Laboratory Investigations

In vitro determination of human dura mater permeability to opioids and local anaesthetics

Richard F. McEllistrem MB FFARCSI,
Ronald G. Bennington BSc, Sheldon H. Roth MSc PhD

The permeability of human thoracic and lumbar dura mater to various compounds in clinical use was determined in vitro. Sections of dura mater, 3 to 4 cm in diameter, obtained at post-mortem were placed between the ports (area = 3 cm²) of two glass chambers (A and B) which fitted tightly together to form a two-chamber apparatus for measuring permeability through the dura. Each chamber contained 5 ml of artificial cerebrospinal fluid. A sample of test drug solution was introduced into one chamber (A) and 50 µl aliquots were withdrawn from the other chamber (B) at predetermined intervals. Permeability was determined by calculating the rate of diffusion (slope) from the plot of mean drug concentration (chamber B) versus time. Dura mater permeability was shown to be a simple diffusion process and to be independent of lipid-solubility and molecular weight. Permeability appeared to increase with age and may have a linear relationship to the initial concentration.

L'objectif de cette étude est d'établir in vitro la perméabilité de la dure-mère humaine à différents produits d'usage clinique

Key words

ANAESTHETICS, LOCAL: bupivacaine, lidocaine;

ANATOMY: dura;

ANALGESICS: fentanyl, morphine, nalorphine, sufentanil.

From the Departments of Anaesthesia, Pharmacology and Therapeutics, University of Calgary and Foothills Hospitals, 3330 Hospital Drive N.W., Calgary, Alberta, Canada T2N 4N1.

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Address correspondence to: Dr. Richard F. McEllistrem, Department of Anaesthesia, Regional Hospital, Limerick, Ireland.

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courant. Des sections de dure-mère de 3 à 4 cm de diamètre obtenues à l'autopsie sont placées entre les fenêtres (aire de surface = 3 cm²) de deux chambres de verre (A et B) réunies étroitement de façon à former un appareil à deux compartiments permettant la mesure de la perméabilité dure-mérienne. Chaque chambre contient 5 ml de liquide céphalo-rachidien artificiel. Un échantillon de la drogue-test est introduit dans la chambre A et des petites quantités de 50 µl sont retirées de l'autre chambre (B) à intervalles prédéterminés. La perméabilité est déterminée par le calcul de la vitesse de diffusion, en portant sur une courbe le tracé de la concentration moyenne de la drogue en rapport avec le temps. Les résultats montrent que la perméabilité de la dure-mère consiste en un simple processus de diffusion, indépendant de la solubilité lipidique et du poids moléculaire. La perméabilité semble augmenter avec l'âge et pourrait avoir une relation linéaire avec la concentration initiale.

Identification of specific opiate receptors in the brain and spinal cord and the subsequent demonstration that spinal (epidural and intrathecal) administration of opiates may lead to segmental spinal analgesia is the basis for epidural narcotic injection.¹⁻³ Studies in animals and humans have demonstrated recently that pre- and postsynaptic receptors in the substantia gelatinosa of the dorsal horn of the spinal cord may be a major site of action of spinal opioids.⁴ The epidural route of administration offers several advantages over direct intrathecal (cerebrospinal fluid) administration, such as accessibility to all spinal segments and the possibility of repeated doses through an indwelling catheter. However, following epidural injection, there are at least three competing processes: transfer across the meninges, vascular uptake, and distribution into extradural adipose tissue. In order to gain access to the cerebrospinal fluid epidural opioids must first penetrate the dura mater. Clinical studies have demonstrated

TABLE Permeability, diffusion rate constant, initial concentration, molecular weight and lipid solubility of the compounds studied on human thoracic and lumbar dura *in vitro*

Anaesthetic drug	Molecular weight (g)	Partition coefficient (25°C)*	Initial concentration (chamber A) ($\mu\text{g} \cdot \text{ml}^{-1}$)	Permeability mean $\pm$ (SE) ($\mu\text{g} \cdot \text{ml}^{-1} \cdot \text{min}^{-1}$)	Diffusion rate constant
Fentanyl	336	813.0	20	0.020 (0.002)	1.0×10^{-3}
Sufentanil	386	1778.0	20	0.009 (0.001)	4.5×10^{-4}
Morphine	285	5.8	100	0.115 (0.003)	1.15×10^{-3}
Morphine	285	5.8	1000	0.984 (0.050)	9.84×10^{-4}
Nalorphine	311	3.9	100	0.110 (0.005)	1.10×10^{-3}
Lidocaine	234	2.9	1000	1.024 (0.051)	1.02×10^{-3}
Bupivacaine	288	27.5	1000	0.943 (0.057)	9.43×10^{-4}

*Data from 13,14.

FIGURE 1 Schematic of the diffusion cell apparatus used for the *in vitro* permeability studies. The two chambers were held together with a spring locking clamp (not shown).

that opioid cerebrospinal fluid bioavailability following epidural injection is approximately 4%.^{5,6} The factors that determine opioid meningeal permeability are still unknown. Previous studies have reported conflicting data and it is still not known whether molecular weight, lipid solubility, or some unknown factor determines meningeal permeability to opioids.⁷⁻¹⁰ The objectives of our study were to quantify the permeability of dura mater to opioids and local anaesthetics used clinically, to evaluate the relative importance of this tissue to epidural analgesia, and to determine which physicochemical properties may be important in this transfer.

Methods

Human thoracic and lumbar dura mater was obtained at post-mortem from 13 subjects (male and female) aged 26 to 79 yr with no history of neurological disorder and in whom there was no reason to suspect damage to these membranes. The specimens were washed to remove any blood and then dry-frozen in plastic containers at -70°C until used. The dura mater was examined using a dissection microscope at $20\times$ power before and after each experiment to rule out damage to the membrane. The dura mater exposed to each compound was matched for age and site of excision (i.e., thoracic or lumbar).

Pieces of dura mater, 3 to 4 cm in diameter, were placed between the ports (area = 3 cm^2) of two glass chambers A and B (Figure 1). These chambers were fitted tightly together by means of a locking clamp and O-ring to form a tight seal. Each chamber contained 5 ml of artificial cerebrospinal fluid (CSF) at pH 7.4 for opioid compounds and pH 6.8 for local anaesthetic compounds to prevent precipitation. The chemical composition of the artificial CSF corresponded to that found *in vivo* ($\text{mmol} \cdot \text{L}^{-1}$): NaCl 134, KCl 4.5, CaCl_2 1.6, KH_2PO_4 1.25, MgSO_4 2, NaHCO_3 16, dextrose 10.¹¹

Specially prepared disposable Sylgard SEPTA were fitted to the ends of each chamber, held in place by black phenolic screw caps with open tops. A sample of test drug solution was introduced into chamber A at time zero and 50 μl aliquots were withdrawn from chamber B at predetermined intervals of 0, 20, 40, 60, 120, and 180 min using a Hamilton syringe. The contents were continuously mixed by means of a Burrell wrist action shaker (Burrell Scientific Inc, PA USA) and all experiments were performed at room temperature. Compounds selected for this study were opioids and local anaesthetics in clinical use which cover a relatively broad range of molecular weights and lipid solubility (Table).

Drug assay

Lidocaine and bupivacaine were analyzed using a Perkin Elmer series 4 high pressure liquid chromatograph (HPLC) fitted with a LC 85 ultraviolet-visible detector set at 215 nm and a 10 cm Whatman ODS 3 column with 5 μ packing. The buffer medium consisted of 40/60 phosphate buffer (0.01 M, pH 6.0)/acetonitrile pumped at a flow rate of $1 \text{ ml} \cdot \text{min}^{-1}$. The assay procedure was accurate to $\pm 1\%$ with precision $\pm 2\%$ and limits of detection at $1 \mu\text{g} \cdot \text{ml}^{-1}$.

Morphine and nalorphine were assayed by gas chromo-

tography (GC) using a Perkin Elmer series 10 GC fitted with a LC17 BAS electrochemical detector and Waters C18 Novapak column. A potential of 0.72 volts relative to a Ag/AgCl reference electrode was utilized for the detector. The mobile phase was 85% KH_2PO_4 (0.07M), hexane sulphuric acid (0.6 mM) and 15% methanol with a flow rate of $1.2 \text{ ml} \cdot \text{min}^{-1}$. The assay procedure was accurate to $\pm 2\%$ with precision $\pm 3\%$ and limits of detection at $10 \text{ ng} \cdot \text{ml}^{-1}$.

Fentanyl and sufentanil aliquots were placed into glass screw top ($16 \times 125 \text{ mm}$) centrifuge tubes containing 1.9 ml CSF plus internal standard. The opioid was extracted using an alkalinized hexane/ethanol mixture, evaporated to dryness using nitrogen. The residue was reconstituted into $200 \mu\text{l}$ ethylacetate, transferred to $300 \mu\text{l}$ autosampler conical vials, evaporated to dryness and finally reconstituted with $100 \mu\text{l}$ ethylacetate and sealed. Quantitative determinations of the extracted samples were performed using a Hewlett Packard GC/MS with autosampler. A DB-1 polymethyl siloxane ($30 \text{ cm} \times 0.25 \text{ mm}$ i.d. $0.25 \mu\text{M}$ film thickness) column was utilized with helium carrier gas ($40.8 \text{ cm} \cdot \text{sec}^{-1}$ at 200°C). Samples were injected at a purge rate of 0.5 min (spitless port) with temperatures of the injection port (280°C), transfer line (300°C), oven (initial 200°C , final 300°C at $20^\circ \cdot \text{min}^{-1}$ rate). The run time was ten minutes. The solvent delay of the mass spectral detector was five minutes. The electron multiplier was 300 volt relative and source temperature was $150\text{--}200^\circ\text{C}$. The assay procedure was accurate to $\pm 13.51\%$ with precision $\pm 14.07\%$ and limits of detection at $0.5 \text{ ng} \cdot \text{ml}^{-1}$.

Statistical analysis

Permeability of dura mater for each compound was determined by calculating the slope from plots of mean drug concentration data from chamber B at different time points. Linear regression analysis was utilized to determine the slope of the line relating concentration versus time data. Permeability is thus given as the slope of the regression. A diffusion rate constant for each compound was obtained by dividing the permeability by the initial concentration in chamber A. Statistical analysis was performed using Student's *t* test.¹² A *P* value < 0.05 was considered statistically significant.

Results

Initial concentrations of compounds injected into chamber A were within clinical concentrations. The correlation coefficient (*r*) for regression used to determine the permeability of each opioid through the dura mater was at least 0.90 in each case.

Ten experiments were performed using fentanyl and

FIGURE 2 Mean fentanyl drug concentration ($\pm$ SE) measured in Chamber B as a function of time, for age 26 yr (O) and age 51–53 yr (Δ). The slope of the line represents permeability.

eight experiments using sufentanil. Although sufentanil is twice as lipid-soluble as fentanyl, sufentanil and fentanyl exhibited similar dural permeability and diffusion rate constants when losses due to adsorption are taken into account (Table).

Twenty-one experiments were performed using morphine and nalorphine. Dura mater permeability to morphine and nalorphine was not different. Permeability of dura mater to morphine is concentration-dependent; increasing the initial concentration by a factor of ten ($0.1 \text{ mg} \cdot \text{ml}^{-1}$ to $1.0 \text{ mg} \cdot \text{ml}^{-1}$) produced approximately a ten-fold increase in permeability ($0.115 \mu\text{g} \cdot \text{ml} \cdot \text{min}^{-1}$ to $0.984 \mu\text{g} \cdot \text{ml} \cdot \text{min}^{-1}$).

Eighteen experiments were performed using lidocaine and bupivacaine. Permeability of dura mater to lidocaine and bupivacaine were not different although bupivacaine is ten times more lipid-soluble than lidocaine.

Dura permeability to fentanyl adjusted for the age of the dura was determined. Six experiments were performed using dura mater from one subject aged 26 yr and four experiments were performed using dura mater from subjects aged 51 to 53 yr. Permeability of dura to fentanyl was greater for older subjects than it was for younger subjects indicating that permeability may be a function of age ($P < 0.005$) (Figure 2).

Discussion

The transfer of opioids across the dura mater into the cerebrospinal fluid is the first step in the production of spinal analgesia following epidural opioid injection. The pharmacokinetic pattern of that transfer is fundamental in determining the time course of epidural analgesia. Data from this study have demonstrated that (1) dural transfer is a simple diffusion process, (2) permeability appears to be independent of lipid solubility and molecular weight, (3) permeability increases with age, and (4) dural permeability to morphine has a linear relationship to the initial concentration.

We developed a modified *in vitro* model of human dura mater permeability to study the pharmacokinetic behaviour of opioid transfer from the epidural space into the cerebrospinal fluid (CSF).⁷ Our model might be criticized because all experiments were performed at room temperature rather than at 37°C. However, although dura mater permeability increased with temperature increase, we would expect an increased temperature to affect all compounds in a similar fashion.⁷ In addition, we assumed that drugs gained access to the CSF solely by passive diffusion through the dura and arachnoid mater, although arachnoid permeability was not measured independently.

It has been postulated that epidural opioids may gain access to their site of action at the dorsal horn of the spinal cord by diffusion through the dura mater, via the arachnoid granulations in the dural cuff region, and following rapid uptake into the dorsal radicular branch of the spinal segmental arteries.¹³ Arachnoid granulations and villi are most numerous in relation to the superior sagittal sinus but they have also been described in the spinal arachnoid mater.¹⁵ Arachnoid granulations are the major site of fluid transfer from the subarachnoid space to the venous system and they appear to function as passive, pressure-dependent one-way-flow valves.¹⁵ It is therefore unlikely that epidural drugs would gain access to the CSF via this route. The spinal arteries in association with spinal nerves penetrate the dura mater and divide into dorsal and ventral branches accompanying the corresponding nerve roots.¹⁶ The dorsal radicular arteries although relatively small, 0.2 to 0.5 mm in diameter, are normally numerous superior to the eighth cervical segment and caudal to the fourth thoracic segment and lumbar epidural opioids may gain rapid access to dorsal horn via this route.¹⁶

Epidural drugs are separated from the CSF by two layers of meninges, dura and arachnoid mater. A recent publication has suggested that the principal barrier to diffusion following epidural administration in dogs and monkeys is the pia-arachnoid mater.¹⁷ To gain access to the CSF, epidural compounds must diffuse through the dura and arachnoid mater. The cranial arachnoid is a

delicate nonvascular membrane between the dura mater and CSF and is made up of leptomeningeal cells. In humans the spinal arachnoid becomes a very distinct membrane and is unlikely to pose an important barrier to diffusion.¹⁵

Comparison of the results must take into account the quantities of lipid-soluble opioids that may be adsorbed into the dura mater itself and onto the glass chamber over the 180-minute sampling time. The highly lipid-soluble opioid, sufentanil, appeared to be adsorbed by dura mater and glass in significantly greater amounts (42%) than fentanyl (18%); other compound demonstrated minor degrees of adsorption of 5.0% or less.

Our data demonstrate that dura mater permeability appeared to be independent of lipid solubility because morphine, fentanyl and sufentanil, which have very different lipid solubilities yielded similar diffusion rate constants. The opioids and local anaesthetic compounds we utilized in this study have similar molecular weights, therefore we were unable to demonstrate a relationship between molecular weight and dural permeability. The diffusion rate constant for all compounds was similar indicating little discrimination by the dura mater.

Our study indicates that permeability of human dura mater to opioids and local anaesthetics is a simple diffusion process. These results could be explained by examining the structure of the dura mater. The spinal dura mater is a continuation of the meningeal layer of the cranial dura.¹⁵ It is not a lipid barrier like the cell membrane but a compact structure composed of branching lamellae enclosing clefts.¹⁸ An abundance of ground substance exists between these lamellae which may account for the ready passage of drugs through the human dura mater.¹⁸ Structural changes in the dura mater associated with aging may account for the increased dural permeability demonstrated in our data.

What are the clinical implications of our findings? Intrathecal potency of opioids is related inversely to lipid solubility.¹⁹ Dura mater permeability is independent of lipid solubility and less lipid soluble opioids such as morphine diffuse through the dura mater as rapidly as the more lipophilic agents fentanyl and sufentanil. Epidural morphine is not adsorbed as rapidly into the epidural venous plexus, nor is it redistributed as quickly into epidural adipose tissues as the more lipophilic opioids, resulting in increased morphine availability for transfer across the dura mater. Therefore epidural morphine may have increased potency at the site of action and may also have increased CSF bioavailability following injection. Clinically, the epidural dose of highly lipid-soluble opioids may have to be surprisingly high to produce spinal analgesia comparable to 5 mg of epidural morphine.

In conclusion, we have demonstrated that the permea-

bility of human dura mater *in vitro* to opioids and local anaesthetics is independent of lipid solubility and molecular weight but may depend on the initial concentration of the drug.

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