Cerebrospinal fluid lactate and pyruvate concentrations in patients with Parkinson's disease and mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS)

1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) induced parkinsonism is reportedly caused by impairment of the activity of complex I of the mitochondrial respiratory chain enzyme in the substantia nigra.^{1,2} Decreases in the activity of the mitochondrial respiratory chain enzyme complex I have been reported in the substantia nigra, skeletal muscle, and platelets of patients with Parkinson's disease.³ These results are controversial except for platelets.³ Patients with mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS) show a decreased mitochondrial respiratory chain enzyme complex I level and high lactate concentrations in CSF and blood.^{4,5}

We examined whether CSF concentrations of lactate and pyruvate were increased in patients with Parkinson's disease or MELAS.

We studied 38 patients with Parkinson's disease (five untreated, 15 treated with levodopa, and 18 treated with amantadine or bromocriptine) and four patients with MELAS. All patients with Parkinson's disease were typical and had two or more symptoms of rigidity, resting tremor, akinesia, and postural instability. The controls had headache, entrapment neuropathy, and cervical spondylosis. All patients were admitted to hospital and informed consent had been obtained. After an overnight fast and bed rest, CSF was collected at 9 00 am from patients and controls, in a lateral recumbent position. Lactate and pyruvate concentrations were determined according to the method of Asanuma *et al.*⁶ Statistical analysis was performed with one way ANOVA and Scheffé's test by StatView IV.

There were no significant differences in the CSF concentrations of lactate and pyruvate or the lactate:pyruvate ratio in the 38 patients with Parkinson's disease versus the controls (table). There was no significant correlation of lactate or pyruvate concentrations with age in either the control group or in patients with Parkinson's disease. No significant difference was found between the subgroup treated with levodopa and the untreated patient subgroup. There were no significant differences between different durations of illness or severity of disease, which was classified into three grades according to Hoehn and Yahr: mild (stages 1 and 2), moderate (stage 3), and severe (stages 4 and 5). The patients with MELAS showed significantly higher lactate and pyruvate concentrations and a higher ratio than patients with Parkinson's disease and controls (table).

In patients with MELAS, high concentration of lactate in the CSF is an important

finding for diagnosis of CNS mitochondrial impairments. Several reports showed normal blood lactate concentrations in Parkinson's disease.⁷⁻⁹ These findings are compatible with investigations of mitochondrial complex I activity in various organs.³ The decrease in mitochondrial respiratory chain enzyme complex I activity of the substantia nigra in Parkinson's disease is very likely to be a characteristic finding of Parkinson's disease.³ It may also be intimately associated with the onset of the disease. Przedborski *et al.*¹⁰ reported that chronic levodopa treatment caused a significant reduction in complex I activity of the mitochondrial respiratory chain. But, in our study, there were no significant differences in lactate concentrations between the patients treated with levodopa and the untreated patients.

Hattori *et al.*⁹ also reported normal CSF lactate concentrations in nine patients with Parkinson's disease. Any slight decrease in complex I activity of the substantia nigra in Parkinson's disease may be localised to the substantia nigra.

We thank Professor T Sato, Juntendo University Hospital, for examination of the MELAS muscle.

MITSUTOSHI YAMAMOTO
HIROSHI UJIKE
KEN WADA
TAKUSHI TSUJI

Department of Neurology, Kagawa Prefectural Central Hospital, 5-4-16, Bancho, Takamatsu 760, Japan

Correspondence to: Dr M Yamamoto.

- Nicklas WJ, Vyas I, Heikkilä RE. Inhibition of NADH-linked oxidation in brain mitochondria by 1-methyl-4-phenylpyridine, a metabolite of the neurotoxin, 1-methyl-1,2,3,6-tetrahydropyridine. *Life Sci* 1985;36:2506-8.
- Mizuno Y, Saitoh T, Sone N. Inhibition of mitochondrial NADH ubiquinone oxidoreductase activity by 1-methyl-4-phenylpyridinium ion. *Biochem Biophys Res Commun* 1987;143:294-9.
- Schapiro AHV. Evidence for mitochondrial dysfunction in Parkinson's disease—a critical appraisal. *Mov Disord* 1994;9:125-38.
- Ichiki T, Tanaka M, Nishikimi MM, *et al.* Deficiency of complex I and mitochondrial encephalomyopathy. *Ann Neurol* 1988;23:287-94.
- Pavilakis SFG, Phillips PC, Dimauro S, *et al.* Mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes: a distinct clinical syndrome. *Ann Neurol* 1984;16:481-8.
- Asanuma K, Ariga N, Aida A, *et al.* A new method for simultaneous autoanalysis of plasma levels of lactic acid and pyruvic acid by means of oxidize system. *Seibutushiryō-Bunseki* 1985;8:16-24.
- Di Monte D, Tetrad JW, Langston JW. Blood lactate in Parkinson's disease. *Ann Neurol* 1991;29:342-3.
- Bravi D, Anderson JJ, Dagani F, *et al.* Effect of aging and dopaminomimetic therapy on mitochondrial respiratory function in Parkinson's disease. *Mov Disord* 1992;7:228-31.
- Hattori Y-N, Yoshino H, Kondo T, Mizuno Y, Horai S. Is Parkinson's disease a mitochondrial disorder? *J Neurol Sci* 1992;107:29-33.
- Przedborski S, Jackson-Lewis V, Muthane U, *et al.* Chronic levodopa administration alters cerebral mitochondrial respiratory chain activity. *Ann Neurol* 1993;34:715-23.

Distribution of tenascin in human malignant gliomas is not related to cell proliferation

The knowledge that radiolabelled antitenascin (TN) monoclonal antibodies are able to induce regression of human glioma xenografts prompted attempts at administration directly into tumours *in vivo*, to deliver cytotoxic radionuclides able to selectively bind to components of human gliomas.^{1,2} It has been shown, however, that TN is unevenly expressed in these tumours, with its distribution being most heterogeneous in anaplastic astrocytomas and glioblastomas.³ To evaluate whether TN expression is related to cell proliferation, surgical samples from 75 malignant gliomas (48 anaplastic astrocytomas and 27 glioblastomas according to the World Health Organisation classification) underwent double staining immunohistochemical evaluations by a procedure reported elsewhere.³ Distribution of TN was studied by using the specific monoclonal antibody BC-4, which was obtained in this laboratory⁴ and employed in clinical trials.² The monoclonal antibody Ki-67, purchased from DAKO (Carpenteria, CA, USA), was used for nuclear labelling of proliferating cells.

In both anaplastic astrocytomas and glioblastomas, Ki-67 labelled nuclei were found to occur at the same rate, with no significant differences being found in tumours strongly stained with anti-TN monoclonal antibody BC-4, compared with tumours that exhibited a weak to moderate staining (table). For TN expression, besides the definite reaction shown by the stroma and the subendothelial matrix, the only constant occurrence was a strong staining of the white matter at the periphery of the tumours. This phenomenon was not related to the presence of proliferating cells, as shown by the usual lack of nuclear labelling by the Ki-67 monoclonal antibody. As a general rule, TN expression was related to the histological texture, rather than the number of proliferating cells in different areas of a single tumour. Staining was most pronounced in areas with a compact texture, even in the presence of few Ki-67 labelled nuclei. By contrast, areas with a loose texture exhibited a less intense staining, even in the presence of several Ki-67 labelled nuclei. This reduced TN expression, unrelated to the occurrence of proliferating cells, was paramount in loosely textured areas of both anaplastic astrocytomas and glioblastomas. Enhancement of staining occasionally occurred in the perivascular conglomerates of cells, without a relation with either Ki-67 nuclear labelling of both gliomatous and endothelial cells, or TN expression in the perivascular stroma. Similarly, a consistent reaction occurring at the boundaries of necrotic areas was not related to the amount of proliferating cells.

Although TN, a polymorphic glycoprotein of the extracellular matrix, has been regarded as an exclusive component of the basement membrane of vessels in human gliomas, it is also present in stromal elements and neoplastic glial cells. However, TN expression in gliomas was found to be uneven, with the degree of anaplasia being usually associated, in immunohistochemical preparations, with a pronounced heterogeneity in both intensity and distribution of staining, which was not related to the cell type.³ The present results confirmed this heterogeneous distribution in malignant

Mean (SD) concentrations of lactate and pyruvate in CSF

Diagnosis (n)	Age (y)	Lactate (L) (mg/dl)	Pyruvate (P) (mg/dl)	L:P
Controls (25)	53.6 (17.4)	13.3 (1.0)	0.87 (0.11)	15.5 (2.3)
Parkinson's disease (38)	66.1 (9.2)	13.1 (1.9)	0.87 (0.15)	15.3 (3.4)
MELAS (4)	32.3 (18.6)	30.7 (4.4)*†	1.17 (0.21)‡	27.0 (6.6)*†

*P < 0.0001; controls v Parkinson's disease.

†P < 0.0001; Parkinson's disease v MELAS.

‡P < 0.01; MELAS v controls or Parkinson's disease.

MELAS = mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes.

Ki-67 labelling indices in human malignant gliomas according to the intensity of staining with the anti-TN mAb BC-4

	Anaplastic astrocytomas (n = 48) % (SD)	Glioblastomas (n = 27) % (SD)
Group 1 (strong staining)	16.3 (8.3) (n = 26)	15.7 (6.8) (n = 14)
Group 2 (weak to moderate staining)	15.5 (6.7) (n = 22)	16.1 (9.4) (n = 13)

There were no significant differences between groups (Student's *t* test).

gliomas, but failed to show a correlation with cell proliferation. Nor was the occasional enhancement of staining in perivascular conglomerates of cells related to either TN expression in the stroma, or endothelial proliferation. It must be stressed that TN is actually represented by different isoforms, with the primary transcript being dependent on the cell cycle, and that the role of TN in tumour progression is regarded as being primarily related to the actively remodelling extracellular matrix on proliferating cells. Furthermore, TN has been shown to play a part in epithelial–mesenchymal interactions, cell spreading and adhesion, and postanoxic and post-traumatic gliosis.³ The variety of factors related to and affecting the TN expression in various tissues might account for the different outcomes resulting from clinical trials. Intracavitary administration of radiolabelled anti-TN monoclonal antibody after debulking of glioblastomas, resulted in some complete remissions with a median follow up of 18 months.¹ In several patients, however, anti-TN treatment was not able to affect the progression of growth, with early recurrences after surgery and immunotherapy. Further testing has been advocated, to select those patients in whom a TN related immunotherapy can be expected to provide beneficial effects. All the more so as TN expression in malignant gliomas is related neither to the cell type, nor to the cell proliferation. Evidence of a widespread distribution of TN in the white matter at the periphery of malignant gliomas, irrespective of its actual expression within the bulk of the tumours, limits the use of cerebral immunoscintigraphy with anti-TN monoclonal antibodies⁵ as a guideline for subsequent immunotherapy. Despite the intrinsic limitations of findings obtained on surgical specimens, a preliminary immunohistochemical screening aimed to the evaluation of the behaviour of both the tumorous stroma and the glial components possibly represents a necessary prerequisite of clinical trials testing the potential benefits of TN related immunotherapy.

This study was partially supported by the National Research Council (CNR) and the Ministry of University and Scientific Research (MURST). We thank Mr Sergio Deseri for his technical help in the preparation of the manuscript.

PATRIZIA CASTELLANI
ANNALISA SIRI
LUCIANO ZARDI
Laboratory of Cell Biology,
Istituto per la Ricerca sul Cancro,
Genoa, Italy.
ANDREA BARBANERA
ALESSANDRA DORCARATTO
GIUSEPPE VIALE
Department of Neurosurgery,
University of Genoa Medical School,
Genoa, Italy.

Correspondence to: Dr G L Viale, Clinica Neurochirurgica dell' Università, Ospedale S. Martino, Largo R. Benzi 10, 16132 Genova, Italia.

1 Riva P, Arista A, Sturiale C, Moscatelli G, Tison V, Mariani M, et al. Treatment of intracranial human glioblastoma by direct intratumoral administration of ¹³¹I-labelled

- anti-tenascin monoclonal antibody BC-2. *Int J Cancer* 1992;51:1–7.
- Riva P, Arista A, Sturiale C, Tison V, Fratterelli M, Lazzari S, et al. Radio-immunotherapy of CNS malignant gliomas by direct intraslesional injection of specific ¹³¹I radio-labelled monoclonal antibodies. *Cancer* 1994;73:1076–82.
- Castellani P, Dorcaratto A, Siri A, Zardi L, Viale GL. Tenascin distribution in human brain tumours. *Acta Neurochir (Wien)* 1995; 136:44–50.
- Balza E, Siri A, Ponassi M, Caocci F, Linnala A, Virtanen I, Zardi L. Production and characterization of monoclonal antibodies specific for different epitopes of human tenascin. *FEBS* 1994;332:39–43.
- Paganelli G, Magnani F, Zito F, Lucignani G, Sudati F, Truci G, et al. Pre-targeting immunodetection in glioma patient: tumor localization and SPECT imaging of [Tc-99m] PnAO biotin. *J Nucl Med* 1994;21:314–21.

Spontaneous intracranial hypotension: MRI and radionuclide cisternography findings

Spontaneous intracranial hypotension (SIH) is a rarely reported syndrome of spontaneous postural headache associated with low CSF pressure. Although postural headache usually occurs after a dural puncture,¹ a spontaneous form of the syndrome does exist. Its characteristic headache is elicited or exacerbated by the upright position and is relieved when the patient is recumbent.² There is no history of previous dural puncture and, in most cases, the aetiology remains obscure.³ The similarity of SIH to the postdural puncture headache syndrome supports the notion that a CSF leak may be the cause of SIH, but this has rarely been demonstrated.^{2–4}

We present a patient with SIH whose gadolinium enhanced MRI disclosed bilateral subdural hygroma and thin diffuse meningeal enhancement. The clinical symptoms were spectacularly resolved when a CSF leak at the dorsal level, shown by radionuclide cisternography, was closed after an epidural blood patch. This case is unusual because meningeal enhancement persisted on the brain MRI at three month follow up whereas the patient was clinically asymptomatic.

Figure 1 Initial brain MRI. Coronal T1 weighted after gadolinium injection, showing bilateral extra-axial fluid collections and thin, intense, and diffuse meningeal enhancement.

Figure 2 Radionuclide cisternography six hours after injection of tracer. Anterior projection of the thoracic spine showing diffusion of the radionuclide into the extra-arachnoid space. The isotope is seen outlining the spinal roots, predominantly left sided (image of "christmas tree").

A 32 year old previously healthy man developed a sudden severe frontal headache with vertigo and vomiting. The headache appeared after a sneezing crisis accompanied by bright rhinorrhea, while he was travelling in a 14 hour plane journey. The headache subsided when the patient lay down. He was admitted to our hospital two days later. General physical examination was normal, there was no meningeal signs, and he was afebrile. Unenhanced brain CT and routine biological examination were normal. Lumbar puncture yielded a clear CSF with no red blood cells, and 1 white blood cell/mm.³ The protein concentration in CSF was slightly raised at 0.92 g/l (normal 0.2–0.4 g/l). All cultures were negative.

The patient was treated symptomatically with bed rest and analgesics and discharged. He was readmitted three weeks later because of continuing and incapacitating postural headache associated with vomiting, anorexia, and vertigo. His clinical examination was unchanged. Brain MRI disclosed bilateral subdural hygroma, small thin ventricles, and thin linear meningeal gadolinium enhancement including membranes along cerebral and cerebellar convexities, inter-hemispheric fissure, and tentorium (fig 1). There was no mass effect. Two blood patches were carried out at 24 hour intervals with epidural injection at the level L4 of 15 ml autologous blood. These were unsuccessful. Because of a doubt about the diagnosis, radionuclide cisternography was performed 10 days later, consisting of an injection at the lumbar level L4–L5 of Indium111-DTPA. It was suggestive of a CSF leak, showing early appearance of activity in the bladder, slow ascent along the spinal axis, no activity over the cerebral convexities after 24 hours, and appearance of extra-arachnoid radioactivity at the dorsal level six hours after injection, visualised as a double straight line (fig 2). A magnetic resonance myelogram did not show any abnor-