Morphology of antemortem dural puncture healing: an ex vivo sheep study

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INTRODUCTION

Closure of holes caused by unintentional puncture of the dural sac with a relatively large-bore Tuohy needle is thought to be a linear process over a relatively short time. Scientists believe that cerebrospinal fluid (CSF) leakage through the puncture hole is responsible for intracranial hypotension, which in turn may be responsible for postdural puncture headache (PDPH).[1]

Our objective was to study the rate and mode of natural closure of such dural puncture holes in an ex vivo sheep model at 1, 3, and 7 days after intentional dural puncture.

METHODS

We randomly assigned 27 adult sheep (*Ovis aries*) to one of three groups and performed an intentional dural puncture at the lumbosacral transition space between the sixth lumbar and first sacral vertebrae (L6/S1)[2] on each animal. Animals were under general anesthesia in the sternal position. We used an 18-gage Tuohy epidural needle (Perican, B. Braun, Melsungen, Germany). After observing CSF flow, we removed the Tuohy needle and placed a metal surgical staple in the skin next to the skin puncture hole to mark the site for future dissection and obtaining samples. The animals were euthanized after 1 day (group 1), 3 days (group 2), and 7 days (group 3).

After full recovery from general anesthesia, the sheep were housed following international animal welfare standards. After the animals were euthanized, they were immediately refrigerated at −20°C (Zanotti freezing chamber, Zanotti s.p.A, Pegognaga, Milano, Italy) for 72 hours.[2]

The frozen animals were prepared such that the samples contained only the area of interest. The dural sacs were extracted without damaging them and immediately immersed in 2.5% glutaraldehyde with phosphate solution buffered. The inner subarachnoid surfaces of the samples were studied with a JSM 6400 Scanning Electron Microscope (JEOL Corporation, Tokyo, Japan) as previously described.[3 4]

The areas and circumferences of the puncture hole lesions were measured using Adobe Photoshop and ImageJ V.1.46a (http://rsb.info.nih.gov/ij/) software. The characteristics of the edges of the lesions on the arachnoid mater and disrupted dural laminas were studied. We describe the statistical analysis in the online supplemental text.

RESULTS

Twenty-four lesions were suitable for the study. Table 1 shows the demographic data, the areas, and the circumference measurements of arachnoid puncture holes. Figure 1 depicts the arachnoid lesions. The lesions in all cases consisted of a dural lesion superimposed on an arachnoid lesion. Closure of the area and circumference over time exhibited a logarithmic pattern (figure 2; estimate, −0.14; SE, 0.055; t value, −2.54; p=0.02 and estimate, −0.065; SE, 0.025; t value, −2.61; p=0.017, respectively).

Areas were smaller on days 3 and 7 than on day 1 (day 1, 1.77 mm^2; day 3, 0.58 mm^2 (p=0.027;

Table 1 Demographic data and area and circumference measurement over time

	Day 1 n=9	Day 3 n=9	Day 7 n=6	Overall n=24
Sex				
Woman	8 (88.9%)	8 (88.9%)	4 (66.6%)	20 (83.3%)
Man	1 (11.1%)	1 (11.1%)	2 (33.3%)	4 (16.6%)
Weight				
Median (min, max)	37.0 (30.0, 48.0)	39.0 (30.0, 57.0)	30.0 (24.0, 46.0)	38.5 (24.0, 54.0)
Area (mm^2)				
Median (min, max)	1.77 (0.338, 4.24)	0.589 (0.443, 1.15)	0.470 (0.338, 1.38)	
Circumference (mm)				
Median (min, max)	5.59 (2.96, 8.46)	3.50 (3.27, 5.39)	3.25 (2.43, 4.74)	

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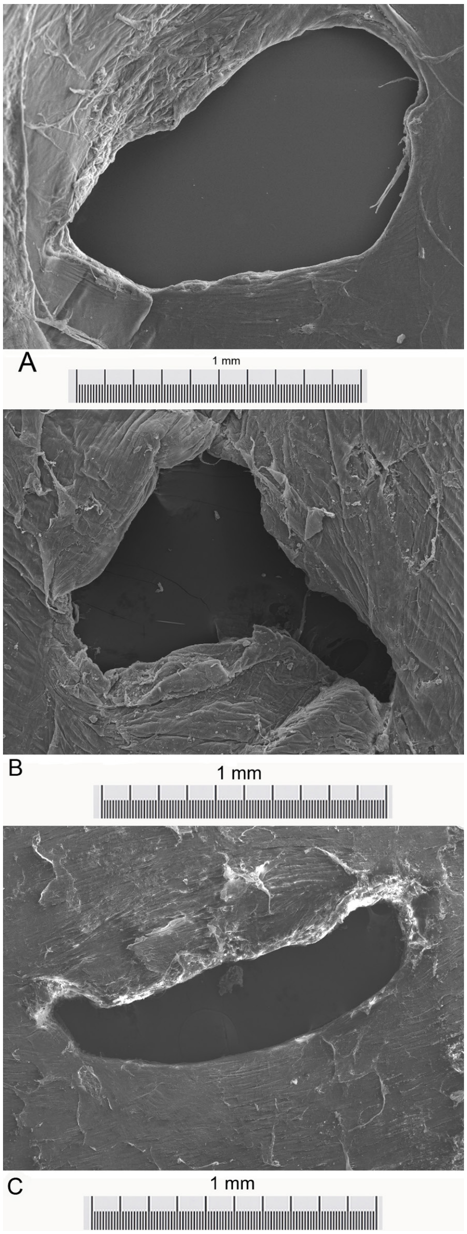

Figure 1 Dural sac lesion seen from the internal surface (arachnoid layer) performed with an 18-gage Tuohy needle. (A) 1 day after lumbar puncture. (B) 3 days after lumbar puncture. (C) 7 days after lumbar puncture. Scanning electron microscopy was used to study the samples. Magnification: A, B, and C = ×70.

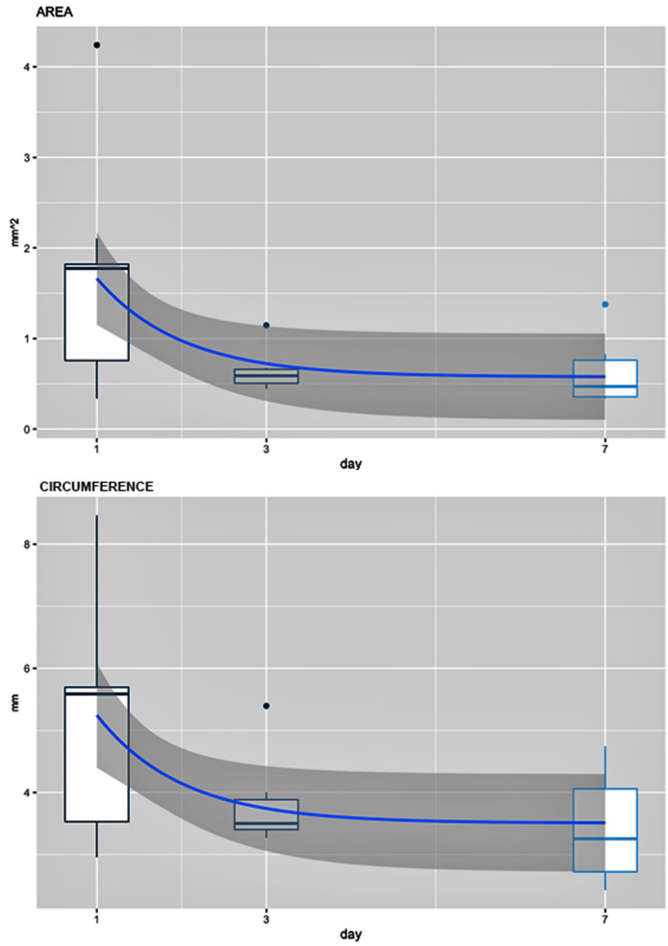

Figure 2 Area and circumference. Median, range, and outliers in days 1, 3, and 7 and logarithmic fit (blue line; gray area represents 95% CI).

95% CI 0.07 to 1.08); day 7, 0.47 mm^2 (p=0.027; 95% CI: 0.08 to 1.84)). No differences between days 3 and 7 were observed (p=0.743; 95% CI −0.55 to 0.66). The areas of the puncture holes were 66.7% and 73.5% smaller on days 3 and 7 than those on day 1. However, there were no differences in circumference between days 1, 3, and 7 (day 1, 5.59 mm; day 3, 3.5 mm (p=0.09, 95% CI −0.34 to 3.4); day 7, 3.25 mm (p=0.09; 95% CI −0.43 to 3.9)). There were also no differences between days 3 and 7 (p=0.65; 95% CI −1.7 to 2.2).

By analyzing the characteristics of the lesions, we observed that the bevel on Tuohy spinal needles produced a small cutting lesion of the dura–arachnoid complex. As the tip advanced, the Tuohy needles produced a precise "clean" cut with limited damage to the dura and arachnoid maters. The tip of the needle appeared to fold the cut tissue inward toward the arachnoid layer, so that dural and arachnoid lesions produced a "crescent moon" shape resembling the letters "U" or "V," as well as a geometrical image resembling an oval or ellipse.

DISCUSSION

The current study showed that the healing process in a dura–arachnoid lesion produced by a Tuohy needle is not linear as expected, but rather a logarithmic process over time. The sheep model is the most relevant animal model concerning tissue regeneration and wound healing for preclinical human studies.[5] We continued the study for only 7 days because we fully expected to find a nearly healed lesion following the natural clinical course of PDPH. However, we found only approximately 65% closure of the puncture holes. Extrapolating from this logarithmic pattern of the closure of puncture holes, we deduce that the closure process will likely take a long time because the logarithmic graph lines almost flatten beyond 7 days.[6] Immediately following needle withdrawal, the edges of the lesion tended to retract due to the viscoelastic properties of the dura mater.[7] Subsequently, it is most likely fibrosis that contributes to closure, which is a much longer process.

The healing of the needle injury to the dura thus followed an inverse logarithmic mathematical model rather than the expected linear pattern.

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