Utility of In-Vitro Proton NMR of Cerebrospinal Fluid in the Diagnosis of Patients with Spina Bifida

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INTRODUCTION

Combination of embryopathy, stretching, ischemia, compression and trauma is responsible for cord dysfunction in spina bifida. Changes in neuronal metabolism leads to changes in the local milieu of CSF in the cord. Change in metabolite profile of CSF in spina bifida in terms of increase in products of anaerobic metabolism, nerve membrane integrity and nerve ischemia has not yet been studied. The objectives of the present study are : (a) to analyze the metabolite profile of CSF of spina bifida patients using in-vitro proton magnetic resonance spectroscopy (^{1}H-MRS), and (b) to compare the levels of metabolites with controls and to establish the correlation of underlying neuronal dysfunction with metabolic changes in spina bifida patients.

PATIENTS AND METHODS

Sixteen infants with spina bifida [meningomyeloceles (n=6), operated cases of spina bifida with retethering of cord (n=10)] presented to the Department of Pediatric Surgery of our Institute were considered for the study. Ten age matched children served as control group. These were the patients who underwent lumbar puncture for suspected meningitis but cytology and biochemical examination were negative for the same. 60µl of D_2O containing 0.5mmol/l TSP (Sodium –3-trimethyl-silyl propionate – 2,2,3,3,-H4 was added to 540 µl of native CSF sample for NMR experiments. TSP served as a chemical shift reference ($\delta = 0.0$) and concentration standard. Typical parameters used for one dimensional (1D) proton NMR experiments were: pulse width 90°, number of data points 32 K, spectral width 5000 Hz, number of scans 48 and relaxation delay of 14 s. Two dimensional (2D) total correlation spectroscopy (TOCSY) of the sample were carried out using standard software package. One-way analysis of variance (ANOVA) was used to assess the differences between the groups. Values of $p < 0.05$ were considered significant.

RESULTS

Assignment of various metabolite resonances was carried out using 2D TOCSY. Concentrations of various CSF metabolites in controls, meningomyelocele (MMC) and retethering groups are shown in Fig. 1a and b. In MMC and retethering groups, significantly higher concentrations were observed for Lac ($p<0.01$), Ace ($p<0.01$), Cho ($p=0.01$), Ala ($p<0.01$) and GPC ($p=0.01$) compared to controls. However, there was no significant change in the concentration of Cr and Glc metabolites in controls and patient groups.

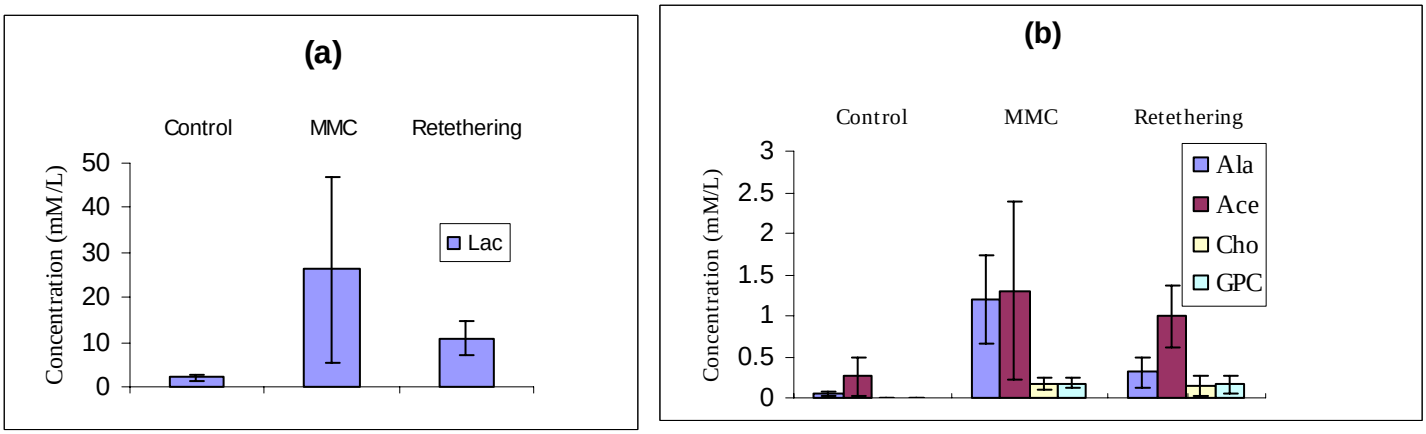

Fig. 1. (a&b) Comparison of the concentration of metabolites in cerebrospinal fluid of controls and patients with spina bifida.

DISCUSSION

In the present work, several significant differences in the metabolite concentrations were observed in the CSF of patients with spina bifida compared to controls. Concentration of Lac, the end product of anaerobic metabolism and Ala were found in excess in patients with MMC and retethered cord. This indicates an increase in anaerobic metabolism at the local site of cord pathology. Braughler et al also noted an increase in the levels of Lac and Ala in the CSF of rat spinal cord following trauma (1). Cho and GPC levels, possible indicators of cell membrane damage, were also found to be raised significantly in MMC and retethered group (2). Absence of resonances from Cho and GPC in controls suggests that several factors like stretching and compression disrupt the neuronal integrity of cord leading to dysfunction in the setting of spina bifida and retethering of cord (MMC and retethering groups of this study). We have also observed significantly high levels of Ace in the CSF of MMC and retethering group. Koshorek et al reported significant alteration in the Ace levels in CSF of patients with lumbar disc herniation by high resolution proton NMR spectroscopy (3). Our data suggest that in-vitro ^{1}H MRS measurements of metabolites in CSF is a promising tool in improving our understanding of the pathophysiology of neuronal dysfunction in patients of spina bifida and for providing additional diagnostic information.

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