Research Article

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Evaluation of early cerebral metabolic, perfusion and microstructural changes in HCV-positive patients: A pilot study

Joanna Bladowska[1,*], Anna Zimny[1], Brygida Knysz[2], Krzysztof Małyszczak[3], Anna Kołtowska[1], Paweł Szewczyk[1], Jacek Gąsiorowski[2], Michał Furdal[4], Marek J. Sąsiadek[1]

[1]*Department of General Radiology, Interventional Radiology and Neuroradiology, Chair of Radiology, Wroclaw Medical University, Borowska 213, 50-556 Wroclaw, Poland;* [2]*Department of Infectious Diseases, Liver Diseases and Acquired Immune Deficiency, Wroclaw Medical University, Koszarowa 5, 51-149 Wroclaw, Poland;* [3]*Division of Psychotherapy and Psychosomatic Medicine, Department of Psychiatry, Wroclaw Medical University, L. Pasteura 10, 50-367 Wrocław, Poland;* [4]*Regional Specialistic Hospital, Department of Cardiology, Kamienskiego 73a, 51-124 Wroclaw, Poland*

Background & Aims: The aim of the study was to evaluate early metabolic perfusion, and microstructural cerebral changes in patients with the hepatitis C virus (HCV) infection and normal appearing brain on plain MR using advanced MR techniques, as well as to assess correlations of MR measurements with the liver histology activity index (HAI).

Methods: Fifteen HCV-positive patients and 18 control subjects underwent single voxel MR spectroscopy (MRS), perfusion weighted imaging (PWI), and diffusion tensor imaging (DTI), using a 1.5T MR unit. MRS metabolite ratios (NAA/Cr, Cho/Cr, mI/Cr) were calculated. PWI values of relative cerebral blood volume (rCBV) were assessed from 8 areas including several cortical locations, basal ganglia, and fronto-parietal white matter. DTI fractional anisotropy (FA) and apparent diffusion coefficient (ADC) values were obtained from 14 white matter tracts.

Results: Compared to controls, HCV-positive patients showed significantly (p <0.05) lower NAA/Cr ratios within frontal and parietal white matters, lower rCBV values within frontal and temporo-parietal cortices, decreased FA values, as well as increased ADC values in several white matter tracts. We also found elevated rCBV values in basal ganglia regions. The increase in mI/Cr and Cho/Cr ratio was correlated with a higher HAI score.

Conclusions: The results of advanced MR techniques indicate neurotoxicity of HCV reflected by neuronal impairment within white matter, cortical hypoperfusion, and disintegrity within several white matter tracts. Hyperperfusion in basal ganglia may be an indicator of brain inflammation in HCV patients. Our findings may suggest a biologic link between HCV-related liver disease and cerebral dysfunction.

Introduction

Numerous studies have documented that the hepatitis C virus (HCV) can invade the central nervous system (CNS), which was supported by detection of HCV specific transcripts and proteins in post-mortem brains of HCV-infected patients [1–3]. It has been reported that HCV is not exclusively hepatotropic, as it can also replicate in leukocytes, including monocytes/macrophages [4]. The infected monocytes may cross the blood-brain barrier and thus provide access of HCV to the CNS in a process similar to that described for HIV, known as a 'Trojan horse' mechanism [3,5]. Moreover, several studies have evidenced that HCV infection is associated with cognitive dysfunction, fatigue, and depression [4–7].

We hypothesized that since HCV can infect the brain and impair CNS function, it may cause alterations in cerebral metabolism, perfusion and white matter integrity, as measured by *in vivo* proton magnetic resonance spectroscopy (MRS), perfusion weighted imaging (PWI) and diffusion tensor imaging (DTI). MRS has enabled the *in vivo* studies of certain metabolites in a variety of pathologic processes that affect the CNS. MRS can show the changes in metabolite profiles in normal appearing white matter (NAWM) and normal appearing gray matter (NAGM) [8]. PWI is a method that brings information on cerebral flow at the capillary

Keywords: HCV; Cerebral metabolism; Cerebral perfusion; Cerebral white matter; Magnetic resonance spectroscopy; Perfusion-weighted imaging; Diffusion tensor imaging.

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* Corresponding author. Address: Department of General Radiology, Interventional Radiology and Neuroradiology, Wroclaw Medical University, Borowska 213 Str., 50-556 Wroclaw, Poland. Tel.: +48 (71) 733 1660; fax: +48 (71) 733 1689.

E-mail address: asia.bladowska@gmail.com (J. Bladowska).

Abbreviations: HCV, hepatitis C virus; MR, magnetic resonance; HAI, histology activity index; MRS, magnetic resonance spectroscopy; PWI, perfusion weighted imaging; DTI, diffusion tensor imaging; NAA, N-acetylaspartate; Cr, creatine; Cho, choline; mI, myo-inositol; rCBV, relative cerebral blood volume; FA, fractional anisotropy; ADC, apparent diffusion coefficient; CNS, central nervous system; HIV, human immunodeficiency virus; NAWM, normal appearing white matter; NAGM, normal appearing gray matter; DSC-MR, dynamic susceptibility contrast MR imaging; WSCT, Wisconsin Card Sorting Test; FLAIR, fluid-attenuated inversion recovery sequence; DWI, diffusion-weighted imaging; SVS, Single Voxel Spectroscopy; PCG, posterior cingulate gyrus; ACG, anterior cingulate gyrus; P-WM, parietal white matter; BG, basal ganglia; FWM, frontal white matter; MCP, middle cerebellar peduncle; ILF, inferior longitudinal fasciculus; IFOF, inferior fronto-occipital fasciculus; GCC, genu of the corpus callosum; SCC, splenium of the corpus callosum; PLIC, posterior limb of internal capsule; SLF, superior longitudinal fasciculus, PC, posterior cingulum; IDU, intravenous drug users.

Research Article

level (microvasculature). Among a few PWI techniques, the dynamic susceptibility contrast MR imaging (DSC-MR) is the most often used one. DSC MRI enables non-invasive measurements of relative cerebral blood volume (rCBV), thus providing information similar to that obtained in PET and SPECT studies but with several advantages, such as lack of ionizing radiation, high spatial resolution, and low relative cost [9]. DTI provides measures of the 3D anisotropic diffusion of water molecules within tissue. By measuring the fractional anisotropy (FA) and apparent diffusion coefficient (ADC) parameters, it is possible to delineate the characteristic changes in brain tissues at the level of cellular microarchitecture, as well as to show the changes in NAWM and NAGM that are not visible on plain MR [10]. The FA parameter reflects the directionality and coherence of water self-diffusion: tissues with highly regular fibers have high anisotropy, whereas those with less regular fibers demonstrate low anisotropy. The ADC is an indicator of the average diffusion a water molecule experiences within a voxel and is independent of the directionality of water diffusion. For example, cerebrospinal fluid exhibits a high ADC because there are no barriers to diffusion, whereas tissues, such as white or gray matter, will exhibit lower ADC because of cellular barriers to diffusion [10]. These advanced MR techniques may offer potentially unique insight into the pathophysiology of CNS involvement by the hepatitis C virus.

The aim of our study was to assess early metabolite, perfusion and microstructural alterations using MRS, PWI, and DTI in HCV-positive patients with mild liver disease, before antiviral therapy, who presented the normal appearing brain on plain MR. The other purpose was to assess correlations of MR measurements with the rate of liver disease.

To the best of our knowledge, this is the first report focusing on the analysis of MRS, PWI, and DTI changes within the normal appearing brain in HCV-positive subjects with only mild liver disease.

Materials and methods

Patients

Fifteen HCV-positive naive patients (6 women and 9 men; mean age 39.5 years, range 19–58 years) and 18 normal control subjects (6 women and 12 men; mean age 34.69 years, range 19–56 years) were enrolled in the study. The HCV patients suffered from chronic viral hepatitis with HCV RNA positive results for longer than 6 months. Plasma viral load (HCV RNA) at the time of imaging was determined, as well as the Cobas TaqMan test version 2.0 with High Pure system isolation, the Roche Diagnostics real-time PCR method for HCV was used.

In all the HCV patients, a liver biopsy was performed. Histopathological examinations of the samples revealed an HAI (histology activity index) inflammation score and HAI fibrosis score of 0–2.

The clinical characteristics of the studied groups are shown in Table 1.

The study was performed in accordance with the guidelines of the local University Ethics Committee for conducting research involving humans. Each patient signed his/her informed consent before participation in the examination.

Neurologic evaluation

All patients underwent physical and neurologic examinations, including determination of their Karnofsky score (max. 100). The inclusion criteria were: lack of neurological abnormalities in standard questionnaires and a Karnofsky score above 80. The exclusion criteria were as follows:

1. Presence of neurological diseases (e.g., inflammatory changes, brain neoplasms);
2. Psychiatric disease history (e.g., depression, schizophrenia, dementia).

Table 1. Clinical characteristics of studied patients.

Subjects	HCV before treatment Genotype 1	Control group
Mean age (yr) range (yr)	39.5 19-58	34.7 19-56
Intravenous drug users	0 (0%)	0 (0%)
Presence of cryoglobulinemia	0 (0%)	0 (0%)
HCV RNA at imaging (IU/ml) mean	58,672-4,485,492 1,324,553	-
Treatment	-	-
Disease duration (months)	>6	-
Liver disease	mild	-
Inflammation (HAI) mean	0-2 1.2	-
Fibrosis (HAI) mean	0-2 0.6	-

Psychological evaluation

Two cognitive tests, Wisconsin Card Sorting Test (WCST) as a measure of executive function and Brickenknap's d2 concentration endurance test as a measure of visual attention, were used in order to assess a possible deterioration of cognitive functions [11,12].

The control group (CG)

The healthy control group with negative anti-HCV antibodies had no history of drug abuse or liver disease and consisted mainly of the hospital staff.

MR imaging protocols

Imaging was performed with a 1.5T Signa Hdx scanner (GE Healthcare) using a 16-channel coil dedicated for head and spine imaging. Conventional sequences included: axial, sagittal, and coronal T2-weighted images, axial T1-weighted, and FLAIR (fluid-attenuated inversion recovery sequence) images, as well as diffusion-weighted imaging (DWI). Only those subjects who had normal signal intensity of the gray and white matter without evidence of cerebral atrophy were included in our study.

Magnetic resonance spectroscopy (MRS)

The MRS examinations were performed using the Single Voxel Spectroscopy (SVS) technique (PRESS sequence). The data acquisition parameters were as follows: TR = 1500 ms, TE = 35 ms, 128 acquisitions, number of excitations = 8. Using the localizing axial T2-weighted images, voxels of $2 \times 2 \times 2$ cm (8 cm^3) in size were placed in the normal appearing brain in the following 5 regions: posterior cingulate gyrus (PCG), anterior cingulate gyrus (ACG), left parietal white matter (PWM), left basal ganglia (BG) (Fig. 1A), and left frontal white matter (FWM). The total acquisition time was 3 min 45 s for each voxel. All MR spectroscopy studies were performed by using the automated single-voxel MR spectroscopy package Proton Brain Examination/Single Voxel (PROBE/SV; GE Medical Systems, Milwaukee, WI). The pre-imaging algorithm of the PROBE software automatically adjusted the transmitter and receiver gains and center frequency. The local magnetic field homogeneity was optimized with the three-plane auto-shim procedure with linear gradient shimming, and the flip angle of the third water-suppression pulse was adjusted for chemical shift water suppression (CHESS) before PRESS acquisition. Each spectrum was automatically fitted to four peaks corresponding to the levels of N-acetylaspartate (NAA) (2.02 ppm), total creatine (Cr) (3.03 ppm), choline-containing compounds (Cho) (3.23 ppm), and myo-inositol (ml) (3.56 ppm). Metabolite intensity ratios of NAA/Cr, Cho/Cr, and mI/Cr were automatically calculated at the end of each PROBE/SV acquisition.

MRS data were post-processed using a software provided by the manufacturer (GE workstation, ADW 4.4). The ratios of NAA, Cho, and mI to creatine (NAA/Cr, Cho/Cr, mI/Cr, respectively) were analyzed.