerebral equilibration (Upton *et al.*, 2003).

The clinical use of morphine is complicated by the presence of the active metabolite morphine-6-glucuronide (M6G). The potency of M6G compared to morphine and its contribution to analgesia following morphine administration have been debated for more than two decades. At present the analgesic equipotency ratio after acute administration of M6G, at doses associated with few side effects, is considered to be 1:3–4, whereas the potency ratio after chronic administration remains to be established (Peat *et al.*, 1991; Thomson *et al.*, 1995; Cann *et al.*, 2002; Romberg *et al.*, 2003, 2004; Skarke *et al.*, 2003; Kilpatrick and Smith, 2005).

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For many opioids there is a time delay in effect with regard to maximum plasma concentration. For morphine and other opioids and anaesthetics, it has been proposed that this delay in effect is similar in magnitude to the time required for equilibration of blood and central nervous system (CNS) concentrations (Upton *et al*., 1997b). This delay in effect can also be the result of drug distribution within brain tissue or rate limiting mechanisms at the receptor level (Bouw *et al*., 2001). The delay in cerebral equilibration may imply that the cerebral kinetics of an opioid becomes an important factor in determining the optimal opioid for clinical use. For example, an opioid used for treating acute or breakthrough pain should possess a rapid time to onset of effect, while in the treatment of chronic pain a longer duration of action is advantageous.

In human studies, it has been shown that the ratio of plasma volume to total volume of M6G is approximately five times higher than the ratio for morphine. M6G has a 6–15 times lower volume of distribution at steady-state and 5–15 times lower clearance (Hanna *et al*., 1991; Lötsch *et al*., 1998). The physico-chemical properties of M6G (log $D_{pH\ 7.4}$ −0.8 at 25°C (Avdeef, 1996) and approximately 0 at 37°C (Van Crugten *et al*., 1991)) indicate that it should not readily penetrate the blood–brain barrier (BBB). However, studies in rats and humans show that M6G penetrates the BBB and is distributed in various regions of the brain such as the cortex, brain extracellular fluid (ECF), and cerebrospinal fluid (CSF) (Shimomura *et al*., 1971; Chapman, 1990; Hanna *et al*., 1990; Frances *et al*., 1992; Aasmundstad *et al*., 1995; Bickel *et al*., 1996; Wu *et al*., 1997; Stain-Texier *et al*., 1999; Bouw *et al*., 2001; Lötsch *et al*., 2002; Tunblad *et al*., 2005).

Shimomura *et al*. (1971) showed that after an intraperitoneal injection of M6G in rats only conjugated morphine was detected in the brain, which suggests that M6G penetrates into the brain unchanged and is not converted to free morphine (Shimomura *et al*., 1971). In an isolated, perfused brain preparation, it was shown that the uptake of M6G, after an intravenous bolus injection, was (unidirectional and) low compared to that of morphine (Bickel *et al*., 1996; Wu *et al*., 1997). Using microdialysis, Bouw *et al*. (2001) showed that half of the duration between administration of M6G and its antinociceptive effect is due to transport across the BBB. Also Aasmundstad *et al*. (1995) and Stain-Texier *et al*. (1999) have used the microdialysis method to show that M6G penetrates into the brain after systemic administration and once in the brain M6G almost exclusively accumulates in the extracellular space. While these studies have provided important insights into the cerebral kinetics and dynamics of M6G, the data are often difficult to equate quantitatively to clinical practice, where M6G has shown promise as a post-operative analgesic when administered either intravenously or subcutaneously (Hanna *et al*., 2005; van Dorp *et al*., 2006). We have previously used a sheep preparation to examine the cerebral kinetics and dynamics of drugs used in the peri-operative period (Upton *et al*., 1997a, 2003). Physiological pharmacokinetic-pharmacodynamic models developed in sheep have been adapted to assess the clinical behaviour of these drugs in man (Upton and Ludbrook, 2005). Hence, the aim of this study was to determine the cerebral kinetics of M6G, after a short intravenous infusion, in chronically instrumented, conscious sheep.

Methods

Animal preparation

Sheep were chosen as an experimental animal as the relative perfusion of their brain and the control of cerebral blood flow (CBF) are similar to that in humans (Upton *et al*., 1994; Doolette *et al*., 1999). All experimental procedures were approved by the Animal Ethics Committee at the University of Adelaide and were conducted in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes. The surgical procedures for preparing the sheep and the details about the experiment have been described previously (Upton *et al*., 1994, 1997a). Briefly, female Merino sheep of similar age and body mass (mean weight 44.3 ± 5.0 kg) were used. Instrumentation was performed under general anaesthesia. Catheters were chronically implanted with their tips in the abdominal aorta (for sampling of arterial blood), in the right atrium (for drug administration and measurement of cardiac output (CO)), and in the dorsal sagittal sinus (for cerebral venous blood sampling) (Doolette *et al*., 1999). A Doppler transducer was placed over the sagittal sinus using a previously validated method to quantify the relative changes in CBF (Upton *et al*., 1994; Doolette *et al*., 1999). After recovery from anaesthesia, the sheep were housed in floor pens for a least a day before the study and their catheters maintained with a saline/heparin lock (0.9%/50 IU ml^{-1}).

Study design

On different study days 5 sheep received an intravenous infusion of M6G 2.2 mg kg^{-1} over a 4-min period. On the day of the study the instrumented sheep was placed in a non-weight-bearing sling inside a metabolic crate and prepared for physiological measurements and blood sampling. After 5 min of baseline measurements, the infusion was commenced at time zero. M6G dihydrate (CeNeS Limited, Cambridge, UK) 4 mg ml^{-1} was prepared in 0.9% isotonic saline by a pharmacist on the same day the study was performed. Infusion rate was adjusted according to the weight of each sheep. The procedures below were performed during each study.

Blood sampling

Before drug infusion, blank blood was obtained for use in the calibration curves for the quantification of M6G. Simultaneous blood samples of 2 ml volume were collected from the arterial and sagittal sinus catheters at 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 start of infusion. The blood samples were collected in 10 ml tubes with heparin as an anticoagulant. After the study the blood was centrifuged at 3250 r.p.m. for 10 min and plasma was separated. The blood samples were stored at room temperature for <90 min before separation of plasma. The plasma samples were stored at −20°C until analysis.

Blood gas, cardiac output and CBF measurements

The CBF index was continuously monitored using a computerized data acquisition system. The relative changes were quantified using Doppler flow probe and a flow meter (Bioengineering, University of Iowa, IA, USA). The recording of the parameter was commenced 5 min before drug infusion (baseline) and throughout the study period.

CO was measured in triplicate immediately before the drug infusion, and at 4, 10, 30 and 60 min after the start of the infusion, using a thermodilution method (Runciman *et al*., 1984). The values were averaged to obtain the mean at each time point. Additional arterial blood samples were taken at 0, 4, 10 and 30 min for blood gas measurements (ABL System 625, Radiometer, Sweden).

High-performance liquid chromatography and assay validation

Plasma samples containing M6G were assayed using a validated high-performance liquid chromatography (HPLC) assay. All assays were calibrated using eight-point standard curves prepared in plasma separated from blood taken from the same animal before drug administration. As a validation procedure, inter-assay variability was monitored with quality controls (QC) before assaying the plasma samples.

The chromatography conditions for the quantification of M6G were adapted from a previous method by Milne *et al*. (1991), which described the quantification of M6G in plasma. Analysis was performed with a Shimadzu (Kyoto, Japan) HPLC system equipped with LC-10AS pump, a SCL-10A VP system controller, SPD-10A VP UV–Vis detector, SIL-10A auto injector, and a Nova-Pak 4 μ C_{18} 5 × 100 mm column with a Waters radial compression module (Waters Assoc., Lane Cove, Australia). The mobile phase (1 l) comprised 25% acetonitrile, 0.01 M NaH_2PO_4 and 230 mg l^{-1} lauryl sulphate (sodium salt), adjusted to pH 2.6 with 85% orthophosphoric acid.

M6G plasma samples were processed using solid phase extraction using Sep-Pak C18 cartridges (Waters Assoc., Lane Cove, Australia). Briefly, in 10 ml flat bottom plastic tubes 1 ml of plasma and 70 μl of hydromorphone HCL (internal standard) were made alkaline with 3 ml of 500 mM bicarbonate buffer pH 9.3. The tubes were vortexed briefly. The cartridges were conditioned by 10 ml of methanol filtered under vacuum followed by 10 ml Milli-Q water. The plasma samples were filtered at maximum rate of 1.5 ml min^{-1} through the cartridges, which was subsequently washed with 20 ml of 5 mM bicarbonate buffer pH 9.3 at rate of 1.5 ml min^{-1} followed by 1 ml of Milli-Q water at one drop per second until the cartridges were dry. This step was repeated. Finally, 200 μl was transferred to an auto injector vial and 100 μl was injected in the HPLC system. Blank plasma samples containing standards (0.25–25 μg ml^{-1}, $n=8$ standards), and QC were included in each run to monitor assay performance and were prepared in duplicate at two different concentrations: low (LQC, 1.0 μg ml^{-1}) and high (HQC, 10 μg ml^{-1}). The extraction recovery was 96.8 ± 7.6% for M6G ($n=5$) and 96.8 ± 4.0% for the internal standard ($n=4$).

Over six assay days, the mean r^2 value was 0.998 ± 0.003 ($n=6$), while inter-assay accuracy and precision (mean ± s.d.) were 99.3 ± 12.5% (LQC) and 96.5 ± 9.6% (HQC). Similarly, intra-assay ($n=6$ replicate samples) accuracy and precision were 99.1 ± 7.4 (LQC) and 105.8 ± 6.6 (HQC). The assay was both precise and accurate at the limit of quantification (0.25 μg ml^{-1}) with inter-assay accuracy and precision being 98.8 ± 7.6% ($n=8$).

Pharmacokinetic analysis

In general terms, a hybrid modelling of kinetics was employed. Empirical forcing functions were used to represent inputs into the brain, and curve fitting of the output of the brain to physiological realistic models used to determine model parameters. The cerebral kinetics of M6G were defined using a non-linear mixed effect approach using NONMEM (version V, level 1.1) with linear interpolation of arterial and CBF inputs for individual sheep, and the conditional first-order approximation (FOCE) with interaction analysis. Structural parameters were assigned a log–normal distribution across the population, and a combined additive and proportional residual error models were also tested. Model comparisons were made using the objective function returned by NONMEM with the log-likelihood ratio test, and standard diagnostic plots.

Three different physiologically based kinetic models of the brain were fitted to the sagittal sinus M6G concentrations obtained. The basic forms of the equations describing these models have been published previously (Huang *et al*., 1998; Upton *et al*., 2003). They are repeated below for convenience. C_{in} and C_{out} are the afferent arterial and efferent sagittal sinus drug concentrations of the brain, respectively, and Q_{CBF} is CBF (Figure 1):

A single flow-limited compartment defined by a single distribution volume and CBF:

$$V_1 \cdot dC_{out}/dt = Q_{CBF} \cdot (C_{in} - C_{out}) \quad (1)$$

A single flow-limited compartment with an apparent first-order loss (PS_{loss}) representing either deep distribution or metabolism:

$$V_1 \cdot dC_{out}/dt = Q_{CBF} \cdot (C_{in} - C_{out}) - PS_{loss} \cdot C_{out} \quad (2)$$

A two-compartment membrane-limited model with a permeability term (PS) representing distribution into a deep compartment:

$$\begin{aligned} V_1 \cdot dC_{out}/dt &= Q_{CBF} \cdot (C_{in} - C_{out}) + PS \cdot (C_2 - C_{out}) \\ V_2 \cdot dC_2/dt &= PS \cdot (C_{out} - C_2) \end{aligned} \quad (3)$$

where V_1 is the volume of the first compartment of the brain (which includes blood), and V_2 and C_2 are the volume of, and concentration in, the second compartment of the brain (if appropriate). The permeability-surface area coefficient (PS) is a term describing loss or exchange of drug from the first compartment. In the case of Equation (2), PS represents a unidirectional loss of drug, such as metabolism or deep distribution, which is essentially irreversible. In the case of Equation (3), PS represents the effective inter-compartmental clearance of drug between the two compartments.

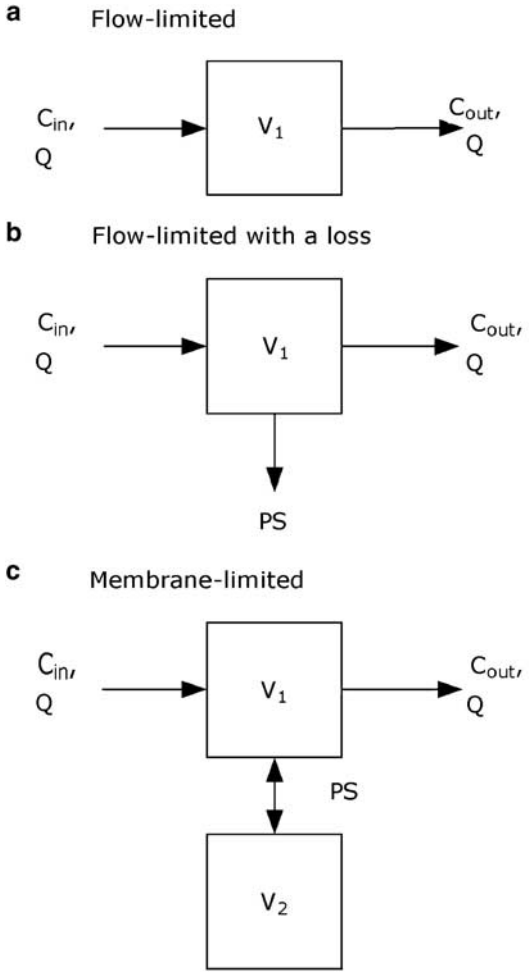

Figure 1 Graphical representation of kinetic models: (**a**) flow limited model (Equation 1), (**b**) single flow-limited compartment with an apparent first order loss of drug (Equation 2), and (**c**) a two-compartment membrane limited model with a permeability term describing distribution into a deep compartment (Equation 3).

Calculated variables

Model parameters were used to calculate secondary variables to facilitate comparison with values reported in the literature for other opioids. Brain equilibration times for M6G were calculated by using the final cerebral kinetic model and parameter values to simulate the time course of the brain concentrations for a step increase in the afferent arterial blood concentration from 0 to 1. The time required for the blood (compartment 1) and brain (compartment 2) concentration to reach 50 and 95% of the arterial blood concentration were calculated.

The apparent permeability of the BBB (PS) was compared with the typical CBF in a sheep (40 ml min^{-1}) (Doolette *et al.*, 1999). The apparent brain:plasma partition coefficient was calculated from V_2 and a nominal real volume of 63 ml for the region of the brain drained by the sagittal-sinus catheter (70% of 90 ml, whole brain) (Doolette *et al.*, 1999).

Drug retention ($R_{\%}$) in the brain was calculated as follows:

$$R_{\%} = (1 - \mathrm{AUC_{sag}}/\mathrm{AUC_{art}}) \cdot 100\% \quad (4)$$

where the total arterial ($\mathrm{AUC_{art}}$) and sagittal sinus ($\mathrm{AUC_{sag}}$) area under the blood concentration–time curves to 75 min were calculated using the trapezoidal rule. The drug retention indicates the amount of drug that had entered the brain via the arterial blood, but had not left the brain via the efferent venous blood by the end of the period of interest. Retention in the brain could be the result of metabolism or deep distribution from which efflux is relatively slow.

Statistical analysis

GraphPad Prism (GraphPad Software version 4.02. for Windows, GraphPad Software, San Diego, CA, USA) was used for all statistical analyses. In the case of CBF, which was continuously measured at 1 s intervals, a 10 s moving average from each individual animal was calculated at each of the pharmacokinetic blood sampling time points. Cardiac output (CO) and relative changes from baseline (100%) in CBF were calculated as mean and 95% confidence intervals (CI). Statistically significant changes from baseline were tested using one-way repeated measures ANOVA with Bonferroni's Multiple Comparison Test with time as the repeated measure. A α-value of 0.05 was set for statistically significant differences. All data are presented as mean $\pm$ s.d. or mean (95% CI).

Results

In individual animals both CBF and CO showed some changes from baseline during M6G infusion. However, no statistically significant time-effect could be demonstrated ($P = 0.93$ for CBF, $P = 0.22$ for CO).

Pharmacokinetic data

Figure 2 shows the mean observed plasma concentrations of M6G. Only a very small amount of M6G was extracted across the brain. The mean (95% CI) peak arterial concentration of M6G was 33.4 μg ml^{-1} (18.4–48.4 μg ml^{-1}) at 4 min, while sagittal sinus $C_{\max}$ was 30. 9 μg ml^{-1} (17.8–44.0 μg ml^{-1}) at 4 min. Maximal concentration gradient of M6G across the brain was during the early infusion period at 1 min.

Initial analyses indicated that the data were heteroscedastic, and that an additive error model was not supported. Furthermore, while between-animal variability was identified for the initial distribution volume (V_1), this was not the case for V_2 or PS. As a consequence, inter-animal variability was removed from these parameters (without a decrease in model fit) and a proportional error model only was employed to obtain the most parsimonious model. The results of the kinetic modelling are summarized in Table 1. $R_{\%}$ of M6G in the brain was minimal (9.8% after 75 min).

The flow-limited model adequately described the data with a population average (value (% s.e. of estimate)) distribution volume of 14.5 (26%) ml with between-animal variability (%CV) 29 (93%)%, and residual unexplained variability of 35 (14%)%. Addition of an irreversible loss (at least over the time-frame of the study) in the flow-limited with loss model was not supported by the data. This model

collapsed into a flow-limited model as the loss term (PS) was estimated to be very small ($<1\times10^{-6}$ ml min^{-1}), while all other model parameters were identical to the flow-limited model. In contrast, the membrane-limited model identified low permeability (PS = 2.5 (85%) ml min^{-1}) from the V_1 (13.7 (27%) ml) compartment into a deep tissue compartment (V_2 18.4 (50%) ml). Between-animal variability in V_1 was 29 (90%)%, and residual unexplained variability was 35 (14%)%. Although the membrane-limited model did not afford a statistical improvement in model fit ($P>0.5$), this was still selected as the most appropriate model, based upon the known pharmacology/physiology of M6G entry into the brain tissue, and the approximate 10% retention of M6G in the brain at 75 min which would not be the case for a flow-limited model with a volume of 15 ml and a CBF of 40 ml min^{-1}.

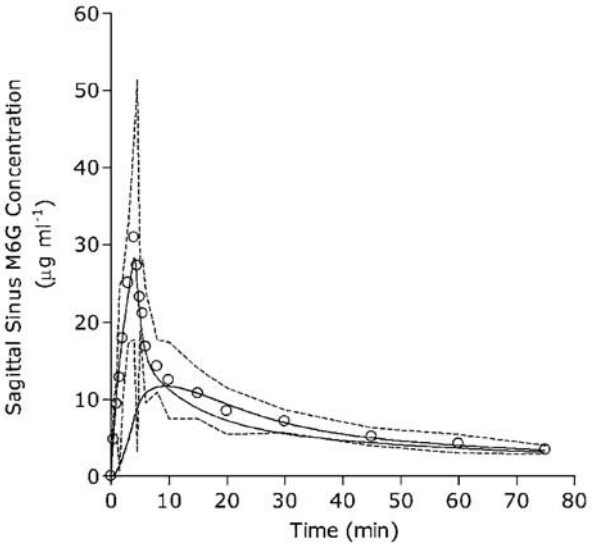

Figure 2 The sagittal-sinus plasma concentrations (open circles) of M6G obtained from five sheep. Data are shown as mean at each time point. Dotted lines are 95% CI, the solid thin line is the fitted sagittal-sinus concentrations, while the solid thick line represents simulated concentrations in the deep brain tissue compartment.

The times to reach 50 and 95% equilibration of the first compartment after M6G administration were 0.3 and 2.5 min, respectively. For the deep compartment, the times were 5.8 and 23.9 min, respectively. Assuming a nominal real volume of the region of the brain drained by the sagittal sinus catheter of 63 ml, the volume of the deep compartment equates to a brain:plasma partition coefficient of approximately 0.3.

Discussion

Cerebral pharmacokinetic data

A membrane-limited model was considered to be the most appropriate model to describe the cerebral kinetics of M6G in sheep. Morphine has previously been studied using the same model (Upton *et al*., 2003) and it was demonstrated that the membrane-limited model best described morphine. Comparing the two opioids using the numbers from Upton *et al*. (2003), the permeability of M6G across the BBB was relatively low (2.5 versus 7.4 ml min^{-1}), as was its cerebral distribution volume (18.4 versus 92.4 ml). This is in agreement with results by others, who also found a lower rate of penetration into the brain for M6G compared to morphine in both animals and humans (Frances *et al*., 1992; Björkman *et al*., 1995; Bickel *et al*., 1996; Wu *et al*., 1997; Stain-Texier *et al*., 1999; Bouw *et al*., 2001; Meineke *et al*., 2002). The threefold lower permeability is further in agreement with the findings from human studies showing a reduced M6G potency relative to morphine by about a factor of 4 (Skarke *et al*., 2003).

The relatively slow permeation of M6G through the BBB was followed by a short time to 50% equilibration of the deep compartment (5.8 min) in comparison with morphine (10.3 min) (Upton *et al*., 2003). Similar results were found by Stain-Texier *et al*. (1999) using a microdialysis technique; they found time to maximum concentration in the cortex and whole brain to be only 15 min in rats. In addition, Björkman *et al*. (1995) showed that rapid and extensive brain uptake occurs within the first 5 min after intravenous administration of morphine, M3G and M6G in pigs. However, the ke_0s for onset/offset of morphine effect

Table 1 Model parameter estimates for brain kinetics of M6G in 5 sheep

Model	*V_1 (ml)*	*PS (ml/min)*	*V_2 (ml)*	*Residual error (% CV)*
Flow-limited				
Parameter value	14.5 (26%)	—	—	35 (14%)
Inter-animal variability (%CV)	29 (93%)	—	—	
Flow-limited with loss[a]				
Parameter value	14.5	2.2×10^{-9}	—	35
Inter-animal variability (%CV)	29	—	—	
Membrane-limited				
Parameter value	13.7 (27%)	2.5 (85%)	18.4 (50%)	35 (14%)
Inter-animal variability (%CV)	29 (90%)	—	—	

Data are the value of the population estimate, with data in parentheses indicating the % s.e. of the parameter estimate indicating the reliability of the parameter estimate.
[a]Covariance step failed and % SE of parameter estimates are not available.

estimated by Sarton *et al*. (2003) and for M6G effect estimated by Romberg *et al*. (2004) contrasts with the above findings and indicates that a very large part of the delay in analgesic effect is not related to passage across the BBB. This has also have been suggested by Bouw *et al*. (2001), but in relation to neuronal dynamics. In the present model, the brain tissue is a 'lumped' compartment. The various subregions of the brain such as ECF and CFS in which opioid receptors may vary in density are beyond the scope of the present work, as the methods used assess the global brain concentrations of the drug and therefore comparisons to results obtained from microdialysis techniques are not possible.

The sheep preparation employed is arguably more representative of human cerebral kinetics than other species, such as rodents, due to a similar $CBF\,g^{-1}$ tissue to humans (sheep $0.44\,ml\,min^{-1}$ per g brain (Upton *et al*., 1997a), humans $0.54\,ml\,min^{-1}$ per g brain). In contrast, rodents have $CBF\,g^{-1}$ tissue several-fold greater. As a consequence identification of a membrane limitation to brain entry will be markedly over-represented in small rodents as drug delivery to the brain is very high compared to a (presumably) similar permeability per gram tissue. As cerebral kinetics depends on the fundamental physiological properties of the brain and its relative blood flow, an animal model that has similar cerebral physiology to humans is an advantage.

Intravenous administration of morphine has been shown to induce analgesia faster than M6G in both animal (Milne *et al*., 1993; Illum *et al*., 1996) and human studies (Hanna *et al*., 1991; Osborne *et al*., 1992; Penson *et al*., 2002). However, the duration of the antinociceptive effects of M6G seems to be longer (Gardmark and Hammarlund-Udenaes, 1998) and it has been suggested that this longer duration of effect of M6G might partly be explained by its slower passage into and out of the brain compartment (Frances *et al*., 1992). Bouw *et al*. (2001) concluded from the results of their study that half of the delay in the effect of M6G can be explained by transport across the BBB, and suggested that the other half is due to drug distribution within brain tissue or rate limiting mechanisms at the receptor level. In the present study, the antinociceptive effect of M6G was not determined; however, the low permeablity coefficient found supports the slower onset of analgesia found by others.

Conclusion

The pharmacokinetic modelling of M6G showed a delayed equilibration between brain and blood of a nature that is primarily limited by permeability across the BBB, in accordance with its physico-chemical properties.

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Conflict of interest

The authors state no conflict of interest.

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