In vivo detection of metabolic changes in a mouse model of scrapie using nuclear magnetic resonance spectroscopy

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In vivo proton nuclear magnetic resonance (NMR) spectroscopy studies of scrapie in a mouse model have shown the appearance of an abnormal peak in the brain early in the incubation period. This abnormal peak was detected weeks before the detection of a protease-resistant form of a membrane protein and vacuolar histopathology in vitro, and several months before clinical signs, and the signal increased in intensity as the disease progressed. In the chronic stage of the disease, a reduction in N-acetyl aspartate levels was observed using in vivo and in vitro proton NMR spectroscopy.

Introduction

Scrapie of sheep, bovine spongiform encephalopathy and Creutzfeldt-Jakob disease of man are transmissible disorders of the central nervous system (CNS) which have long incubation periods followed by rapid neurological degeneration and a fatal outcome. Clinical signs, post-mortem brain pathology and detection of a protease-resistant form of a neuron membrane protein are used to confirm diagnosis of these disorders. However, it has not been possible to detect these disorders in vivo in either humans or animals infected, but not visibly affected, by the disease.

For three decades, mice have been used to investigate the pathology of and the pathogens (variously termed virinos, prions or slow viruses) responsible for scrapie and other infectious degenerative diseases of the CNS (Dickinson & Outram, 1988; Fraser, 1979b). Diagnostic features of the brains of mice terminally infected with scrapie include vacuolation of the neuropil (Fraser, 1979a; Scott & Fraser, 1984; Beck & Daniel, 1987) and the accumulation of protease-resistant clumps or fibrils of a neuron membrane protein, PrP (PrP*) (Merz et al., 1981; Bolton et al., 1982). The degree and location of vacuolar degeneration, the incubation period and other biological characteristics of the disease in mice depend primarily on the strain of scrapie, route of infection and Sinc genotype of the mouse (Hope et al., 1986; Dickinson et al., 1968; Dickinson, 1975; Fraser & Dickinson, 1973; Fraser, 1979a, b). The Sinc locus, which has two alleles, s7 and p7, probably contains the PrP gene (Westaway et al., 1987) and has a major effect on the incubation period (Hunter et al., 1987).

Nuclear magnetic resonance (NMR) spectroscopy is a non-invasive method of obtaining chemical information which has been applied to the biochemical study of living systems (Gadian, 1982). In vivo proton NMR spectroscopy methods have been used recently to investigate cerebral metabolism in human subjects, both in healthy adults (Frahm et al., 1990), and in patients presenting with focal lesions (Gill et al., 1990; Wolinsky et al., 1990) and diffuse disorders (Menon et al., 1990a, b). Metabolic processes have also been studied in a wide variety of animal models of the disease (Gadian et al., 1986; Bates et al., 1989b; Prichard & Shulman, 1986) and perfused organs (Cohen, 1983; Hollis, 1982), and in simpler systems such as isolated cell suspensions (Hollis, 1982; Navon et al., 1983), tissue extracts (Bates et al., 1989a; Gill et al., 1990) and body fluids (Bell et al., 1989).

To assess some of the metabolic changes that take place in the CNS of animals with scrapie, we used in vitro and in vivo proton NMR spectroscopy to study the development of scrapie in a mouse model of the disease.

Methods

Animal model. The test animals used in this study were VM/Dk mice infected by intracerebral (i.c.) inoculation with a standard dose of the ME7 strain of scrapie [ME7/VM model, 6.5 i.c. 1D_{50} units (negative log
per 0.02 ml dose). In this mouse model the clinical course is stereotyped, characterized by a long incubation period (340 to 370 days), the detection of PrP* in the brain by in vitro immunoblotting 100 to 110 days post-inoculation (p.i.), with vacuoles appearing in the hypothalamus and the septal nuclei of the paraterminal body around 170 to 190 days p.i., and severe vacuolation throughout the brain (but notably in the cortex) during the clinical phase of disease (Fraser & Dickinson, 1973). As a control, an equal number of VM/Dk mice of the same age and sex as the test mice were examined. Initially, control and test mice were examined 325 days p.i., near the clinical phase of the disease, using in vitro and in vivo NMR spectroscopy. Mice were then examined using in vivo NMR spectroscopy 52, 108 and 192 days p.i.

After in vivo measurements, mice were killed and the brains extracted for in vitro NMR spectroscopy analysis of metabolites.

**1H NMR spectroscopy studies.** In vitro 1H NMR spectroscopy measurements of metabolites in perchloric acid (PCA) extracts (Gill et al., 1990) of brain were obtained at 500 MHz on a JEOL spectrometer (pulse angle 45°, repetition time 3.5 s). The water resonance was suppressed by the use of homogated decoupling centred at the water frequency. The spectra were referenced to sodium 3-trimethylsilyl-2,2,3,3-tetradeuteropropionate (TSP) which was added during the extraction procedure, both for chemical shift calibration and quantification. Total metabolite concentrations were obtained by calculating peak height ratios relative to TSP and correcting for the number of protons contributing to each signal.

In vivo NMR spectra were obtained at 4.7 T using an Oxford 30 cm bore, superconducting magnet controlled by a Spectroscopy Imaging Systems Corporation (SISCO)-200 operating console (Queen Mary and Westfield College, London, U.K.). Mice were kept under anaesthesia with 0.75 to 1% halothane in oxygen flowing at a rate of 500 ml/min for the duration of the study. Non-localized 1H NMR spectra (200 MHz) were recorded using a 6 mm surface coil placed over the head. The water signal was suppressed using a spin-echo variant of the binomial pulse sequence (1331)-TE/2-(2662)-TE/2-Acq (Gadian et al., 1986), with the excitation maximum set for N-acetyl aspartate (NAA) at 2.02 p.p.m. Each spectrum was accumulated in 512 data collections using a 16-step phase cycle, a long echo time (TE) of 420 ms and a repetition time of 1.8 s. The data acquisition time was therefore 15.6 min. The data were processed with a sine bell weighting function (time constant 0.03 s) before Fourier transformation. Peak area ratios were measured relative to the creatine plus phosphocreatine compounds (Cr) peak.

**Results**

The aliphatic region of a representative in vitro spectrum from a PCA extract of brain from a control mouse 325 days p.i. is illustrated in Fig. 1(a). Resonances were assigned to NAA, Cr, choline-containing compounds (Cho), taurine (Tau), inositol (Inos), glutamine and glutamic acid (Gln and Glu), and lactate (Lac). The total concentration of NAA was 5.35 ± 0.35 μmol/g wet weight (wt) (Table 1).

A representative spectrum from the PCA extract of the brain from a test mouse near the clinical phase of the disease, 325 days p.i., is illustrated in Fig. 1(b). Signals from the same metabolites present in the spectrum of a PCA extract of a control brain were identified. The total concentration of NAA was significantly reduced in test animals compared to controls (test, 4.00 ± 0.52 μmol/g wet wt, control, 5.35 ± 0.35 μmol/g wet wt, P < 0.001; Table 1). No significant change in the concentrations of other metabolites was observed, including Inos, Tau, Cho and the total Cr pool, in the group of test animals compared to the controls.

Since data from the spectra of the brain extracts from control and test mice at 325 days p.i. showed significant changes in NAA levels only, the data collection parameters for in vivo studies were selected for detection of NAA. The excitation profile of the water suppression pulse was selected to be maximum in the region of the spectrum encompassing NAA. A long TE value of 420 ms was used owing to the relatively long T2 value for NAA (Gadian et al., 1986; Frahm et al., 1989). A representative in vivo spectrum from a control mouse at 325 days p.i. is illustrated in Fig. 2(a), and shows features...
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Fig. 2. \textit{In vivo} proton NMR spectra obtained at 200 MHz from (a) control mouse and (b) scrapie-infected mouse 325 days p.i. Resonances are assigned to NAA, Cr and Cho. The broad resonance with a weighted mean chemical shift of 1.8 p.p.m. (*) was seen only in infected mice. NAA/Cr was reduced in the infected mice.

similar to those previously reported, except for the absence of signal from subcutaneous fat owing to the longer TE value used (Gadian et al., 1986). Resonances from NAA, Cho and Cr were assigned. The linewidths for these resonances were broader than the corresponding signals in the \textit{in vitro} spectrum, being dominated by magnetic field inhomogeneities. The relative ratios of these signals were distorted by the frequency dependence of the solvent suppression sequence (Gadian et al., 1986) and, therefore, were different from the ratios from the \textit{in vitro} spectra. Resonances from compounds with short T\textsubscript{2} values or complex spin–spin coupling patterns (Frahm et al., 1989) were not detected at the long TE value used.

A typical \textit{in vivo} spectrum from a test mouse at 325 days p.i. is illustrated in Fig. 2(b) and shows a significant reduction in NAA/Cr, consistent with the \textit{in vitro} findings. An additional broad, multi-component resonance was detected, with a weighted mean chemical shift of approximately 1.8 p.p.m., which was not seen in the spectrum of the PCA extract of brain.

The time course studies showed no significant change in NAA levels (either total NAA concentration or NAA/Cr) at 52, 108 and 192 days p.i. in either the \textit{in vivo} or \textit{in vitro} spectra (Table 1), although a small reduction in NAA levels was observed in the \textit{in vitro} spectra at 192 days p.i.

The resonance, centred at approximately 1.8 p.p.m., was observed \textit{in vivo} in the spectra of three of the four test animals 52 days p.i., but in none of the controls (Fig. 3a, b); this peak was present in all the test animals 108 and 192 days p.i., as well as 325 days p.i., but in none of the corresponding controls. The signal intensity of the peak increased relative to Cr as the disease progressed (Table 1). No corresponding signal was seen in any of the spectra of PCA extracts of brain from test animals.

Discussion

Near the clinical phase of the disease, a reduction in NAA levels was observed and an additional resonance centred at 1.8 p.p.m. was detected in the ME7/VM murine model of scrapie. NAA is an amino acid believed to be located in neurons (Birken & Oldendorf, 1989) and a reduction in relative NAA levels has been observed by \textit{in vivo} NMR spectroscopy in a number of neurodegenerative diseases in humans which is thought to reflect
neuronal loss (Menon et al., 1990a, b). A reduction in NAA levels in this mouse model of scrapie near the clinical phase of the disease would be consistent with the occurrence of severe vacuolar degeneration and neuronal damage (Fraser & Dickinson, 1968). Preliminary analysis of spectra from PCA extracts of brain from a number of scrapie–murine models, including the ME7, 22L or 79A strains in C3H mice and the 87V or 22C strains in VM/Dk mice (J. Hope, J. D. Bell, S. C. R. Williams & I. J. Cox, unpublished results) indicated that the extent of NAA reduction corresponded with the severity of vacuolar degeneration present in these murine models (Fraser & Dickinson, 1968). These data support the idea that there appears to be an association between decreased NAA levels and neuronal loss, vacuolation and/or other neuronal damage.

The in vivo NMR spectra from test animals showed the presence of a broad resonance centred at 1.8 p.p.m., which was not detected in spectra from age and sex matched controls. This resonance appeared early in the incubation period and increased in intensity as the disease progressed. Observation of this signal preceded in vitro detection of PrP* and histopathology by weeks, and clinical signs by several months in this murine model. This signal has not been observed by in vivo NMR spectroscopy in the brain of rodents with other neurological conditions, such as histidinaemia in mice (Gadian et al., 1986) and hyperammonaemia in rats (Bates et al., 1989b).

The nature of the signal at 1.8 p.p.m. observed in the in vivo spectra of test animals is not clear. No corresponding signal was observed in the PCA extracts, suggesting it may arise from a lipid or membrane-related compound(s). It is unlikely that this resonance arises from subcutaneous fat for a number of reasons. First, its chemical shift is different from that of subcutaneous lipid (which normally resonates at 1.2 to 1.5 p.p.m.). Second, the resonance is present in spectra acquired with a relatively long TE (420 ms), at which stage signals from subcutaneous lipid (T2 47 ± 5 ms) are markedly attenuated. Finally, chemical shift imaging (using a selective pulse to excite the region at 0.3 to 2.3 p.p.m.) demonstrates signal arising from inside the brain of test mice at 325 days p.i. (J. D. Bell, S. C. R. Williams & I. J. Cox, unpublished results). Therefore, the peak may arise from lipids which have a mobile component and are in an environment different from that normally expected, or from mobile membrane-related compounds. Since this resonance was observed before biochemical changes in the PrP protein, and the onset of vacuolation and clinical signs, it may be a valuable in vivo marker for scrapie. Further work is in progress to determine the origin of the signal and to establish its specificity for the spongiform encephalopathies.

In conclusion, an intense multi-component resonance has been observed in scrapie-infected mouse brain by in vivo NMR spectroscopy. This signal appears early in the incubation period and increases in intensity as the disease progresses. In the late phase of the disease, a reduction in the NAA/Cr ratio is also observed, probably reflecting neuronal loss and/or damage. NMR techniques may be of value in the non-invasive assessment of transmissible degenerative encephalopathies.

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References


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