Effect of Needle Puncture on Morphine and Lidocaine Flux through the Spinal Meninges of the Monkey In Vitro

Implications for Combined Spinal-Epidural Anesthesia

Christopher M. Bernards, M.D.,* Dan J. Kopacz, M.D.,† Maher Z. Michel, M.B., Ch.B.‡

Background: Combined spinal–epidural anesthesia is a technique growing in popularity. However, there have been no attempts to investigate the risk of epidural drug reaching the subarachnoid space in high concentration by passing through the meningeal hole left by the spinal needle. This study begins to address this question by quantitating the flux of morphine and lidocaine through the spinal meninges of the monkey in vitro after puncture with three different-sized needles.

Methods: Spinal meningeal tissue from anesthetized monkeys was mounted in a diffusion cell and drug flux was measured through intact tissue and through tissue punctured with a 27-G Whitacre, a 24-G Sprotte, and an 18-G Tuohy needle.

Results: The flux of morphine through the meningeal tissue was significantly increased by puncture with each of the study needles. The flux of lidocaine was significantly increased only by puncture with the 24-G Sprotte and 18-G Tuohy needles. The flux of morphine through intact tissue was less than the flux of lidocaine through intact tissue. In contrast, the flux of morphine and lidocaine were the same through tissue punctured with the study needles. The magnitude of the drug flux through the needle puncture was a function of the diameter of the study needle.

Conclusions: Epidural anesthesia after accidental or intentional puncture of the spinal meninges has occasionally resulted in high spinal blocks and total spinal anesthesia. This study suggests that drug movement through the meningeal hole is responsible for this complication and that the risk may be decreased by using the smallest possible needle to puncture the meninges. (Key words: Anesthetic techniques; combined spinal–epidural. Anesthetics; local: lidocaine. Anesthetics, opioid: morphine. Complications: dural puncture. Equipment, needles: Tuohy; Sprotte; Whitacre.)

COMBINED spinal–epidural anesthesia (CEA) is a technique by which a spinal block and an epidural catheter are placed simultaneously in the same patient. This technique is growing in popularity because the spinal block produces a rapid onset of anesthesia, while the epidural block allows the flexibility to extend or prolong the block intraoperatively and to provide epidural analgesia postoperatively.1–5

However, there is a potential risk that the meningeal hole made by the spinal needle may allow dangerously high concentrations of subsequently administered epidural drugs to reach the subarachnoid space. Numerous clinical reports of high or total spinal block during epidural anesthesia in which an accidental “wet-tap” preceded successful epidural placement lend credence to this concern.4–6 as does a recent report of cardiorespiratory arrest after CSEA for cesarean section.7

The goal of this study was to determine if meningeal puncture results in a significant increase in transmeningeal flux of epidurally administered drugs. To address this question, we used a well-controlled in vitro meningeal permeability model to quantitate the flux of lidocaine and morphine through the spinal meninges of the monkey before and after puncture with an 18-G Tuohy needle, 24-G Sprotte needle, and a 27-G Whitacre needle.

Materials and Methods

Monkey tissue (Macaque nemestrina) was obtained from 4–7 kg animals of both sexes scheduled to be

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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.

**Tissue Preparation**

Spinal cords from *M. nemestrina* monkeys were removed en block and an incision was made simultaneously through all three meningeal layers (dura, arachnoid and pia) along the ventral surface of the spinal cord. Together, the dura, arachnoid and pia mater were carefully reflected from the spinal cord preserving their normal anatomic relationships. The meningeal tissue used for these experiments came from the L1–L2 or L2–L3 cord segments and did not include nerve roots or portions of dural cuff.

All three meninges, in their normal anatomic relationships, were placed between two halves of a temperature controlled diffusion cell with a 0.785-cm² connecting port. Ten milliliters bicarbonate-buffered mock cerebrospinal fluid (pH 7.38–7.42; 295 mOsm) was placed in fluid reservoirs on both sides of the meningeal tissue. For control studies with intact tissue, oxygen (95%) and carbon dioxide (5%) were bubbled through each fluid reservoir throughout the experiment to maintain normal pH and to oxygenate meningeal cells. For tissues punctured with one of the study needles, oxygen (95%) and carbon dioxide (5%) were bubbled through the fluid reservoirs for 10 min to saturate the mock cerebrospinal fluid; the reservoirs then were covered with porous tape, and 95% O₂ and 5% CO₂ were blown over the mock cerebrospinal fluid to maintain oxygen and carbon dioxide saturation. This was done to prevent excessive stirring, which might lead to bulk flow of study drug through the puncture site.

**Flux Measurements**

Experiments using intact tissue (n = 6) and experiments using punctured tissue (n = 6) were performed in specimens from different animals. For the meningeal puncture studies, three pieces of tissue were obtained from each animal and were mounted in three different diffusion cells. Thus we were able to study all three needles simultaneously in tissue from the same animal. With the tissue mounted in the diffusion cell, it was punctured with the study needle inserted perpendicular to the long axis of the tissue. The Tuohy needle was inserted with its bevel perpendicular to the long axis of the tissue.

After mounting the tissue, we allowed 20 min for the chambers to equilibrate to 37°C. For both control and meningeal puncture studies, the study drugs and a radioactively labeled tracer were added to the fluid reservoir on the dura mater side of the diffusion cell at time zero: morphine sulfate, 2.5 mg base and ³H-morphine 0.0075–0.015 μCi (specific activity 62 Ci/mmol and radiochemical purity 99%) and lidocaine hydrochloride, 2.9 mg base and ¹⁴C-lidocaine 0.008–0.016 μCi (specific activity 42 mCi/mmol and radiochemical purity 98.3%). Thereafter, at 5-min intervals for 45 min, 100-μl samples were removed simultaneously from the donor reservoir on the dura mater side of the tissue and from the recipient reservoir on the pia mater side of the diffusion cell. Samples were collected into borosilicate glass scintillation vials for later scintillation counting.

Flux was determined by measuring the amount of drug in the recipient reservoir at each sample time. The amount of drug in the recipient reservoir was divided by the amount of drug in the donor reservoir to determine the fraction of the drug from the donor reservoir that had diffused through the tissue at each time point. These data were then plotted versus time and the best fit line through the data points was determined by the method of least squares. The slope of this line is equal to the drug's flux, expressed as percentage per minute. The flux was then divided by the surface area of the tissue to yield the flux per squared centimeter (percentage per minute per squared centimeter). Surface area is equal to the area of the port connecting the two fluid reservoirs. By expressing flux as a percentage of the amount of drug in the donor reservoir, we are able to compare the relative flux rate of morphine and lidocaine even though they are used clinically in very different concentrations.

**Drug Analysis**

All samples were placed in borosilicate glass vials containing 5–10 ml Hydrofluor scintillation fluid (National Diagnostics, Manville, NJ). Samples were counted in a Packard liquid scintillation counter (Tri-Carb 2000, Downers Grove, IL) for 30 min or until the standard deviation of depositions per minute was less than 2%. This scintillation counter is able to distinguish between ³H and ¹⁴C present in the same sample. Background counts from mock cerebrospinal fluid without any radioactivity were subtracted from total depositions per minute to obtain corrected depositions per minute.

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Statistical Analysis
Differences in transmeningeal drug flux between intact tissue (control) and tissue punctured with the study needles were determined by one-way analysis of variance. Student's unpaired t test was used for post hoc testing. Bonferroni's correction for multiple comparisons was applied, and a P value less than 0.017 was considered statistically significant. One-way analysis of variance was also used to determine if the relationship between drug flux and needle diameter was statistically significant.

Results
The determination coefficients ($r^2$) for linear regression lines used to determine drug flux were in each case greater than 0.869 indicating a good fit of the data to a linear model. The between-group analysis of variance yielded a P value of 0.0001 for each drug indicating a significant effect of needle puncture on drug flux.

Table 1 shows the experimentally determined flux of lidocaine and morphine through intact tissue and through tissue punctured with each of the study needles. Meningeal puncture with each of the needles significantly increased the flux of morphine and the increase in flux was greater for larger needle diameters. The flux of lidocaine was not increased significantly by the 27-G Whitacre needle, but was increased significantly by puncture with the 24-G Sprotte and the 18-G Tuohy needles. As with morphine, the larger diameter Tuohy needle increased flux more than did the smaller diameter Sprotte needle.

Drug flux through the meningeal hole made by each needle was calculated by subtracting the average drug flux through intact tissue from drug flux through tissue punctured with each of the study needles. Figure 1 plots the relationship between drug flux through the needle hole and needle diameter for both morphine and lidocaine. A linear regression equation was fit to each set of data points and resulted in nearly identical equations with determination coefficients of 1.0 and 0.999 (fig. 1). This indicates that the fluxes of morphine and lidocaine were the same through tissue that had been punctured with the study needles. In contrast, the flux of lidocaine through intact tissue was significantly greater than the flux of morphine through intact tissue (table 1).

Discussion
The data indicate that needle puncture does result in a significant increase in drug flux through the spinal meninges of the monkey in vitro. The increase in flux was found to be related to the diameter of the needle used to puncture the tissue. In addition, the flux of both drugs was the same through holes made by needles of the same diameter (fig. 1). This finding contrasts with the fact that the flux of lidocaine through intact tissues was found to be significantly greater than mor-

Table 1. Morphine and Lidocaine Flux through Intact and Punctured Meningeal Tissue

<table>
<thead>
<tr>
<th>Flux (%)·min$^{-1}$·cm$^2$</th>
<th>Intact</th>
<th>27 ga Whitacre</th>
<th>24 ga Sprotte</th>
<th>18 ga Tuohy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morphine</td>
<td>0.006 ± 0.001</td>
<td>0.011 ± 0.003*</td>
<td>0.052 ± 0.016*</td>
<td>0.291 ± 0.04*</td>
</tr>
<tr>
<td>Lidocaine</td>
<td>0.022 ± 0.001</td>
<td>0.029 ± .01</td>
<td>0.073 ± 0.013*</td>
<td>0.296 ± 0.04*</td>
</tr>
</tbody>
</table>

Data are mean ± SE; n = 6.

* P < 0.016.

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phine in this study and in a previous study from this laboratory. This suggests that the rate of flux through
the needle hole is dependent on hole size and not on any physicochemical property of the study drugs. Con-
sistent with this is the fact that the equations describing the relationship between morphine and lidocaine flux
through the needle holes and needle diameter are nearly identical (fig. 1). The clinical implication of
these findings is that other opioids and local anesthetics can be expected to behave similarly.

The data clearly show that needle puncture increases drug flux through the spinal meninges because flux
through the low resistance needle hole is much faster than through the higher resistance intact meningeal
tissue. However, the important question is whether or not this faster flux through the needle hole results in
a clinically relevant increase in the amount of drug reaching the subarachnoid space. Whether or not drug
movement through the meningeal hole is clinically relevant will in turn depend upon whether or not the
rate at which drug moves through the meningeal hole is significantly faster than the rate at which the drug is
able to diffuse through the intact tissue. This point is made clear by the fact that the flux of morphine through
tissue punctured with a 27-G Whitacre needle was significantly greater than the flux of morphine through
the intact tissue. In contrast, the flux of lidocaine through tissue punctured with this same needle was
not significantly greater than the flux of lidocaine through intact tissue. The reason for this apparent in-
consistency is that the flux of lidocaine through intact tissue is much faster than the flux of morphine.
Thus, the amount of lidocaine diffusing through the tissue is much greater than the amount that is able to cross
through the meningeal hole made by the 27-G needle.

Thus, drug movement through the meningeal hole contributes relatively little to net transfer across the
tissue. In contrast, the flux of morphine through the intact tissue is slow enough that drug movement
through the meningeal hole contributes significantly to net drug transfer across the tissue. The clinical im-
PLICATION of this is that meningeal puncture with small needles may have little effect on the subarachnoid
concentration of epidural drugs that readily cross the me-

In addition, the data indicate that the clinical impact of a meningeal hole will depend on the surface area
of meningeal tissue exposed to drug in vivo. Drug flux
through the intact meningeal tissue is reported as a rate
per square centimeter because net flux is dependent
upon the meningeal surface area exposed to the drug.
Therefore, the net amount of drug that reaches the sub-
arachnoid space by diffusing through the intact menin-
ges in vivo depends upon the surface area exposed to
drug and thus the volume of solution injected. In con-
trast, drug flux through the needle hole is essentially
fixed by the size of the hole and thus in vitro flux
should approximate in vivo flux. The clinical conse-
cquence of this depends upon the size of the meningeal
surface area exposed to drug in vivo. If a very large
meningeal surface area is exposed to drug, then the
amount of drug that traverses intact meningeal tissue
may be very large relative to the fixed amount of drug
that may traverse a "small" meningeal hole. In this
instance, the clinical impact of drug movement through
the meningeal hole may be negligible. In contrast, if
the exposed meningeal surface area is small (e.g., after
injection of an opioid in a small volume) and the needle
hole is large, then the amount of drug that traverses
the low resistance hole may be very much greater than
the amount of drug that would have been able to reach
the subarachnoid space by diffusing through the intact
meninges. Unfortunately, the size of the meningeal
surface area exposed to drug after epidural injections
of various volumes is unknown.

However, in an effort to quantitate the potential im-
 pact of each of the three study needles, one can cal-
culate the meningeal surface area that will result in
transfer of an amount of drug equal to that which moves
through the meningeal hole made by each needle. For
example, with morphine, the meningeal surface area
that results in the same drug flux as the 27-G Whitacre
needle is 0.83 cm², for the 24-G Sprotte needle this
area is 7.7 cm², and for the 18-G Tuohy needle this
surface area is 47.5 cm². It can be seen that a relatively
small meningeal surface area would need to be exposed
to epidural drug to render the effect of a 27-G Whitacre
needle negligible, whereas the surface area that would
need to be exposed to epidural drug to render the
Tuohy needle hole negligible is quite large. The clinical
implication for CSEA is that the net amount of drug
that reaches the subarachnoid space when a large sur-
face area of meningeal tissue is exposed to drug (i.e.,
a large volume of epidural solution is injected) and a
small diameter spinal needle is employed is likely not
significantly affected by drug movement through the
needle hole. Conversely, when a small meningeal sur-
face area is exposed to drug (i.e., a small volume of
epidural solution is injected) and a large diameter nee-
dle is employed, the net amount of epidural drug that

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reaches the subarachnoid space may be very much increased by the needle hole.

This reasoning assumes that the site of epidural injection and the location of the needle hole are in close proximity. However, if the injection site is located at some distance from the needle hole, then a small volume drug injection may not result in any drug movement through a needle hole of any size. Unfortunately, the site of injection relative to the meningeal puncture site is difficult to predict or to control especially when using an epidural catheter.9

This in vitro model of transmeningeal drug movement is meant to mimic the clinical situation of drug diffusion from the epidural space to the subarachnoid space. The principal advantage of this model is that it allows quantitation of drug flux with an accuracy that is not possible in vivo. In addition, the model uses fresh meningeal tissue and includes the arachnoid mater, which is the principal diffusion barrier across the spinal meninges.10 This represents a significant improvement over previous in vitro models of transmeningeal drug movement, which have used cadaveric tissue that did not include the arachnoid mater.11

The principal limitation of the model is that it does not reproduce the dynamics of the epidural space. In particular, the model does not reproduce pressure gradients across the meninges or drug loss from the epidural space into other sites (e.g., epidural fat and epidural venous drainage). Loss of drug to extrapudial sites would result in a gradual decrease in the concentration gradient across the meninges and thus a decreasing rate of drug flux into the subarachnoid space. Because drug concentration across the meninges is relatively constant in this model, the reported flux rate represents the initial rate of drug transfer across the meninges in vitro. This initial rate would be expected to decrease with time as drug concentration falls in the epidural space in vitro.

Perhaps more important, the model does not reproduce any pressure gradient between the epidural space and the subarachnoid space. Much has been written in the past about the existence of negative pressure in the epidural space.12 In fact, the hanging-drop test for identification of the epidural space relies on this purported negative pressure. However, more recent studies have shown that this negative pressure is an artifact produced by tenting of the dura mater and movement of the ligamentum flavum as the epidural needle is advanced into the epidural space.13-14 This artifactitious negative pressure does not exist in vivo when an epidural catheter is used for injection as is the case with CSEA. Thus, the lack of a negative pressure gradient across the tissue in this model is appropriate.

An additional concern is that a positive pressure gradient exists between the epidural space and subarachnoid space in vitro as solution is injected into the epidural space. If such a gradient did exist as drug was injected into the epidural space, it might well result in bulk flow of solution across the meningeal hole. However, injection of solution into the epidural space results in simultaneous increase in both subarachnoid and epidural pressures as the meninges are compressed by the volume of epidural solution.15,16 The result is that a positive pressure gradient between the epidural space and spinal cord does not develop during injection of epidural drugs, in fact subarachnoid space pressure tends to be slightly greater than epidural space pressure.15 Thus, the absence of a pressure gradient in our model does not appear to be a major limitation.

In conclusion, the goal of this study was to quantitate the relative rate of drug movement from the epidural space to the subarachnoid space through holes made in the spinal meninges by needles of various sizes. We found that the rate of drug movement through the meningeal hole made by each needle is dependent upon needle size and not on any physicochemical property of the study drugs. Further, the data suggest that the clinical impact of drug transfer through the meningeal hole will depend upon the rate at which the epidural drug is able to cross through the intact tissue and the surface area of the meninges exposed to the drug. The potential clinical importance of this finding is that it suggests that smaller diameter spinal needles might be safer for CSEA than larger diameter needles. Given that the spinal needle is typically inserted through a larger Tuohy needle positioned in the epidural space, use of small diameter spinal needles for CSEA should not present a particularly difficult technical challenge. Whether smaller spinal needles will prevent some of the complications seen with CSEA requires additional clinical studies to corroborate these in vitro data.

References