NeuroImage 61 (2012) 1165–1175

Contents lists available at SciVerse ScienceDirect

NeuroImage

journal homepage: www.elsevier.com/locate/ynimg

Focal cortical damage parallels cognitive impairment in minimal hepatic encephalopathy

Carmina Montoliu [b,1], Gabriel Gonzalez-Escamilla [a,1], Mercedes Atienza [a], Amparo Urios [b], Olga Gonzalez [d], Abdallah Wassel [e], Roberto Aliaga [f], Remedios Giner-Duran [d], Miguel A. Serra [e], Jose M. Rodrigo [g], Vicente Belloch [f], Vicente Felipo [c], Jose L. Cantero [a,*]

[a] Laboratory of Functional Neuroscience, Spanish Network of Excellence for Research on Neurodegenerative Diseases (CIBERNED), University Pablo de Olavide, Seville, Spain
[b] Fundación Investigación Hospital Clínico Universitario de Valencia-INCLIVA, Spain
[c] Laboratorio de Neurobiología, Centro Investigación Príncipe Felipe de Valencia, Spain
[d] Servicio de Digestivo, Hospital Arnau de Vilanova, Valencia, Spain
[e] Grupo Hepatología, Servicio Aparato Digestivo, Hospital Clínico de Valencia, Spain
[f] ERESA, Unidad de RM, Valencia, Spain
[g] Departamento de Medicina. Universidad de Valencia, Spain

ARTICLE INFO

Article history:
Accepted 13 March 2012
Available online 21 March 2012

Keywords:
Minimal hepatic encephalopathy
Cognitive impairment
Early diagnosis
Cortical thickness
Hepatic cirrhosis

ABSTRACT

Little attention has been paid to cortical integrity in patients with minimal hepatic encephalopathy (MHE), although cognitive functions affected in early stages of liver disease are mainly allocated in different neocortical structures. Here we used cortical surface-based analysis techniques to investigate if patterns of cortical thinning accompany the mildest form of HE. To aim this goal, cortical thickness obtained from high-resolution 3T magnetic resonance imaging (MRI) was measured in patients with no MHE (NMHE), MHE, and healthy controls. Further correlation analyses were performed to examine whether scores in the critical flicker frequency (CFF) test, and blood ammonia levels accounted for the loss of cortical integrity in different stages of liver disease. Finally, we assessed group differences in volume of different subcortical regions and their potential relationships with CFF scores/blood ammonia levels. Results showed a focal thinning of the superior temporal cortex and precuneus in MHE patients when compared with NMHE and controls. Relationships between blood ammonia levels and cortical thickness of the calcarine sulcus accounted for impaired visual judgment in patients with MHE when compared to NMHE. Regression analyses between cortical thickness and CFF predicted differences between controls and the two groups of HE patients, but failed to discriminate between patients with NMHE and MHE. Taking together, these findings provide the first report of cortical thinning in MHE patients, and they yield novel insights into the neurobiological basis of cognitive impairment associated with early stages of liver diseases.

Introduction

Hepatic cirrhosis (HC) is characterized by the development of regenerative nodules surrounded by fibrotic scar tissue in response to chronic liver injury (Lefton et al., 2009). Most HC patients remain asymptomatic up to development of liver decompensation, frequently followed by a wide spectrum of neuropsychiatric abnormalities that reduces the quality of life and impacts daily functioning (Heidelbaugh and Bruderly, 2006; Menon and Kamath, 2000). This syndrome, so-called hepatic encephalopathy (HE), is thought that results from alterations of neuronal function and metabolism, morphological changes of astrocytes, and neuronal loss (Butterworth, 2010; Häussinger and Görg, 2010), leading to functional deficits of varying severity, ranging from subtle emotional, cognitive and motor dysfunctions to severe coma (Ferenci et al., 2002). HE has been associated with increased mortality (Stewart et al., 2007) and is a condition potentially reversible with medical treatment (Riordan and Williams, 1997); therefore, identification and treatment should not be delayed at this stage of liver disease.

Minimal hepatic encephalopathy (MHE) represents the mildest form of HE and it is associated with a poor outcome prognosis for many patients (Stewart and Smith, 2007). MHE leads to psychomotor slowing and subtle cognitive deficits that mainly affect attention, memory and executive abilities (for a review, Weissenborn et al., 2001a), impacting on the capacity to perform daily activities requiring awareness and cognitive integrity (Bajaj, 2008; Wein et al., 2004). Although diagnostic criteria and markers of MHE have evolved considerably in the last decade (e.g., Bajaj et al., 2011; Randolph et al., 2009; Amodio et al., 2005; Kircheis et al., 2002), poor sensitive of these markers impedes to reliably detect this condition in clinical settings.

* Corresponding author at: Laboratory of Functional Neuroscience, University Pablo de Olavide, Ctra. de Utrera Km 1, 41013, Seville, Spain. Fax: +34 954 349151.
E-mail address: jlcanlor@upo.es (J.L. Cantero).
[1] Contributed equally to this work.

1053-8119/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
doi:10.1016/j.neuroimage.2012.03.041

Neuropsychological approaches have critically contributed to describe the pattern of cognitive deficits in MHE (e.g., Mattarozzi et al., 2005; Randolph et al., 2009; Stewart et al., 2010), although factors such as age, education level, and arousal state during testing act as potential confounders affecting the diagnosis of MHE. The critical flicker frequency (CFF) test, an age and education-independent probe, has shown not only high levels of sensitivity and specificity to identify MHE patients (Romero-Gomez et al., 2007; Sharma et al., 2007; Kircheis et al., 2002), but also a considerable diagnostic accuracy in assessing the recovery of MHE (Sharma et al., 2010). Therefore, research combining neuropsychological and CFF tests with novel biomarkers is clearly needed to establish a multidimensional approach aimed at increasing our ability to detect mild stages of HE.

Brain damage caused by HE and its reversal with restoration of liver function has also been unveiled in humans with in vivo neuroimaging techniques (Rovira et al., 2008; Naegele et al., 2000). Studies carried out in HC patients have shown hyperintensities in the globus pallidus and substantia nigra on T1-weighted images (Krieger et al., 1996), likely revealing increased accumulation of manganese in basal nuclei (Spahr et al., 1996). But it still remains unclear whether hyperintensities of globus pallidus are unique features of HC or they extend to HE patients. Osmoregulatory dysfunctions in response to hyperammonemia have been further revealed by proton MR spectroscopy (MRS) in both HC and HE conditions. Thus, increased levels of brain ammonia were found to correlate with an elevated glutamine/glutamate peak coupled with decreased myo-inositol and choline resonances (Lee et al., 1999; Häussinger et al., 1994; Kreis et al., 1992). However, the lack of differences in MRS patterns between MHE and overt HE patients (Lee et al., 1999) has lowered the value of this technique to establish different stages of chronic liver disease.

Previous research mostly assumes that neurocognitive deficits associated with MHE have a subcortical origin, but it is uncertain to date if a loss of cortical integrity plays an additional role in this subtle cognitive impairment (Kato et al., 2000; Burra et al., 1999; Taylor-Robinson, et al., 1999). Computational neuroanatomy analysis techniques applied to high-resolution cerebral MRI have substantially contributed to in vivo detection and quantification of changes in the neocortical mantle (Duncan et al., 2004; Good et al., 2001). This approach has revealed patterns of cortical thinning in prevalent neurological (Butman and Floeter, 2007; Charil et al., 2007; Singh et al., 2006) and psychiatric disorders (Makris et al., 2007; Lyoo et al., 2006; Kuperberg et al., 2003) emphasizing its value to identify potential cortical lesions during the natural course of HE.

The main goal of the present study is to examine if specific patterns of cortical thinning underlie MHE. Secondly, as visual judgment is impaired in MHE patients (Zafiris et al., 2004), we evaluate whether a loss of cortical integrity parallels this functional impairment in this subclinical population. Thirdly, relationships between changes in blood ammonia levels and cortical gray-matter are also evaluated to determine their diagnostic value in different stages of chronic liver disease. Finally, we investigate group differences in volume of different subcortical regions and their potential relationships with CFF scores/blood ammonia levels in order to determine if patterns of cortical thickness provide a similar or better diagnostic index than the volume of basal ganglia in discriminating patients with and without MHE.

Materials and methods

Participants

Thirty-four patients with HC and 17 control subjects were enrolled in the study after signing an informed consent. Physical examination, standard laboratory tests, and a standardized battery of psychometric tests aimed at detecting MHE were administered to all participants. The diagnosis of HC was based on clinical, biochemical, and ultrasonographic data. Exclusion criteria were overt HE or history of overt HE, infection, recent (<6 weeks) antibiotic use or gastrointestinal bleeding, history of recent (<6 weeks) use of drugs affecting cognitive function (e.g., benzodiazepines, anti-epileptic and/or psychotropics), history of shunt surgery or transjugular intrahepatic portosystemic shunt for portal hypertension, electrolyte imbalance, renal impairment (serum creatinine >1.5 mg/dL), presence of hepatocellular carcinoma, or severe medical problems (e.g., congestive heart failure, pulmonary disease, cerebrovascular disease, neurological or psychiatric disorder).

HC patients were divided into those with MHE (n = 17) and those who did not show clinical signs of MHE (NMHE) (n = 17) based on psychometric criteria (see Neuropsychological assessment and CFF test section). Patients were diagnosed with chronic liver cirrhosis during the past ten years, and cerebral MRI was performed at the time of recruitment for the experiment. Liver diseases were discarded in controls (n = 17) by clinical, analytical, and serological tests. Demographic characteristics, etiology of liver disease, and psychometric scores of study populations are shown in Table 1. Study protocols were previously approved by the Scientific and Ethical Committees of Hospitals involved in the study, and procedures followed were in accordance with the ethical guidelines of the Helsinki Declaration.

Laboratory tests

Blood samples were obtained from each participant after overnight fasting. Laboratory assessment included hemogram (hemoglobin, total white blood cells and platelets), kidney and liver function tests (bilirubin, aspartate amino transferase, alanine aminotransferase, alkaline phosphatase, albumin, prothrombin time, and creatinine). Table SM1 (Supplementary material) shows results of laboratory tests for each group.

Blood samples were collected onto ice, and ammonia determinations were performed immediately after collection. Ammonia levels were determined with the Ammonia Test Kit II for the PocketChem BA system (Arkay, Inc., Kyoto, Japan) following manufacturer's specifications. This test is commonly used to establish blood ammonia levels for screening purposes, although it is inadequate for clinical diagnosis due to its poor reliability.

Table 1
Demographic, clinical profile, and CFF scores.

	Controls	NMHE	MHE
Demographic			
# of cases	17	17	17
Gender (M/F)	6/11	13/4	10/7
Age	57.3 ± 6.1	55.5 ± 7.8	60.7 ± 8.1
Clinical profile			
Alcohol	–	11	10
HVB	–	1	0
HVC	–	3	6
HVC + Alcohol	–	2	0
HVB + Alcohol	–	0	1
Ascites	–	2	2
Child Pugh A/B/C	–	12/5/0	10/6/1
MELD	–	10 ± 3	8 ± 2
CFF scores			
CFF	42.9 ± 2.5	41.6 ± 2.9[§a]	38.3 ± 4.1[*a]

Values are expressed as mean ± SEM. Group differences were determined by one-way ANOVA followed by *post-hoc* t-tests ($p<.05$). HC patients without minimal hepatic encephalopathy (NMHE) and with minimal hepatic encephalopathy (MHE); (M/F) = Male/Female; HVB = hepatitis virus B; HVC = hepatitis virus C; MELD = model for end-stage liver disease; CFF = critical flicker frequency test. *Significant differences between controls and MHE, §significant differences between NMHE and MHE. [a]$p<.01$.