ASPECTS OF THE NATURAL HISTORY OF LIVER DISEASE IN HAEMOPHILIA

A GRANT APPLICATION TO

ACTION RESEARCH FOR THE CRIPPLED CHILD

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1. BACKGROUND

Over the last ten years, the increasing availability of concentrates of coagulation factors VIII and IX has revolutionised the management of haemophilia. Patients are now treated with frequent infusions of these concentrates from a very early age, bleeding episodes can usually be prevented or rapidly aborted, and children who have had the benefits of intensive modern therapy have greatly improved chances of avoiding the permanent joint and muscle damage which is so characteristic of older patients.

A large proportion of haemophiliacs receiving frequent treatment with factor concentrates have chronic abnormalities of plasma liver function tests[1,2]. In our own practice, the prevalence of abnormalities on random testing is now greater than 90%, with infants and children being affected as commonly as older patients[3]. Histological studies of liver biopsy samples at several Centres, including our own, have shown a spectrum of abnormalities which include chronic active hepatitis (CAH), chronic persistent hepatitis (CPH), and cirrhosis [4,5,6]. Because the natural history of liver disease in haemophiliacs is unknown, the prognostic significance of liver function test and histopathological abnormalities is uncertain. If analogous to similar abnormalities in non-haemophiliacs, the problem is a serious one. The possibility exists that the life expectancy of children with haemophilia, having progressively improved over the last 30 years, will start to diminish in the late 1980's.

It is widely believed that one important pathogenic factor in the liver disease of haemophiliacs is the transmission by factor concentrate infusions of the infective agents responsible for non-A, non-B (NANB) hepatitis[7,8,9]. Factor concentrates are prepared from plasma pools usually obtained from many thousands of donors. In patients receiving treatment with these concentrates for the first time - in the case of severely-affected haemophiliacs this will usually be within a few months of birth - it is our experience that the incidence of post-infusion acute NANB hepatitis approaches 100%. It seems clear that the majority of concentrates must be contaminated with at least one agent. Although the acute illness may be mild, it is of concern that there is a high risk of chronicity - none of ten patients we followed prospectively after a first infusion of concentrate had normal liver function tests after 6 months[6]. The risk of NANB infection may not be confined to the patients themselves. Many haemophiliacs are now treated at home, and their wives, children and other household contacts may be at risk through sexual contact or accidental inoculation of infected blood.

Because the antigenic characteristics of NANB agents have not been adequately elucidated, reliable serological tests for their detection have not been available. It has therefore not been possible either to screen donors or concentrates for contamination by NANB agents, or to make immunological studies of the natural history of NANB-induced disease. Over the last two years, we have used 'acute' and 'convalescent' Phase sera from haemophiliacs and other patients infected with NANB agents in attempts to establish a specific immunoassay. Initial attempts were based on an immunodiffusion system[10], but this subsequently proved insufficiently sensitive or reproducible to be useful. Very recently, we have established a radio-immunoassay (RIA) which appears to be specific for one type of NANB hepatitis which is commonly associated with infusion of factor VIII concentrates[11]. The prevalence of the antigen detected by this assay in normal blood donors is about 3%. The assay is now available for application to the study of NANB infection in haemophiliacs, and such studies form a major part of this grant proposal.

Since biochemical liver function tests do not correlate well with histopathology, accurate diagnosis of chronic liver disease is dependent on liver biopsy. This procedure may carry an increased risk in haemophiliacs, and is only performed where there are the strongest clinical indications. There is a need for non-invasive techniques of assessment which may be performed serially. We wish to carry out an evaluation of the usefulness of two possible techniques. These are (a) assay of serum procollagen III peptide (PC III) and (b) quantitative ultrasound scanning. Serum levels of PC III, a cleavage product of newly synthesized collagen, are raised in patients with hepatitis B virus-induced chronic active and chronic lobular hepatitis (CAH and CLH)[12], which are progressive lesions leading to development of cirrhosis, and are normal in patients with CPH. Preliminary observations suggest that this assay may also be useful in differentiating chronic NANB virus-induced CAH and CLH from CPH and may indicate whether lesions continue as CAH or ameliorate with the passage of time. If PC III levels stay high, this may indicate that progression to cirrhosis with its resultant complications is likely. Although conventional ultrasound scanning is useful in the assessment of gross changes of advanced liver disease, it is not capable of sufficient resolution to replace biopsy. Our preliminary experience with a newly-developed technique of quantitative scanning (carried out in collaboration with colleagues at the Royal Marsden Hospital) shows sufficient promise to justify more extensive studies of the value of the procedure in assessing structural damage within the liver.

While little is yet known about natural histories of NANB infection and liver disease in haemophiliacs, the very high incidence of acute hepatitis after a first exposure to factor concentrates is a matter of considerable clinical concern.

There is evidence that pooled normal immunoglobulin may prevent or modify the course of post-infusion NANB hepatitis in non-haemophiliacs[15], and we have therefore used pooled immunoglobulin, given immediately before or at the same time as the first dose of concentrate, to treat patients at high risk. Follow-up is at present incomplete, but initial results suggest that some protection against infection can be obtained. If beneficial effects are confirmed, we shall wish to establish a formalized trial by collaboration with other Centres.

Treatment of established NANB-induced chronic liver disease is a more difficult problem. Several of our patients with potentially progressive lesions such as CAH who have shown a progressive deterioration in their clinical state have been treated with steroids on an empirical basis. Detailed studies of the effects of these agents need to be carried out using the newer techniques of assessment described above. If preliminary results are encouraging, a prospective controlled trial will be established.

2. OBJECTIVES

2.1 To use a newly-developed radioimmunoassay for immunological markers of non-A, non-B hepatitis to study (a) the natural history of acute and chronic NANB hepatitis in haemophiliacs; (b) the antigen/antibody content of different preparations of clotting factor concentrates; (c) the transmissability of NANB infection to household contacts of haemophiliacs; (d) the antigen/antibody content of different preparations of pooled immunoglobulin.

2.2 To evaluate (a) serum procollagen peptide III assay and (b) quantitative ultrasound scanning as predictors of natural history and hepatic histopathology in haemophiliacs with chronic liver disease.

2.3 (a) To make a detailed evaluation of patients treated with pooled immunoglobulin to prevent post-concentrate hepatitis. If appropriate, to establish a collaborative multi-centre trial to more fully assess efficiency.
(b) To make a detailed evaluation of the response to steroid therapy in patients with severe chronic liver disease. If appropriate, to establish a multi-centre controlled trial to assess the possible benefits of steroid therapy.

3. METHODS OF PROCEDURE

(a) RIA for NANB antigen or antibody. This is a solid phase sandwich assay in which the 1st layer antibody and the I^{125} labelled second layer antibody, are gamma globulin fractions from patients with convalescent NANB hepatitis. The antigen detected by this assay is present in acute NANB hepatitis but not in acute type A and B hepatitis.

3. Continued

The antibody assay is a competitive RIA in which the test serum is allowed to compete with I^{125} anti-NANB antigen for binding to caesium chloride-purified NANB antigen bound to a solid phase.

(b) RIA for procollagen III peptide [12,15]. This is a conventional fluid phase RIA.

(c) Patients to be studied are registered at the Royal Free Hospital Haemophilia Centre. All age groups are represented. About 200 patients receive infusions of blood products in any one year. All patients receiving infusion therapy are reviewed at frequent intervals and detailed information about types and amounts of blood products given, and sequential changes in biochemical and immunological tests, is available for a period of several years in most patients, and a lifetime in many. Patients recognized to have chronic abnormalities of liver function tests are reviewed in combined 'Haemophilia-Hepatitis' clinics, where yearly barium swallow and ultrasound examinations are carried out to assess possible progression to cirrhosis and portal hypertension. Patients currently under our care have a wide spectrum of hepatic abnormality, and probably represent all stages of the evolution of haemophilic liver disease. A large bank of stored sera is maintained. To facilitate storage and analysis of data, a microcomputer-based record system is currently being developed.

(d) Studies utilising the newly-developed RIA for immunological markers of NANB hepatitis

The assay will be applied to sequential blood samples obtained from patients with acute and chronic NANB hepatitis, in an attempt to define the natural histories of these diseases, determine how long antigenaemia persists, and the frequency and timing of seroconversion. Correlations will be made with the types and quantities of infused blood, blood products, biochemical abnormalities, the clinical state of the patients, and (where available) histopathological appearances of liver biopsy specimens. Different concentrates of factors VIII and IX will be examined for antigen/antibody content, and the results correlated with the findings in patients who are treated with these concentrates. The effects of procedures designed to render concentrates non-infective may also be examined. Samples obtained in a pilot study of household contacts of haemophiliacs will be tested to investigate the hypothesis that sub-clinical transmission of NANB hepatitis may occur. Preparations of normal pooled immunoglobulin will be examined for antigen/antibody content in an attempt to predict the likely efficacy of these preparations in the prevention of factor concentrate-transmitted disease. An assessment will also be made of the ability of immunoglobulin to neutralize the antigenic content of factor concentrates in vitro.

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(e) Procollagen peptide III assays and quantitative ultrasound scanning

Serial PC III assays and scanning will be carried out in patients recognized to have chronic liver disease.

(e) continued.
The results of these tests will be correlated with the clinical state of the patients, infusion history, fluctuations in liver function tests, NANB serology, barium swallow and conventional ultrasound results, and (where available) the histopathological appearances of liver biopsy specimens. An assessment will be made of the value of these non-invasive techniques in prediction of natural history.

(f) Clinical studies using pooled immunoglobulin
A detailed evaluation will first be made of patients who have been treated with pooled immunoglobulin. These are patients who had never previously received treatment with factor concentrates. If this evaluation suggests that immunoglobulin is capable of favourably modifying the course of post-transfusion hepatitis, a formalized multi-centre trial will be established, patients will be allocated to treatment with either concentrate alone, or concentrate with immunoglobulin. In the latter case, immunoglobulin obtained from a commercial source will either be given intra-venously immediately before the concentrate infusion, or pre-mixed with concentrate. Serial blood samples will be obtained for 9 months after the concentrate infusion. In addition to the currently available conventional liver function tests, NANB serology and PC III levels will also be examined to more fully document the effects of immunoglobulin. It will probably be sufficient to assess response in 10 patients (5 immunoglobulin-treated and 5 controls) in the first instance.

(g) Response to steroids in chronic liver disease
A detailed analysis of response, using all the techniques and assays described in the preceding sections, will be made in patients who are treated with steroids. If the results of this analysis suggest benefit, a multi-centre trial will be established.

4. FURTHER WORK EXPECTED

The proposed investigation is expected to result in significant improvements in the management of patients with congenital coagulation disorders and members of their families. Application of the NANB assay to blood donor selection and methods of plasma product preparation should allow development of safer and more effective therapeutic materials. The availability of non-invasive methods of assessment of liver damage will allow methods of prevention and treatment to be more adequately evaluated.

5. REASONS FOR SUPPORT REQUESTED

The work which has lead to the development of a RIA for NANB antigen/antibody was supported by the Medical Research Council. This work now having been successfully concluded, we wish to apply the assay in the ways described in this application. We have no other financial support.

The medical registrar will be involved in the day-to-day supervision of patients included in the clinical studies, and in the collection and analysis of clinical and laboratory data.

The MLSO will carry out RIAs for NANB antigen and antibody and for procollagen peptide III. He will also collect, separate and store serum specimens.

Consumable expenses include the costs of:

a. isotopes

b. plastic beads and tubes for NANB RIA

c. commercial reagents for procollagen peptide III RIA, and diagnostic tests for hepatitis A and B viruses.

6. ETHICAL CONSIDERATIONS

With the exception of the two possible multi-centre clinical trials of immunoglobulin and steroids, the proposed studies are regarded as components of routine patient management and surveillance. No application to our ethical practices committee has therefore been made. All patients are fully informed of the nature and the reasons for investigations.

It is not yet known whether there will be sufficient justification to proceed to the proposed multi-centre clinical trials. Application for ethical committee approval will be made when the need for this becomes apparent.

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