LETTERS TO THE EDITOR

Ferritin in the cerebrospinal fluid of patients with Parkinson's disease

Transition metal ions, particularly iron, copper and manganese are capable of producing oxygen-containing free radicals^{1,2} which have the potential for causing tissue damage. The normal brain contains large concentrations of copper and iron, located in large part within the basal ganglia.³ The biochemical forms and physiological functions of these metal ion deposits are largely unknown. We have previously reported finding elevated copper ion concentrations and normal total iron concentrations in the cerebrospinal fluid (CSF) of patients with untreated, idiopathic Parkinson's disease (PD).⁴ Others have found increased total iron concentrations in the zona compacta of post mortem substantia nigra from patients with PD.⁵ Iron in a non-protein-incorporated state is potentially cytotoxic.¹ In tissues it is probably stored in an inert form within the protein, ferritin.⁶ We have measured the concentration of ferritin in the CSF and serum of patients with PD and a control group.

Informed consent for undergoing a lumbar puncture to obtain CSF was obtained from patients with untreated, idiopathic PD. These patients were aware that the test was being done for research purposes and that it did not carry serious risk of long-term ill effects but that they might suffer from back discomfort during the procedure and self-limiting headaches afterwards. Patients having a lumbar puncture as part of their investigation for a variety of diverse conditions made up the control population. The two groups were matched for age [mean age (SD) 54.8 (11.3) yrs for PD and 51.0 (12.7) yrs for controls] and sex (male:female ratio 16:8 for PD and 14:7 for controls). All the CSF samples were acellular (fewer than five red or white cells per mm³) and had a normal total protein content.

CSF and serum ferritin were measured using the Becton Dickinson Ferritin (¹²⁵I) Radioimmunoassay kits following methodology described in the manufacturers' literature (for serum assays). Neat, unconcentrated serum and CSF were used in these assays. The 200 µl of standard solution, serum or CSF are vortex mixed with 700 µl of ¹²⁵I-labelled human liver ferritin tracer and 100 µl of rabbit anti-human liver ferritin antibody. The mixture is incubated for 90 minutes at 37°C and then vortex mixed with 500 µl of precipitating antiserum (goat anti-rabbit antiserum). After centrifuging at 1000 × g for 15 minutes the γ activity of the supernatant is counted. Serum and CSF ferritin concentrations are measured by reference to a standard curve prepared by following the above procedure using four different standard concentrations of ferritin. Satisfactory precision and accuracy have been established.

There was no significant difference between the PD and the control groups for

Figure Total concentrations of ferritin measured by radioimmunoassay in the CSF of patients with idiopathic Parkinson's disease and a control population.

either, the serum or the CSF ferritin concentrations (fig). Statistical evaluation was carried out using the Wilcoxon rank sum test with an appropriate correction for the large number of shared values at the lower end of the measurements (for CSF ferritin measurements). The limit of detection of the assay for CSF was 1 µg/l. Five patients in the PD group and 11 in the control population had undetectable levels of ferritin in the CSF. There was no correlation between CSF ferritin and serum ferritin. A positive correlation was shown between CSF ferritin concentration and the age of the patients ($r = 0.48$, $n = 45$, $p < 0.01$ —Spearman rank correlation) but there was no correlation with CSF total protein concentration, severity of PD (as assessed by Webster or North Western Universities Disability rating scale scores), rate of progression of the disease (severity divided by the duration of symptoms) or with total CSF iron concentration.

We have found normal concentrations of ferritin in the CSF and serum of patients with PD. In previous studies⁴ we found normal total iron content in the CSF in PD. However there is no simple correlation between iron and ferritin concentrations suggesting complex interactions between them. Each ferritin molecule has the capacity to store up to 4000 iron (III) ions some or all of which can be released from the apoprotein by reduction to iron (II) ions,⁶ for example in the presence of ascorbate, high concentrations of which are present in CSF.⁷ Iron (II) ions in low-molecular weight complexes (so-called "free iron") are potentially highly damaging in the presence of molecular oxygen by virtue of production of reactive oxygen metabolites including the hydroxyl free radical¹ whereas iron (III) stored in ferritin is largely inert. The consequences of the presence of reactive forms of iron can be sought by measuring products of free radical-mediated damage,

such as lipid peroxidation products. These are present in normal concentrations in the CSF⁸ in PD although elevated levels have been reported in post-mortem brains of patients with PD.⁹ Many assays of lipid peroxidation are of low sensitivity and uncertain specificity. Newer techniques for measuring the products of nucleic acid or amino acid damage by oxygen-containing free radicals¹⁰ may help resolve the issues of whether transition metal-catalysed free radical-mediated mechanisms are important in the pathophysiology of PD, and whether evidence of the operation of such mechanisms can be obtained from CSF studies.

HS PALL
S BRAILSFORD
AC WILLIAMS
Queen Elizabeth Hospital, Birmingham
J LUNEC
Wolfson Institute, Medical School,
University of Birmingham, Birmingham
DR BLAKE
The London Hospital Medical School,
Whitechapel, London

Correspondence to: Professor Williams

We acknowledge the financial support of the West Midlands Regional Health Authority and the Arthritis and Rheumatism Council.

- Halliwel B, Gutteridge JMC. Oxygen toxicity, oxygen radicals, transition metals and disease. *Biochem J* 1984;219:1-14.
- Donaldson J, McGregor D, La Bella F. Manganese neurotoxicity: a model for free radical-mediated neurodegeneration? *Can J Physiol Pharmacol* 1982;60:1398-405.
- Cummings JN. The copper and iron content of brain in the normal and in hepatolenticular degeneration. *Brain* 1948;71:410-15.
- Pall HS, Williams AC, Blake DR, et al. Raised cerebrospinal fluid copper concentration in Parkinson's disease. *Lancet* 1987;ii:238-41.
- Dexter DT, Wells FR, Lees AJ, et al. Increased nigral iron content and alteration in other metal ions occurring in brain in Parkinson's disease. *J Neurochem* 1989;52:1830-6.
- Worwood M. Ferritin in human tissues and serum. *Clinics in hematology* 1982;11(2):275-307.
- Halliwel B, Gutteridge JMC. Oxygen radicals and the nervous system. *Trends in Neurosciences* 1985;8:22-6.
- Pall HS, Williams AC, Blake DR, Winyard P, Lunec J. Lipid peroxidation and Parkinson's disease. *Lancet* 1986;ii:870-1.
- Dexter D, Carter C, Agid F, et al. Lipid peroxidation as a cause of nigral cell death in Parkinson's disease. *Lancet* 1986;ii:639-40.
- Aruoma OI, Halliwel B, Dizdaroglu M. Iron ion dependent modification of bases in DNA by the superoxide radical generating system hypoxanthine/xanthine oxidase. *J Biol Chem* 1989;264(22):13024-8.

The effects of repeated administration of a long acting TRH analogue (RX77368), on TSH, T₃, T₄ and prolactin in patients with motor neuron disease.

In 1983, Engel *et al*¹ noted the effects of large doses of TRH in patients with motor neuron disease (MND). Beneficial effects on bulbar function, spasticity and cramps have been documented in patients with MND receiving a long acting TRH analogue (RX77368) in single and repeated intravenous infusions,^{2,3} and with oral administration.⁴

We have documented the endocrine effects of a single dose of RX77368 in patients with MND³ and of two consecutive doses in normal individuals.⁵ A marked effect of RX77368 on the release of both TSH and prolactin from the pituitary was seen, but the response of TSH to a second infusion was diminished

compared with the first, whereas the prolactin response was unaltered. We now report the endocrine effects of repeated intravenous infusions of RX77368 in MND.

Seven patients (six male, one female, age range 43–67) with MND³ were given six intravenous infusions of RX77368 on Mondays and Thursdays of three successive weeks, starting at 9.30 am on each occasion. The dose of RX77368 was 0.2 mg/kg, given over two hours. If side effects were unacceptable,^{2,4} the dose was reduced on subsequent infusions. Two patients failed to complete all the infusions, one having five, the other four. Of the other patients, three had dose reductions, to 0.15 or 0.1 mg/kg because of side effects. Blood samples were taken for measurement of T₄, T₃, TSH and prolactin

Figure Each point represents blood samples from up to seven patients. Samples were taken before each infusion, one hour through, immediately after, then four and six hours after each infusion. Further samples were taken each day until the next infusion. There was a three to four day interval between infusions. Error bars represent standard error of the mean. Cross-hatched bars represent RX77368 infusions, 0.1–0.2 mg/kg over two hours. Base line TSH is suppressed between infusions compared with pre-treatment ($p < 0.05$), and peak TSH is greater after the first infusion than after subsequent infusions ($p < 0.05$). Peak values of T₃ and T₄ after the infusion follow the same pattern as TSH, being greatest after the first infusion ($p < 0.05$ in each case).

before, after one hour and at the end of the infusion, then at four and six hours after the infusion, and every morning at 9 am until the next infusion.

All hormones were measured as previously described,⁵ except that prolactin standards were obtained from the Division of Molecular Endocrinology, Hammersmith Hospital, London. The detection limit for prolactin was 40 mU/l. Results were analysed using analysis of variance with Duncan's test for multiple comparisons. Correlations between groups were described using Spearman's test. The study was approved by the local ethics committee.

Each time point has been ascribed a number in the order in which blood samples were taken, and is identified by this number in the graphs (fig). Samples were taken during and immediately after the infusions more rapidly than at other times, and thus the time scale of the points in the graph is not constant.

The first infusion caused a significantly greater TSH response than the subsequent infusions, each of which caused a response similar to each other. The basal trough levels of TSH between infusions were significantly lower than the TSH concentration before the first infusion, despite the fact that trough T₄ and T₃ concentrations were not significantly different from pre-treatment levels.

The marked prolactin response to each infusion was similar, and trough levels of prolactin were no different to pre-treatment levels.

The thyroid hormone responses to RX77368 reflect the TSH response. Trough levels of T₄ and T₃ between infusions were, however, not significantly different from pre-treatment levels.

Peak T₄ and T₃ responses correlate with peak TSH response ($p < 0.005$ and < 0.01 respectively).

There is continuing interest in the possible therapeutic use of TRH analogues in MND.^{2–6} If long term studies are planned, it is important to know the long term endocrine effects, and particularly whether thyrotoxicosis might occur.

A single infusion of RX77368 produces endocrine effects that are not dissimilar to those of native TRH.³ However, the biological effects of the analogue were not the same as TRH, in that the TSH released seemed to be of lesser biological potency.⁵ This study demonstrated again the reduction in TSH response to a second infusion,⁵ but there was no further decline in TSH response after repeated administration. The dose of RX77368 is far in excess of the maximally effective dose of RX77368 for hormonal release, so that the response seen still represents the maximum endocrine response to the analogue.³

It is notable that thyroid hormone concentrations return to an unaltered baseline before the next infusion of RX77368. Despite the normal T₄ and T₃, the subsequent infusions all produced lesser TSH responses than the first infusion. In addition, there was a permanent suppression of plasma TSH between infusions, suggesting a reduction in the tonic stimulatory effect of endogenous TRH on the thyrotrophs. This reduction might be caused by reduced endogenous TRH release, modification of numbers of TRH receptors on the cell surface, modification of intracellular transduction systems, or a delayed effect of the transient increase of thyroid hormones on TSH gene transcription. The reduction of TSH response, does

not, however, influence the effect of RX77368 on the release of prolactin, further evidence that the TRH stimulated post receptor events in lactotrophs and thyrotrophs are differentially regulated.

If RX77368 was administered on the present dose schedule over extended periods of time, thyrotoxicosis should not be a significant side effect. The baseline thyroid function would not alter, though there would be periods during and immediately after infusions when the peripheral thyroid hormones would be significantly elevated. The possibility thus exists of using RX77368 in such a way as to avoid the hazard of thyrotoxicosis.

This work was supported by the Motor Neuron Disease Association of Great Britain and by the Special Trustees of Westminster and Roehampton Hospitals. RX77368 was kindly supplied by Reckitt and Colman Ltd, Pharmaceutical Division, Hull, United Kingdom. We thank the Department of Chemical Pathology, Westminster Hospital for the radioimmunoassays.

DAVID JA ECKLAND
H MODARRES-SADEGHI*
STAFFORD L LIGHTMAN
ROBERTO J GUILLOFF*

Departments of Neuroendocrinology and Neurology,*
Charing Cross and Westminster Medical School,
Westminster Hospital, London

- Engel WK, Siddique T, Nicoloff JT. Effects on weakness and spasticity in amyotrophic lateral sclerosis of thyrotropin releasing hormone. *Lancet* 1983;ii:73–5.
- Modarres-Sadeghi H, Rogers H, Emami J, Guilloff RJ. Subacute administration of a TRH analogue in motor neurone disease. An open study. *J Neurol Neurosurg Psychiatry* 1988;51:1146–57.
- Guilloff RJ, Eckland DJA, Demaine C, Hoare RC, Macrae KD, Lightman SL. Controlled acute trial of a thyrotrophin releasing hormone analogue (RX77368) in motor neurone disease. *J Neurol Neurosurg Psychiatry* 1987;50:1359–70.
- Modarres-Sadeghi H, Guilloff RJ. Comparative safety and efficacy of intravenous and oral administration of a TRH analogue (RX77368) in motor neurone disease. (In press).
- Eckland DJA, Lightman SL. The TSH, T₄, T₃ and prolactin responses to consecutive infusions of a potent and stabilized thyrotrophin releasing hormone analogue, RX77368, in man. *Eur J Clin Invest* 1988;18:405–9.
- Guilloff RJ. Use of TRH analogues in motor neurone disease. In: Metcalfe G, Jackson I, eds. Thyrotrophin releasing hormone—biomedical significance. *Ann NY Acad Sci* 1989;553:399–421.

Carotid dissection: a new false localising sign

Spontaneous internal carotid artery dissection typically presents with ipsilateral head and neck pain, oculosympathetic paresis, transient ischaemic attacks and stroke.¹ Lower cranial nerve palsies are uncommon.² We describe a carotid dissection with tenth and twelfth cranial nerve involvement and hemiplegia which clinically mimicked medullary infarction.

A hypertensive 41 year old man developed right frontal and orbital pain. There was no history of trauma but there was a history of alcohol abuse. Three days later on awakening, the patient was unable to swallow. He also noticed weakness, numbness, and clumsiness of the left hand and leg. On physical examination his blood pressure was 140/90. There were no head, neck or orbital bruits. Mental status examination was normal. There was