METHODS

Spatial Characteristics of Cisterna Magna in Rats and Novel Technique for Puncture with a Stereotactic Manipulator

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The actual size and sites for puncture of the cisterna magna in adult Wistar rats were estimated on acrylic models. We proposed a new method for obtaining cerebrospinal fluid from narcotized rats. This method consists in controlled introduction of a needle into the cisterna magna through the skin and/or dura mater above this structure using a stereotactic micromanipulator. Experiments on rats with focal and global cerebral ischemia showed that this technique ensures single and repeated sampling of at least 50 μl liquor not containing blood admixtures.

Key Words: *spatial characteristics of the great cerebral cistern; puncture technique; cerebrospinal fluid; rats*

Sampling of the liquor from laboratory animals (*e.g.*, rats) is an involved technique for studying biological fluids. Liquor is usually sampled from the cisterna magna (CM), because sampling from the lateral cerebral ventricle provides only small amounts of the liquor (15 μl), which is insufficient for studies of bioactive substances and evaluation of other parameters.

There are 3 methodical approaches to obtaining cerebrospinal fluid (CSF) from CM: (1) from awake animals through a preimplanted cannula [3]; (2) though a cannula inserted close to the dura mater above the cistern [4]; and (3) direct puncture of CM through the skin on the dorsal surface of the neck in narcotized animals [4,6].

The methods for liquor sampling from awake animals have serious disadvantages, including high risk of damage to the nervous tissue during CM cannulation, possible plugging of the cannula, necessity of preestimation of stereotactic coordinates of CM in animals of various species and ages, and low efficiency. According to published data only 43% liquor samples were transparent or weakly bloody [4]; cannulas were occluded in 6% experiments; the liquor was to obtained in 23% rats; 3% animals died on the next day after cannulation; and state of 3% animals worsens after liquor sampling. Most liquor samples contain blood admixtures (more than 28%), which attests to brain trauma and makes it difficult to interpret the experimental results.

Sampling of the liquor from narcotized animals is an efficient physiological method. This technique satisfies international requirements for experiments on laboratory animals. Narcotization is an additional intervention, which is characterized by low risk of complications compared to cannulation of CM. This highly effective method (90-100%) allows sampling of 60-100 μl liquor [4]. A disadvantage of this technique is manual puncture of CM. Therefore, the efficiency and quality of manipulations depend entirely on researcher's skill, *e.g.* its ability to determine accurately the moment of puncturing the dura mater above CM. The major difficulty is passing through several tissue layers. The first and most dense layer is the skin. These pecu-

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liarities increase the risk of damaging brain tissue during insertion of a needle into CM.

Here we studied spatial characteristics of CM in rats and developed a new nontraumatic method for its puncture with a stereotactic micromanipulator.

MATERIALS AND METHODS

Two experimental series were performed on male Wistar rats weighing 150-450 g. In series I spatial characteristics of CM were evaluated on acrylic models (5 rats, 250-300 g). The animals were narcotized with 100 mg/kg ketamine and fixed in a stereotactic device (Fig. 1). The angle between the head and body was 90°; punctured surfaces were positioned horizontally. The skin from spinous process projecting at the initial part of the vertebral column and skull bones was cut along the median line (10-15 mm). The tissues were mechanically displaced from the midline of muscular fasciae on the neck until dura mater appeared. The dura mater looked like a stretched membrane and had mat surface (rhombus). The parietal bone, vertebral column, and occipital protuberances formed the cerebral, caudal, and lateral corners of the rhombus, respectively. The membrane was perpendicularly punctured at the middle point of the midline (depth 1 mm). The maximum amount of the liquor was sampled. The needle was removed. A median longitudinal incision (1.5 mm) was made through the site of puncture in the dura mater. The residual liquor was removed from CM with a cotton plug. The cavity of CM was filled with freshly prepared acryl emulsion using a micropipette. After induration of acryl, CM models were separated from surrounding tissues and examined in transmitted light. Profiles of CM were visualized on the screen and sketched in the frontal and sagittal surfaces. The actual size of CM was measured on models with a micrometer.

Schematic images and data on the size of CM were used to determine the exact point of puncture on the skin on the neck and dura mater above CM (open technique) and address point inside CM. Introduction of a needle point into the address point during sampling of the liquor provides minimum risk of damage to CM walls.

In series II the technique was tried on 37 intact rats. The method of CM puncture with a stereotactic manipulator was tested on 253 rats with pathological changes in the central nervous system. Neurospecific proteins GFAP and NSE were assayed after single and repeated sampling of the liquor from CM (1-week interval). We measured the volume of samples and determined the incidence of complications (samples with blood admixtures, bleeding, and changes in the general state of animals). The procedure yielding at least 50 μl transparent liquor and not producing complications was considered to be successful.

RESULTS

Examination of models showed that CM is a funnel-like structure. Its longitudinal axis was positioned at an angle of 52° to the horizontal surface (Fig. 2, X). The top of this funnel corresponded to the surface of stretched brain membrane above CM (dorsal wall of CM). Flattened processes were oriented laterally at this level. They provided additional space for accumulation of the liquor (Fig. 2, Y). The bell of the "funnel" passed into the spout (narrowest part of CM, Fig. 2, Y, Z). The width of a gap was 1.3 mm. Point A positioned at a depth of 1.5 mm and equally distant from the lateral wall of CM served as an address point for puncture (Fig. 2, Y). Point A_1 laid 1.5 mm proximal to the median point (B_1). In this region the dura mater was easily swayed under pressing with a button probe. In the sagittal surface, point A (address point) was localized at a distance of 1.8 mm or less from the inner anterior and posterior surface of CM (segments CA and AB, Fig. 2, X). It can be hypothesized that frontal deviation of a needle from the address point is at higher risk of damage to CM wall than sagittal deviation. Thus, the puncture needle must follow the median orientation to obtain high-quality samples of CSF. Point A_1 on the dura mater and point A_2 on the skin (1.5 mm proximal to the most movable point B_2 on the skin above CM) are the exact points for harmless puncture of CM (Fig. 2).

Puncture of CM and control over the depth of introduced needle were performed using a Narishige stereotactic micromanipulator. The injection needle

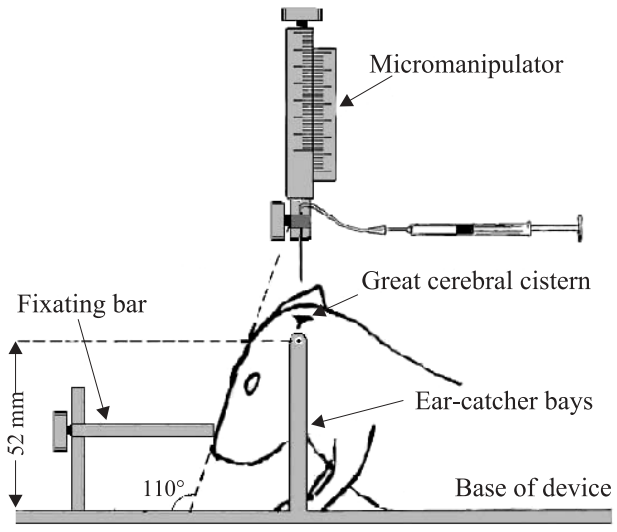

Fig. 1. Fixation of rats in a stereotactic device during puncture of the great cerebral cistern.

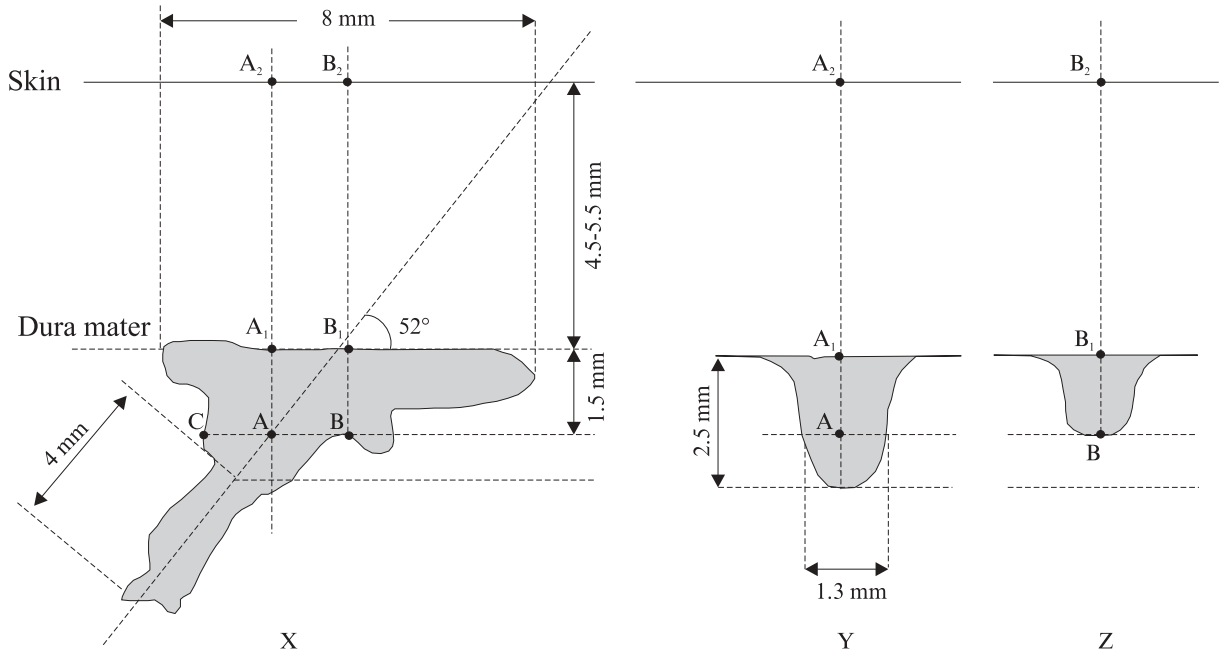

Fig. 2. Parameters of the great cerebral cistern and points of puncture in rats. X, median sagittal profile. Y, frontal profile at the midpoint on the dura mater. Z, frontal profile at the optimal puncture point. A, address point of puncture. A_1, projection of the address point on the dura mater. A_2, projection of the address point on the skin. B_1, midpoint on the dura mater. B_2, midpoint on the skin. B, position of a needle point during puncture through points B_1 and B_2. C, projection of point A_1 on the anterior wall of the cistern.

connected to a microsyringe (250 µl) via an elastic volume-graduated tube (50-100 and 150 µl) was fixed in the micromanipulator. The manipulator and the needle were oriented vertically (Fig. 1). The outer diameter of the needle was ≤0.7 mm. The needles were sharpened in such a manner that the slant of a pricking part did not exceed 1 mm. The section of the needle was oriented along the median longitudinal surface of CM to reduce the risk of trauma.

CM was punctured by the open method immediately before perfusion of rat brain fore pathomorphological examination. After visualization of the dorsal surface of the dura mater above CM the point with the maximum mobility was determined by light pressing with a button probe. In most cases this point was located on the midline (Fig. 2, B_1). The exact point for CM puncture was derived from this point and stained with a dye (A_1). It was localized 1.5 mm proximal to point B_1 (Fig. 2, X). The needle was oriented toward point A_1 and introduced into the address point A in CM (depth 1 mm). The piston of the microsyringe was moved to mark 60-70 µl. CSF was sampled. Usually, the first portion of the liquor (50-60 µl) rapidly flows into the collecting system due to intracerebral pressure. Additional amounts of the liquor can be obtained by drawing out the piston by 20-30 µl at 1-2-min intervals.

For transcutaneous puncture of CM, the hair was removed from the dorsal surface of the skin in rats. We palpated the zone between the parietal bone and vertebral column. The most movable point on this surface (B_2) was determined with a button probe. The exact point of puncture (A_2, Fig. 2) was positioned 1.0-1.5 mm proximal to point B_2. This point was marked with a marker. The exact point of puncture should be positioned on the midline using a micromanipulator. This line passes through the spinous process projecting at the skin in the initial part of the vertebral column. Transcutaneous puncture of CM can be accompanied by displacement of tissues on the neck and change in the angle of needle entry, which is related to hardness of the skin. To avoid this, we made a small cut on the skin (1 mm) in the exact point of CM puncture. The needle was introduced to a depth of 1.5, 2, and 3 mm, which depended on the weight of rats (<200, 200-300, and 300-400 g). The needle was moved at 200-µ increments. CSF was sampled at each position of a needle (similarly to the open puncture technique of CM). When the liquor appeared in the connecting tube, the needle was introduced into CM by 100-200 µ. CSF was sampled with a microsyringe at a flow rate of no more than 40 µl/min. The needle was removed immediately after this procedure. The cut was sutured. The amount of the liquor was estimated with a microsyringe. Samples were stored in a refrigerator at -70°C. The system for sampling of CSF was washed with a 10-fold volume of sterile NaCl. Puncture of CM from the dura mater and transcutaneous puncture lasted 10 and 4 min, respectively (except for the time of narcotization).

The liquor from CM was sampled 600 times over 2-3 months after modeling of perinatal hypoxic/ischemic damage to the central nervous system and focal ischemia (middle carotid artery occlusion). The samples were also taken from control and intact rats [1,2]. Samples of the liquor were obtained in all experiments. Single puncture of CM allowed us to obtain 50-220 μl CSF. The amount of the liquor did not decrease during repeated punctures at 2-week intervals. Moderate xanthochromia was observed only in 10 samples (1.7%). Probably, it was associated with blood entry into the needle during passing through the tissues on the neck.

The major addition to the technique for sampling of CSF from CM in narcotized rats is that we estimated the exact puncture points from spatial characteristics of CM, evaluated the address point to insert the puncture needle, and performed controlled introduction of the needle from tissues of the neck into CM with a stereotactic micromanipulator. This approach reduces the risk of damage to the brain tissue surrounding CM. The proposed method for puncture of CM from the skin to the dura mater allows a researcher to evaluate admissibility of the risk depending on the conditions and goal of the experiments. This simple study does not require calculation of stereotactic coordinates of CM and the use of additional devices.

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