The frequency of gastrointestinal side-effects in the APD-treated patients is similar to that described in other studies.25,26 Patients receiving glucocorticoids are already at risk of peptic ulceration and 2 of the patients in the control group had dyspepsia. Such side-effects could limit the use of APD, though no patient was withdrawn from the trial on this account.

This is the first report describing the use of APD in the treatment of steroid osteoporosis. Its results are in accord with those from other animal and human studies. Thus, bisphosphonates have been found to increase bone mass in normal rats24 and pigs25 and to inhibit the bone loss accompanying castration26 or glucocorticoid treatment28 of animals. Preliminary reports have suggested that bone mineral content increases in patients receiving APD for treatment of Paget's disease26 or osteoporosis.29 Bisphosphonates are also of value in disease26 and juvenile osteoporosis. The present study differs from those cited, however, in that it is prospective and includes a randomly allocated treatment control group. It thus provides the best evidence to date for a positive effect of bisphosphonates on bone mass. Although this study includes only steroid-treated patients, it is possible that its findings may have relevance to other types of osteoporosis associated with increased bone resorption.

We thank Prof O. L. M. Bijvoet for the APD, the nursing staff of the endocrinology department, Auckland Hospital, for their help, Mr Alan Stewart for statistical advice, and Mrs Brown Bennett and Ms Ormie Wijeyesinghe for secretarial assistance.

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17. Wijeyesinghe for secretarial assistance.

CHRONIC ENTEROVIRUS INFECTION IN PATIENTS WITH POSTVIRAL FATIGUE SYNDROME

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Summary

76 patients with the postviral fatigue syndrome (PVFS) and 30 matched controls were investigated. Virus isolation was attempted from concentrated faecal samples by direct culture and after acid dissociation of virus from antibody. Positive cultures of enteroviruses were obtained from 17 (22%) patients and 2 (7%) controls. An enterovirus-group-specific monoclonal antibody, 5-D8/1, directed against the VP1 polypeptide, was used to detect entroviral antigen in the circulation, either free or complexed with antibody. VP1 antigen was detected in the serum of 44 (51%) of a further group of 87 PVFS patients. The number of patients positive for VP1 antigen was greater (42/44) when IgM complexes were detectable than when they were not (2/23). 1 year later, the 17 patients of the first group of 76 with positive cultures were again studied. The same virus was again isolated from 5

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(29%), 13 (76%) had detectable IgM responses to enteroviruses, and 9 (53%) were positive for VP1 antigen in the serum. These results show that chronic infection with enteroviruses occurs in many PVFS patients and that detection of enterovirus antigen in the serum is a sensitive and satisfactory method for investigating infection in these patients.

Introduction

An association between previous infection with Coxsackie B viruses and the chronic postviral fatigue syndrome (PVFS), also called myalgic encaphalomyelitis, has been suspected.\(^1\) Similar associations have been reported between these viruses and recurrent perimyocarditis and chronic peripheral muscle disease,\(^2,4\) mainly on the basis of retrospective findings of significantly increased titres of neutralising antibodies to Coxsackie B viruses.\(^6\) These viruses are myotropic and involved in acute muscle disease.\(^6\) Persistent enterovirus infection has now been demonstrated by the detection of genomic sequences of Coxsackie B virus in muscle biopsy samples from patients with dermatomyositis, polymyositis, and chronic myocarditis.\(^7,8\) Chronic enterovirus infection has also been reported in cases of chronic myopathy and myositis.\(^8,9\) Significant levels of IgM antibodies specific for Coxsackie B virus have been found in patients with chronic PVFS,\(^10\) which strengthens the serological evidence for a role of enteroviruses in this syndrome. We report here chronic enterovirus infection in a group of patients with PVFS.

Subjects and Methods

Subjects

The patients had a history of at least 6 months and up to 12 years of excessive muscle fatigue on exercise accompanied by myalgia, with or without an acute viral infection at onset. All patients had dysphasia, difficulty with concentration, and short-term memory, and most had difficulty in accommodation. No patient had evidence of neurological disease, and physical examination was normal. When possible the patients enlisted a neighbour of similar age and sex to act as a control.

Isolation of Viruses from Faeces

Stool specimens were obtained from 76 PVFS patients (group A) and 30 controls during the third week of May, 1986. Specimens reached the laboratory within 24 h of collection and were stored at \(-70\,^\circ\)C. 100 ml of a 20% faecal suspension was prepared from every specimen and the supernatant clarified.\(^11\) Supernatants were centrifuged at 150,000 g for 2 h and the pellets resuspended in 4 ml of ‘Medium 199’ (Gibco). Virus was cultured by two techniques, followed by electron microscopy, and confirmed by indirect immunofluorescence with a monoclonal antibody, 5-D8/1, directed against characteristic morphology of enterovirus-induced CPE and by radioimmunoprecipitation to be VP1.\(^12\) Stool samples were obtained 12 months later from all the patients from whom enteroviruses were isolated, and the entire process of virus isolation and identification repeated. 36 normal healthy individuals matched by age, sex, and geographical location were included at this time as a new set of controls.

Detection of Immune Complexes

Circulating IgM immune complexes in serum samples obtained from a different series of 87 PVFS patients (group B) were estimated by precipitation with 2% polyethylene glycol (PEG). Precipitated IgM was measured by single radial immunodiffusion against monospecific antibody to human IgM.\(^13\) Positive samples were included at this time as a new set of controls.

Detection of Enterovirus-specific IgM Antibodies

Enterovirus-specific IgM responses in patients with positive stool cultures were measured by the \(\mu\)-antibody-capture ELISA for Coxsackie B IgM.\(^14\) These assays were done 12 months after the initial virus isolation.

Characterisation of Monoclonal Antibody 5-D8/1

The monoclonal antibody 5-D8/1, produced against heat-inactivated Coxsackie B5 virus, reacted with the VP1 polypeptide of all tested enteroviruses except hepatitis A virus. This antibody specifically detects a single enteroviral protein, and the antibody does not react with any virus that is not an enterovirus (fig 1).\(^15\) In a separate study, 5-D8/1 was used to screen 130 field isolates of a wide range of enteroviruses in a dot-blot enzyme immunoassay. 95% of the isolates were conveniently and correctly identified as enteroviruses.\(^16\)
Detection of Enterovirus Antigen in Serum

The technique for the detection of antigens that are free or in circulating immune complexes was modified by use of peroxidase-labelled 5-D8/1 as detector instead of rabbit polyclonal antibody. Briefly, 100 µl samples of serum were incubated with 10 µl of peroxidase-labelled monoclonal antibody for 5 days at 4°C. PEG in EDTA buffer, pH 7.6, was added to a final concentration of 2%. After a further 2 days at 4°C the samples were centrifuged, the precipitate washed with 2% PEG, and its enzyme content measured. Results were expressed as the percentage of the labelled antibody that was precipitated. 87 group B sera were studied with sera from matched normal controls.

VP1 antigen detection tests were also done on the sera of all group A patients with positive cultures after acid centrifugation and compared with the results from 16 group A patients who were culture negative.

Results

Virus Isolation

Results of attempts to isolate virus from the 76 patients in group A with PVFS and 30 controls are shown in Table I. 12 of the 17 enteroviruses isolated after acid centrifugation produced visible CPE only after a second passage in cell culture. In May, the month when the first stool samples were collected, some acute enterovirus infections (both overt and subclinical) would be expected in the population. This is shown by the positive findings after direct culture without acid centrifugation in 2 PVFS patients and 2 controls. While acid dissociation and centrifugation resulted in the isolation of viruses from a further 15 PVFS patients, neither of the 2 controls became positive.

3 of the 4 isolates recovered by direct culture were identified as Coxsackie B5 (table II), which suggests a predominance of this particular serotype at the time the samples were obtained. The remaining direct isolate was an echovirus type 11. Table II shows that subgroups of enteroviruses other than Coxsackie B are also associated with PVFS. Despite their characteristic CPE, enteroviral morphology under electron microscopy, and positive reaction with 5-D8/1, 2 isolates could not be serotyped by National Institutes of Health, World Health Organisation, and Colindale antiserum pools, which cover all enteroviruses other than types 68–71.

The 17 patients with positive stool cultures were studied again after 12 months. 5 (29%) were still yielding the same virus (assuming that the untyped enterovirus isolated from patient 14 in 1987 was the same as that isolated in 1986). Neither of the 2 patients from whom virus was isolated previously by direct culture, before acid centrifugation, was positive in 1987. The 2 controls who had been negative for VP1 antigen were still negative when tested 12 months later.

IgM Responses to Enteroviruses

13 of the 17 culture-positive group A patients (76%) still had detectable enterovirus IgM antibodies 12 months after the isolation of virus from their faeces (table II). Most patients (9/13) showed heterotypic responses, a well-known finding in enterovirus infections. Both patients from whom virus was isolated by direct culture in 1986 had no detectable enterovirus-specific IgM response in 1987.

IgM Complexes and Enterovirus Antigens in Serum

IgM circulating immune complexes were detected in 64 of 87 group B sera (74%) from the second group of patients and 42 of the 64 had detectable enterovirus antigen in the serum (fig 2). 2 of 23 patients (9%) without IgM complexes also had detectable antigen in their circulation, possibly complexed with IgG. Thus a total of 44 (51%) patients were positive for enterovirus antigen. All positive patients were retested after 4 months and 39 (89%) were still positive. No controls were positive for enterovirus antigen. The

<table>
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<th>VP1 antigen</th>
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NT = not tested.
patients had detectable VP1 in the serum, but the level of VP1 antigen was detected by direct culture (fig 3). 7 of 16 culture-negative patients still had positive cultures, of the same serotype as those with negative cultures had detectable antigen, it was present at low concentration. The fact that 89% of the patients with detectable levels of VP1 were still positive 4 months later indicates a prolonged infection.

The methods we used to culture the virus effectively removed bound neutralising antibodies from the virus. Overall the technique yielded positive cultures in 17/76 PVFS patients, and 2/30 normal controls. This suggests that enterovirus infection plays an important role in the aetiology of PVFS. This is supported by our finding that acid centrifugation of the faecal samples enhanced the recovery of viruses from the PVFS patients but had no effect on samples from the controls.

Attempts to isolate virus from faecal samples of PVFS patients by direct culture, the conventional method used in clinical virology, were consistently negative, probably because antibodies (predominantly IgA) in the gut neutralise the virus. Acidification, however, dissociates the antibody from the virus, which is acid resistant.11 The now viable viruses are then pelleted by centrifugation through 30% sucrose leaving the antibody above. Where viruses were cultured before acidification, no VP1 antigen was detected in serum. This suggests transient viral carriage rather than systemic infection.

When investigations were repeated 1 year later, 5 of 17 patients still had positive cultures, of the same serotype as before. 13 of the 17 still had enterovirus-specific IgM antibodies and 9 had detectable VP1 antigen in their serum. This is strong evidence for the persistence of virus infection for at least 1 year.

Viral antigen detection in the serum is a more sensitive test for demonstrating enteroviral infection in PVFS than virus isolation. In fact clinical monitoring of these patients showed that the correlation between clinical improvement and disappearance of both VP1 antigen and IgM complexes from the circulation was high (data not shown).

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References continued at foot of next page
PLACEBO-CONTROLLED TRIAL OF TOPICAL INTERFERON IN LABIAL AND GENITAL HERPES

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Summary
The efficacy of topical interferon-β (IFN-β) treatment was assessed in 25 patients with herpes of the lips or genitals who completed a 2-year follow-up in a double-blind placebo-controlled trial. IFN-β gel (10^6 U/g) 4 times daily (about 2 x 10^6 U) applied locally during eruptions (about 10 days) reduced the mean number of recurrences (p < 0.007) and the duration of eruptions (p < 0.007); in the placebo group these indices did not change significantly. Reduction of symptoms and severity was noted in 11 of 12 patients on IFN-β and in only 1 on placebo. No important side-effects were recorded. Topical IFN-β may therefore be advantageous as a time-limited local treatment of recurrent herpes simplex virus infections of the genitons and lips.

Introduction
TREATMENT OF HERPES SIMPLEX VIRUS (HSV) DERMAL INFECTIONS MUST TAKE ACCOUNT OF THE RECURRENT NATURE OF THE DISEASE.1 The latency state of the virus in sensory ganglia afferent to the facial and genital areas is poorly understood, as is the trigger that reactivates virus replication in the skin innervated by these ganglia.2 Patients with frequent herpes outbreaks may well have some impairment in cellular immunity that allows periodic virus reactivation.3,4 Latent virus seems to be widespread in the population: in necropsy studies in the USA, HSV-1 was found in 45% of unselected trigeminal ganglia and HSV-2 in 10% of sacral ganglia.5 The rising number of patients with genital herpes6 and the high incidence of labial herpes,7 the severe discomfort, the danger of spread to sexual partners and to babies at birth, and the associated cancer risk,8 emphasise the need for an effective and convenient treatment.

Patients and Methods

Interferon Ointment
Human IFN-β (‘Frone’, InterYeda, Israel) was produced from poly(rI: rC)-superinduced foreskin fibroblasts and purified to more than 10^7 units/mg protein, the preparation containing essentially the IFN-β species.9,10 The IFN-β gel was made with 1 g of a carboxymethylcellulose carrier gel. Identical tubes of 5 g containing either IFN-β gel or placebo carrier gel were manufactured. The tubes were stored at 4°C, with a stability of 1 year.

Trial Design
The study was conducted at the Soroka Medical Center. Of the 30 patients enrolled, 2 from the IFN-β group and 3 from the placebo group did not complete the 2-year follow-up and were therefore excluded from the analysis. These patients’ history of herpes did not differ from that of the patients who completed the follow-up. The evaluable population consisted of 6 men and 5 women with documented recurrent genital and 2 men and 12 women with labial herpes, mean age 35 (range 15-59) who had been followed for 1–27 years (mean 9.7, median 6). Patients were enrolled when physical examination indicated acute herpes infection and serological tests showed HSV neutralising titres of over 20. Samples were obtained from vesicular lesions by means of ‘Virolut’ swabs and cultured on vero cells in M199 medium for 2–10 days until

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The antiviral nucleoside acyclovir reduces recurrences if taken systemically. However, its high efficacy depends on prophylactic administration since the recurrence rate rebounds to high levels when treatment is interrupted.9,12 Topical acyclovir is not active against recurrences, although it reduces virus growth and hastens healing.13-15 On the other hand, topical application of human fibroblast interferon (IFN-β) in labial and genital herpes seems to reduce the rate of recurrences and to alleviate symptoms, severity, and duration of eruptions.16 In a preliminary trial, IFN-β cream applied 6 times daily during eruptions and twice daily between episodes reduced by more than 5-fold the rate of recurrences over 15 months in 9 of 12 patients with facial herpes and 9 of 19 with genital herpes, 80% of all patients having at least a 2-fold reduction.16 Without continuous therapy resumption of treatment at the first prodromal signs prevented eruptions in 50% of cases, mainly in patients whose HSV isolates showed high sensitivity to IFN in vitro.18 Since treatment by local applications to the herpetic lesions would have an important practical advantage over long-term systemic treatments, we sought to confirm these early findings in a double-blind trial of IFN-β ointment versus placebo.

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