Effect of glutamine/glutamate increase on the N-acetylaspartate quantification in patients with hepatic encephalopathy assessed by 1H-MRS

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Introduction

The accurate determination of the N-acetylaspartate (NAA) concentration by ^{1}H MRS in patients with liver failure is a crucial index for the assessment of hepatic encephalopathy (HE), and brain recovery before and after liver transplantation respectively. The main features of ^{1}H-MRS brain spectra from patients with HE is an increased glutamate and glutamine (Glx) signals, and decreased myo-inositol, choline and N-acetylaspartate signals (1, 2). However, β,γ-Glx resonances are very close to the methyl-NAA resonance and interfere with NAA quantification at TE < 70 ms. We evaluated the overestimation of NAA concentration (dNAA) due to β,γ-Glx peaks in six HE patients and ten healthy age-matched controls, and compared NAA overestimation in controls and patients with that calculated from a set of model solutions containing NAA and Glx in variable amounts. We found a substantial difference of NAA overestimation due to overlapping Glx in controls and in patients; a difference that could not be explained by experiments with model solutions.

Methods

Two sets of model solutions were prepared containing NAA (SetA: 23mM, SetB: 11.5 mM), glutamate, glutamine at different concentration (SetA and SetB:from 14 to 55 mM) and 3-(Trimethylsilyl)propionic acid-d_4 sodium salt (TSP). Six patients with hepatic encephalopathy from mild to severe assessed according to ref.3 and ten sex- and age-matched healthy volunteers were recruited (informed consent was obtained from each subject). All ^{1}H-MRS measurements were performed in a 1.5 T General Electric Medical Systems (Milwaukee, Wisconsin) Signa Horizon LX whole-body scanner using a 25 cm diameter quadrature birdcage head coil. In vivo: voxel (3x3x2cm) was placed in the mid-brain parietal-occipital grey matter. In vitro: voxel (2x2x2cm) was placed in the middle of the solution container. Absolute concentrations of NAA were measured by acquiring spectra at 5 echo times (TE = 35, 70, 100, 144, 288 ms; TR = 4000 ms; number of acquisitions = 32), and using water as internal standard. Peak areas were calculated using the time domain fitting program MRUI (3). NAA absolute quantification was performed using two TE sets: one with all acquired TEs, and a second set without the point at TE=35 ms. The difference of NAA concentrations obtained by the two TE sets was taken as a measure of the overestimation (dNAA) due to contamination of β,γ-Glx peaks as their resonance is maximal at TE=35 ms in our experimental conditions. The relationship between dNAA and total area of the β,γ-Glx peaks, as measured at TE = 35 ms and normalized to the internal water area (Glx-35), was evaluated in HE patients, in controls and in model solutions.

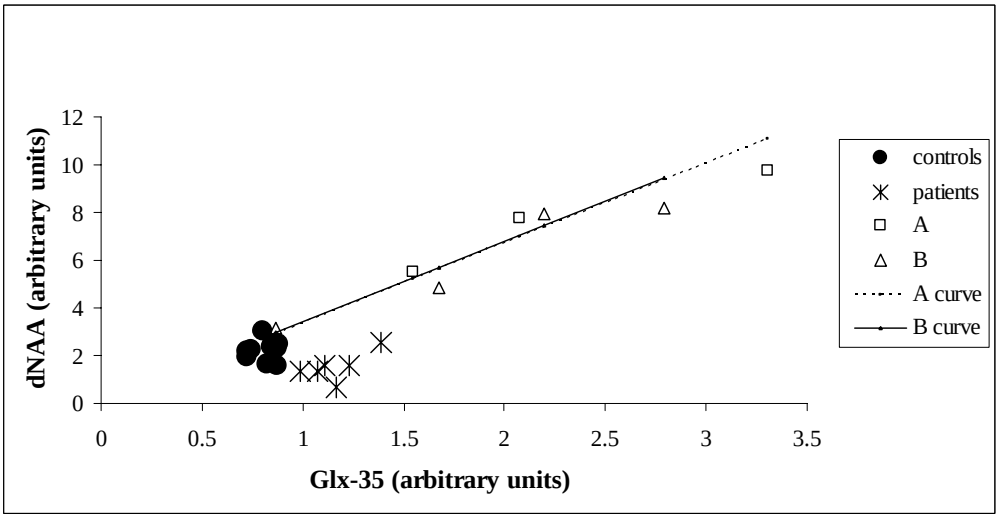

Figure 1. Effect of variable amounts of Glx on the quantitative assessment of NAA in model solutions, healthy volunteers, and patients with hepatic encephalopathy.
Two different sets (A, B) of model solutions were used as specified in the Methods section.
Overestimation of NAA (dNAA) is reported as difference of NAA concentration calculated by the full set of TEs data (35, 70, 100, 144, 288 ms) minus the concentration calculated from the same data set after withdrawing the TE 35 ms time point (i.e. 70, 100, 144, 288 ms).
Glx-35 is the total area of the β,γ-Glx peaks, as measured at TE = 35 ms normalized to the area of internal water.

Results

Plotting NAA overestimation (dNAA) as a function of Glx-35 both model solutions gave regression lines with an overlapping coefficient (dNAA/glx-35) SetA= 3.4 ± 0.5 a. u., SetB= 3.4 ± 0.4 a.u. (Fig.1). HE patients shows a 39% increase of the Glx-35 signal compared to controls. However, this result is not matched by an increase of the NAA overestimation, as it would be expected by the linearity of dNAA vs Glx -35 obtained from the model solutions. On the contrary there is a 30% decrease of dNAA in HE patients compared to controls. The mean of the ratio of dNAA/Glx-35 in HE patients is 1.3 ± 0.4 a.u. and in controls is 2.6 ± 0.6 a.u.

Discussion

Our results shows that the degree of contamination of Glx on NAA estimated in healthy controls was comparable to that obtained by the model solutions, while HE patient showed a smaller degree of contamination, although they had a higher Glx content. This puzzling result suggests the presence of other metabolites resonating between 2.0-2.5 ppm, involved in the brain biochemical modifications of HE patients, and GABA could be one of the possible candidates.

References

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