GABA LEVELS IN CEREBROSPINAL FLUID (CSF) OF A PEDIATRIC POPULATION MEASURED BY LC/ MS
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Abstract
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Studies indicate that low levels of brain GABA (gamma-aminobutyric acid) are associated with poor seizure control. However, precise measurement of GABA levels in the developing brain remains especially challenging due to their extremely low levels in cerebrospinal fluid (CSF). Described is a robust stable isotope dilution ESI-MS/MS method that was applied to the measurement of CSF GABA levels in infants (ages 4 - 24 months, n=30) with seizures and age-matched controls (n=30) without seizures.

The method uses 130U of CSF and the deuterium labeled GABA ([D6]-GABA). A-Aminobutyric acid (2,3,4,5,6-pentafluorophenyl) triacontyamine as internal standard. A liquid-liquid extraction was performed and the aqueous phase containing the analyte (GABA) was recovered and taken down to a stream of nitrogen. Danyl derivatization of the extract was prepared and analyzed using an Alliance 2690 HPLC (Waters Corp, Milford, MA) coupled to Waters Premier electrospray tandem mass spectrometry (ESI-MS/MS) with multiple reaction ion monitoring (MRM) using argon as the collision gas and with detection in positive ion mode. Detector responses were linear (R²=0.99) over a range of 0.1 to 100 ng/mL. Recovery of GABA from CSF samples was 98 ± 3%, the lower limit of quantification was 0.1 ng/mL. The inter- and intra-assay precisions were in the range 2.4 - 6.7%. Samples analyzed 32 days post-preparation gave similar responses to freshly prepared samples indicating stability of the danyl derivative. This method has the sensitivity to determine GABA concentrations of 1 to 100 ng/mL in CSF. Mean (± SEM) CSF GABA concentrations in infants with seizures were 18.4 ± 2.5 ng/mL, which was significantly lower (p=0.001) than in age-matched controls (28.8 ± 2.1 ng/mL). This method has potential for pre-screening patients in the paediatric stage and for monitoring patients’ responses in evaluating treatment strategies.

Introduction

Epilepsy remains a global challenge accounting for 1% of the global disease burden.[1,2] The syndrome currently affects over 2.3 million people in the United States, with 30% of the cases coming from the pediatric population. In the USA, 60% of the 300,000 newly diagnosed cases each year are children under the age of 5 years.

A disease biomarker capable of identifying patients with epilepsy at an early stage would provide a valuable means of initiating treatment possible to manage the disease.[3,4] Gamma-aminobutyric acid (GABA) is a major presynaptic inhibitory neurotransmitter that is widely distributed in the brain and helps to regulate excessive brain excitation that could otherwise lead to neurological and psychiatric disorders.[5,6,7]

GABA levels are closely related to overall brain GABA levels and these are minimally affected by changes in cerebrospinal GABA concentrations. Low GABA levels in the brain are associated with several neurological disorders including seizures.[5,6,7]

We therefore investigated the potential of using the CSF GABA level as a clinical biomarker for evaluating patients with epilepsy.

Precise and accurate measurement of GABA in CSF is challenging due to the extremely low levels especially in infants, small sample volumes, and background interferences from biological matrices that further limit sensitivity. The goal of this work was to develop a HPLC tandem mass spectrometry method to determine GABA concentrations in cerebrospinal fluid that would be applicable to low volume CSF typical obtained from pediatric patients and to apply this to evaluating CSF GABA in infants and children with and without epilepsy.

References


Methodology

LC/MS/MS Conditions:

Mass Spectra

Quantification of GABA

The concentration of GABA in CSF was determined from measurement of the ratio of peak areas of GABA/[2H6]GABA constructed for accurate concentrations of GABA over the dynamic range 0.1 - 10 ng/mL.

Limit of detection (LOD) of the method was 0.01 ng/mL and lower limit of quantification (LOQ) was 0.1 ng/mL.

Calibration curves for pure standards, standards spiked to water (0.1 mL) or spiked to a pool of CSF (0.1 mL) were linear and parallel.

Clinical Application

This tandem LC-MS method was applied to a study to establish age- and gender-related, age-matched sub-study to compare GABA CSF levels in seizure and non-seizure infant, age range 4 - 24 months, the seizure group comprised 38 infants (19M, 19F, mean age 18.6 ± 3.6 months) and there were 42 infants in the non-seizure group (19M, 23F, mean age 19.3 ± 3.6 months).

Age-related differences in CSF GABA concentrations

A significant age-related difference in the mean CSF GABA concentrations was observed with a sharp increase in GABA occurring after 4-months of age. For infants <4 months of age CSF GABA levels were significantly lower than in infants aged 4-24 months. This was also observed in children up to 13 years of age. These findings indicate that CSF GABA may be a useful and specific biomarker to screen patients with neurological disease and for monitoring patients’ responses in evaluating treatment strategies.

Gender differences in CSF GABA concentrations

Consistent throughout was a lower CSF GABA level in seizure patients when compared with non-seizure patients and this was true for both males and females. However, there was no significant gender differences in the level of CSF in seizure status.

Summary

Described is a robust sensitive and specific method for the determination of cerebrospinal fluid gamma-aminobutyric acid (GABA) concentration suitable for analysis of GABA at low concentrations in the CSF of pediatric patients. Age-related differences were detected in CSF GABA levels, and a significantly lower level of CSF GABA was observed in infants and children with seizures. The method has potential application in screening patients with neurological disease and for monitoring patients’ responses in evaluating treatment strategies.

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LC-MS/MS analysis