Research Article

JOURNAL OF HEPATOLOGY

Cirrhosis

Cerebrospinal fluid metabolomics highlights dysregulation of energy metabolism in overt hepatic encephalopathy

Nicolas Weiss[1,2], Pierre Barbier Saint Hilaire[3], Benoit Colsch[3], Foucauld Isnard[3], Suleiman Attala[3], Augustin Schaefer[1], Maria del Mar Amador[4], Marika Rudler[1,5], Foudil Lamari[6], Frédéric Sedel[7], Dominique Thabut[1,5,†], Christophe Junot[3,*,†]

[1]*Brain Liver Pitié-Salpêtrière (BLIPS) Study Group, Groupement Hospitalier Pitié-Salpêtrière-Charles Foix, Paris, France;* [2]*Unité de réanimation neurologique, Fédération de neurologie 1, pôle des maladies du système nerveux, Groupement Hospitalier Pitié-Salpêtrière-Charles Foix, Assistance Publique – Hôpitaux de Paris et Institut de neurosciences translationnelles IHU-A-ICM, Paris, France;* [3]*CEA, iBiTec-S, Service de Pharmacologie et d'Immunoanalyse, Laboratoire d'Etude du Métabolisme des Médicaments, MetaboHUB-Paris, Université Paris Saclay, 91191 Gif-sur-Yvette cedex, France;* [4]*Neurometabolic Unit and University Pierre and Marie Curie, Paris, Groupe Hospitalier Pitié Salpêtrière-Charles Foix, 83 Boulevard de l'Hôpital, 75013 Paris, France;* [5]*Unité de Soins Intensifs d'Hépato-gastroentérologie, Groupement Hospitalier Pitié-Salpêtrière-Charles Foix, Assistance Publique – Hôpitaux de Paris et Université Pierre et Marie Curie Paris 6, Paris, France;* [6]*Department of Metabolic Biochemistry, Groupe Hospitalier Pitié Salpêtrière-Charles Foix, 83 Boulevard de l'Hôpital, 75013 Paris, France;* [7]*Medday Pharmaceuticals, ICM-Brain and Spine Institute-iPEPS, Groupe Hospitalier Pitié Salpêtrière-Charles Foix, 83 Boulevard de l'Hôpital, 75013 Paris, France*

See Editorial, pages 1077–1078

Background & Aims: Hepatic encephalopathy (HE) is a neurological complication observed in patients with liver disease and/or porto-systemic shunt. The proportion of cirrhotic patients developing overt HE is about 20%, and 60–80% of cirrhotic patients exhibit mild cognitive impairment potentially related to minimal HE. However, the pathophysiological mechanisms of HE remain poorly understood. In this context, metabolomics was used to highlight dysfunction of metabolic pathways in cerebrospinal fluid (CSF) samples of patients suffering from HE.

Methods: CSF samples were collected in 27 control patients without any proven neurological disease and 14 patients with symptoms of HE. Plasma samples were obtained from control patients, and from cirrhotic patients with and without HE. Metabolomic analysis was performed using liquid chromatography coupled to high-resolution mass spectrometry.

Results: Concentrations of 73 CSF metabolites, including amino acids, acylcarnitines, bile acids and nucleosides, were altered in HE patients. Accumulation of acetylated compounds, which could be due to a defect of the Krebs cycle in HE patients, is reported for the first time. Furthermore, analysis of plasma samples showed that concentrations of metabolites involved in ammonia, amino-acid and energy metabolism are specifically and significantly increased in CSF samples of HE patients. Lastly, several drugs were detected in CSF samples and could partially explain worsening of neurological symptoms for some patients.

Conclusion: By enabling the simultaneous monitoring of a large set of metabolites in HE patients, CSF metabolomics highlighted alterations of metabolic pathways linked to energy metabolism that were not observed in plasma samples.

Lay summary: CSF metabolomics provides a global picture of altered metabolic pathways in CSF samples of HE patients and highlights alterations of metabolic pathways linked to energy metabolism that are not observed in plasma samples.

Keywords: Metabolomics; Cerebrospinal fluid; Hepatic encephalopathy; Cirrhosis; Liquid chromatography; High-resolution mass spectrometry; Blood-brain barrier.

Received 9 October 2015; received in revised form 11 July 2016; accepted 22 July 2016; available online 9 August 2016

* Corresponding author. Address: Laboratoire d'Etude du Métabolisme des Médicaments, DSV/iBiTec-S/SPI, bat 136, CEA-Saclay, Gif-sur-Yvette cedex 91191, France. Tel.: +33 1 69 08 43 66.

E-mail address: christophe.junot@cea.fr (C. Junot).

† These authors share senior authorship.

Introduction

Hepatic encephalopathy (HE) encompasses a spectrum of neurological and neuropsychiatric abnormalities observed in patients with liver disease and/or porto-systemic shunt, after exclusion of other known brain diseases. According to the classification of the World Congress of Gastroenterology held in 1998 in Vienna, three HE categories should be defined: type A is associated with acute liver injury, type B is associated with a porto-systemic shunt without associated liver disease, and type C is associated with cirrhosis or portal hypertension [1]. The latter type is the most frequent since cirrhosis is a major public health problem in developed countries with, in France, 700,000 reported cirrhotic patients associated with 15,000 deaths per year and an at-risk population of 12 million people [2]. The prognosis of cirrhosis has improved dramatically in recent years by refinement of prophylactic strategies and better management of complications (gastrointestinal bleeding,

ascites). Furthermore, the recent and widespread indication of early trans-jugular intra-hepatic porto-systemic shunt has decreased mortality with, however, an increased occurrence of HE by direct hepatic shunting [3]. Thus, the proportion of cirrhotic patients developing overt HE has largely increased and is currently at least 20% [4].

The pathophysiological mechanisms of HE are still poorly understood. It is generally assumed that HE is caused by an association of increased ammonia levels and inflammation. Ammonia, the degradation product of nitrogen compounds, is metabolized by the liver into urea in healthy individuals. Ammonemia is increased in cirrhotic patients, and reflects altered liver function or porto-systemic shunting. Outside the liver, ammonia can be metabolized into glutamine by glutamine synthase in muscle cells and in astrocytes of the central nervous system. Glutamine is osmotically active and high glutamine levels are responsible for astrocyte swelling, and then afterwards brain edema, which are compensated for by the release from astrocytes of taurine and myoinositol, which act as osmoregulators [5].

However, although these mechanisms are well documented and accepted, there is no correlation between the severity of HE and ammonia or glutamine blood levels [6–8]. In animal models and in humans, some studies have shown that high ammonia levels induce HE only if systemic inflammatory response syndrome is present [9–11]. Thus, it is widely accepted that sepsis is able to trigger HE in cirrhotic patients as a result of altered nitrogen metabolism and also by releasing pro-inflammatory mediators [12].

Finally, impaired blood-brain barrier (BBB) permeability and transport have been pointed out as a mechanism involved in the occurrence of HE manifestations [13]. For example, in response to increased glutamine concentrations, an accelerated transport of methionine and aromatic amino acids (AAAs, including tryptophan, phenylalanine and tyrosine) across the BBB has been observed [14,15].

Cerebrospinal fluid (CSF) is a medium of choice to approximate intra-cerebral metabolism because of its proximity to neuronal and glial cells, and its biochemical analysis could thus be of value in understanding the pathophysiology of HE. However, there are few published studies regarding the metabolic composition of CSF in HE. In 1974, Vergara *et al.* reported an increase of ammonia concentration in CSF with large variations between patients [16]. They also detected elevated levels of glutamine, glutamate, and α-ketoglutarate (αKG). Increased concentrations of neurotransmitters such as serotonin and dopamine [17], amino acids like tryptophan, tyrosine, phenylalanine and methionine, and bile acids were also reported by other authors [14,18]. However, these data are sparse, and restricted to few metabolites.

By enabling the concomitant detection of a wide range of metabolites, metabolomics could address this issue. This is a data-driven and a multidisciplinary approach combining analytical chemistry for the acquisition of metabolic fingerprints, and biostatistics, informatics and biochemistry for mining and interpretation of data. Metabolomics has already been successfully applied to highlight disease biomarkers in many medical fields [19]. By using metabolomics, we aimed to provide a global picture of the metabolic phenotypes of HE patients.

In this study, we applied liquid chromatography coupled to high-resolution mass spectrometry–based metabolomics to CSF samples of HE patients. By these means, we observed multiple alterations of metabolic pathways. Some of them have already been described, whereas others are highlighted for the first time.

Patients and methods

This study was performed in the hepatology intensive care unit (ICU) of La Pitié-Salpêtrière Hospital between November 2012 and June 2013 for CSF samples, and in 2015 for plasma samples. Samples were collected after written informed consent, in accordance with the local ethics committee of La Pitié-Salpêtrière Hospital, Paris, France.

Patients

CSF study

We prospectively collected CSF samples from 14 patients admitted to the hepatology ICU for symptoms of HE. They underwent lumbar puncture to rule out meningitis or meningoencephalitis. Patients with a previous neurological history other than HE or with a do-not-resuscitate order were excluded. The severity of cirrhosis was evaluated by the Child-Pugh score [20] and the model for end-stage liver disease (MELD) score [21]. Leukocyte count, hemoglobin level, platelets, prothrombin time, international normalized ratio (INR), liver enzymes, creatinine level, sodium level, albumin, C-reactive protein (CRP), and ammonemia were recorded. HE was assessed using the West Haven (WH) score [1]. Overt HE, so-called HE patients in the present study, was defined by a WH score ranging from 2 to 4 [22].

Patient samples were compared with those of 27 CSF control samples collected from adult patients seen in the Neurometabolic Unit of La Pitié-Salpêtrière Hospital and free of any neurological disease after extensive etiological work-up.

Plasma study

Plasma samples were collected from 9 healthy control samples, 12 overt HE and 13 cirrhotic patients without overt HE. The severity of cirrhosis was evaluated by the Child-Pugh and the MELD scores, whereas the severity of HE was assessed by the WH score. Leukocyte count, hemoglobin level, platelets, prothrombin time, INR, liver enzymes, creatinine level, sodium level, albumin, CRP, and ammonemia were also recorded. Clinical data are given as absolute numbers and percentages for qualitative variables and median and interquartile range for quantitative variables.

Metabolomic analyses

Chemicals and reagents, the procedure for extraction of CSF and plasma metabolites, experimental settings for liquid chromatography coupled to high-resolution mass spectrometry, data processing tools and statistical analyses are detailed in the Supplementary material.

Results

Between November 2012 and June 2013, 14 patients underwent lumbar puncture. Of the 14 CSF samples, 12 were from HE cirrhotic patients (type C) without trans-jugular intrahepatic porto-systemic shunt, and 2 from non-cirrhotic HE patients: a liver transplant patient (PV2) who developed HE before transplantation and displayed delirium 4 months after transplantation, and a patient with a rare cause of portal hypertension (hepatoportal sclerosis) (MP11). Diagnosis of meningitis or meningoencephalitis was ruled out for all patients. CSF samples from HE patients were compared with those of 27 control patients. Patient characteristics are shown in Table 1.

Three complementary liquid chromatography coupled to high-resolution mass spectrometry based methods were used to provide optimal coverage of the CSF metabolome. The annotation procedure and also MS/MS experiments led to the identification and relative quantification of 122 metabolites. Analytical information and identification status of annotated metabolites are displayed in Supplementary Table 1 and in the Supplementary material.