A NEW PROCEDURE FOR LUMBAR PUNCTURE IN THE MOUSE (INTRATHECAL INJECTION)  
PRELIMINARY REPORT  

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ABSTRACT  

Mice and other small animals are often used in experimental studies on  
central nervous disorders. If the intrathecal administration of certain sub-  
stances is required in such experiments, the substances are usually adminis-  
tered by cisternal puncture under general anesthesia. In work on metabolic  
aspects of diseases, however, general anesthesia is disadvantageous since it  
influences cerebral metabolic conditions.  

The present authors thus developed a technique for intrathecal adminis-  
tration by lumbar puncture in the mouse under local anesthesia. Although  
it may appear that correct positioning of a mouse could be difficult under  
local anesthesia, the authors were able to ensure a satisfactory posture by  
pulling the bilateral ears and tail gently in the craniocaudal direction. In  
order to puncture the mid-point of the spinous processes accurately in the  
mouse, we observed the spinous processes directly through a lumbosacral  
incision in the skin.  

In order to test the reliability of our technique, trypan blue and 5%  
glucose were administered to mice intrathecally.  

Based on macroautograms obtained at 30 min after intrathecal adminis-  
tration of 25 µl of trypan blue, the intrathecal injection of trypan blue by  
our technique was judged to be successful in 95% of the twenty mice.  

Twenty-five µl of 5% glucose was administered intrathecally to mice and  
the intracerebral water content was estimated at 10, 20, 30, 40, 50, 60 min  
after the administration following decapitation.  

No marked change in intracerebral water content was observed at 10 min  
after the intrathecal administration, but at 20 min a significant rise in water  
content was found (p<0.05). The water content tended to decrease there-  
after and at 30 min it dropped to the pre-administration level.  

It is considered therefore that the intrathecally administered glucose  
affected the intracerebral water content in some definite way, thereby demon-
strating the reliability of our method.

Compared to cisternal puncture, the present technique for lumbar puncture offers several advantages.
1) It can be carried out under local anesthesia.
2) Practically, it is a simpler procedure than cisternal puncture.
3) It is reliable, giving a high success rate of over 95%.

INTRODUCTION

The central nervous system is subject to highly specialized anatomical and metabolic conditions, including the blood-brain barrier and metabolic compartmentation. Transport of certain substances into the brain tissue is thus limited when they are administered into the bloodstream, and the influence of certain substances on the brain differs according to whether the administration into the bloodstream or into the cerebrospinal fluid (CSF). The need thus exists for a technique to introduce substances into the intrathecal space, i.e., into the CSF, under abnormal conditions such as in meningitis.

Mice and other small animals are often used in experimental studies on central nervous disorders. If the intrathecal administration of certain substances is required in such experiments, the substances are usually administered by cisternal puncture under general anesthesia. In work on metabolic aspects of diseases, however, general anesthesia is disadvantageous since it influences cerebral metabolic conditions.

The present authors thus developed a technique for intrathecal administration by lumbar puncture in the mouse under local anesthesia. The effectiveness of the method was demonstrated by the intrathecal administration of trypan blue and 5% glucose.

METHODS

a) Lumbar Puncture

The two important points in lumbar puncture are (1) a correct body position which prevents rotation or bending of the vertebral column, and (2) puncture at the mid-point of the spinous processes. Although it may appear that correct positioning of a mouse could be difficult under local anesthesia, the authors were able to ensure a satisfactory posture by pulling the bilateral ears and tail gently in the craniocaudal direction. In general, it is almost impossible to puncture the mid-point of the spinous processes accurately in the mouse percutaneously. This problem was solved, however, by observing the spinous processes directly through a lumbosacral incision in the skin.
Fig. 1 Apparatus for lumbar puncture and intrathecal injection.
A: Lid of cage.
B: Pean's forceps.
C: Forceps with laterally bent tips for hooking open the incision.
D: Scissors.
E: Microsyringe.
F: #27 G winged needle (for lymphangiography).

Fig. 2 Step in lumbar puncture. Series A, plan view; series B, oblique view.

1-A, B: Grip the ears of the mouse with Pean's forceps, hold the tail by hand and pull in the craniocaudal direction.
2-A, B: Make a skin incision in the lumbosacral region and retract the skin.
3-A, B: With the spinous process in direct view, puncture the mid-point.
Fig. 1 shows the apparatus used, and Fig. 2 illustrates the steps in our lumbar puncture procedure. A pair of Pean’s forceps (Fig. 1, B) and a pair of forceps modified for hooking open the incision (Fig. 1, C) were fixed to the lid of the cage (Fig. 1, A) with a rubber band. The ear flaps anesthetized with Xylocaine Spray \textsuperscript{R} were then gripped with the Pean’s forceps and the tail was pulled gently by hand (Fig. 2-1). In doing this, it is important not to pull sharply since the axis of the body can be bent by a forceful maneuver. The skin in the lumbosacral region anesthetized with Xylocaine Spray \textsuperscript{R} was incised to a length of 1.5–2 cm and retracted laterally (Fig. 2-2) with the modified forceps. The spinous processes were thus brought into direct view. Their comparative shortness in the mouse means that they grow out almost at right angles to the spinal canal. Puncture was therefore made with a needle set vertically at mid-point of two spinous processes (Fig. 2-3). Furthermore, as shown diagramatically when the needle tip is slightly penetrating the intervertebral disc. Accordingly, if the lumbar puncture is conducted properly, the needle will be held by the spinous processes and intervertebral disc and there will be little risk of disturbance of the needle with slight body movements.

b) Intrathecal administration

Since the needle is fixed after such precise lumbar puncture, intrathecal administration can be performed with relative ease. Intrathecal injection was carried out manually by attaching a winged needle to a microsyringe. During this procedure, if the speed of infusion is too fast, or if the volume is too large, the mouse may become irritable and show convulsions, and death may even
occur. Particular care is required with the administration of stimulants (e.g. glutamic acid).

We injected at a speed of less than 25 μl/min and a maximum total volume of 25 μl. No abnormalities were observed in mice at this speed and volume.

RESULTS

In order to test the reliability of our technique, trypan blue and 5% glucose were administered to mice intrathecally.

a) Trypan Blue

Twenty-five μl of trypan blue was administered intrathecally to twenty ICR mice (8W). A macroautogram obtained at 30 min after the administration is shown in Fig. 4. Intrathecally administered trypan blue was observed to stain the spinal cord and the brain. Based on such macroautograms, the intrathecal injection of trypan blue by our technique was judged to be successful in nineteen out of the twenty mice (95%).

b) 5% Glucose

Twenty-five μl of 5% glucose was administered intrathecally to ICR (8W) and the intracerebral water content was estimated at 10 (n=20), 20 (n=20), 30 (n=19), 40 (n=19), 50 (n=18), 60 (n=20) min after the administration following decapitation. The results are shown in Fig. 5. No marked change in intracerebral water content was observed at 10 min after the intrathecal administration, but at 20 min a significant rise in water content was found (P<0.05).
The water content tended to decrease thereafter and at 30 min it dropped to the pre-administration level. At 40 min there was a further slight reduction in water content, and subsequent levels fluctuated near the pre-administration level.

It is considered therefore that the intrathecally administered glucose affected the intracerebral water content in some definite way, thereby demonstrating the reliability of our method.

**DISCUSSION**

In order to obtain CSF for examination or to introduce substances into the subarachnoid space, lumbar, cisternal or ventricular puncture may be carried out. In experiments using small animals, cisternal puncture under general anesthesia is preferred but it represents a rather complicated procedure. General anesthesia (i.e. the anesthetic itself) is known to exert a strong effect on cerebral metabolism. For example, barbitrates display a protective action against hypoxia. General anesthesia is thus undesirable when studying the metabolic aspects of central nervous disorders.

Compared to cisternal puncture, the present technique for lumbar puncture offers several advantages.

1) It can be carried out under local anesthesia.
2) Practically, it is a simpler procedure than cisternal puncture.
3) It is reliable, giving a high success rate of over 95%.
However, it should be remembered that substances introduced into the intrathecal space by lumbar puncture reach the brain by flow in the CSF, and during their passage to the brain they may absorbed and metabolized to some extent by the spinal cord. Somewhat larger amounts of substances should therefore be administered by lumbar puncture compared to cisternal puncture.

CONCLUSION

A technique for intrathecal administration by lumbar puncture in the mouse under local anesthesia was successfully developed. The reliability and ease of operation of the technique were demonstrated in experiments using trypan blue and 5% glucose.

REFERENCES