NEW CEREBROSPINAL FLUID SAMPLING BY LUMBAR PUNCTURE IN RATS
-REPEATED MEASUREMENTS OF NITRIC OXIDE METABOLITES IN CSF-

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INTRODUCTION: Nitric oxide (NO) facilitates transmission of nociceptive signals in the spinal cord of animals. We previously reported that concentrations of CSF NO metabolites (NOx: plus NOx; NOx) in patients with degenerative lumbar diseases (DLD) are significantly higher than those in painless control subjects (ref. 1), and preoperative NOx may be quantitative predictor of postoperative pain relief in degenerative lumbar diseases (ref. 2). However, it is still obscure whether or not the elevated NOx correlate with pain severity or the duration of spinal nerve compression. Therefore, the animal model is required to repeatedly obtain CSF from lumbar level, in which the compressive level and duration of the nerve root or cauda equina are manipulable. The purposes of this study were to develop a simple method for sampling CSF, and to repeatedly measure the concentrations of CSF NOx in naïve rats.

MATERIALS AND METHODS: Twelve young (13 weeks old, weight: 420-500g) and 7 middle-aged (40 weeks old, 660-780g) male Wistar rats were used. The rats were anesthetized by intraperitoneal injection of mixture of ketamine hydrochloride, xylazine hydrochloride and normal saline. A skin incision 2 cm in length was made over L6 to S1. The interspinous ligament at L6/S1 and cranial half of the S1 spinous process were removed carefully, and a needle (27 G) was inserted into subarachnoid space through the ligamentum flavum and for oblique and shallow angled. These procedures allowed for easy identification of the insertion point on the ligamentum flavum and for oblique and shallow insertion of the needle into the subarachnoid space without injury to the vessels on the vertebral body (Figure). The CSF was spontaneously collected in the needle cup. The purpose of this study was to develop a simple method for sampling CSF, and to repeatedly measure the concentrations of CSF NOx in naïve rats.

RESULTS: The success rate regarding CSF collection was 96%, and the amount of the CSF was 50-70 µl. Surgical time was 21 ± 0.6 minutes. The NOx in young rats (6.5 ± 0.2 µM) was significantly higher (p<0.05) than that in middle-aged rats (5.6 ± 0.3 µM). Four CSF samplings were successfully obtained in 10 of 12 13-wk-old rats and in 6 of 7 40-wk-old rats. CSF NOx, sampled at the 1st, 2nd, 3rd, and 4th week were 6.4 ± 0.5, 6.5 ± 0.6, 6.3 ± 0.4, and 6.5 ± 0.6 µM in 13-wk-old rats, and 4.9 ± 0.4, 6.2 ± 1.0, 6.4 ± 2.3, and 4.9 ± 1.0 µM in 40-wk-old rats, respectively. There were no significant differences in CSF NOx between each sample in 13-wk-old rats or in 40-wk-old rats.

DISCUSSION: This is a first report to measure CSF NOx in rats, the concentrations of which are greater than those in human that we reported previously (ref. 1). In the NO-measuring system (ENO-20), 10 µl is a minimum amount for measurement of NOx, so that collecting amount in our technique is sufficient to measure NOx. Direct lumbar puncture to collect CSF in rats was first reported by De La Calle and Paiño (ref. 3). Their CSF sampling procedure, however, has two technical problems. One is that the lumbar canal in rats is too narrow for perpendicular needle puncture of the dural sac. Second, the blood contamination rate is high (24.9%) because the needle is inserted until it comes into contact with the vertebral body, resulting in bleeding from injury of the vessels there. We therefore modified their techniques as follows: The posterior elements, including the interspinous ligament at L6/S1 and the S1 spinous process, were completely resected and the puncture needle was angled. These procedures allowed for easy identification of the insertion point on the ligamentum flavum and for oblique and shallow insertion of the needle into the subarachnoid space without injury to the vessels on the vertebral body (Figure). These modifications led to not only a significant decrease in the blood contamination rate (11%), but also a high success rate of CSF sampling (96%). Therefore, our method is a reliable and simple sampling technique for a small amount of CSF to measure NOx etc. Using this technique, the involvement of CSF NOx on the pathomechanism underlying persistent pain in DLD may be elucidated in the future.