An In Vitro Study of Dural Lesions Produced by 25-Gauge Quincke and Whitacre Needles Evaluated by Scanning Electron Microscopy

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Background and Objectives: A study using scanning electron microscopy showed that although the laminas forming the dura mater are concentric and parallel to the surface of the medulla, the fiber layers' orientations are different in each sub-lamina, dispelling the conventional knowledge that all the fibers of the dura are arranged in a parallel direction. Thus, this study evaluated the dural lesions produced by Whitacre and Quincke spinal needles in the external and internal surface of the dura mater of the lower spine area in an attempt to gain more insight into the pathophysiology of postdural puncture headaches (PDPH).

Methods: The T11-L4 dural membranes from 5 fresh (immediately after extraction of organs for transplantation), male patients declared brain dead, ages 23, 46, 48, 55, and 60 years, were excised by anterior laminectomy. Morphologic orientation of the membrane and normal pH were maintained with an apparatus designed for this purpose. One hundred punctures (20 on each sample) at 90-degree angles were done with a new needle each time, 50 with 25-gauge Whitacre and 50 with 25-gauge Quincke needles. Half of the punctures with the Quincke needles were done with the bevel in parallel direction to the axis of the spinal cord, and the rest with the bevel perpendicular to it. Fixation in solutions of 2.5% glutaraldehyde phosphate buffer, followed by dehydration with acetone, was done 15 minutes after the punctures. After acetone was removed at ideal conditions of temperature and pressure, the specimens were then metalized with carbon followed by gold and inspected under a scanning electron microscope.

Results: Twenty-five of the Whitacre and 23 of the Quincke punctures were found for evaluation. There were no differences in the cross-sectional area of the punctures produced by the Whitacre or Quincke needles on the dura. The area of the dural lesions produced by 25-gauge Quincke needles, 15 minutes after they have been withdrawn, was 0.023 mm² (confidence interval [CI] 95%, 0.015 to 0.027) in the external aspect (epidural surface) and 0.034 mm² (CI 95%, 0.018 to 0.051) in the internal aspect (arachnoid surface) of the dural sac. The area of the lesions produced by the 25-gauge Whitacre needles was 0.026 mm² (CI 95%, 0.019 to 0.032) and 0.030 mm² (CI 95%, 0.025 to 0.036) in the external and internal surfaces of the dural sac, respectively. There were no significant differences in the cross-sectional areas of the punctures produced by the 25-gauge Whitacre or 25-gauge Quincke needles. Moreover, with Quincke needles the dural lesions closed in an 88.3% (CI 95%, 86.3 to 92.4) and 82.7% (CI 95%, 74.1 to 90.9) of their original sizes in the epidural and arachnoid surfaces, respectively. With Whitacre needles the closure occurred in an 86.8% (CI 95%, 83.8 to 90.3) and 84.8% (CI 95% 81.7 to 87.3) in the dural and arachnoid surfaces, respectively. However, there were differences in the morphology of the lesions. The Whitacre needles produced coarse lesions with significant destruction in the dura's fibers while the Quincke needles produced a "U"-shaped lesion (flap) that mimics the opened lid of a tin can, regardless of the tip's direction.

Conclusions: The needles produced lesions in the dura with different morphology and characteristics. Lesions with the Quincke needles resulted in a clean-cut opening in the dural membrane while the Whitacre needle produced a more traumatic opening with tearing and severe disruption of the collagen fibers. Thus, we hypothesized that the lower incidence of PDPH seen with the Whitacre needles may be explained, in part, by the inflammatory reaction produced by the tearing of the collagen fibers after dural penetration. This inflammatory reaction may result in a significant edema which may act as a plug limiting the leakage of cerebrospinal fluid. Reg Anesth Pain Med 2000;25:393-402.

Key Words: Meninges: Dura mater, Postdural puncture headache, Spinal anesthesia, Scanning electron microscopy; Spinal anesthesia equipment: Quincke needle, Whitacre needle.
The incidence of postdural puncture headaches (PDPH) is significantly reduced by the use of either small-gauge or noncutting tip (pencil-point) needles.\textsuperscript{1,2} It is widely believed that the use of these “pencil-point” needles for spinal anesthesia is associated with less trauma to the dural fibers, thus decreasing the leakage of cerebrospinal fluid (CSF). However, there is no clear evidence to support this assumption. Moreover, for many years anesthesiologists have accepted Labat’s postulate that puncturing the dural membrane with the bevel of the needle oriented in a direction parallel to the axis of the spinal cord decreases the number of fibers severed by the needle, and thus the incidence of PDPH.\textsuperscript{3} Franksson and Gordh\textsuperscript{4} supported this theory by showing that the use of a small-gauge needle was associated with a decrease in the number of dural fibers broken by the needle’s tip and a decrease in the leak of CSF through the puncture. They also noted that fewer fibers were broken when the bevel of the needle was inserted in a longitudinal fashion than when it was done transversely in this in vitro model. However, there is conflicting information in this area. Recent studies using scanning electron microscopy of the human dura mater showed that the fibers of the dura mater neither run in a longitudinal direction nor are arranged in a parallel fashion.\textsuperscript{5,6} In fact, the external surface of the dura is formed by collagen fibers that run in random longitudinal, horizontal, and oblique directions, confirming earlier observations by Fink and Walker.\textsuperscript{9} Thus, based on anatomical basis, and on a preliminary study,\textsuperscript{10} this information questions the justification, based on clinical experience, of inserting a cutting tip spinal needle into the dura mater in direction “parallel” to its fibers. However, the morphology of the hole\textsuperscript{11-13} may vary with the direction of the needle when penetrating the dura, as well as the type of needle used to influence the development of PDPH. Thus, we believed that it was important to evaluate the dural lesions produced by Whitacre and Quincke spinal needles in the external and internal surface of the dura mater of the lower spine area in an attempt to gain more insight on the pathophysiology of PDPH.

**Methods**

After family consent and institutional review board approval, the low thoracic and lumbar dural sac and its neural content were removed from 5 donors immediately after their deaths. The subjects, 23, 46, 48, 55, and 60 years of age, were treated in the Intensive Care Unit for cerebral hemorrhage and subsequently diagnosed as brain dead. None had a history of sepsis, collagen, endocrine, or neurologic disorders. Mechanical ventilation and vital functions support were provided until organ extraction (heart, liver, kidneys, and corneas) for transplant purposes was performed. Once mechanical ventilation was discontinued, evisceration of the abdominal organs, dissection of the paravertebral muscles, and laminectomy from the 8th thoracic vertebra until the joint of the first sacrum with the 5th lumbar vertebra was completed. At these levels, the intervertebral discs were sectioned and the vertebral bodies T7-8 and L5-S1 were removed in block, and the outlets of the spinal nerves dissected. The dural sac was removed in block from the 11th thoracic until the outlet of the 5th lumbar spinal nerve. Before the extraction from the medullar channel, the dural sac position was marked with different suture stitches to properly identify it once it was removed.

**In Vitro Procedures**

After dissection from the spinal cord, the dural membrane orientation and morphology, as well as normal pH (through a buffered phosphate solution), were maintained with an apparatus designed for this purpose to avoid radial traction.\textsuperscript{10} One hundred punctures were done, 20 in each of the specimens, at 90 degrees to the long axis of the vertebra with a new needle each time, 50 with 25-gauge Whitacre (Becton Dickinson, Madrid, Spain) and 50 with 25-gauge Quincke (Becton Dickinson) needles. Half of the punctures with the Quincke needles were done with the bevel in the “parallel direction to the fibers of the dura mater,” and the rest with the bevel perpendicular to the cord. Fifteen minutes after the punctures were performed, small pieces from each of the dural sacs were fixed by immersion for 4 hours in 2.5% glutaraldehyde with a phosphate solution buffered to a pH of 7.28 to 7.32, and then dehydrated through repeated immersions in acetone until a concentration of 100% was attained. The acetone from the samples was exchanged with CO$_2$ in a closed pressurized chamber (Balzers CPD 030-Critical Point Dryer; Bal Tec AG, Forstentum, Lichtenstein) until the critical point of pressure (73.8 bar) and temperature (31°C) was reached. A carbon layer was then deposited on the samples to a thickness of less than 200 Armstrong with a Balzers MED 010 Mini Deposition System followed by a gold plating with a Balzers SCD 004 Sputter Coater equipment (both by Balzers, Bal-Tec AG).\textsuperscript{14} Quality control for the fixation technique was checked against the fixation characteristics of biological cells of well-known morphology and size (e.g., erythrocytes), because these cells are present in the majority of the specimens of the meninges.\textsuperscript{15}
Measurments

The specimens were studied both in the outer epidural surface and the inner subarachnoid surface with a JEOL JSM 6400 scanning electron microscope (JEOL Corporation Ltd, Tokyo, Japan).

For this study, we selected the 25-gauge needles because they are the most frequently used needles for spinal anesthesia in our hospitals. The punctures were accomplished always on the posterior surface of the dura mater since this is the accessible zone in clinical practice. Moreover, this zone of the dura mater was far away from the dissection zone because we used an anterior approach for laminectomy. The punctures were controlled by simultaneous observation of enlarged images obtained by an operative stereoscopic microscope Zeiss S 21 OPMI 111 (Carl Zeiss, Oberkochen, Germany) and video camera (Video Dye Camera 2CCD Sony Mod. DXC-930 P; Sony Corporation, Tokyo, Japan) in a Sony Trinitron Dye Mod HM-1430 AND monitor. The lesion’s area produced by the dural punctures was measured from photographs of the lesions, once they had been amplified 600 times. Since all lesions were irregularly shaped geometrical figures, their area was calculated using the multiple triangles addition technique. The total error in the measurement method in the surface of each lesion was analyzed and calculated at 2%. Determinations were accomplished in square microns and were expressed in mm$^2$ rounding of accordance to the grade of numerical meaning up to 1/1,000 millimeter. The dural lesion closing was measured 15 minutes after withdrawing the needle, and it was calculated as the differences of areas between the cross-section of the needle and that of the dural lesion divided by the cross-section of the needle and expressed as a percentage. For this purpose, needles were measured after being covered with gold through scanning electron microscopy.

Statistical Analysis

To prove the normal distribution of these data, a standardization test to the values obtained from the measurements was applied. The quantitative variables are expressed as the mean of the measurements in all groups and their confidence intervals were estimated to 95%. The matching between the results of different groups was accomplished through the Student t-test for independent groups. The hypothesis contrast was accomplished with the 2-tailed method. Statistical significance was determined at a P value $\leq .05$.

Results

Twenty-five of the 50 punctures done with the Whitacre, 10 in the external and 15 in the internal surfaces, were found for evaluation. Twenty-three of the 50 punctures done with the Quincke needles, 13 in the external and 15 in the internal surfaces, were found for evaluation. Nine of those were done with the needle inserted in a direction “parallel to the fibers” and 14 with the needle inserted in a perpendicular direction to the axis of the cord. The surface of the dural lesions produced by 25-gauge Quincke needles, 15 minutes after the puncturing was done, was 0.023 mm$^2$ (confidence interval [CI] 95%, 0.015 to 0.027) in the external surface (epidural) (Fig 1), and 0.034 mm$^2$ (CI 95%, 0.018 to 0.051) in the internal surface (subarachnoid) of the dural sac (Fig 2). The lesions produced by the 25-gauge Whitacre needle was 0.026 mm$^2$ (CI 95%, 0.019 to 0.032) and 0.030 mm$^2$ (CI 95%, 0.025 to 0.036) in the external (Fig 3) and internal surfaces, respectively (Fig 4). When the area of the dural lesions found in the external and internal surfaces produced by the 2 types of needles was compared, no significant differences were found. We also compared the external and internal sides of the lesions produced by the same needle, and no differences were found. The percentage of lesion closing area with the Quincke needles, 15 minutes after puncture, was 88.3% (CI 95%, 86.3 to 92.4) and 82.7% (CI 95%, 74.1 to 90.9) in the external and internal surfaces, respectively. With the Whitacre needles, the percentage of closing was 86.8% (CI 95%, 83.8 to 90.3) and 84.8% (CI 95%, 81.7 to 87.3) in the epidural and arachnoid surfaces, respectively. The size of the lesions produced by the Quincke needles after puncturing with the 2 directions of the bevel was also compared. The mean area of these lesions produced with the Quincke needles on the external surface was 0.023 mm$^2$ (CI 95%, 0.019 to 0.026) and 0.024 mm$^2$ (CI 95%, 0.014 to 0.034) for the parallel and perpendicular bevel directions to the axis of the spinal cord, respectively. In the internal surface, the size of the lesions was 0.035 mm$^2$ (CI 95%, 0.009 to 0.079) and 0.034 mm$^2$ (CI 95%, 0.010 to 0.058) for the parallel and perpendicular bevel directions to the axis of the spinal cord, respectively. There were no statistical differences found between the 2 types of puncture methods used.

However, there were differences in the morphology of the lesions.

Description of the Morphology of the Dural Lesions Produced With Both of the Spinal Needles

External (Epidural) Surface. The Whitacre needles produced lesions in the dura that were either rounded, oval, elliptical, or star-shaped. The
borders of all the lesions evaluated were coarse due to the tearing of the collagen fibers (Fig 3). It is noteworthy that the lesions' cross-sectional area decreased or even disappeared in the deeper laminae of the dural membrane. In contrast, lesions produced by the Quincke needles had either a semilunar shape (Fig 1) or a "U" shape. In either case, all the lesions formed a flap of dural membrane that reminded us of a partially opened lid of a can and the borders of the lesions had a clean-cut appearance. The orientation of the needle had no influence on the morphology of the lesion (Fig 1).
fact, there were no differences in the cross-sectional area of the lesions when comparing the 2 approaches for the needle insertion.

**Internal (Subarachnoid) Surface.** Lesions produced by the Whitacre needles in the internal surface of the dural membrane (Fig 4) had a similar morphology and characteristics to those in the external surface (Fig 3). Significant destruction of the collagen fibers in the borders of the lesions produced by a tearing effect of the needle was also
noted. In contrast, the borders of the lesions produced by the Quincke needles were always smooth regardless of the orientation of the bevel of the needles when penetrating the dura, i.e., parallel to the longitudinal axis of the cord or perpendicular to it (Fig 2). The collagen fibers in the borders of the lesions were always neatly cut as it was appreciated at 200× magnification.

Quality Control of the Needles. We have noticed in previous evaluations of spinal needles via electronic scanning microscopy that important differences among needles from different manufacturers can exist. In some instances, differences among the needles from the same manufacturer can be found. The needles that we used in this study could be considered "high-quality," because they did not have fractures or metallic splinters in their tips (Figs 5 and 6). The Whitacre needles did not have metallic protrusions around the lateral holes (Fig 5). The Quincke needles had a double bevel without saw-tooth-type defects. In both needles, the openings at the tip were thoroughly sealed by the stylet and they did not extend beyond the tip of the needle (Fig 6).

Discussion

Conventional wisdom is that the use of "pencil-point" needles for spinal anesthesia is associated with less trauma to the dural fibers. By deductive reasoning, clinicians have explained that the lower incidence of PDPH is the result of less CSF leaking through the smaller holes produced by these "less traumatic" needles. However, just as it was noted by Hart and Whitacre\textsuperscript{16} back in 1951, to date there is no direct evidence to support this reasoning.\textsuperscript{17} Our findings suggest that the Whitacre needles produced more traumatic lesions to the dural membrane, as suggested by the large tears seen on the borders of the orifices made by these needles. In contrast, the borders of the lesions produced by the Quincke needles were always smooth and clear-cut. Moreover, there were no differences in the cross-sectional areas of the lesions produced by these 2 types of needles of similar diameters. Thus, based on the assumption that the incidence of PDPH is the result of a significant loss in CSF volume through the hole made by the spinal needle, these findings led us to hypothesize that the lower incidence of PDPH seen with the use of Whitacre needles may be the result of an "edematous plug" formed after a more severe inflammatory reaction in the area of the puncture. This hypothesis is based on the observation that blunt trauma is associated with more edema and swelling than the trauma produced by a sharp object. Clearly, this hypothesis will have to be tested in an in vivo model, and we are currently working on this next step.

The morphology of the lesions produced by the Whitacre needles in the internal side of the dural

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**Fig 3.** Dural lesion produced with a 25-gauge Whitacre needle seen from the external (epidural) surface. Note the “flaps” of collagen fibers at the borders of the lesions. SEM. Original magnification ×200; bar, 100 µm.
membrane could be explained by the tearing produced after a "tenting" effect produced by the Whitacre needles on the dural membranes.

In light of our "edematous plug" hypothesis, we are concerned with the results of some studies that showed that not all the cases of CSF hypotension were associated with PDPH, questioning the CSF loss and PDPH cause-and-effect relationship. A recent study evaluated, through magnetic resonance imaging, 11 patients who underwent routine lumbar punctures for CSF leaks at the puncture site and the incidence of PDPH. The investigators' goal was to determine if the maximum volume of leakage correlated with the appearance of headaches. They found no correlation between the amount of fluid loss and the occurrence of headache. Thus, they...
concluded that CSF leaks into the paraspinal area after dural puncture, and the volume of CSF loss, had no impact on the incidence of PDPH. However, there are 3 studies that correlated the volume of CSF loss and the incidence of headache. Moreover, a more recent in vitro study of CSF leakage after dural punctures found a lower loss of CSF after dural punctures with “pencil-point” needles than with Quincke needles, adding more confusion to the subject. However, it is difficult to reconcile the results of all these studies in view of the differences in the techniques used for the preparation of the dural samples, resulting in the potential inclusion of partial or total thickness of the arachnoid membrane with the dural membrane preparation. The time interval between the death of the donor and the preparation of the sample and the variable thickness of the dural membrane in different areas of the spinal cord may also alter its viscoelastic properties, thus rendering variable results in the size of the dural lesions and the amount of CSF leakage. Consequently, these studies suggest that the appearance of PDPH may be the result of several factors coming into play at a critical point, and it is not clear which of these factors is the most important.

Fig 5. Whitacre needle. (A) Magnification of the point needle at 200×, SEM. (B) Magnification of the point of the needle at 50×, SEM.
In conclusion, our results suggest that the use of Whitacre spinal needles for spinal anesthesia results in more tissue destruction in the dural membrane when compared with the use of Quincke needles of similar size. This difference may play an important role in the pathogenesis of PDPH.

References

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