Increased availability of central benzodiazepine receptors in patients with chronic hepatic encephalopathy and alcohol related cirrhosis

R Jalan, N Turjanski, S D Taylor-Robinson, M J Koepp, M P Richardson, J A Wilson, J D Bell, D J Brooks

Abstract

Background/aims—To measure cerebral benzodiazepine receptor binding using \(^{11}\)C-flumazenil positron emission tomography in patients with stable chronic hepatic encephalopathy, who were also characterised by proton magnetic resonance spectroscopy.

Methods—Six abstinent patients of mean age 61 years with alcohol related cirrhosis and grade I–II hepatic encephalopathy and 11 matched healthy volunteers were studied. Each patient’s encephalopathy was defined according to clinical, psychicometric, electroencephalographic, and magnetic resonance spectroscopy criteria. Using positron emission tomography, the brain volume of distribution of \(^{11}\)C-flumazenil was obtained; this reflects benzodiazepine receptor availability. Proton magnetic resonance spectra were acquired at 1.5 T using a multivoxel technique; peak area ratios were calculated for choline, glutamine/glutamate, N-acetylaspartate, and creatine resonances.

Results—The mean volume of distribution of \(^{11}\)C-flumazenil was significantly higher in the cortex, cerebellum, and the basal ganglia in the patients compared with controls (p<0.001). In the patient group, the mean glutamine/glutamate to creatine ratio was significantly increased and the mean choline to creatine ratio was significantly decreased in all brain areas, compared with healthy volunteers. However, the N-acetylaspartate to creatine ratio was unchanged compared with controls.

Conclusions—The spectroscopy results reflect the cerebral metabolic derangement associated with hepatic encephalopathy. Stable grade I–II chronic hepatic encephalopathy in alcohol related cirrhosis may be associated with increased cerebral benzodiazepine receptor availability. However, a direct effect of previous chronic exposure to alcohol cannot be excluded.


Keywords: benzodiazepine receptors; chronic hepatic encephalopathy; \(^{11}\)C-flumazenil; in vivo proton magnetic resonance spectroscopy; positron emission tomography

Hepatic encephalopathy is the term used to describe the neuropsychiatric abnormalities which result from liver failure and/or portosystemic shunting. It is probable that up to 80% of patients with chronic liver disease are affected, but in the majority of these patients, the syndrome is subclinical, with impairment of reaction times in activities of daily living such as driving and operating machinery. In about 30% of individuals, the syndrome may become clinically overt, characterised by global depression of the central nervous system (CNS), with psychomotor retardation and reversible alterations in behaviour, mood, personality, and disturbances in consciousness.

Patients with hepatic encephalopathy show impairment of psychometric performance and sometimes slowing of the electroencephalograph (EEG) mean cycle frequency (mcf). In vivo proton magnetic resonance spectroscopy (H MRS) techniques have been used to provide objective, non-invasive biochemical information on cerebral metabolic processes in such patients. Previous studies have shown that an increase in the glutamine/glutamate MR resonance correlates with clinical severity of encephalopathy.

The pathogenesis of hepatic encephalopathy is unknown, but interest has focused on the potential pathogenetic role of circulating toxins and on changes in the functional state of cerebral neurotransmitter systems. \(\gamma\)-Aminobutyric acid (GABA) is one of the main cerebral inhibitory neurotransmitters, and alterations in the activity of the GABAergic system have been causally implicated in the genesis of the condition.

The GABA$_\text{A}$ receptor is a hetero-oligomeric complex that contains a GABA binding site, a benzodiazepine binding site (BzR), and a chloride ionophore. Binding of the neurotransmitter GABA to this receptor induces opening of the chloride channel, resulting in neuronal...
**Table 1** Patient characteristics

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (y)</th>
<th>Diagnosis</th>
<th>Child-Pugh class</th>
<th>Albumin (g/l)</th>
<th>Urea (mmol/l)</th>
<th>Oesophageal varices</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>39</td>
<td>ARC B</td>
<td>B&lt;sub&gt;i&lt;/sub&gt;</td>
<td>41</td>
<td>2.3</td>
<td>Yes (grade 2)</td>
</tr>
<tr>
<td>2</td>
<td>64</td>
<td>ARC C</td>
<td>C&lt;sub&gt;i&lt;/sub&gt;</td>
<td>35</td>
<td>5.2</td>
<td>Yes (grade 2)</td>
</tr>
<tr>
<td>3</td>
<td>66</td>
<td>ARC B</td>
<td>B&lt;sub&gt;i&lt;/sub&gt;</td>
<td>32</td>
<td>3.5</td>
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<tr>
<td>4</td>
<td>59</td>
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<td>B&lt;sub&gt;i&lt;/sub&gt;</td>
<td>30</td>
<td>4.3</td>
<td>Yes (grade 3)*</td>
</tr>
<tr>
<td>5</td>
<td>52</td>
<td>ARC B</td>
<td>B&lt;sub&gt;i&lt;/sub&gt;</td>
<td>35</td>
<td>5.4</td>
<td>Yes (eradicated)*</td>
</tr>
<tr>
<td>6</td>
<td>68</td>
<td>ARC C</td>
<td>C&lt;sub&gt;i&lt;/sub&gt;</td>
<td>36</td>
<td>5.9</td>
<td>Yes (eradicated)*</td>
</tr>
</tbody>
</table>

Albumin normal range: 35–51 g/l.
Urea normal range: 2.5–6.6 mmol/l.

*Oesophageal varices obliterated by band ligation and/or oesophageal sclerotherapy.
ARC, alcohol related cirrhosis.

membrane hyperpolarisation. BzR agonists potentiate the effects of GABA<sub>e</sub>, while BzR antagonists do not possess intrinsic activity, but act by blockade of endogenous agonist.10

Postmortem studies on patients dying in hepatic coma have shown increased cerebral benzodiazepine content.11 An upregulation of BzRs could explain the increased sensitivity to benzodiazepines that is characteristic of patients with hepatic encephalopathy and the improvement in arousal following administration of BzR antagonists which has been reported in some studies.12 13

Positron emission tomography (PET) provides a means of studying the integrity of neurotransmitter systems in vivo.14 11C-flumazenil (11C-FMZ) is a weak partial agonist that binds specifically and reversibly to the central benzodiazepine receptor site in the GABA<sub>e</sub> complex.15 The total flumazenil (FMZ) volume of distribution (VD) represents both specific and non-specific binding in the area under study. Non-specific binding represents a small fraction in the receptor rich areas, therefore 11C-FMZ VD reflects the availability of BzR binding sites.

We hypothesised that central benzodiazepine receptor binding is abnormal in patients with hepatic encephalopathy. Therefore, in this study, we used 11C-flumazenil and PET to measure BzR binding in the brains of patients with stable grade I–II chronic hepatic encephalopathy. Results were related to clinical and psychometric parameters. 1H MRS was also undertaken as an objective measure of cerebral metabolic derangements in the patient cohort.

**Patients and methods**

The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki with a priori permission being obtained from the Ethics Committee of the Imperial College School of Medicine, London (REC 4047) and the UK Administration of Radiation Substances Advisory Committee (ARSAC). All patients were outpatients and were studied on the morning of two consecutive days. Informed consent was obtained 24 hours prior to the study and a detailed neurological examination was performed to exclude any focal neurological signs. On day 1, the patients underwent 11C-FMZ PET. On day 2, in vivo 1H MRS of the brain was performed.

**PATIENTS**

Six patients with cirrhosis were studied (four men, two women; mean age 61 years, range 50–70). Table 1 summarises patient characteristics. All patients had biopsy proven alcoholic cirrhosis and had been abstinent for at least six months prior to the study. Patients were excluded from the study if they had renal failure (creatinine greater than 150 µmol/l) or if they were receiving any neurotropic medications. All patients were receiving lactulose for treatment of hepatic encephalopathy.

The baseline mental state was assessed immediately before the PET study, using the West-Haven criteria.16 Neuropsychometric evaluation involved the number connection tests (NCT) A and B16, Digit Symbol Test of the Wechsler Adult Intelligence Score,17 and the Digit Copying Subtest of the Kendrick battery.18 Baseline EEGs were performed using conventionally placed electrodes and the mean cycle frequency (mcf) was obtained. These tests were all performed a maximum of 24 hours before the PET study and are summarised in table 2.

Blood was also drawn for standard biochemical and haematological parameters of liver function and a Pugh's score and Child's grade (as modified by Pugh), reflecting the severity of hepatic dysfunction were calculated for each subject.19

**HEALTHY VOLUNTEERS**

Eleven healthy controls (eight men, three women; mean age 55 years, range 46–71) underwent 11C-FMZ PET, and 10 healthy controls (six men, four women; mean age 51 years, range 40–62) underwent 1H MRS.

**11C-FMZ POSTERION EMISSION TOMOGRAPHY**

11C-FMZ PET studies were performed in 3D mode using a 953b Siemens/CTI camera. The reconstructed spatial resolution of this scanner, for 31 simultaneously acquired slices, is 8.5 × 8.5 × 5.2 mm at full width half maximum.20

**Table 2** Neuropsychological status of the patients studied prior to positron emission tomography

<table>
<thead>
<tr>
<th>Patient</th>
<th>Mental grade</th>
<th>Asterixis</th>
<th>NCT A</th>
<th>NCT B</th>
<th>Digit copying</th>
<th>Digit symbol</th>
<th>EEG mcf</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1+</td>
<td>1+</td>
<td>35</td>
<td>85</td>
<td>133</td>
<td>65</td>
<td>6 cps</td>
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<tr>
<td>2</td>
<td>2+</td>
<td>1+</td>
<td>157</td>
<td>240</td>
<td>91</td>
<td>30</td>
<td>6.5 cps</td>
</tr>
<tr>
<td>3</td>
<td>1+</td>
<td>0</td>
<td>107</td>
<td>195</td>
<td>70</td>
<td>43</td>
<td>7.5 cps</td>
</tr>
<tr>
<td>4</td>
<td>1+</td>
<td>0</td>
<td>90</td>
<td>180</td>
<td>133</td>
<td>65</td>
<td>9 cps</td>
</tr>
<tr>
<td>5</td>
<td>1+</td>
<td>2+</td>
<td>46</td>
<td>190</td>
<td>95</td>
<td>46</td>
<td>8 cps</td>
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<tr>
<td>6</td>
<td>2+</td>
<td>2+</td>
<td>115</td>
<td>250</td>
<td>98</td>
<td>66</td>
<td>8.5 cps</td>
</tr>
</tbody>
</table>

Mental grade according to West Haven criteria: 0, no abnormality; 1+, trivial lack of awareness; 2+, lethargy and disorientation.16

Asterixis grades: 0, none; 1+, rare flapping motion; 2+, occasional, irregular flapping motion.20

NCT, number connection test A and B. Normal range for NCT A: up to 35 seconds; NCT B: up to 81 seconds.

EEG mcf, mean cycle frequency (normal range more than 8.9 cycles per second).

Digit copying and digit symbol tests. Normal range for digit copying: score in two minutes, more than 95; digit symbol: number completed in two minutes, more than 55.
Each patient’s head was immobilised while in the scanner using a comfortable moulded head holder and was aligned with the glabella-inion line parallel to the detector rings using a laser beam. Correct positioning of the patient in the scanner was verified with a two minute transmission scan. A 10 minute transmission scan was collected using a retractable external source of 60Ga in Ga:Ge to correct for attenuation of 511KeV γ radiation by the brain and skull. Before scanning, a 22 gauge cannula was inserted into the radial artery after subcutaneous infiltration with 0.5% bupivacaine. High specific activity 11C-FMZ (370 MBq, EDE 2.6 mSv) was injected intravenously in tracer doses. The tracer was prepared by a modification of the method of Maziere et al. Arterial blood was continuously sampled for determination of the metabolite corrected arterial input function. Radioactivity in arterial blood was assayed continuously using an online bismuth germanate (BGO) detection system. Discrete samples taken at 2, 3, 4, 5, 10, 20, 35, 60, and 90 minutes after injection were used to calibrate the BGO detector and to derive the metabolite corrected plasma curve from the measured whole curve. Fractional concentrations of hydrophilic metabolites and of unchanged lipophilic 11C-FMZ were determined by solid phase extraction of plasma followed by high pressure liquid chromatography (HPLC). The derivation of a plasma input function from the data obtained from arterial blood was carried out as described previously. Scanning began 30 seconds before tracer injection, collecting 20 serial time frames, over a 90 minute study period.

Data analysis

The 20 frames of the dynamic images were realigned with one another by an automated “least square” technique which reduced any movement artefact during the scan. The data were then processed with spectral analysis to generate pixel by pixel parametric images of the volume of distribution (VD) of 11C-FMZ from the brain uptake and plasma input functions. Cerebral 11C-FMZ binding is 90% specific and FMZ VD reflects the binding of available BzR (Bmax). Image analysis was performed using Analyze software (version 7.5.4 BRU, Mayo Foundation, Rochester, Minnesota) on SUN SPARCstations (Sun Microsystems, Inc., Mountain View, California). Regions of interest (ROIs) were defined with Gaussian clustering using the multispectral voxel classification feature of Analyze, version 7.5. Two different FMZ images of the same dynamic 11C-FMZ PET scan (an early blood flow weighted and a late receptor weighted image of 11C-FMZ binding), were used as “multispectral” images and segmented using Gaussian clustering. This automated algorithm allocates each voxel into three different clusters: (1) high blood flow and high FMZ VD; (2) high blood flow and moderate FMZ VD; and (3) low blood flow and low FMZ VD. These clusters correspond to: (1) cerebral cortex; (2) cerebellar cortex, thalamus, and basal ganglia; and (3) white matter and CSF, respectively. The Gaussian clustering technique calculates means and standard deviations for each cluster, and then models probability distribution of each cluster as a Gaussian function with these parameters. Each voxel is then classified by calculating the probability that it belongs to a particular cluster. A voxel is included in one cluster if it lies within five standard deviations from the cluster’s centre. An automated postprocessing iterative relaxation technique smoothes the segmentation, so that classification of voxels which are of uncertain cluster membership will be influenced by adjoining voxels and no voxel is left unclassified. Cerebral cortex was defined over 17 consecutive axial planes in each subject, thalamus over seven planes, and cerebellum over six planes.

Statistical analysis

Mean values for 11C-FMZ VD of the cerebral cortex, thalamic, and cerebellar voxels were calculated from the 11C-FMZ VD image. The results of 11C-FMZ VD obtained from the patients were compared with those of controls using Student’s unpaired two tailed t test.

IN VIVO 1H MRS

Cerebral 1H MR spectra were acquired using a 1.5 T Picker magnet (Picker International, Cleveland, Ohio). An enveloping quadrature transmit/receive coil tuned to 64 MHz was used for all the examinations. Details of methodology used are described elsewhere. Briefly, a T1 weighted image was acquired to position a transverse 2 cm slice at the level of the basal ganglia. In the selected slice a 2D CSI technique was used to acquire spectra (TR 1500 ms, TE 130 ms) from contiguous voxels from all brain areas with a nominal voxel size of 4 cc.

Data analysis

All data were analysed by an observer who was unaware of the clinical status of the patient. All spectra were manually phased and processed with an exponential filter of 120 ms in the time domain. Spectra localised to the cortex, cerebellum, and basal ganglia were analysed in each patient. A knowledge based algorithm was used for removal of the water peak and also for baseline flattening. Cerebral creatine (Cr) was taken as the internal reference. Peak area ratios of N-acetyl aspartate (NAA), choline (Cho), and glutamine/glutamate (Glx) were measured relative to the Cr resonance using the NMR1 spectral processing program (New Methods Research, Inc., E Syracuse, New York) on a SUN SPARClstation 10 (Sun Microsystems, Inc., Mountain View, California).

Statistical analysis

All the data were expressed as mean and standard deviation unless otherwise stated. Differences between groups were tested using the Mann-Whitney U test for the spectroscopy data. Correlations were measured using Pearson analysis. In all cases, a p value of less than 0.05 was considered statistically significant.
Results

11C-FMZ PET

The mean volume of distribution of 11C-FMZ in the cerebral cortex was 5.4 (0.3) in the patients and 4.3 (0.5) (p<0.001) in the healthy volunteers (fig 1). In the cerebellum, 11C-FMZ VD was 4.7 (0.4) in the patients and 3.7 (0.4) in the controls (p<0.001); in the basal ganglia, 11C-FMZ VD was 3.3 (0.4) in the patients and 2.2 (0.3) in the controls (p<0.001). In two patients the cerebellum was only partially in the field of view, preventing calculation of the cerebellar 11C-FMZ VD. Table 3 shows individual patient values for 11C-FMZ VD. Metabolism of the parent 11C-FMZ in the plasma was significantly slower in patients compared with controls (fig 2).

There was no significant correlation between the concentration of plasma albumin and cortical or basal ganglia 11C-FMZ VD.

IN VIVO 1H MRS

Figure 3 shows 1H MR spectra from a patient and a healthy volunteer. Table 4 summarises mean values of NAA/Cr, Cho/Cr, and Glx/Cr ratios obtained from the patients and the controls. There was no difference in the mean value of the NAA/Cr ratio in the patient and volunteer populations. The mean Cho/Cr ratio was significantly lower in the cortex, cerebellum, and the basal ganglia in the patients compared with the controls (p<0.05, p<0.05, and p<0.05 respectively). The mean Glx/Cr ratio was significantly higher in the cortex, cerebellum, and the basal ganglia in the patients compared with the controls (p<0.01, p<0.01, and p<0.001 respectively).

Discussion

Research to date has focused on the neurotoxic or neuroactive effects of circulating gut derived toxins and on disturbances in the activity of certain neurotransmitter systems, including the GABAergic system.28 It is of note that no increase in BzR density has been observed in the brains of animals with fulminant liver failure and hepatic encephalopathy,28 but in this study we have shown that 11C-FMZ VD is increased in the cortex, cerebellum, and the basal ganglia of a relatively uniform population of patients with alcohol related cirrhosis, portal hypertension (indicated by presence of oesophageal varices), and stable chronic grade I–II hepatic encephalopathy. This observed change in 11C-FMZ VD is probably related to an increase in BzR receptors. The differences between our study and previous animal data are likely to be owing to the fact that we studied patients with chronic liver disease and stable chronic hepatic encephalopathy, whereas the animal studies were performed with models of acute hepatic decompensation.28

The peripheral metabolism of FMZ was slower in our patient population than in healthy volunteers (fig 2). However, the input function for the calculation of brain VD is the arterial plasma concentration of the parent tracer (11C-FMZ). This calculation therefore, takes into account the metabolic fraction and also the partition of counts between red cells and plasma, but it does not account for protein

<table>
<thead>
<tr>
<th>Subject</th>
<th>Cortex</th>
<th>Cerebellum</th>
<th>Basal ganglia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1</td>
<td>5.9</td>
<td>4.5</td>
<td>3.6</td>
</tr>
<tr>
<td>Patient 2</td>
<td>5.2</td>
<td>5.1</td>
<td>3.1</td>
</tr>
<tr>
<td>Patient 3</td>
<td>6.2</td>
<td>–</td>
<td>3.9</td>
</tr>
<tr>
<td>Patient 4</td>
<td>5.5</td>
<td>–</td>
<td>3.5</td>
</tr>
<tr>
<td>Patient 5</td>
<td>4.8</td>
<td>4.3</td>
<td>2.8</td>
</tr>
<tr>
<td>Patient 6</td>
<td>5.0</td>
<td>5.1</td>
<td>2.8</td>
</tr>
<tr>
<td>Controls: mean (SEM)</td>
<td>4.3 (0.5)</td>
<td>3.7 (0.4)</td>
<td>2.2 (0.3)</td>
</tr>
</tbody>
</table>
Table 4 Comparison of metabolite ratios in patients and controls

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Controls</th>
<th>Patients</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAA/Cr</td>
<td>2.2 (0.4)</td>
<td>2.4 (0.5)</td>
<td>NS</td>
</tr>
<tr>
<td>Cho/Cr</td>
<td>0.7 (0.3)</td>
<td>0.4 (0.3)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Glx/Cr</td>
<td>0.1 (0.1)</td>
<td>0.5 (0.4)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>NAA/Cr</td>
<td>2.1 (0.5)</td>
<td>2.2 (1.0)</td>
<td>NS</td>
</tr>
<tr>
<td>Cho/Cr</td>
<td>0.9 (0.3)</td>
<td>0.6 (0.1)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Glx/Cr</td>
<td>0.1 (0.1)</td>
<td>0.5 (0.4)</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Results expressed as mean (SEM). Cho, choline containing compounds; Cr, creatine; Glx, glutamine/glutamate; NAA, N-acetylaspartate.

Figure 3 Proton magnetic resonance spectrum from a healthy volunteer and a patient with grade I hepatic encephalopathy. Cho, choline; Cr, creatine; Glx, glutamine and glutamate; NAA, N-acetylaspartate.
unlikely that the increased \( ^{11}C\)-FMZ VD that we observed in our abstinent patients with hepatic encephalopathy is directly related to a substitutive effect of alcohol or the effects of abstinence causing increased receptor availability. Further studies should be performed in other homogeneous groups of patients with hepatic encephalopathy, such as primary biliary cirrhosis.

In line with our previous publications, we have also shown in this study that in this group of patients there is a significant increase in the mean Glx/Cr ratio.\(^{20,41}\) Other investigators have previously shown an elevation in this multi-component glutamine-glutamate resonance, which in patients with hepatic encephalopathy, reflects an increase in cerebral glutamine.\(^{1,41,42}\) Glutamate is also the substrate for GABA synthesis catalysed by the enzyme glutamic acid decarboxylase. However, direct linkage of glutamine and GABA metabolism is unlikely, because the former is synthesised in astrocytes, while the Cho/Cr ratio is decreased in the cerebral cortex, cerebellum, and basal ganglia. Although an effect of chronic alcohol consumption cannot be completely excluded, these results suggest that in patients with hepatic encephalopathy and MRS proven metabolic derangement, there is a brain GABAergic system dysfunction as reflected by increased brain BzR availability. Further confirmatory studies must be performed in patients with cirrhosis who do not have a previous history of alcohol excess.

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