Localised 1H-MR spectroscopy for metabolic characterisation of diffuse and focal brain lesions in patients infected with HIV

I L Simone, F Federico, C Tortorella, C F Andreula, G B Zimatore, P Giannini, G Angarano, V Lucivero, P Picciola, D Carrara, A Bellacosa, P Livrea

Abstract

Objectives—To evaluate the role of proton MR spectroscopy (1H-MRS) in detecting metabolic changes in diffuse or focal lesions in the brain of patients infected with HIV.

Methods—Sixty HIV seropositive patients (25 with HIV related encephalopathies, 20 with toxoplasmosis, eight with progressive multifocal leukoencephalopathies (PMLs), and seven with lymphomas) and 22 HIV seronegative neurological controls were examined with a combined MRI and 1H-MRS technique using a Siemens 1.5 Tesla Magnetom. Spectra (Spin Echo sequence, TE 135 ms) were acquired by single voxel, localised on focal lesions in toxoplasmosis, PML, lymphomas, and HIV encephalopathies and on the centrum semiovale of neurological controls. Choline (Cho), creatine (Cr), N-acetyl aspartate (NAA), lactate, and lipids were evaluated in each spectrum and NAA/Cr, NAA/Cho, and Cho/Cr ratios were calculated.

Results—A significant decrease in NAA/Cr and NAA/Cho ratios were found in all HIV diagnostic groups in comparison with neurological controls (p<0.003), suggesting neuronal or axonal damage independent of brain lesion aetiology. However, the NAA/Cr ratio was significantly lower in PML and lymphomas than in HIV encephalopathies (p<0.02) and toxoplasmosis (p<0.05). HIV encephalopathies, lymphomas, and toxoplasmosis showed a significant increase in the Cho/Cr ratio in comparison with neurological controls (p<0.03) without between group differences. The presence of a lipid signal was more frequent in lymphomas (71%) than in other HIV groups (Fisher’s test, p=0.00003). The presence of mobile lipid resonance together with a high Cho/Cr ratio in lymphomas may be related to an increased membrane synthesis and turnover in tumour cells. A lactate signal (marker of inflammatory reaction), was found in all but one patient with PML lesions (75%), but had a lower incidence in the other HIV diagnostic groups (Fisher’s test, p=0.00024).

Conclusion—1H-MRS shows a high sensitivity in detecting brain involvement in HIV related diseases, but a poor specificity in differential diagnosis of HIV brain lesions. Nevertheless, the homogeneous metabolic pattern that characterises PML suggests the usefulness of 1H-MRS as an adjunct to MRI in differentiating CNS white matter lesions, such as HIV encephalopathies, from PML.

Keywords: proton magnetic resonance spectroscopy; magnetic resonance imaging; HIV infection

The involvement of the CNS is a common feature of HIV infection, as the HIV induced state of cellular immune suppression makes the CNS vulnerable to opportunistic infections and tumours as well as susceptible to damage from HIV itself.1

Brain MRI is acknowledged as a sensitive tool for the detection of brain abnormalities in patients infected with HIV. Although some neuroradiological findings are not pathognomonic, they may suggest the aetiological diagnosis of focal or diffuse brain lesions. Focal lesions of different sizes, in different phases of evolution (cerebritis, granuloma, and abscess), and in preferential sites (basal ganglia and corticomedullary junctions), as detected by MRI, may be suggestive of toxoplasmosis, whereas pronounced gadolinium (Gd) enhanced periventricular or subependimal lesions indicate lymphomas.2 White matter involvement during HIV infection may appear on MRI either as asymmetric finger-like multifocal lesions suggestive of progressive multifocal leukoencephalopathy (PML),3 or as symmetric, regularly bordered lesions suggestive of HIV related encephalopathy.4 Nevertheless, it is generally accepted that conventional MRI is not always sensitive enough for the detection of the earliest stages of brain infection by HIV,5 or specific enough for the differential diagnosis of single lesions (for example, toxoplasmosis or lymphoma).6

Complementary to structural and morphological information obtained by brain MRI, the recent technique of localised in vivo proton MR spectroscopy (1H-MRS) has been proposed as a sensitive method for the characterisation of the metabolic state of the brain in normal and in pathological conditions. Several 1H-MRS studies have shown locally altered metabolite concentrations in acute and chronic brain ischaemia,7 multiple sclerosis,8 9 brain tumours,10 epilepsy,11 neurodegenerative disorders such as Huntington’s chorea,12 Parkinson’s disease, and Alzheimer’s dementia.13 14 15 A decrease of N-acetyl aspartate (NAA) in the brain of patients with HIV at various stages of AIDS dementia complex has been interpreted as a
Table 1 Clinical and immunological parameters in HIV infected (HIV+) patients

<table>
<thead>
<tr>
<th>Diagnosis (No)</th>
<th>Sex M/F</th>
<th>Age (range)</th>
<th>Risk</th>
<th>CDC classification</th>
<th>CD4 cells/mm³</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-related encephalopathies (25)</td>
<td>15/10</td>
<td>16-48</td>
<td>17 drug abusers</td>
<td>IV</td>
<td>1-296</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1 homosexual</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1 haemophiliac</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5 partners of HIV+</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1 blood transfused</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIV-toxoplasmosis (20)</td>
<td>16/4</td>
<td>24-47</td>
<td>13 drug abusers</td>
<td>IV</td>
<td>3-88</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2 homosexuals</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4 haemophiliacs</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1 partner of HIV+</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4 drug abusers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIV-lymphomas (7)</td>
<td>4/3</td>
<td>26-38</td>
<td>2 partners of HIV+</td>
<td>IV</td>
<td>6-244</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1 blood transfused</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIV-PML (8)</td>
<td>6/2</td>
<td>26-64</td>
<td>6 drug abusers</td>
<td>IV</td>
<td>4-156</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1 homosexual</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1 blood transfused</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

marker of neuronal or axonal injury induced by HIV. Furthermore, low NAA has been found in patients with HIV with clinical or psychometric evidence of CNS involvement but without abnormalities on MRI, suggesting a higher sensitivity of 1H-MRS compared with MRI in the detection of early brain damage induced by HIV. Only very few 1H-MRS studies have been carried out on patients with HIV with focal brain lesions. The diagnostic accuracy of this technique has been investigated in patients with toxoplasmosis, lymphomas, PML, cryptococcomas, and cytomegalovirus encephalitis, but discordant results have been obtained. Whereas Chang et al found that using 1H-MRS the metabolic patterns of toxoplasmosis and lymphomas are completely differentiated, Chinn et al reported that the two lesions could not be differentiated by this method.

In the present study we used a localised single voxel 1H-MRS technique to evaluate the brain metabolism in CNS diseases which complicate HIV infection.

Materials and methods
PATIENTS
Sixty patients seropositive to HIV antibodies (41 men and 19 women) were examined. The mean group age was 32.4 (SD 6.7) (range 16–64) years. Forty one patients were intravenous drug misusers, four were homosexuals, five were haemophiliacs, five were thought to have contracted the HIV through heterosexual contacts with high risk partners, and five had had blood transfusions. All patients belonged to the Centers for Disease Control group IV.

Seven patients with AIDS related brain lymphomas and 28 with opportunistic brain infections including eight with PML and 20 with toxoplasmosis were studied. Diagnosis of lymphoma was made by means of specific MRI periventricular lesions and in three out of seven patients it was confirmed by biopsy. Multifocal unenhancing lesions in white matter on MRI suggested PML; specific JC virus DNA sequences were detected by polymerase chain reaction in CSF samples from five of the eight patients with PML. Diagnosis of toxoplasmosis was performed according to MRI findings or to the efficacy of empirically employed antitoxo-

MRI AND ‘1H-MR SPECTROSCOPY’
A combined MRI and 1H-MRS investigation was performed with a Magnetom Siemens instrument operating at 1.5 Tesla. MRI axial 5 mm multislices were acquired in proton density and T2 weighted spin echo (SE) sequences (TR 2200 ms, TE 12–80 ms); axial, sagittal, and coronal T1 weighted SE (TR 600 ms, TE 20 ms) images were obtained before and after 0.2 mmol/kg intravenous gadopentetate dimeglumine (Gd-DTPA; scans at five and 20 minutes after Gd administration). Water suppressed spectra were acquired from brain localised single volumes of interest (VOIs) by using SE sequence, with TR 1500 ms and TE 135 ms. The VOI was located in toxoplasmosis, lymphoma, PML, and HIV related encephalopathy lesions, and in the centrum semiovale of neurological controls. Voxel size was optimised to the size lesions, ranging from 3.37 cm³ (1.5×1.5×1.5 cm) to 8 cm³ (2×2×2 cm), and the acquisition number was 512 or 256 in small or large VOIs respectively. A correction for inducing phase distortion eddy currents was performed in each spectrum, and Gaussian and exponential time domain filters were applied before Fourier transformation.
The main proton metabolites identified in each spectrum were NAA at 2 ppm, phosphocreatine-creatine (Cr) at 3 and 3.9 ppm, and compounds containing choline (Cho) at 3.2 ppm. An additional signal acquired in the opposite phase by using SE sequence with TE 135 ms was defined as lactate on the basis of its chemical shift at 1.1 to 1.3 ppm and 7 Hz coupling constant. Large resonances at 0.9 to 1.4 ppm were assigned to methylene and methyl groups of lipid. Figure 1 shows a representative 1H-MR spectrum acquired on normal white matter of a neurological control.

STATISTICAL ANALYSIS
Spectroscopic data were expressed as metabolite ratios calculated from integrated peak areas under NAA, Cho, and Cr peaks. As HIV involves the whole brain and so renders unreliable the comparison with the controlateral normal appearing hemisphere, metabolite ratios of brain pathological lesions in patients with HIV were compared with those of normal white matter of neurological controls.

Differences between means of metabolite ratios were evaluated by the non-parametric Mann-Whitney U test, because of non-Gaussian distribution of the data. Distribution of frequency of metabolic changes was tested by Fisher's exact test. The ratios NAA/Cr, NAA/Cho, and Cho/Cr were considered pathological if they were more than 2 SD outside neurological control means. Frequency of detection of lactate and lipid signals was evaluated in each HIV spectrum. All test results were regarded as significant at p<0.05. Multivariate general linear analysis was used to make a discriminant analysis.

Results
Figures 2–5 show representative 1H-spectra acquired from patients with HIV encephalopathy, toxoplasmosis, lymphoma, or PML lesions.

Table 2 shows the means of NAA/Cr, NAA/Cho, and Cho/Cr ratios in patients with HIV and neurological controls.

The NAA/Cr ratio was significantly decreased in patients with HIV encephalopathies (p=0.0019), toxoplasmosis (p=0.00001), lymphomas (p=0.0026), and PML (p=0.00001) in comparison with neurological controls. Fur-
thermore, PML and lymphoma lesions were both characterised by a lower NAA/Cr ratio than HIV encephalopathies (p=0.0003, p=0.013 respectively) and toxoplasmosis (p=0.0032, p=0.05 respectively); toxoplasmosis lesions showed a decrease in NAA/Cr when compared with HIV encephalopathies (p=0.038). No difference in NAA/Cr ratio was found between PML and lymphomas. Low values of the NAA/Cr ratio were found in all but one patient with PML (87.5%) and in five of the seven patients with lymphomas (71%), but in only four of the 25 with HIV encephalopathies (16%) and in four of the 20 with toxoplasmosis (20%) (Fisher’s test p=0.000002, table 3).

The NAA/Cho ratios were significantly decreased in patients with HIV encephalopathies (p=0.0009), toxoplasmosis (p=0.00001), lymphomas (p=0.0001), and PML (p=0.0001) when compared with neurological controls. Moreover, the NAA/Cho ratios were lower in patients with lymphoma (p=0.0008), toxoplasmosis (p=0.036), and PML (p=0.012) lesions than in those with HIV encephalopathies; lymphomas showed a decrease of NAA/Cho in comparison with toxoplasmosis (p=0.009). Low values for NAA/Cho ratios were found in six of the seven patients with lymphomas (86%), in eight of the 25 with HIV encephalopathies (32%), in 13 of the 20 with toxoplasmosis (65%), and in five of the eight with PML (62.5%) (Fisher’s test, p=0.0003, table 3).

A significant increase in Cho/Cr ratios were found in patients with HIV encephalopathies (p=0.013), toxoplasmosis (p=0.013), and lymphomas (p=0.022) in comparison with neurological controls. No differences were found between the four HIV diagnostic groups. Raised Cho/Cr values were detected in four of the seven patients with lymphomas (57%) and in eight of the 20 with toxoplasmosis (40%), but in only three of the 25 with HIV encephalopathies (12%) and in two of the eight with PML (25%) (Fisher’s test, p=0.0007, table 3).

The pathological evidence of lipid signal was more frequent in patients with lymphomas (71%) than in those with toxoplasmosis (35%) and HIV encephalopathies (8%); no lipid peak...
A lactate signal was detected in six of the eight patients with PML (75%), but was less frequent in the other HIV diagnostic groups (two of the seven patients with lymphomas, four of the 20 with toxoplasmosis, and four of the 25 with HIV encephalopathies) (Fisher’s test, p=0.00024, table 4). No relation existed between MRI Gd enhancement in brain lesions and lactate presence.

TOXOPLASMOSIS SUBGROUP

According to clinical data and MRI findings at the time of the first observation, patients with toxoplasmosis were grouped as acute (13) and remission phase (seven). A raised lactate signal was exclusively detected in those with acute toxoplasmosis, although the frequency was low (four of 13 patients). The NAA/Cr ratio was lower in those with acute than in those with remission toxoplasmosis (p=0.05). No significant differences were found between groups for NAA/Cho and Cho/Cr ratios. Furthermore, patients with both acute and remission lesions showed a decreased NAA/Cr (p=0.00001 and p=0.0016 respectively) and NAA/Cho (p=0.00001 and p=0.0037 respectively) and an increase of Cho/Cr (p=0.037 and p=0.05 respectively) in comparison with neurological controls (table 5).

FOLLOW UP

In patients with toxoplasmosis serial 1H-MRS investigations were performed in a follow up ranging from 30 days to eight months. All patients were treated with antitoxoplasm therapy. Five patients with acute lesions showed a significant decrease of NAA/Cr at the first examination (mean 1.05 (SD 0.14); p=0.0006 v normal controls), three of whom recovered to normal values over eight months. Three out of eight lesions showed evidence of lactate, which remained raised in one patient over 40 days. A high Cho/Cr ratio was found in four lesions at the first study (mean 2.48 (SD 0.8); p=0.0018 v neurological controls), recovering during the follow up to control values in two lesions and rising in the other two (1.76 and 2.78 respectively) in line with clinical worsening.

Four patients with HIV related encephalopathy were followed up from six months to two years. Two patients showed a decrease in the NAA/Cr ratio (0.86 and 1.16), progressively falling during the follow up. In the other two patients a high Cho/Cr ratio was found (2.32 and 1.72), whereas the NAA values were normal.

Using NAA/Cr, NAA/Cho, and Cho/Cr values as combined variables in the four HIV groups, discriminant analysis showed that 1H-MRS had an overall diagnostic accuracy of
Table 5  H-MRS metabolites in HIV toxoplasmosis group

<table>
<thead>
<tr>
<th>Actual groups</th>
<th>No of cases</th>
<th>NAA/Cho (mean (SD))</th>
<th>NAA/Cr (mean (SD))</th>
<th>Cho/Cr (mean (SD))</th>
<th>Lactate n (%)</th>
<th>Lipids n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neurological controls (22)</td>
<td></td>
<td>2.25 (0.51)</td>
<td>2.09 (0.51)</td>
<td>1.12 (0.26)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>HIV toxoplasmosis - acute phase (13)</td>
<td></td>
<td>1.38 (0.39)*</td>
<td>1.56 (0.56)*</td>
<td>1.7 (0.9)*</td>
<td>4/13 (31)</td>
<td>5/13 (38)</td>
</tr>
<tr>
<td>HIV toxoplasmosis - remission phase (7)</td>
<td></td>
<td>1.27 (0.65)*</td>
<td>1.44 (0.42)*</td>
<td>0/7 (0)</td>
<td>2/7 (29)</td>
<td></td>
</tr>
</tbody>
</table>

Comparison of means by Mann-Whitney U test: * different from neurological controls; † different from HIV toxoplasmosis - remission phase. Only the significant differences observed are presented; for statistical details see results.

Table 6  Result of discriminant analysis

<table>
<thead>
<tr>
<th>Actual groups</th>
<th>No of cases</th>
<th>Predicted group membership (n (%))</th>
<th>(1)</th>
<th>(2)</th>
<th>(3)</th>
<th>(4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) HIV-related encephalopathies</td>
<td>25</td>
<td>14 (56)</td>
<td>8 (32)</td>
<td>3 (12)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>(2) HIV-toxoplasmosis</td>
<td>20</td>
<td>7 (35)</td>
<td>6 (30)</td>
<td>3 (15)</td>
<td>4 (20)</td>
<td></td>
</tr>
<tr>
<td>(3) HIV-PML</td>
<td>8</td>
<td>0 (0)</td>
<td>1 (12.5)</td>
<td>6 (75)</td>
<td>1 (12.5)</td>
<td></td>
</tr>
<tr>
<td>(4) HIV-lymphomas</td>
<td>7</td>
<td>0 (0)</td>
<td>1 (14.3)</td>
<td>2 (28.6)</td>
<td>4 (57.1)</td>
<td></td>
</tr>
</tbody>
</table>

Per cent of “grouped” cases correctly classified: 50%.

50% (30 of 60 lesions). Nevertheless, considering the diagnostic power in each HIV group, it seemed very low (30%) for toxoplasmosis (only six of 20 lesions correctly classified) whereas it was higher for PML (75%) (six of eight lesions correctly classified (table 6)).

Discussion

In recent years, the increasing interest in vivo 1H-MRS in the study of CNS involvement in HIV has provided much information on metabolic changes in AIDS-dementia complex as well as in the early stages of HIV infection. The very few 1H-MRS studies performed until now on focal brain lesions by opportunistic infections or CNS tumours in patients with HIV disagree on the potential role of 1H-MRS in the differential diagnosis of various focal lesions. The results of our study indicate that significant metabolic changes occur in brain proton spectra both in diffuse and focal lesions of different aetiology in patients infected with HIV.

A recurrent finding of this study was the decrease in NAA/Cr ratio found in patients with HIV related encephalopathies as well as in patients with lymphoma, toxoplasmosis, and PML lesions. It is likely that a reduction in the NAA/Cr ratio depends on a selective reduction of NAA; as Cr is assumed to be a relatively stable brain metabolite both in normal and pathological conditions, it is often used as an internal standard in the in vivo evaluation of relative concentrations of metabolites. Although the role of NAA in CNS is not well established, most researchers agree that this amino acid, located exclusively in neurons and their processes, may be a marker of neuronal viability. A decrease in NAA is detected in many diseases characterised by neuronal damage, such as stroke and dementia, by axonal injury, such as multiple sclerosis. In agreement with several 1H-MRS studies, our data indicate that HIV encephalopathies showed a significant decrease of NAA in comparison with neurological controls. This confirms the histopathological findings of neuronal, dendritic, and axonal loss indirectly induced by HIV. Furthermore, we found that PML and lymphoma lesions were both characterised by lower levels of NAA than toxoplastic-
Oedema is a prominent feature of acute toxoplasmosis lesions and it is possible that the relative number of neurons/unit of volume is at first reduced and later increases as the oedema is absorbed. By contrast, HIV encephalopathies were characterised by an irreversible and progressive NAA loss.

Our findings confirm the high sensitivity of 1H-MRS in detecting brain metabolic impairment which seems to be consistent with histopathological features of HIV related brain diseases. Nevertheless, according to previous results, but in disagreement with another report,23 the discriminant analysis showed an overall low diagnostic power of the 1H-MRS technique to distinguish different brain lesions in patients with HIV. Diagnosis based on MRI is sometimes very difficult in the presence of focal lesions, in particular of toxoplasma abscesses and brain lymphomas. Although our data showed that lymphomas were characterised by lower NAA/Cr and NAA/Cho ratios and by more frequent lipid signals in comparison with toxoplasmosis, the few patients studied with lymphoma make the 1H-MRS unable to differentiate the two HIV related diseases.19 By contrast, the metabolic pattern of PML lesions seems to differ from those of the other HIV groups, as it is highly characterised by low NAA, constant presence of lactate, and absence of lipid signal. It is known that in the late stages of PML white matter abnormalities on MRI (T1 hypointensity and T2 hyperintensity) are usually well differentiated from those found in HIV encephalopathies, whereas in the early stages of PML T1-weighted lesions may appear isointense and MRI features of the two diseases may be confused.20 Therefore we suggest that the 1H-MRS technique could support MRI in the differential diagnosis of CNS lesions involving white matter during HIV infection.

Our opinion is that 1H-MRS, combined with MRI findings and clinical and laboratory indices, could help to define diagnosis, but above all it may be useful to monitor the course of progression of disease and to evaluate the efficacy of specific therapy in longitudinal studies.


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