Detection of Epithelial Cell Transfer in Spinal Areas by Light Microscopy and Determining Any Tissue Coring via Cell Culture During Combined Spinal-Epidural Interventions

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Background and Objectives: Epithelial tissue coring by spinal needles during subarachnoid injections may cause intraspinal epidermal tumors. Previous studies have investigated tissue transfer with different needle types during subarachnoid or epidural injection. This study deals with the transfer of epithelial tissue during combined spinal-epidural (CSE) anesthesia.

Methods: We studied 68 American Society of Anesthesiologists I to III adult patients. CSE anesthesia was induced under aseptic conditions at the L2-3 or L3-4 interspace with patients in the lateral decubitus position. Cerebral spinal fluid, spinal needle stylet, fluid used to flush the interior of the spinal needle, fluid used to wash the exterior of the spinal needle, fluid used to flush the interior of the epidural needle, and fluid used to wash the exterior tip of the epidural needle were examined under light microscopy (n = 30 patients) or incubated in a cell-culture medium (n = 38 patients). Samples were incubated in cell-culture medium alone (n = 13) or in a cell-culture medium for 3 weeks and then in a medium with epidermal growth factor (n = 25). As a positive control, skin tissue samples were taken by punch biopsy from 10 randomly chosen patients who underwent CSE interventions. These samples were incubated in an enriched medium serum.

Results: Light microscopy revealed that there was cell transfer in all phases in various rates: samples 1, 2, 3, 4, 5, and 6 contained epithelial cells and debris in ratios of 6.9%, 20.7%, 6.9%, 20.7%, 26.7%, and 33.3%, respectively. Epithelial cell colonization was detected in the cell-culture samples taken from the control group but not in the samples taken from the CSE group.

Conclusions: We could not reproduce the cells or cell debris obtained during the CSE interventions in vivo, which can be explained by a possible structural deformation of cells or the inadequacy of the amount of cells that were transferred. *Reg Anesth Pain Med 2006;31:539-545.*

Key Words: Combined spinal epidural intervention, Tissue coring, Cell transferring, Light microscopy, Cell culture.

Iatrogenic intraspinal lumbar epidermoid tumors after repeated subarachnoid antibiotic injections were first described in the 1950s.[1-3] It was suggested that epithelial tissue coring by spinal needles during subarachnoid injections might be responsible for this rare type of tumors.[1] Because epidermoid tumors show a fairly slow progression, it does not seem possible to design studies to show that the tumor is one of the complications of the direct spinal interventions. However, various studies have been performed to show whether spinal needles carry epithelial tissues during the interventions or not. Campbell et al.[4] reported that the frequency of tissue coring by spinal needles may reach up to 75%, but they were not able to detect tissues in the cerebral spinal fluid (CSF). In another study, the presence of benign squamous epithelial cells in CSF was shown.[5] In this study performed on cadavers, Puolakka et al.[6] showed that maximum tissue coring occurred during lumbar puncture using Quincke type spinal needles. Another study examining tissue coring during caudal injections in chil-

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dren showed no nucleated cells with mitotic activity.[7] There are studies on epithelial tissue coring in the epidural or subarachnoid space. To the best of our knowledge, there are no studies in the literature showing tissue coring during the application of the combined spinal-epidural (CSE) technique, in which the spinal needle is advanced through the epidural needle.

Our hypothesis is that epithelial tissues are transplanted into the spinal area during CSE anesthesia analgesia. We used light microscopy to determine at every stage of the procedure in which needle the epithelial cell transfer occurs and what the odds of this are. We used enriched cell-culture medium to determine if there were any epithelial tissues with mitotic activity.

Methods

After the approval of the study by the Local Ethics Committee of the Cerrahpasa Medical Faculty, Istanbul University, patients with the American Society of Anesthesiologists I to III who underwent either lower-abdominal or lower-extremity or urinary surgery and had no contraindications to CSE anesthesia analgesia were included in the study.

All the patients were duly informed, and consent from each was obtained. Exclusion criteria were as follows: refusal to give consent; bleeding diathesis, systemic, or local infections; or undergoing an anticoagulation treatment. Sixty-eight patients who were included in the study for CSE anesthesia were divided into 2 groups: light microscopy and enriched culture medium. The patients were chosen randomly by drawing lots. Among the same patients, another random choice was made, and 10 patients were chosen as the control group. Punch biopsy samples were taken from the surgical incision area of these patients.

Application of CSE Anesthesia Analgesia

Spinal interventions were performed while the patients were in lateral position and awake by the same experienced anesthesiologist. After the hands were disinfected and sterile gloves worn, the area was cleaned broadly with povidine iodine solution 3 times by using a painting penset. Then, new sterile gloves were worn, and the application area was covered with sterile cloths. Local anesthesia was performed by injecting 2% lidocaine into the skin and the subcutaneous area via interspinous spaces of L2-3 or L3-4. A Combined Spinal Epidural kit (Espocan, Docking System, Perifix Soft Type; B. Braun, Melsungen AG, Germany; Spinocan: 27 gauge, Perican: 18 gauge) was used in all the patients. Aseptic conditions were maintained. All the tubes into which the samples would be deposited and the combined spinal-epidural catheters were placed on the preparation table by an assistant wearing sterile gloves. On collecting the samples, the tubes were closed with sterile air tight rubber plugs.

An 18-gauge epidural needle with stylet and with bevel directed upward was inserted through the skin and subcutaneous tissue using the loss-of-resistance technique with 0.9% NaCl. Then, a 27-gauge spinal needle was advanced through the epidural needle. After ensuring CSF flow, the spinal needle stylet was placed into a tube with 1 mL of a special solution (saccamano fixator for light microscopy and DMEM F12 for cell culture) and was sealed and marked as sample 2. The first 6 or 7 drops of CSF that flowed spontaneously through the spinal needle were collected into a second tube, which was marked as sample 1. A predetermined local dose of anesthetic (1-1.5 mL 0.5% bupivacaine) was injected into the subarachnoid space. After the injection, the spinal needle was taken out and was flushed with 1 mL of the predetermined solution. This fluid was collected into a tube and marked as sample 3. Particular care was taken to prevent the tip of the spinal needle from touching the collected sample. The spinal needle was placed into another tube. After the addition of 1 mL of the predetermined solution into it, this tube was marked as sample 4. The tip of the epidural needle was then turned in a cranial direction, and the epidural catheter was inserted. The epidural needle was removed, and 1 mL of the same solution was passed from inside the needle and the solution was collected into a tube. This sample was marked as sample 5. Care was taken to prevent the tip of the epidural needle from touching the collected sample. The tip of the epidural needle was placed into another tube. After the addition of 1 mL of the same solution into it, the tube was marked as sample 6.

Cytologic Examination

A total of 30 patients were studied during the cytologic examination of the samples. The samples taken from the patients were collected into test tubes containing 1.0 mL of Saccamano's fixator (2% MTO-Carbowax 1540; Supelco, Bellefonte, PA) and 50% absolute ethanol. The samples were then centrifuged at 2,000 rpm for 5 minutes. Supernatants were removed. Six hundred microliters of suspended pellets were divided into 2 parts with 300 μL each. Cytocentrifuge was performed at 1,600 rpm for 6 minutes. Preparations of cytocentrifuge were air dried, and Wright-Giemsa staining was applied. By using both the low-power (10×) and