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Cerebral immune activation in chronic hepatitis C infection: A magnetic resonance spectroscopy study☆

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***Background/Aims*: Abnormal cerebral metabolism and cognitive impairments have been reported in patients with chronic hepatitis C (HCV) but studies have failed to demonstrate a relationship between these findings.**

***Methods*: Twenty-five HCV-positive patients with histologically-mild liver disease were studied with cerebral proton magnetic resonance spectroscopy (MRS), using acquisition parameters to quantify myo-inositol (mI) and other metabolites in frontal white matter (FWM). Patients underwent automated attention and working memory tests (Cognitive Drug Research test system).**

***Results*: The mean mI/creatine ratio in the HCV+ve patients (0.64, SD 0.21) was significantly higher ($p = 0.02$) than in healthy controls (0.52, SD 0.10). On cognitive testing, the HCV+ve patients showed impairments in 2/4 composite scores, reflecting working memory and attention, compared to normative data from healthy volunteers ($p < 0.005$) and HCV−ve controls ($p = 0.03$). There was a significant association between elevated FWM mI/creatine and prolonged working memory reaction times ($R = 0.72$, $p = 0.002$).**

***Conclusions*: Elevated FWM mI/creatine is a feature of HIV-related minor cognitive-motor disorder. It is associated with infection and immune activation of microglial cells. The similar findings in this study suggest that cerebral immune activation may also occur in HCV infection. This may underlie the mild neurocognitive impairment and neuropsychological symptoms observed in a proportion of patients.**

***Keywords*: Hepatitis C; Cognitive; Magnetic; Spectroscopy; *myo*-inositol**

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Abbreviations: HCV, hepatitis C virus; MRS, magnetic resonance spectroscopy; mI, *myo*-inositol; FWM, frontal white matter; SD, standard deviation; HIV, human immunodeficiency virus; HRQL, health related quality of life; CNS, central nervous system; Cho, Choline; Cr, Creatine; NAA, *N*-acetyl aspartate; MCMD, minor cognitive motor disorder; HAD, HIV-associated dementia; IVDU, intravenous drug use; MDU, major recreational drug use; CDR, cognitive drug research; MDMA, 3,4-methylenedioxymethamphetamine; TNF, tumour necrosis factor; LPS, lipopolysaccharide; BBB, blood brain barrier; HPA, hypothalamic pituitary axis; IL, interleukin.

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1. Introduction

Patients with chronic hepatitis C (HCV) infection and human immunodeficiency virus HIV/HCV co-infection frequently report neuropsychological symptoms. Numerous studies have documented high levels of fatigue, depression and impaired health-related quality of life (HRQL) in patients infected by HCV [1–10]. A number of studies have also reported an adverse impact of HCV on cognitive function [11–16]. Slowed processing speed and impaired working memory are the most common findings. There has been considerable debate as to whether there is a biological basis to these findings and a number of studies have suggested that social and psychological factors are more important than the viral infection *per se* [17–21]. However, a biological basis for neurocognitive impairment in HCV infection is suggested by reports of both HCV replication within the central nervous system (CNS) [22–24] and of altered cerebral metabolism, as measured by *in vivo* proton magnetic resonance spectroscopy (^{1}H MRS) [11,15,16,25]. Using different techniques, four MRS studies have variously reported elevations in basal ganglia and central white matter choline (Cho)-containing compounds [11,16,25] and reductions in grey and white matter *N*-acetyl aspartate (NAA) [15,16] in patients with histologically-proven mild liver disease due to HCV infection. The studies to date have not been able to demonstrate an association between MRS findings and neurocognitive performance.

It should be noted that *in vivo* MRS is a readily available, standardized technique, which gives information on a number of cerebral metabolites, depending on the acquisition parameters employed. Cerebral ^{1}H MRS has been readily applied to HIV-associated minor cognitive-motor disorder (MCMD) and HIV-associated dementia (HAD) more extensively than any other viral infection [26,27]. In HIV disease, an early and common finding is increased myo-inositol (mI) in the frontal white matter [28,29]. Other findings include elevated Cho and decreased NAA in both the white matter and basal ganglia [30]. These latter abnormalities parallel the findings to date in HCV infection.

myo-Inositol (mI) is an intracellular metabolite involved in the synthesis of phosphoinositides and it also plays a significant role as an osmolyte in the regulation of cellular swelling within the brain [31]. Increased mI is associated with microglial activation and astrogliosis [32,33]. Elevated levels of mI in HIV infection are thought to relate to central nervous system (CNS) inflammation, which may underlie neuronal dysfunction [27,29,34,35]. Of all readily measurable MR metabolites, elevations in white matter mI are the most consistently associated with abnormal cognitive processing in early HIV disease [36]. To date, there have been no reports of increased cerebral mI in HCV infection.

This *in vivo* cerebral ^{1}H MRS study was designed to address the hypothesis that increases in cerebral mI occur in HCV-infected patients as a result of immune activation within the brain and that this cerebral metabolite abnormality is associated with mild cognitive impairment.

2. Patients and methods

Twenty-five patients with histologically-defined mild chronic hepatitis C were recruited from a viral hepatitis clinic in a tertiary referral centre (St. Mary's Hospital, London). The patients had been referred for assessment of their liver disease. The mean age (standard deviation – SD) was 45.0 (8.3) yrs and the percentage of male subjects was 54%. Liver biopsy on all patients had been performed no more than 18 months prior to the study. All individuals had *mild* inflammation only, in the absence of cirrhosis or significant fibrosis. The median Ishak necroinflammatory score was 2 and the median Ishak fibrosis score was 1 [37]. All patients were viraemic at the time of the study, as defined by a positive PCR for HCV RNA (Roche Amplicor version 2). The method of viral transmission was related to injection drug use (IVDU) in 65% of cases and to infected blood products or undefined sources in 35% of cases. All patients were carefully questioned for a history of major recreational drug usage (MDU). Individuals were asked whether they had ever used any of five substances (heroin, methadone, LSD, cocaine, ecstasy). They were only classified as non-users if a history of MDU was unequivocally absent. None of the patients had used any recreational drugs in the year preceding the study.

2.1. Cerebral magnetic resonance spectroscopy

Cerebral proton (^{1}H) MRS was performed using a 1.5 T Eclipse™ spectroscopy system (Philips Medical Systems, Cleveland, OH, USA). An enveloping quadrature transmit/receive coil tuned to 64 MHz was used for all examinations. T_1-weighted MR images were acquired in the transverse plane in order to exclude structural brain disease and to position the voxel of interest (T_1-weighted field echo, repetition time (TR) 21 ms, echo time (TE) 6 ms). A single 8 cm^3-sized voxel was positioned in the frontal white matter at the level of the centrum semiovale and ^{1}H MR spectra were obtained using a short-echo automated PRESS sequence (TE 40 ms, TR 1500 ms). Spectral analysis was performed within the time domain using the MRUI software package (available from: www.mrui.uab.es/mrui), incorporating the AMARES algorithm [38] and metabolites were expressed as ratios to cerebral creatine (Cr) (Fig. 1). The total examination time was 30 min. MR spectra were analyzed by a single observer in each case, who was blinded to the clinical status of the patients. Metabolite ratios were compared against age- and sex-matched historical reference data, acquired from 17 healthy hospital staff, using the same parameters and equipment. The mean age (45.1 years, SD 5.7) and the percentage of male subjects (40%) did not differ significantly from the test subjects.

2.2. Cognitive testing

The Cognitive Drug Research (CDR) [39] computerized cognitive assessment system was administered to the subjects, under standardized conditions by a trained individual who followed a protocol. The CDR system measures the speed of responses (with millisecond accuracy), as well as the accuracy of responses on a range of simple cognitive tasks. The battery employed in this study comprised tests of attention and working memory, domains where cognitive impairments have previously been reported in this patient population (simple reaction time, choice reaction time, digit vigilance, numeric and spatial working memory tasks) [11]. The data were used to generate four composite scores, which have previously been shown to reflect the following processes: power of attention, quality of working memory, speed of memory processes and ability to sustain attention [40]. The results were