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TRANSMISSION OF NON-A, NON-B HEPATITIS BY HEAT-TREATED FACTOR VIII CONCENTRATE

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Summary In-vitro and animal studies have shown that viral agents can be removed from or inactivated in clotting factor concentrates by physical or chemical treatment. However, clinical data have as yet not substantiated the results of these studies. 13 haemophilia A patients who had not been treated previously with blood or blood products were given a dry-heated factor VIII concentrate and were tested serologically over the next 12 months. Hepatitis developed in 11 patients (84%) and was invariably of type non-A, non-B. Morbidity was not related to the lot of the therapeutic material or to the number of infusions. The incubation period was either 5 or 8–11 weeks, and only 1 patient had symptoms. Aminotransferase elevation showed both monophasic and biphasic patterns. During the follow-up period signs of the disease disappeared in 10 patients (90%). These findings contrast with the absence of non-A, non-B hepatitis in chimpanzees given the same heated concentrate. Thus, clinical studies in first-exposure haemophiliacs are essential for the true evaluation of the safety of new "treated" concentrates.

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Introduction

CLOTTING factor concentrates manufactured from thousands of units of pooled plasma are likely to transmit viral infections to haemophiliacs. The risk of post-transfusion hepatitis B is reduced but not abolished by screening donors for hepatitis B surface antigen (HBsAg), and HBV vaccination may reduce this risk even further.[1] However, non-A, non-B (NANB) hepatitis, with an attack rate close to 100% in haemophiliacs not previously exposed to blood or blood derivatives (first-exposure, or "virgin", patients), remains a formidable problem.[2,3] Moreover, there is epidemiological and serological evidence that concentrates transmit human parvovirus[4] and the human T-cell lymphotropic virus HTLV III/LAV.[5–7]

During the past few years, several manufacturers have developed physical and chemical methods of eliminating or reducing concentrate infectivity with minimum loss of clotting factor activity.[8] One commercial manufacturer heated lyophilised factor VIII (FVIII) concentrate at 60°C for 72 h, a process thought to reduce concentrate infectivity, since no case of NANB hepatitis was found in chimpanzees treated with this preparation even when an NANB inoculum was added before the heating process.[9] However, the heating process does not completely inactivate HBV. Although deliberate contamination of the concentrate with small amounts (300 infectious doses) of HBV before heating did not cause hepatitis B in animals, contamination with extremely large amounts of HBV (30 000 infectious doses)[9] was followed, after a lag period, by hepatitis B and the appearance of hepatitis B markers. Since this concentrate has been

CLINICAL CHARACTERISTICS AND INFUSION DATA FOR THE PATIENTS REGULARLY FOLLOWED-UP

Patient	Age (yrs)	F VIII level (%)	Body weight (kg)	Type of treatment	Total concentrate dose (U)	No of infusions	Lot	Hepatitis incubation period (weeks)	ALT peak (x upper normal limit)
1	2	1	10	Prophylaxis	20 100	67	820628A	Not assessable	8
2	1	1	11	Demand	8700	21	820628A	No hepatitis	2
5	3 months	1	6	Demand	3600	13	820628A	Not assessable	
11	11	2	50	Surgery	1650	1	820817A	10	53
12	15	16	80	Demand	22 000	11	820817A	8	44
13	3	1	12	Demand	1260	3	820817A 820628A	No hepatitis	9
14	22	11	82	Surgery	19 980	17	820628A	5	91
15	1	1	9	Demand	2130	4	820817A	8	7
16	58	18	70	Surgery	66 720	15	820817A	8	7
18	1	1	12	Demand	3000	6	820628A	11	53
19	1	1	12	Demand	4500	15	820628A 840120A	Not assessable	71
20	10	1	40	Demand	3600	2	840120A	5	48
22	1	1	10	Demand	620	2	830121A 833010A 820817A	8	17

marketed without infectivity studies being done in man, we have conducted a multicentre prospective clinical investigation to assess its likelihood of transmitting hepatitis to previously untreated haemophilia A patients.

Patients and Method

Concentrate

Five different lots of a heated FVIII concentrate ('Hemofil T', Hyland Therapeutics, Glendale, California) were used in this study. Each lot was made from pooled plasma collected in 1982, 1983, and 1984 from approximately 5000 North American plasmapheresis donors.

Patients

Haemophilia centres in Milan, Heidelberg, London, and Paris enrolled patients who needed treatment with FVIII concentrate. Only patients highly susceptible to post-transfusion hepatitis were considered—ie, those who had never received blood or blood products. Other inclusion criteria were normal serum levels of aminotransferases, no history or current evidence of liver disease, no medication likely to raise serum levels of liver enzymes, no HBV serum markers (except for anti-HBs in the 1 vaccinated patient [number 21]), and patient willingness to cooperate in a study demanding periodic blood sampling and visits to clinics over a 12-month period. 21 patients with severe, moderate, or mild haemophilia A met these criteria and gave their written informed consent.

Follow-up Procedure

Serum samples were obtained and full physical examinations were done before treatment, and then every 2 weeks during the first month, every 3 weeks for 6 months, and thereafter monthly until the end of the year's follow-up. Liver function tests included serum bilirubin, aminoaspartate transferase (AST), and aminoalanine transferase (ALT), and were done in the central laboratories of each participating centre by automated spectrophotometric methods at 37°C.[10] Serum samples were also tested for HBsAg (anti-HBs) and hepatitis A IgM antibody (anti-HA) by the use of commercial radioimmunoassay kits (Abbott Laboratories, North Chicago, USA). Cytomegalovirus IgM antibody (anti-CMV) was detected by complement-fixation test or by enzyme-linked immunoabsorbent assay (Behring, Marburg, West Germany). Serum IgG antibody to the Epstein-Barr virus capsid antigen was measured by indirect immunofluorescence. At each visit, patients were questioned for symptoms of hepatitis and any other illness, and records were taken of current drug treatment. Portions of sera were also stored at −20°C for future studies. Diagnosis of post-transfusion hepatitis was made when ALT values greater than 2·5 times the upper normal limit at each laboratory were found on at least two consecutive occasions during the follow-up period. NANB hepatitis was diagnosed when no markers indicating recent hepatitis A or B, cytomegalovirus, or Epstein-Barr virus infections were detected and where no clinical or laboratory evidence of any other cause for increased ALT activity could be found.

Results

21 patients were included in the study. 13 were followed up regularly as planned; 7 missed some visits critical for the evaluation of post-transfusion hepatitis (5th and/or 11th week); and 1 was followed-up regularly for 37 weeks, then defaulted.

Of the 13 patients who were regularly followed up (see accompanying table) 9 were given FVIII on demand for treatment of acute bleeding episodes, 3 during surgical procedures, and 1 for prophylaxis. 9 received FVIII from the same concentrate lot throughout the whole follow-up period and 4 were given FVIII from two or three different lots (table). Clinical efficacy of the concentrate was as good as expected from the doses given and the FVIII level achieved. There were no immediate adverse reactions to the concentrate.

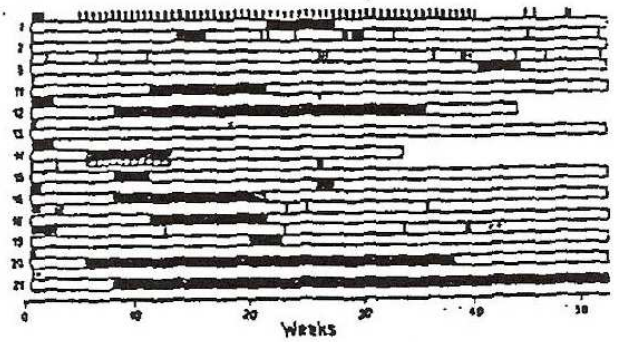
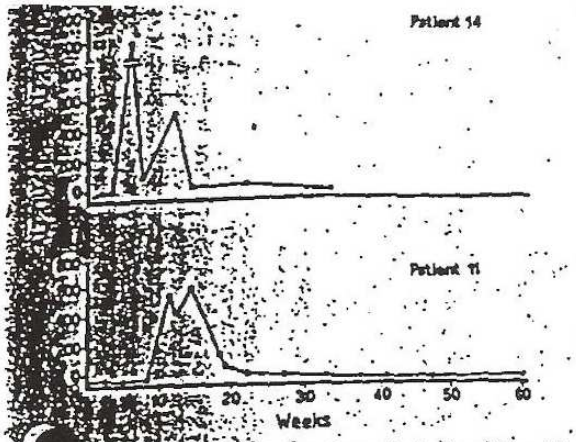


Fig 1—Pattern of non-A, non-B hepatitis in 14 patients infused with the heated factor VIII concentrate.

Each horizontal bar represents results for one patient. The length of th open bar indicates duration of follow-up. Solid bars indicate ALT more tha 2·5 times the upper normal limit. The hatched bar indicates jaundice. Eac vertical stroke indicates the infusion of one concentrate dose (for lack of spac the number of vertical strokes does not correspond to the number of infusion in patients 12, 14, and 16 (see table for exact numbers). Lot changes ar indicated by black dots.



n of ALT changes in 2 patients who had non-A, non-B

Upper panel: shorter incubation, biphasic pattern (patient 14).
Lower panel: longer incubation, monophasic pattern (patient 11).
[illegible] dose of concentrate.

NANB hepatitis developed in 11 (84%) of the 13 patients who were regularly followed up (fig 1). In the others, ALT values were either intermittently raised, but not to the arbitrary cut-off defined for hepatitis (patient 2), or reached this upper limit only in isolated instances (patient 13). The incubation period for hepatitis could be assessed in 8 patients given single or multiple infusions only during the first three weeks of the study (nos 11, 12, 14, 15, 16, 18, 20, 21). The incubation period (the interval between the first infusion of the product and the first abnormal ALT result) was 5 weeks in 2 cases (nos 14 and 20) and 8–11 weeks in 6 (nos 11, 12, 15, 16, 18, 21). 7 patients (nos 1, 11, 12, 15, 16, 19, 21) showed a monophasic pattern of ALT elevation and 4 showed biphasic rises (nos 5, 14, 18, 20) (fig 2). In patients in whom hepatitis developed, ALT rises were 7 to 91 times (median 33) the upper limit of normal values. Serum bilirubin ranged from 0 [illegible] (median 1·2) mg/dl. In all but 1 patient (patient 1) [illegible] hepatitis did not produce symptoms. Patient 14 had [illegible] and jaundice (peak bilirubin 10·7 mg/dl), which lasted for 8 weeks. During the follow-up period, ALT values returned to normal in 10 (90%) of the 11 patients who had hepatitis. Patients 14 and 12 were not followed up after ALT levels returned to normal at 32 and 44 weeks.

Among the 8 patients with incomplete follow-up, 2 had NANB hepatitis, 3 had sporadic ALT rises, and 3 showed no evidence of ALT elevation (not shown). All concentrate lots transmitted hepatitis (table). The frequency of hepatitis was not related to number of infusions (fig 1).

Discussion

The primary purpose of this study was to assess whether hepatitis could be transmitted by heat-treated FVIII concentrates. The enrolment of only patients previously untreated with blood or blood products is of critical importance for the accurate assessment of post-transfusion hepatitis because previous exposure may confer protection against new attacks of NANB hepatitis.[2,3] Our decision to select only first-exposure patients meant that only a small number of patients with haemophilia A could be recruited. In addition, the adoption of strict criteria for follow-up, involving frequent and regular blood sampling, reduced the number of patients suitable for analysis to 13. However, studies of post-transfusion hepatitis are only meaningful when serial biochemical tests are done regularly, since ALT rises during NANB hepatitis are often short-lived[2,11,12] (fig 1) and hence might be missed with irregular follow-up.

There were many similarities between the clinical and biochemical patterns of NANB hepatitis seen in our study and those seen in haemophiliacs given unheated FVIII. Hepatitis occurred in 84% of our patients, a rate close to that (100%) previously observed in first-exposure haemophiliacs infused with unheated commercial concentrates.[2,3] Short (5 weeks) and longer (8–11 weeks) incubation periods were observed, as were monophasic and biphasic ALT patterns (fig 2). However, none of our patients had the very short incubation periods (1–2 weeks) that have previously been reported.[2,3] Two viral agents have been implicated in NANB hepatitis on the basis of cross-challenge studies in chimpanzees.[13,14] More recently, retrovirus or retrovirus-like agent(s) have also been implicated in NANB hepatitis transmitted by plasma products.[15] Our data show that these putative agents were not completely inactivated by heating the FVIII preparation to 60°C for 72 h.

The secondary objectives of this study were to ascertain the severity and tendency to chronicity of the post-transfusion hepatitis and any possible relation between infection and concentrate lot or dose. Occurrence of hepatitis was clearly not related to the lot number or to the number of infusions. The 90% recovery rate during the 12-month follow-up was similar to that which has been reported in first-exposure haemophiliacs given unheated FVIII.[2] Only 1 of our 11 patients became jaundiced and had symptoms. Whether the hepatitis in our patients was truly attenuated by the heat treatment of FVIII can only be established by a controlled study, but we did not think it justifiable to include a control group of patients treated with unheated FVIII, since chimpanzee studies have suggested that a safer product was available.[9] The high prevalence of NANB hepatitis and the absence of HBV transmission in our subjects are in contrast with the HBV transmission and absence of NANB hepatitis in chimpanzees given the same heated concentrate.[9] These differences indicate that the animal model is not reliable for NANB hepatitis transmission studies[16] and that prospective studies in first-exposure haemophiliacs are essential for the evaluation of the safety of new "treated" concentrates. HBV added in large doses to the concentrate withstood the heating procedure, and delayed-onset hepatitis B occurred in chimpanzees.[9] There are two possible explanations for the apparent absence of hepatitis B among our patients. Perhaps the concentrates contained a low bioburden which could be inactivated, or maybe NANB viral infections interfered with HBV expression.[17,18] There have been reports that first-exposure haemophiliacs in whom NANB hepatitis developed after exposure to unheated FVIII concentrates do not have signs of HBV infection.[2,3]

Our finding that NANB hepatitis is transmitted by a heated concentrate should not be taken as evidence that heat treatment is equally ineffective for other viral agents. We have seen, for instance, that none of these patients seroconverted to the retrovirus considered to be the putative agent of AIDS, whereas the rate of seroconversion was high in a group similar to ours in terms of amount of concentrate transfused but who received an unheated preparation.[19] Although this finding needs to be confirmed it is consistent with the observation of the thermolability of AIDS retroviruses.[20]

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