

SHORT REPORT

Proton magnetic resonance spectroscopy pattern of progressive multifocal leukoencephalopathy in AIDS

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Abstract

The objective was to determine whether the use of intermediate echo times (135 ms) in proton magnetic resonance spectroscopy (¹H-MRS) detects a homogenous pattern in progressive multifocal leukoencephalopathy (PML) in HIV-1 infected people, and to confirm the results of previous studies.

Six patients infected with HIV-1, with PML established by biopsy, and six healthy age and sex matched volunteers were evaluated to define their spectroscopic pattern. ¹H-MRS spectra performed at 1.5 T were obtained with the STEAM sequence: TE/TM/TR, 20 ms/13.7 ms/2000 ms; 2500 Hz, size 2048 points, 256 acquisitions (STEAM-20) and with the PRESS sequence; TE/TR, 135 ms/2000 ms; 2500 Hz, size 2048 points, 256 acquisitions (PRESS-135). A single voxel was placed on the lesions and on the parieto-occipital white matter of controls. The peaks of N-acetylaspartate (NAA), choline (Cho), myoinositol (mI), lactate, and lipids were considered, and the results were expressed using creatine as reference.

Spectra of PML lesions were characterised by significantly reduced NAA, lactate presence, and by significantly increased Cho and lipids compared with control group values.

These results indicate that ¹H-MRS detects a homogenous pattern in PML lesions. Recent studies, together with this, suggest that ¹H-MRS may help in the diagnostic approach to patients with suspected PML lesions associated with AIDS. (*J Neurol Neurosurg Psychiatry* 1999;66:520-523)

Keywords: proton magnetic resonance; AIDS; progressive multifocal leukoencephalopathy

Progressive multifocal leukoencephalopathy (PML) is an opportunistic infection caused by JC virus (JCV) which is characterised by demyelination of the CNS in patients with severely impaired cell mediated immunity; PML affects up to 7% of patients with AIDS and can be the presenting AIDS defining illness.^{1,2}

Neurological and radiological findings are non-specific.^{3,4} Serological studies are not helpful in the diagnosis of PML¹ and JCV polymerase chain reaction in CSF may be limited by the use of different methods and scarce clinical experience.⁵ Although a definitive diagnosis can only be established by brain biopsy, this method is non-diagnostic in 4% to 36% of patients with AIDS.⁶ Thus a non-invasive diagnostic method would obviate the need for brain biopsy in PML suspected lesions provided it is sufficiently specific and sensitive.

Proton magnetic resonance spectroscopy (¹H-MRS) obtains non-invasive "metabolic biopsies" of living tissues, and correlates metabolic alterations with pathology.⁷ The aim of the present study was to evaluate the homogeneity of the spectroscopic pattern of PML lesions established at biopsy at short (20 ms) and intermediate (135 ms) echo times to establish whether this method provides a sensitive pattern for its future diagnostic use. In comparison with previous studies,⁸⁻¹⁰ we used slightly different acquisition parameters to maximise detection of cerebral metabolites.

Methods

PATIENTS

Studies were performed in six patients with HIV-1 with pathologically established PML lesions and in six healthy age matched controls. HIV-1 infection was documented by enzyme linked immunosorbent assay (ELISA) and confirmed by western blot analysis.¹¹ The diagnosis of AIDS was based on the clinical criteria established by the Centers for Disease Control.¹² In all cases, neurological examination, laboratory data, MRI with and without contrast, and ¹H-MRS were recorded. The indication for biopsy was determined according to radiologically detectable lesions with no regression tendency in patients having anti-toxoplasmic therapy, or white matter focal lesions. All six patients included in this study gave their written consent for brain biopsy, MRI, and ¹H-MRS.

Control subjects were healthy volunteers matched by age and sex selected from the hospital staff.

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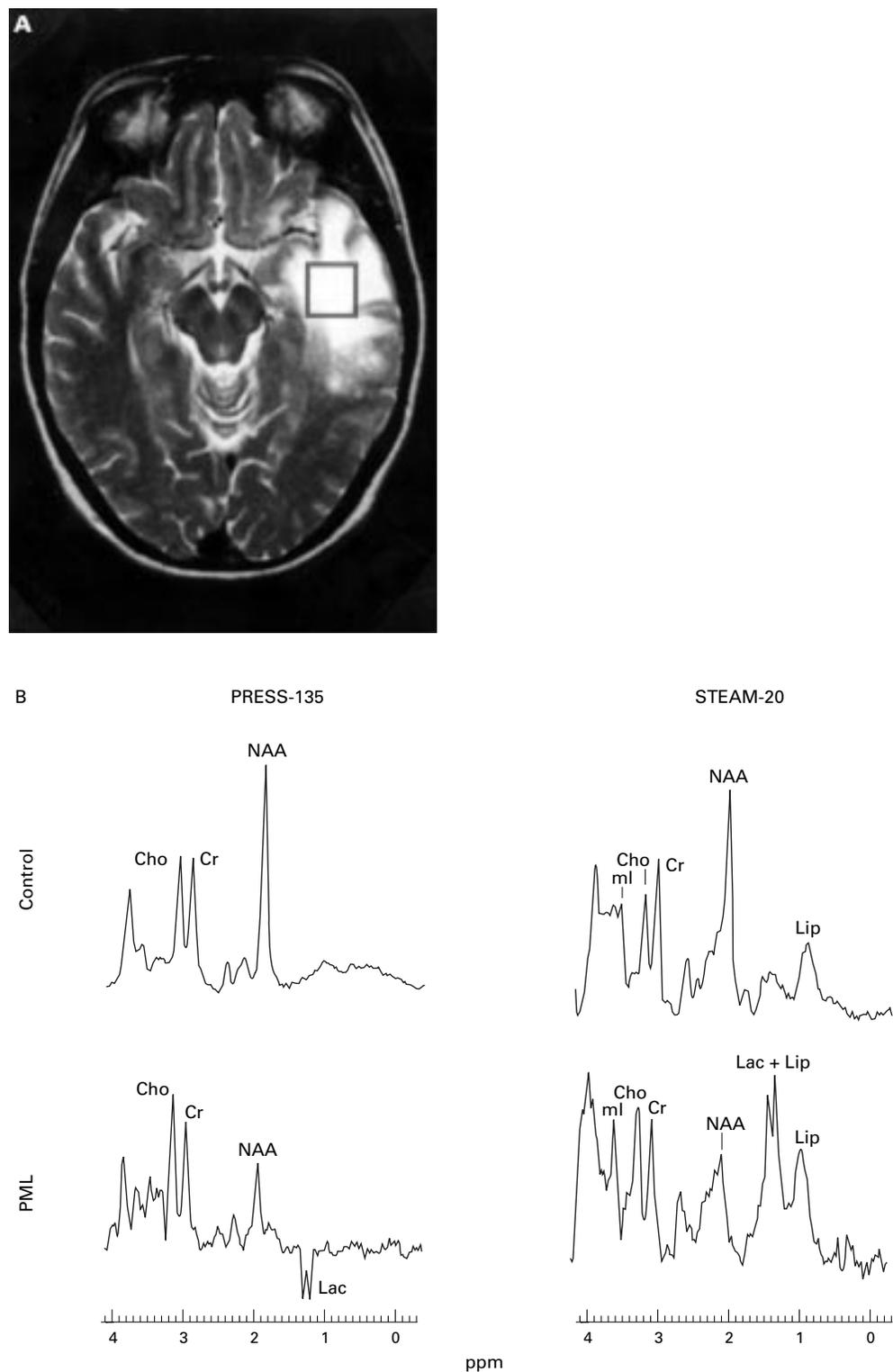
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Received 3 March 1998 and
in revised form
11 September 1998
Accepted 22 September
1998



(A) T2 weighted axial MRI shows a characteristic temporal PML lesion with the voxel placement centred in the lesion. (B) *In vivo* localised ¹H-MRS spectra from white matter control subjects (Control) and PML lesions in HIV-1 infected patients (PML). In comparison with normal white matter, note the strong increase of lipids (Lip) and lactate (Lac), the mild increase of choline (Cho) and myo-inositol (ml), and the evident decrease of N-acetylaspartate (NAA) in PML lesions.

MR STUDIES

Both, MRI and ¹H-MRS, were performed before brain biopsy. Localised ¹H-MRS was conducted immediately after MRI. MRI studies were performed using a Signa System 1.5 T (General Electric Medical Systems, Milwau-

kee, WI, USA) and a quadrature head coil as previously described.¹³ The MRI study was based on coronal, sagittal, and axial projections using spin echo sequences.

¹H-MRS consisted of a "stimulated echo acquisition method" (STEAM) pulse sequence

(TR/TE 1,600/20 ms; TM 13.7 ms; 256 acquisitions; 2048 data points; 2500 Hz spectral width) and a “point resolved spectroscopy” (PRESS) sequence (TR/TE 1600, 135 ms, 256 acquisitions, 2048 data points, and 2500 Hz spectral width). Both sequences were preceded by three chemical shift water suppression pulses (50 Hz bandwidth). These pulse sequences were obtained with the single voxel proton brain examination (PROBE/SV) system, which automatically optimises magnetic field homogeneity over the selected voxel and adjusts the water suppression pulses. A single voxel was placed on the lesions, adjusting the voxel size to the size and shape of the lesions (figure A), and on parieto-occipital white matter in controls (8 cm³).

Data processing was performed on a workstation (SPARCstation 20; Sun Microsystems, Mountain View, CA, USA). Peak areas were estimated with an analysis of free induction delays as described by Saunders *et al.*¹⁴ Thus the amplitudes of the time domain signals, which correspond to the area under each resonance in the frequency domain were measured. The main steps of this analysis involve removal of residual water using the Hankel Lanczos singular value decomposition (HLSVD) algorithm¹⁵ and non-linear least squares fitting using the variable projection (VARPRO) method.¹⁶

Results were expressed as ratios using creatine as reference. Measurements were performed at the following resonances:

N-acetylaspartate (NAA) (2.01 ppm), creatine (Cr) (3.04 ppm), choline (Cho) (3.22 ppm), myoinositol (mI) (3.55 ppm), lactate (1.33 ppm), and lipids (0.90 ppm).

Statistical analysis was performed with Student's *t* test.

PATHOLOGICAL DIAGNOSIS

Diagnosis of PML was established by brain biopsy in all patients as previously described.^{17,18} The lesion used for pathological evaluation was the same as that on which ¹H-MRS had previously been performed. Time between ¹H-MRS examination and pathological analysis ranged from 4 to 8 days.

Results

CLINICAL FINDINGS

Two men and four women, with a mean age of 38.6 (SD 7.0) years (range 30–51 years) were studied. Risk factors for HIV-1 infection were intravenous drug misuse (four patients) and heterosexual transmission (two patients). In two patients, PML was the AIDS defining illness. The mean CD4 count was 40.5 (SD 24.1) mm³ (range 8–76 mm³). Two patients presented with Balint's syndrome, one with cortical blindness, and three with both aphasia and right hemiparesis.

MR FINDINGS

Brain MRI showed a single lesion in two patients and multiple lesions in four patients, all located in the subcortical white matter. Lesions consisted of areas of hypointensity on

¹H-MRS findings

<i>n</i> =6/6	PML (mean (SD))	Control subjects (mean (SD))	<i>p</i> Value
STEAM-20:			
Lipids (0.9)/Cr	1.61 (0.42)	0.23 (0.40)	0.01
Lactate+lipids / Cr	1.31 (0.38)	0	(*)
NAA / Cr	0.78 (0.21)	1.37 (0.10)	0.01
Cho / Cr	1.23 (0.19)	0.92 (0.11)	0.01
mI / Cr	0.76 (0.12)	0.65 (0.07)	NS
PRESS-135:			
Lactate/ Cr	0.39 (0.17)	0	(*)
NAA/Cr	0.77 (0.09)	2.01 (0.22)	0.01
Cho/Cr	1.53 (0.26)	1.17 (0.22)	0.04

Metabolite ratios calculated from the STEAM-20 and PRESS-135 spectra of parieto-occipital white matter of control subjects and patients with PML lesions. Values are expressed as mean (SD).

(*) Differences were qualitative.

T1 and hyperintensity on T2 weighted images, without mass effect or gadolinium enhancement (figure A).

Spectroscopic examinations were well tolerated by all patients and volunteers, and good quality data were recorded in all studies. Figure B shows representative spectra from PML lesions and normal white matter. PRESS sequence allowed an unequivocal assignment of lactate due to its characteristic phase modulation and removal from lipid resonances at the echo time used. The short echo time used in the STEAM sequence allowed the observation of lipid resonances. By comparison with normal white matter, the main differences found in PML lesion spectra (table) were the presence of lactate in all cases at 1.33 ppm, which in STEAM-20 also involves lipids, a significant decrease in the NAA/Cr ratio, and a significant increase in Cho/Cr, and lipids (STEAM-20 spectra)/Cr ratio values.

PATHOLOGICAL FINDINGS

In all patients image guided biopsies were diagnostic showing areas of demyelination with axonal sparing, inclusions within swollen oligodendrocytes, astrocytosis, and enlarged multinucleated astrocytes with bizarre and mitotic nuclei. In situ hybridisation (ISH) for JCV showed DNA in the nuclei of oligodendrocytes and astrocytes in all the patients.

Discussion

In all PML lesions results showed a homogeneous spectroscopic pattern which was characterised by a decrease of NAA/Cr ratio and an increase of lac/Cr, Cho/Cr, and lipids/Cr ratios compared with healthy control subjects. This pattern, in agreement with the results of Chang *et al.*^{9,10} could be interpreted as a decrease of NAA, lactate presence, and an increase in Cho and lipids. However, as creatine concentration may be decreased in PML,^{9,10} the other metabolites would seem to be higher than the actual concentration. The findings of Chang *et al.*^{9,10} and ours suggest that ¹H-MRS may support the diagnostic approach in patients with HIV with suspected PML.

We used the 135 ms echo time in the PRESS sequence. This intermediate echo time has the advantage of offering better signal to noise ratio

in resonances of normal brain metabolites, and a higher visibility and unequivocal assignment of lactate due to characteristic phase modulation. Precisely, the use of long echo times produces a strong reduction of resonance intensity that at low concentrations of the metabolites responsible may be indistinguishable from the spectral noise level.

In our study, ¹H-MRS results were obtained from pathologically established PML lesions. Because ¹H-MRS detects abnormalities in asymptomatic patients infected with HIV,¹⁹ we compared spectra of PML lesions with those in normal brain parenchyma of healthy subjects and not with their contralateral hemisphere.

Our findings are consistent with neuropathological data. As NAA is a neuronal marker,⁷ its decrease in PML lesions could be the result of the axonal loss shown by biopsy. Lactate was detected in all PML lesions, both in short and long echo time. The lactate presence is related to cellular hypoxia and decreased mitochondrial ATP synthesis.⁷ In PML, the origin of the lactate peak may arise from the presence of large numbers of microglial phagocytes and necrotic oligodendrocytes as described in acute multiple sclerosis plaques.²⁰ Increase in Cho resonance (glycerophosphocholine, phosphocholine, choline) and lipids may reflect the accumulation of myelin breakdown products and rapid cell membrane synthesis.⁷ Both processes, myelin breakdown and enlarged bizarre astrocytes (which were seen undergoing mitosis and malignant in aspect), have been found in all our neuropathological analyses of PML biopsies. PML lesions showed raised mI, though not significantly, compared with normal controls. As mI is present within neuroglial cells,²¹ its increase may reflect the astrocytic proliferation in all our six patients.

In summary, our results reflect the neuropathological processes and show a homogeneous and uniform spectroscopic pattern in PML lesions established by biopsy. Even though ours is a small series, these data suggest that ¹H-MRS may help as a non-invasive examination to differentiate between PML and other focal brain lesions in HIV infected patients. However, further work will be necessary to evaluate the discriminative ability of our indices in the study of HIV related intracerebral lesions.

This study was supported by a grant from Sant Pau-Citran.

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