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The Spinal Nerve Root Sleeve Is Not a Preferred Route for Redistribution of Drugs from the Epidural Space to the Spinal Cord

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It has been frequently suggested that the spinal nerve root sleeve is a preferred route for redistribution of drugs from the epidural space to the spinal cord. To determine if this supposition is true, the authors measured the rate at which morphine, fentanyl, and lidocaine diffuse through dog and monkey meningeal specimens with and without a root sleeve. Two meningeal specimens of intact dura–arachnoid–pia mater were removed from each animal and placed in separate temperature-controlled diffusion cells. One specimen included a spinal nerve root sleeve; the other did not. The permeability of the tissues to each drug was then determined by placing the study drug in one of the reservoirs of the diffusion cell and measuring the rate at which the drug diffused through the tissue and accumulated in the second reservoir. There was no difference in permeability between specimens with and without a nerve root sleeve for any drug in either species. Lidocaine was found to diffuse through the tissue significantly faster than fentanyl in both the dog and monkey even though fentanyl is nearly 48 times more lipid soluble than lidocaine. Morphine diffused through the tissue significantly slower than both lidocaine and fentanyl. The authors conclude that the spinal nerve root sleeve is not a preferred route of entry for drugs moving from the epidural space to the spinal cord. In addition, despite hypotheses to the contrary, lipid solubility does not appear to be the overriding determinant of meningeal permeability. (Key words: Analgesics, opioids: morphine; fentanyl. Anesthetics, local: lidocaine. Spinal cord, meninges: dura mater; arachnoid mater; permeability; pia mater. Spinal cord: spinal nerve root sleeve.)

ANALGESIC DRUGS that have sites of action in the spinal cord (*e.g.*, opioids and α-agonists) are commonly administered *via* the epidural space. In order for epidurally administered drugs to reach the spinal cord, they must cross the spinal meninges—dura, arachnoid, and pia mater. There are three routes commonly proposed for redistribution of drugs from the epidural space to the spinal cord—diffusion through the meninges, diffusion through spinal nerve root sleeves, and diffusion into radicular arteries with subsequent transport to the spinal cord.[1–3]

The idea that drugs might diffuse preferentially through the spinal nerve root sleeve is based on studies that suggest that the root sleeve may be a weak link in the diffusion barrier presented by the meninges. Anatomically, the root sleeve is composed of dura mater and arachnoid mater with arachnoid villi penetrating the dura mater to lie either freely in the epidural space or within epidural veins[4] (fig. 1). Physiologic studies have demonstrated that colloidal particles and red blood cells placed within the cerebrospinal fluid (CSF) can exit the subarachnoid space by way of the arachnoid villi.[5,6] These observations led to speculation that drugs might move from the epidural space to the spinal cord by the same mechanism. However, there have been no studies aimed at quantifying the contribution of the root sleeve to the net redistribution of any drug from the epidural space to the spinal cord.

The authors have developed a reliable *in vitro* method for quantifying the diffusion of drugs across fresh dog and monkey meningeal tissue.[7] In the current study we have used this method to determine whether or not the spinal nerve root sleeve does contribute disproportionately to the net flux of drugs across the meninges.

Materials and Methods

Studies were approved by the University of Washington Animal Care Committee, and American Association for Laboratory Animal Care guidelines were followed throughout.

TISSUE SOURCES

Monkey tissue (*Macaque nemestrina*) was obtained from animals scheduled to be killed as part of the tissue distribution program of the University of Washington Regional Primate Research Center. All meningeal specimens were removed from animals anesthetized with thiopental/ketamine and without muscle relaxants. Animals of both sexes weighing 6–23 kg were used.

Dog tissue was obtained from animals used in acute experiments by other investigators. None of these acute experiments involved the spinal cord, meninges, or administration of drugs used in this study (morphine, fen-

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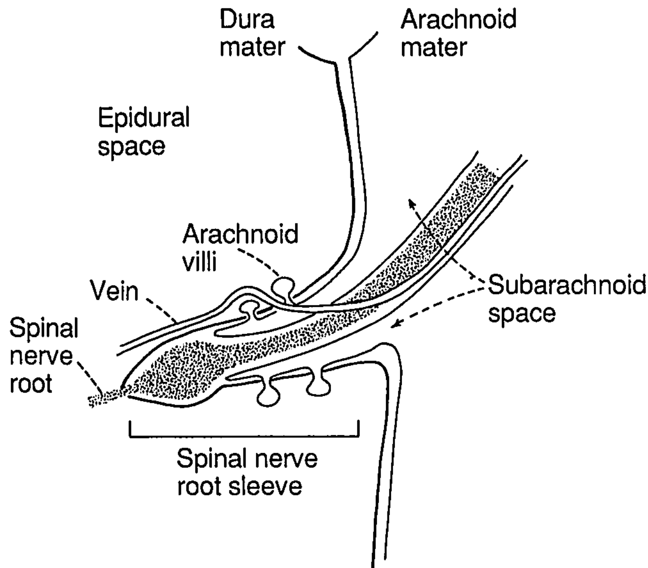

FIG. 1. Spinal nerve root sleeve histology. Arachnoid villi protrude through dura mater either to lie freely in the epidural space or to invaginate into epidural vein. The subarachnoid space is continuous with the arachnoid villi. (Based on data from Welch and Pollay, 1963.)

tanyl, and lidocaine). Tissue was removed from these animals anesthetized with halothane 1–2%–nitrous oxide 66%. Animals of both sexes weighing 14–20 kg were used.

EXPERIMENTAL SET-UP

Tissue Preparation

Spinal cords of five *M. nemestrina* monkeys and eight mongrel dogs were exposed from T5 to L5 by multilevel laminectomy. The cord was removed *en bloc* and an incision was made along the ventral surface through all three meningeal layers (dura, arachnoid, and pia mater). Together the dura, arachnoid, and pia mater were carefully reflected from the spinal cord, preserving their normal anatomic relationships. Two adjacent specimens (each approximately 1 cm^2) of meningeal tissue (intact dura, arachnoid, and pia mater) were obtained from each animal and mounted in diffusion cells. One specimen from each animal included the associated nerve root and root sleeve; the other specimen did not. The meningeal tissue used for these experiments came from the T12–L1, L1–L2, or L2–L3 cord segments in all cases.

Meningeal specimens from each animal (one with a root sleeve and one without) were placed in separate temperature controlled diffusion cells with a 0.785-cm^2 connecting port between the two reservoirs of each cell (fig. 2). Ten milliliters bicarbonate-buffered mock CSF (*p*H = 7.38–7.42; 295 mOsm) was placed in the fluid reservoirs on either side of the meningeal tissue. Each fluid reservoir was vigorously stirred by a magnetic stirrer to minimize unstirred layer effects.[8] Oxygen (95%) and carbon dioxide (5%) were bubbled through each fluid reservoir to maintain normal *p*H and to oxygenate meningeal cells. Mock CSF contained 72 mg/dl glucose to meet energy requirements of the meningeal cells. We allowed 20 min for the chambers to equilibrate to 37° C before beginning any experiments.

Flux Measurements

The fluxes of morphine, lidocaine, and fentanyl were determined across dog meninges, while only fentanyl and lidocaine fluxes were measured across monkey meninges. The flux of each drug was determined using radiolabeled drugs (^{3}H-morphine: specific activity = 50 Ci/mmol, radiochemical purity = 99%; ^{3}H-fentanyl: specific activity = 11.7 Ci/mmol, radiochemical purity = 98.6%; and ^{14}C-lidocaine: specific activity = 42 mCi/mmol, radiochemical purity = 98.3%). Morphine and lidocaine were purchased from New England Nuclear; fentanyl was a gift from Janssen Biochimica/Biotech. Morphine flux was measured alone, whereas lidocaine and fentanyl flux were measured simultaneously. (It is possible to measure the flux of both drugs simultaneously because ^{3}H-fentanyl and ^{14}C-lidocaine can be distinguished from one another by the β counter used for this study.) To verify that the substance measured by this radiotracer method was in fact the study drug, we repeated each experiment once and measured drug concentration by both liquid scintillation counting and gas chromatography–mass spectroscopy (fentanyl), high-performance liquid chromatography–electroconductivity detection (morphine), or gas chromatography–nitrogen/phosphorous detection (lidocaine).

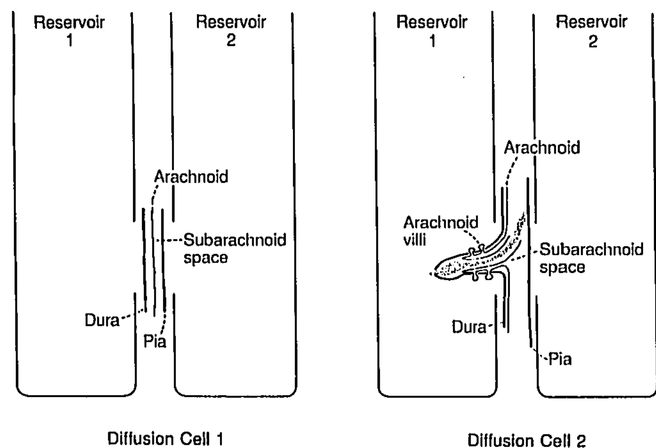

FIG. 2. Diffusion cells used to determine drug flux. Fluid reservoirs of diffusion cell 1 are separated from one another by meningeal specimen without nerve root sleeve (intact dura, arachnoid, and pia mater). Fluid reservoirs of diffusion cell 2 are separated by a meningeal specimen (dura, arachnoid, and pia mater) that includes a spinal nerve root sleeve. The root sleeve is drawn larger than scale for purposes of illustration.

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The order of drug flux measurement (*i.e.*, morphine first or lidocaine–fentanyl first) was the same for both meningeal specimens from the same animal but was randomized across animals.

At time zero the study drug(s) (0.1-mg morphine base and 2.5 μCi ^{3}H-morphine, or 10-μg fentanyl base and 2.5 μCi ^{3}H-fentanyl; and 0.4-mg lidocaine base and 5.0 μCi ^{14}C-lidocaine) was added to the fluid reservoir on the dura mater side (reservoir 1) of each tissue specimen. Thereafter, at 5-min intervals for 45 min, 200-μl samples were removed from reservoir 1 and reservoir 2 (on the pia mater side of the diffusion cells) and placed in borosilicate glass vials for later β counting. Immediately after the first flux experiment, the diffusion cells were thoroughly rinsed five times with 15-ml volumes of mock CSF to remove any residual drug(s), and the experiment was repeated with the second drug(s).

Calculation of Permeability

Flux was determined by plotting the drug concentration in reservoir 2 *versus* time. The slope of the line through these data points was determined by linear regression and is equal to the test drug's flux through the meninges. Permeability was then calculated from the equation:

$$P = Q_t/[C_1 - C_2]_t \cdot A$$

where P = permeability (centimeters per min); Q_t = flux at any time t (micrograms per minute); $[C_1 - C_2]$ = concentration gradient across the meninges at any time t (micrograms per milliliter); and A = cross-sectional area of meninges available for diffusion, *i.e.*, area of the port connecting the two halves of the diffusion cell (centimeters squared).

All experiments were conducted over a time period during which the concentration gradient of the drug under study decreased by less than 3% from its value at time zero. This condition assures that flux is predominantly unidirectional and that the concentration gradient across the tissues is nearly constant throughout the experiment. As a result, the concentration gradient across the tissues can at all times be closely approximated by the initial concentration gradient at time = 0 (*i.e.*, $C_1 - C_2 = C_1$).

Therefore permeability can be calculated from the equation:

$$P = Q/C_1 \cdot A$$

The permeability of intact dura–arachnoid–pia mater specimens, with and without a spinal nerve root sleeve, was then estimated for each drug from 1) the experimentally determined drug flux, 2) the measured initial drug concentration in reservoir 1, and 3) the measured port area (0.785 cm^2) connecting the two reservoirs.

Drug Analysis

All samples were placed in borosilicate glass vials containing 5–10 ml Hydrofluor® scintillation fluid. Samples were counted in a Packard liquid scintillation counter (Tri-Carb 2000) for 40 min or until the standard deviation of depurations per minute was ≤ 2%. Background counts from mock CSF without any radioactivity were subtracted from total depurations per minute to obtain corrected depurations per minute.

Statistical Analyses

For each drug, differences in permeability between meningeal specimens with and without a spinal nerve root sleeve were evaluated for statistical significance by an unpaired Student's *t* test. Differences between drugs with respect to the rate at which they penetrated meningeal specimens were also assessed by an unpaired Student's *t* test. Differences were considered significant at the $P \leq 0.05$ level. All experimental results are reported as mean ± standard deviation.

Results

Data from parallel drug concentration measurements (liquid scintillation counting *vs.* chromatographic methods) were analyzed by linear regression to determine that the drug concentration measured by liquid scintillation counting reflected the study drug's true concentration and not that of other chemicals (*e.g.*, radiation fragments). The resultant regression line slopes and coefficients of determination (r^2) were: morphine slope = 1.025, r^2 = 0.943; fentanyl slope = 1.11, r^2 = 0.977; and lidocaine slope = 0.89, r^2 = 0.994. These results indicate good agreement between the radiotracer method used throughout the study and the appropriate chromatographic method.

Figure 3 shows representative plots of depurations per minute per sample at each sample time for all three study drugs. Depurations per minute was converted to micrograms of drug, and the regression line through the resultant data points was used to determine drug flux (micrograms per minute). The coefficients of determination (r^2) for regression lines used to calculate the flux of each drug through each study tissue averaged 0.970 ± 0.031 with a range of 0.81–0.998.

Table 1 lists the permeability coefficients for morphine, fentanyl, and lidocaine through meningeal tissue with and without a spinal nerve root sleeve. There was no significant difference between specimens with and without a nerve root sleeve with respect to the penetration rate of any drug in either species. Therefore, we averaged the two meningeal permeability coefficient measurements for each drug in each animal in order to compare differences

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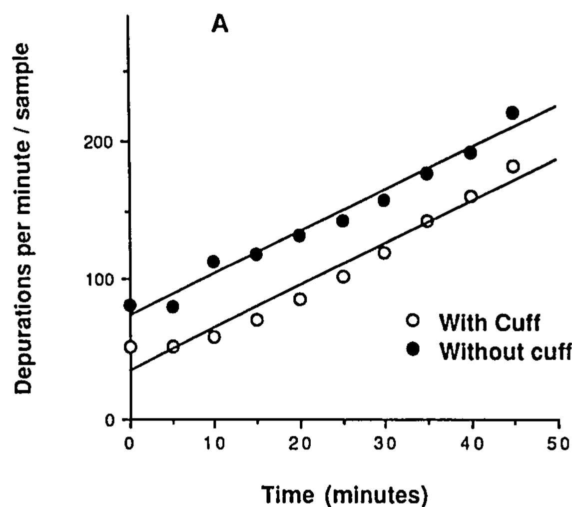
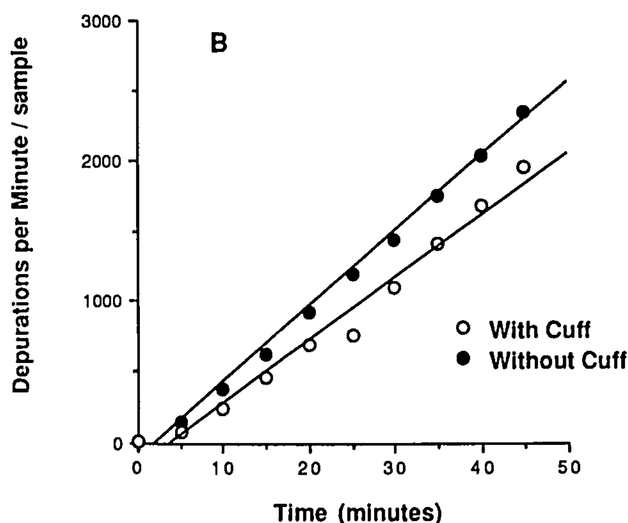
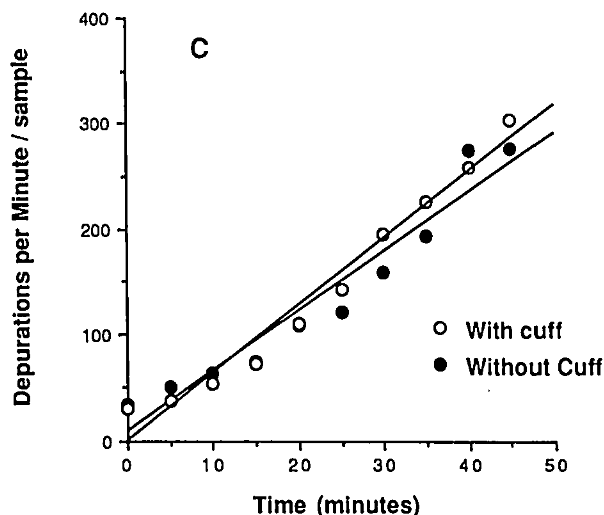


FIG. 3. Representative plots of depurations per minute *versus* time. Each data point represents a single 200-μl sample. Each plot shows the depurations per minute *versus* time data for tissue specimens with and without a root cuff taken from the same dog. The slope of the resultant regression lines were used to determine drug flux. *A*: Morphine. *B*: lidocaine. *C*: fentanyl.





in permeability coefficients between drugs (fig. 4). Lidocaine was significantly more permeant than fentanyl through monkey meninges (lidocaine: 1.45 ± 0.15 cm/min × 10^{-3}; fentanyl: 0.89 ± 0.14 cm/min × 10^{-3}). In the dog, lidocaine was also significantly more permeant than fentanyl, and morphine was significantly less permeant than the other two drugs (lidocaine: 2.61 ± 0.57 cm/min × 10^{-3}; fentanyl: 1.91 ± 0.71 cm/min × 10^{-3}; morphine: 0.76 ± 0.41 cm/min × 10^{-3}).

The order in which each drug's permeability coefficient was measured through dog meninges (*i.e.*, morphine first or lidocaine–fentanyl first) had no effect on the results. Lidocaine's permeability coefficient averaged 2.52 ± 0.77 × 10^{-3} cm/min when measured first and 2.71 ± 0.39 × 10^{-3} cm/min when measured second, $P > 0.05$. Fentanyl's permeability coefficient averaged 2.05 ± 0.98 × 10^{-3} cm/min when measured first and 1.77 ± 0.41 × 10^{-3} cm/min when measured second, $P > 0.05$. Morphine's permeability coefficient averaged 0.59 ± 0.49 × 10^{-3} cm/min when measured first and 0.94 ± 0.25 × 10^{-3} cm/min when measured second, $P > 0.05$.

Discussion

The data indicate that the spinal nerve root sleeve does not contribute disproportionately to the diffusion of morphine, lidocaine, or fentanyl across the meninges of the dog or monkey. That this is true for three drugs with different physicochemical properties (*e.g.*, lipid solubility and molecular weight), suggests that the nerve root sleeve is not important for the meningeal transfer of any drug. In addition, the fact that our results were qualitatively the same (with respect to the relative rate of drug transfer and the absence of a contribution by the root sleeve) in two very different mammalian species suggests that these findings should be qualitatively applicable to humans.

Arachnoid villi, like the remainder of the arachnoid, are composed of flattened cells connected to one another

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TABLE 1. Permeability Through Meninges With and Without Spinal Nerve Root Sleeve

	Permeability (cm/min × 10^{-3})		
	Meninges With Root Sleeve	Meninges Without Root Sleeve	Significance
Dog			
Lidocaine	2.39 ± 0.66	2.84 ± 0.54	NS
Fentanyl	1.64 ± 0.65	2.18 ± 1.06	NS
Morphine	0.74 ± 0.54	0.79 ± 0.45	NS
Monkey			
Lidocaine	1.4 ± 0.28	1.5 ± 0.36	NS
Fentanyl	0.92 ± 0.20	0.85 ± 0.29	NS

Values are mean ± SD. n = 8 for all measurements in dog tissue; n = 5 for all measurements in monkey tissue.
NS = not significant.

by tight junctions.[9,10] Physiologically, arachnoid villi transport CSF (and any substance dissolved or suspended in CSF) from the subarachnoid space to sites outside of the central nervous system.[11] This fact has prompted speculation that the arachnoid villi might be a preferred route by which epidural drugs reach the CSF and thence the spinal cord. Our finding that this is not the case has several likely explanations. First, under conditions of normal CSF pressure, transport of CSF across arachnoid villi takes place by micropinocytosis. Micropinocytotic vesicles are formed at the subarachnoid space with subsequent transcellular movement away from the subarachnoid space and into structures lying outside of the dura mater.[11-14] Since this process moves material out of and not into the CSF, it is not possible for drugs to pass from the epidural space to the subarachnoid space by this mechanism. In pathologic situations with increased CSF pressure (*e.g.*, > 650 mmHg), the cell membranes of the arachnoid villi have been shown to invaginate to produce transcellular pores that allow bulk flow of CSF out of the subarachnoid space.[11] This mechanism would not be expected to facilitate redistribution of drugs from the epidural space to the subarachnoid space in the usual clinical setting because it occurs only with increased CSF pressure. In addition, even if transcellular pores were present at normal CSF pressures, bulk flow of CSF would be down the hydrostatic pressure gradient from the subarachnoid space to the epidural space, and this would oppose retrograde movement of drug molecules from the epidural space.

The lack of a significant effect of the spinal nerve root sleeve may also be due to the fact that drug flux across the meninges is proportional to the surface area available for diffusion. The surface area of the arachnoid villi comprises only a very small fraction of the meningeal surface area available for diffusion. Therefore, the root sleeve would be expected to contribute proportionately less to overall drug flux.

Our knowledge of which physicochemical properties of drug molecules (*e.g.*, molecular weight and lipid solubility) govern their rate of meningeal diffusion is incomplete. Speculation has long centered on the primacy of lipid solubility in controlling the rate of drug flux across the meninges. However, our results would suggest that this is not the case. The octanol–water distribution coefficients (*i.e.*, the partition coefficient measured at *p*H 7.4, 37° C) of the drugs we studied are 1.17 (morphine),[15] 110 (lidocaine),[16] and 955 (fentanyl),[15] whereas their relative permeabilities through dog meninges in this study were 1 (morphine), 2.5 (fentanyl), and 3.4 (lidocaine). Clearly, lipid solubility, as measured by the octanol–water partition coefficient, is not the principal determinant of meningeal permeability. This is consistent with our previous results with monkey meninges showing that alfentanil was only 3.7 times more permeant than morphine even though alfentanil's octanol–water partition coefficient is 130 times greater than that of morphine.[7] Physicochemical properties, other than lipid solubility, that govern a drug's meningeal diffusion rate have not been delineated, but possible candidates include molecular weight and molecular radius.

A possible criticism of our meningeal permeability model would be that the tissues are not stable *in vitro* and, consequently, that differences in permeability coefficients between drugs are related to changes in the tissue over time and not to real differences between the drugs. Clearly, that is not the case for differences between fentanyl and lidocaine, since their permeability coefficients were measured simultaneously through the same tissue specimens in each case. In addition, the order in which the permeability coefficients of these drugs was measured did not significantly affect the results, further indicating

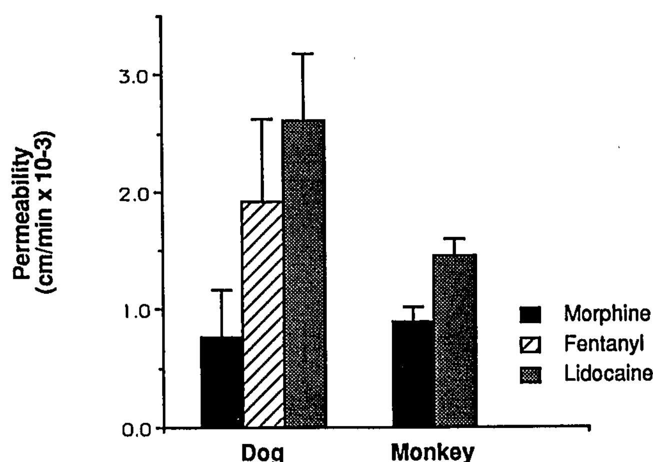

FIG. 4. Permeability coefficients for morphine, fentanyl, and lidocaine through dog and monkey meninges. The permeability coefficient for each drug was significantly different ($P < 0.05$) than that of the other drug(s) tested in that species.

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that the tissue is stable over time. Finally, in our previous study using this model we demonstrated that there was no difference in morphine permeability coefficient measurements made several hours apart in the same tissue.[7] We conclude, therefore, that the differences in permeability coefficients between drugs reported here reflect true differences in diffusion rates and not changes in the meningeal specimens over time.

A second criticism of our model might be that arachnoid villi do not function *in vitro* as they do *in vivo*. However, Welch and Pollay have demonstrated that particulate matter and red blood cells do move through arachnoid villi *in vitro* just as they do *in vivo*.[17]

In summary, we have used an *in vitro* model of meningeal permeability to evaluate the possibility that the spinal nerve root sleeve is a preferential route for diffusion of morphine, fentanyl and lidocaine across the spinal meninges. We found that the nerve root sleeve did not contribute disproportionately to the meningeal permeability of any of the drugs tested in either the dog or monkey. In addition, our results provide further evidence that the lipid solubilities of drugs may not correlate well with their meningeal diffusion rates.

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