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Flux of Morphine, Fentanyl, and Alfentanil through Rabbit Arteries In Vivo

Evidence Supporting a Vascular Route for Redistribution of Opioids between the Epidural Space and the Spinal Cord

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Background: **It has been suggested that opioids may move from the epidural space to the spinal cord by way of the spinal radicular arteries. However, there are no data that address this proposed mechanism. The goal of the current study was to determine whether the radicular arterial supply of the spinal cord is a viable route for movement of opioids between the epidural space and spinal cord.**

Methods: **The carotid and femoral arteries of anesthetized rabbits were exposed, ligated distally, and cannulated proximal to the ligature. A fluid reservoir was placed around the study vessel and filled with saline buffered to pH = 7.4 or 9.0. The study drug (morphine, fentanyl, or alfentanil) and a radiolabeled tracer were added to the reservoir. Blood was collected as it flowed through the arterial segment bathed by the fluid reservoir and analyzed by scintillation counting to determine how much drug diffused through the arterial wall per minute.**

Results: **Relative flux rates through the carotid artery at pH = 7.4 were alfentanil flux > fentanyl > morphine. Increasing the pH to 9.0 resulted in a significant decrease in fentanyl's flux, but no significant change in alfentanil's or morphine's flux. In addition, the data demonstrate a biphasic relationship between octanol:buffer distribution coefficient and transarterial flux rates.**

Conclusions: **Because the critical step in transporting drug *via* radicular arteries is diffusion through the radicular artery wall, these data support the idea that drugs may gain direct access to the spinal cord by diffusing into the radicular arteries as they traverse the epidural space en route to the spinal cord. (Key words: Analgesics, opioids: alfentanil; fentanyl; morphine. Arteries: carotid; diffusion; femoral.)**

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EPIDURAL administration of opioids to produce selective spinal analgesia requires that the opioids redistribute from the epidural space to their sites of action in the spinal cord dorsal horn. However, the mechanism by which opioids move from the epidural space to the spinal cord is poorly understood. Three routes have commonly been proposed: diffusion through the meninges, diffusion through the spinal nerve root sleeve, and diffusion into radicular arteries with subsequent vascular redistribution directly to the spinal cord (fig. 1).[1–3]

Using an *in vitro* meningeal permeability model, we have previously demonstrated that drugs can diffuse through the spinal meninges and that the spinal nerve root sleeve is not a preferred route for drug redistribution between the epidural space and the spinal cord.[4–6] However, there are no experimental data that address the proposal that opioids can gain access to the spinal cord dorsal horn by diffusing into the radicular arteries that supply the spinal cord.

The goal of the current study was to determine whether the radicular arterial supply of the spinal cord is a viable route for movement of opioids between the epidural space and spinal cord. Because the critical step in this mechanism is diffusion through the wall of the radicular artery, we sought to determine whether or not opioids were able to diffuse through arterial walls in an *in vivo* rabbit model.

Materials and Methods

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

Thirty-four New Zealand white rabbits (3.25–5.1 kg) of both sexes were anesthetized by inhalation of halo-

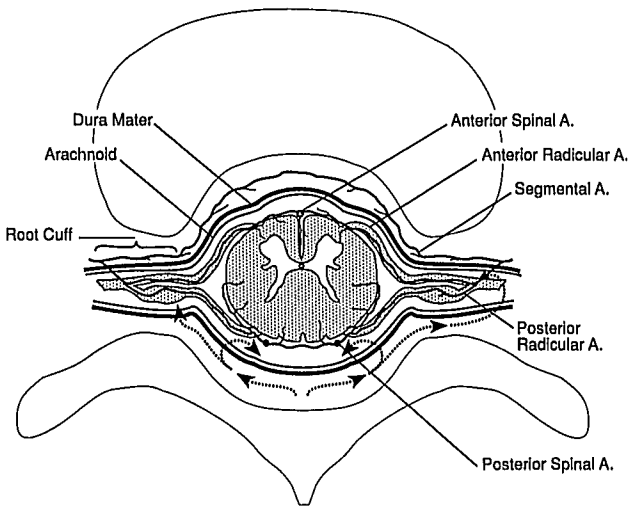

Fig. 1. Cross section of the spinal cord and epidural space indicating the proposed routes by which opioids redistribute from the epidural space to the spinal cord: diffusion through the meninges, diffusion through the spinal nerve root cuff, and diffusion into radicular arteries with subsequent transport directly to the spinal cord.

thane (1–2%) and N_2O (66%) in oxygen. The trachea was intubated *via* a tracheostomy and the lungs were mechanically ventilated with a Harvard pump. Expired CO_2 was continuously monitored (Capnogard model 1250; Novametrix, Wallingford, CT) and arterial blood gases were measured to verify the accuracy of expired CO_2 measurements. Ventilation was adjusted, as indicated by end-tidal CO_2 measurements, to maintain normocapnia. A femoral vein was cannulated to permit fluid and drug administration. Anesthesia was maintained throughout the study with halothane (1–2%) and N_2O (66%) in oxygen.

Surgical Preparation

Figure 2 shows a diagram of the experimental preparation. Approximately 4 cm of both carotid arteries and, in some animals, both femoral arteries were exposed by blunt dissection. The rabbit's femoral and carotid arteries were chosen for this study because they approximate the size of human radicular arteries, which measure 1–2 mm in diameter.[7] A fluid reservoir measuring 14 mm in diameter and 15 mm in length was fashioned from a plastic test tube. Approximately 2.5-mm slits were cut in opposite walls of the reservoir and the reservoir placed around the vessel to be studied. The reservoir was then sealed around the vessel with silicon stopcock grease (Light Laboratory Lubricant #802; Scientific Industries, Inc., New York, NY). This material was chosen because its absorption of the drugs under study was minimal. The distal end of the artery was then ligated so that no blood flowing through it returned to the animal. The vessel was cannulated between the distal ligature and the fluid reservoir and the cannula connected to a roller pump (Gilson Minipuls 3; Gilson Medical Electronics, Villiers-le-Bel, France) by means of Viton™ tubing (Gilson Medical Electronics). This tubing was chosen because pilot work demonstrated that it did not absorb the study drugs. The roller pump allowed precise control of the rate at which the heart pumped blood through the artery being studied. The reservoir was filled with 1 ml 37° C bicarbonate buffered saline (295 mOsm) which was pH adjusted to either 7.4 or 9.0. At the completion of surgery, the animals were paralyzed with pancuronium bromide (0.1 mg/kg/h) and anticoagulated with 1,000–3,000 units of heparin. Heparin was administered to prevent clotting of blood in the roller pump tubing.

Flux Measurements

The flux of morphine, fentanyl, and alfentanil through the arterial wall was measured using tritiated drugs as a radiotracer (^{3}H-morphine, specific activity 87 Ci/mmol, radiochemical purity 95.8%; ^{3}H-fentanyl, specific activity 11.07 Ci/mmol, radiochemical purity 99.8%; ^{3}H-alfentanil, specific activity 12.5 Ci/mmol, radiochemical purity 97.8%). Radiolabeled fentanyl and alfentanil were gifts of Janssen Biochemica/Biotech (Olen, Belgium). Radiolabeled morphine was purchased from New England Nuclear (Boston, MA).

At time 0, the study drug (1 mg morphine base, 0.8

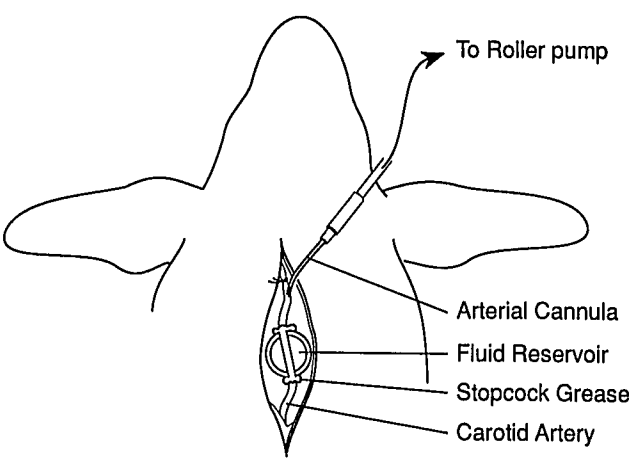

Fig. 2. Schematic of experimental preparation demonstrating the fluid reservoir surrounding the carotid artery, which is cannulated distal to the reservoir for blood collection.

μCi ^{3}H-morphine; 5 μg fentanyl, 0.54 μCi ^{3}H-fentanyl; 50 μg alfentanil, 0.23 μCi ^{3}H-alfentanil) was added to the reservoir surrounding the artery. The roller pump was started at a rate of 1 ml/min and 2-ml fractions were collected over 14 min for later drug analysis. After measuring drug flux, in most animals the temperature of the solution in the reservoir was measured (n = 26) and the diameter of the vessel was measured with a caliper marked in 0.1-mm graduations (femoral artery, n = 12; carotid artery, n = 29). The experiment was then repeated using the next vessel to be studied in that animal.

Calculation of Drug Flux

Flux was determined by plotting the cumulative amount of drug recovered *versus* time. The slope of the line through these data points was determined by least squares linear regression and is equal to the drug's flux through the arterial wall. During the period of flux measurement, the concentration of drug in the reservoir decreased by less than 10% for all study drugs, thus assuring a nearly constant concentration gradient across the arterial wall. Flux is expressed as the percentage of the total amount of drug present in the reservoir that crossed the arterial wall per minute. This was done so that the results would be independent of drug concentration in the reservoir, which, in turn, makes it possible to compare the relative flux of drugs that are used clinically in very different concentrations.

Calculation of Distribution Coefficient

Flux was measured at pH 7.4 and 9.0 to assess the effect of changing the study drugs' octanol:buffer distribution coefficient on drug flux. Altering pH changes the fraction of ionized and nonionized drug in solution, which, in turn, alters the drug's octanol:buffer distribution coefficient. The octanol:buffer distribution coefficient of morphine, fentanyl, and alfentanil at a pH of 7.4 and 9.0 was calculated using the method described by Sanchez *et al.*[8] Specifically,

$$Q = (P^0 + \beta P^+)/(1 + \beta),$$

where Q is the distribution coefficient at the pH of interest, P^0 is the partition coefficient of the unionized drug species, P^+ is the partition coefficient of the ionized drug species, and β is 10^{pKa-pH} of interest.

As described by Sanchez *et al.*, P^o and P^+ can be obtained from a plot of β *versus* $Q(1 + \beta)$, which yields a straight line the slope of which is equal to P^+ and the Y-intercept of which is equal to P^o. For this study, the data for the β *versus* $Q(1 + \beta)$ plot were obtained from previously reported measurements of morphine's distribution coefficient at pH = 7.1 and 7.7,[9] fentanyl's measured distribution coefficient at pH = 2.2 and 9.8,[10] and alfentanil's measured distribution coefficient at pH = 2.2 and 9.8.[10] The pK_as used for these calculations were: morphine = 7.93,[9] fentanyl = 8.43,[10] and alfentanil = 6.5.[10]

Drug Analysis

Whole blood samples (200 μl) were placed in borosilicate glass vials and digested with 500 μl Solvable™ tissue solubilizer (National Diagnostics, Mannville, NJ) at 50° C for a minimum of 24 h. The samples were then decolorized by incubation with 100 μl 30% H_2O_2 at 50° C for a minimum of 24 h. Formula 989™ (National Diagnostics, Mannville, NJ) liquid scintillation cocktail (10–20 ml) was added to each sample and the samples were then allowed to sit for a minimum of 72 h to eliminate chemilluminescence. Samples were counted in a Packard liquid scintillation counter (Tri-Carb 2000, Downers Grove, IL) for 30 min or until the standard deviation of depurations per minute was < 2%. Background counts from whole blood without any radioactivity were subtracted from total depurations per minute to obtain corrected depurations per minute.

Statistical Analysis

Analysis of Variance was used to test for differences among the drugs with respect to their flux through the carotid artery, and unpaired Student's *t* test was used as a *post hoc* test. Unpaired Student's *t* test was used to test for differences between femoral and carotid arteries with respect to drug flux, and for differences in drug flux through the carotid artery at pH = 7.4 *versus* pH = 9.0. Differences were considered statistically significant at the $P < 0.05$ level. All results are reported as mean ± SE.

Results

Figure 3 shows representative plots of the cumulative percentage of study drug crossing the carotid arterial wall and entering the blood stream for each drug. The slope of the linear regression lines through plots like these were used to determine drug flux. The coefficients of determination (r^2) for regression lines used to calculate drug flux averaged 0.975 ± 0.003 with a range of 0.841–0.997, indicating excellent fit of the data to a linear model.

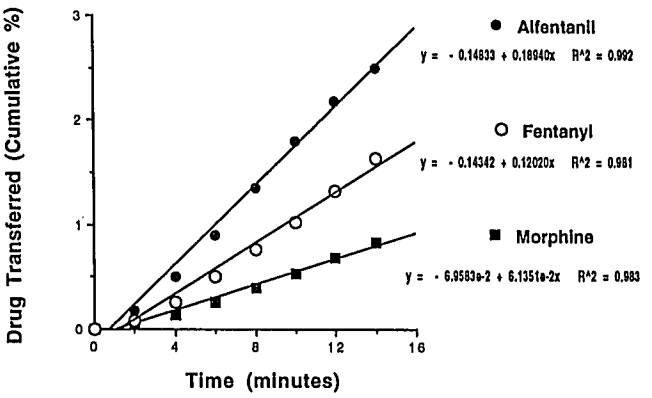

Fig. 3. Representative plots of the cumulative percentage of study drug crossing the carotid arterial wall and entering the blood stream for alfentanil, fentanyl, and morphine. The equation of the linear regression line through each set of data points and the determination coefficient (r^2) is provided. The slope of this line is equal to the drugs' flux.

Figure 4 shows the flux of morphine, fentanyl, and alfentanil through the carotid artery at pH = 7.4. The flux of alfentanil (0.185 ± 0.018%/min) was significantly greater than the flux of fentanyl (0.135 ± 0.011%/min). The flux of morphine (0.058 ± 0.006%/min) was significantly less than the flux of both fentanyl and alfentanil at pH = 7.4.

Figure 5 shows the effect of increasing the solution pH to 9.0 on the flux of morphine, fentanyl, and alfentanil through the carotid artery. The flux of morphine (0.068 ± 0.01%/min) and of alfentanil (0.183 ± 0.03%/min) at pH = 9.0 did not differ significantly from their flux at pH = 7.4. In contrast, the flux of fentanyl at pH = 9.0 (0.090 ± 0.014%/min) was significantly less that its flux at pH = 7.4 (P = 0.037).

The flux of morphine through the femoral artery (0.055 ± 0.008%/min) at pH = 9.0 was not significantly different than the flux of morphine through the carotid artery (0.068 ± 0.010%/min). In contrast, the flux of fentanyl and alfentanil at pH = 9.0 was significantly greater through the carotid compared to the femoral artery (fentanyl: femoral 0.053 ± 0.011%/min, carotid 0.090 ± 0.014%/min, P = 0.0456; alfentanil: femoral 0.081 ± 0.006%/min, carotid 0.183 ± 0.025%/min, P = 0.0026). The diameter of the femoral and carotid arteries was 1.06 ± 0.04 mm and 2.15 ± 0.02 mm, respectively.

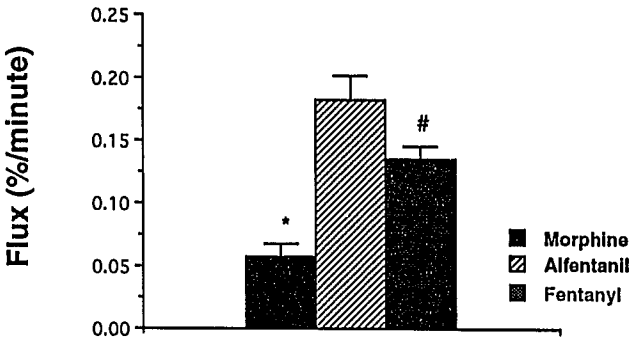

Fig. 4. Flux of morphine, alfentanil, and fentanyl through rabbit carotid artery at pH = 7.4. *indicates flux significantly less than both alfentanil and fentanyl; #indicates flux significantly less than alfentanil. Results are mean ± SE.

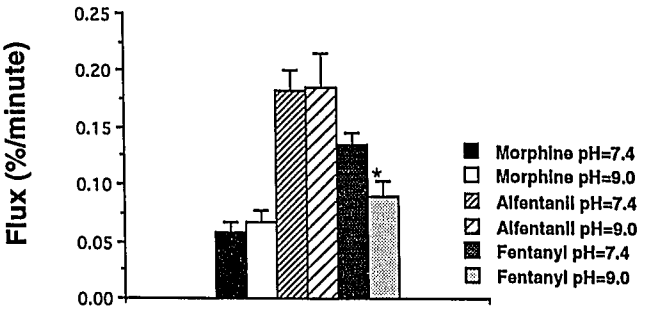

Fig. 5. Effect of raising the solution pH to 9.0 on the flux of morphine, fentanyl, and alfentanil through the carotid artery. Only the flux of fentanyl changed significantly (increased) after raising pH. *indicates significant difference in flux at pH = 7.4 *versus* pH = 9.0. Results are mean ± SE.

Figure 6 shows the relationship between experimentally measured carotid artery flux and the octanol:water distribution coefficient of morphine, alfentanil, and fentanyl at pH = 7.4 and 9.0. There is a biphasic relationship between log distribution coefficient and drug flux. As hydrophobicity increases, drug flux increases

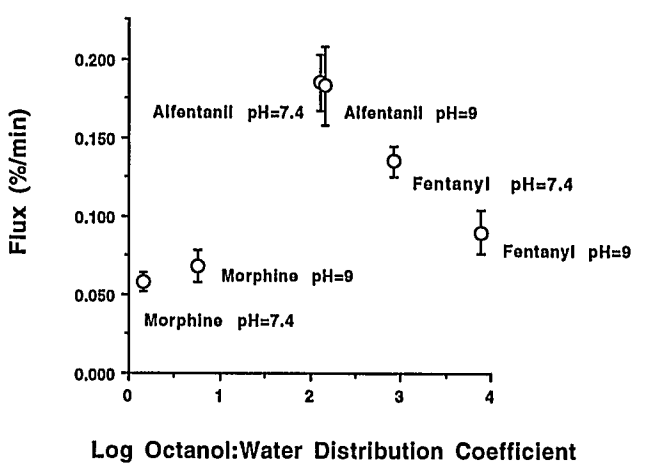

Fig. 6. Effect of octanol:buffer distribution coefficient on flux of morphine, alfentanil, and fentanyl through the carotid artery. The relationship is a biphasic one in which the peak flux occurs at a log distribution coefficient of approximately 2.

significantly to reach a maximum at a log octanol buffer distribution coefficient value of 2.11 (alfentanil at pH = 7.4). As log distribution coefficient increases further, drug flux decreases significantly.

The temperature of the buffer in the reservoir averaged 36.3 ± 0.34° C at the completion of each study, indicating near-constant temperature throughout the experiment.

Discussion

The data indicate that morphine, fentanyl, and alfentanil are able to diffuse through the carotid and femoral arterial walls of the rabbit *in vivo*. Because mammalian arterial walls differ little between species,[11] these drugs can reasonably be expected to diffuse through the similarly sized radicular arteries of humans with comparable facility. In so far as these data demonstrate that opioids can diffuse through arterial walls, they support the suggestion that opioids can be redistributed to the spinal cord by way of radicular artery blood flow.

The data also indicate that the flux of fentanyl and alfentanil was greater through the larger carotid artery compared with the smaller femoral artery. One might expect that flux would be slower through the larger carotid because of the thicker vessel wall. However, because flux is proportional to surface area, the larger surface area of the carotid may more than compensate for differences in diffusion path length. The potential clinical relevance of this finding is that the flux of these drugs may be even greater through larger arteries with a correspondingly greater surface area, *e.g.*, the artery of Adamkiewicz. Because the artery of Adamkiewicz provides much of the blood supply to the lumbar cord segments, it could theoretically deliver larger quantities of opioid to the spinal cord than do the radicular arteries.

Cousins and Mather speculated that the flux of fentanyl through the radicular arterial wall should be greater than the flux of morphine because of its greater "lipid solubility."[1] These authors further speculated that the more rapid movement of fentanyl into the radicular arteries may explain its more rapid onset of analgesia when compared with morphine. As they predicted, the flux of fentanyl was significantly greater than the flux of morphine through the rabbit's carotid and femoral arterial walls. However, the relationship between a drug's hydrophobicity and its flux through the arterial wall was not linear. Because octanol:water distribution coefficient changes as the ionized fraction of drug changes, measuring drug flux at pH = 7.4 and pH = 9.0 made it possible to assess the impact of octanol:water distribution coefficient, a measure of relative hydrophobicity, on drug flux. As indicated in figure 6, the relationship between octanol:water distribution coefficient and drug flux was biphasic, *i.e.*, drugs with an intermediate octanol:buffer distribution coefficient had significantly greater flux than drugs of greater or lesser distribution coefficient. The impact of octanol buffer distribution coefficient is seen most clearly in the case of fentanyl. At pH = 9.0, fentanyl is the most hydrophobic drug studied (log octanol:buffer distribution coefficient = 3.89), yet its flux was not significantly different than that of morphine at pH = 7.4, which is the most hydrophilic drug studied (log octanol:buffer distribution coefficient = 0.15). By lowering the buffer pH to 7.4, fentanyl's log octanol:buffer distribution coefficient is decreased from 3.89 to 2.93 and its flux increases significantly. In contrast, changing the buffer pH from 9.0 to 7.4 moved morphine's log octanol:buffer distribution coefficient toward a more extreme value (from 0.76 to 0.15) and its flux decreased slightly. Changing pH did not significantly alter the log octanol:buffer distribution coefficient of alfentanil (log octanol:buffer distribution coefficient = 2.11 at pH = 7.4 and 2.16 at pH = 9.0) and, correspondingly, its flux did not change. An alternative explanation is that the change in buffer pH alters the permeability barrier presented by the arterial wall and that a change in the nature of the permeability barrier accounts for the pH-dependent change in drug flux. This seems unlikely, because the change in pH did not alter the flux of alfentanil and the pH-related changes in morphine's and fentanyl's flux were in opposite directions.

The finding of a biphasic relationship between octanol:buffer distribution coefficient and drug flux is consistent with findings in other tissues. Bernards and Hill have demonstrated that this same biphasic relationship between octanol:buffer distribution coefficient and drug flux exists for the spinal meninges as well.[6] Likewise, in studies of both skin permeability[12,13] and corneal permeability,[14] the relationship between hydrophobicity and drug permeability was also found to be biphasic. Also, Hansch *et al.* have pointed out that there is an optimal octanol:buffer distribution coefficient for blood brain barrier penetration and that the optimal value for log octanol:buffer distribution coefficient is approximately 2.[15] Interestingly, in this study, drug flux was maximal for alfentanil, which has an octanol:buffer distribution coefficient of 2.11 at pH = 7.4.

The potential clinical significance of this information is that it may be possible to alter the rate of drug penetration through tissues *in vivo* by delivering them in a buffer of the appropriate pH.

It is reasonable to question whether or not trauma from dissection and exposure of the study vessels altered their permeability. To prevent direct vessel injury during the dissection, care was taken not to grab the section of vessel to be studied and not to dissect away any tissue that did not readily separate with blunt dissection. The vessels were bathed in saline throughout the dissection to prevent desiccation and, except for a brief period (30–60 s) during cannulation, pulsatile blood flow was maintained through the lumen of the vessels at all times. In addition, the fact that flux differed significantly among the study drugs suggests that the vessels maintained their integrity as a "selective" permeability barrier and were not damaged by the dissection. Likewise, the fact that concentration *versus* time data produced such linear plots demonstrates that the permeability characteristics of the vessels were not changing over time.

In summary, the results of this study support the idea that drugs may gain direct access to the spinal cord by diffusing into the radicular arteries as they traverse the epidural space en route to the spinal cord. However, these results do not prove that this mechanism operates *in vivo*. Whether or not radicular arteries do, in fact, serve to move sufficient drug to the spinal cord to contribute to analgesia depends on several unknown variables, including the number of radicular arteries contributing to spinal cord blood flow in an individual subject, the radicular artery surface area available for drug diffusion, the percentage of radicular artery blood flow reaching the spinal cord dorsal horn, and the threshold concentration of opioid in the human dorsal horn necessary to produce analgesia. However, these data do demonstrate that the critical step in transporting drug *via* radicular arteries, namely, diffusion through the radicular arterial wall, is possible. In light of these data, additional studies in an *in vivo* epidural model are warranted.

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