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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	33
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
21	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 tranferase (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.

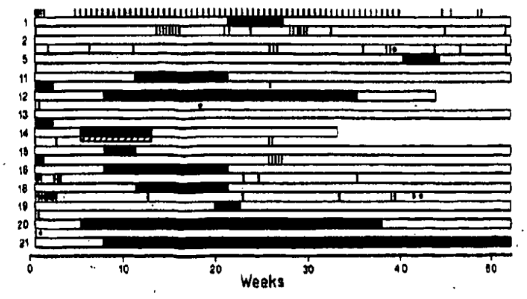

Fig 1—Pattern of non-A, non-B hepatitis in 14 patients infused with the heated factor VIII concentrate.

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

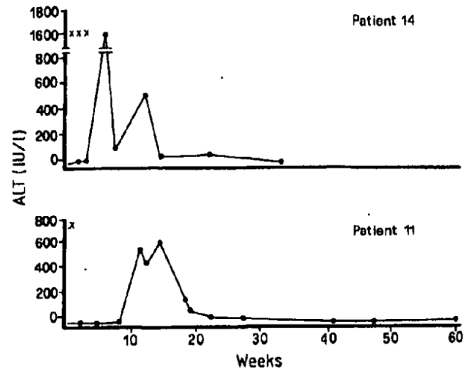

Fig 2—Pattern of ALT changes in 2 patients who had non-A, non-B hepatitis.

Upper panel: shorter incubation, biphasic pattern (patient 14). Lower panel; longer incubation, monophasic pattern (patient 11). Crosses indicate a 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 value 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 0·4 to 10·7 (median 1·2) mg/dl. In all but 1 patient (patient 14), the hepatitis did not produce symptoms. Patient 14 had anorexia 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 concentrate. 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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QUININE AND SEVERE FALCIPARUM MALARIA IN LATE PREGNANCY

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Summary Quinine dihydrochloride was given intravenously to 12 women with severe falciparum malaria in the third trimester of pregnancy. The initial dose consisted of 10 or 20 mg salt/kg over 4 h and was followed by 10 mg salt/kg every 8 h until patients were fit to swallow, when quinine sulphate tablets were given. Uterine activity showed little or no change despite rising quinine concentrations. Of 3 patients in labour, 2 proceeded normally while a third had a successful caesarean section for fetal distress. Late (type II) decelerations of the fetal heart rate were recorded in 6 patients before treatment but in most patients signs of fetal distress diminished as the maternal temperature fell. Hypoglycaemia and hyperinsulinaemia developed in 7 patients, in 2 before quinine was started. The important toxic effect of quinine in late pregnancy is not an oxytocic action but rather its capacity to release insulin.

Introduction

> "La plupart des auteurs admettent aujourd'hui qu'une femme enceinte, affectée de paludisme, est plus exposée à avorter si on ne lui donne pas de quinine que si on lui en donne."
>
> Laveran, 1907[1]

FALCIPARUM malaria can be a devastating complication of pregnancy.[2-4] In Thailand it is the commonest cause of maternal mortality.[5] 50% of pregnant women who become unrousable during the course of falciparum malaria die, and the fetus is stillborn in most of these cases irrespective of the maternal outcome (Warrell et al, unpublished).

In Southeast Asia, quinine has become indispensable for the treatment of severe chloroquine-resistant malaria, but its safety in pregnancy is uncertain. It was used successfully in pregnancy before synthetic antimalarials were developed[3,6,7] but it has also been given to induce abortion[8] and to augment labour.[9] It has been blamed for stillbirth[10] and acute renal failure in pregnancy.[11] Pregnant women seem particularly prone to quinine-induced hypoglycaemia.[12] In Thailand we are obliged to use quinine for all cases of severe malaria because there is no other effective drug available for intravenous use. We report here an investigation of the toxicity of quinine in women who had severe falciparum malaria in late pregnancy.

Patients and Methods

Patients

In 1982 and 1983, patients more than 29 weeks pregnant admitted to Pra Pokklao Provincial Hospital, eastern Thailand, were selected for study if they had *Plasmodium falciparum* malaria which was sufficiently severe to demand treatment with intravenous quinine. Patients or their relatives gave written informed consent to investigation and treatment. The study was approved by the ethical committee, Faculty of Tropical Medicine, Mahidol University, Bangkok. Patients were excluded if they had taken quinine within the previous 3 days or had detectable quinine in their blood on admission.

Clinical Assessment

Patients were admitted to the obstetric intensive-care unit and were seen by both an obstetrician and physician. History and physical examination, including a detailed obstetric assessment with an estimate of gestational age, were recorded on standard forms. A doctor remained with the patient throughout the study.

Treatment

The initial dose of quinine dihydrochloride (Government Pharmaceutical Organisation, Thailand) given was either 10 mg or (in 2 cases) 20 mg of the salt/kg (equivalent to 8·3 and 16·7 mg base/kg, respectively) diluted in 500 ml of normal saline and infused over 4 h; this was followed by further 4 h infusions of 10 mg of the salt/kg every 8 h. As soon as they could swallow, patients took quinine sulphate tablets until they had completed 7 days of quinine treatment.

Investigation

A 'Teflon' catheter was inserted into an antecubital vein and kept patent with heparinised saline. Blood was taken for baseline parasite count, haematocrit, white-cell count, blood urea nitrogen, serum creatinine, albumin, globulin, total and direct bilirubin, and serum aspartate aminotransferase.

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liver involvement on an isotope liver scan. Postoperatively he was given chemotherapy (cisplatin, vinblastine, and bleomycin). Tumour markers studied before chemotherapy were α-fetoprotein 919 IU/l and β-subunit of human chorionic gonadotropin 1279 IU/l. He was given four courses of chemotherapy and then two further courses of cisplatin, etoposide, and doxorubicin. Doxorubicin was substituted for bleomycin because of a reducing transfer factor on serial lung function testing. After three courses of chemotherapy tumour markers had returned to normal. However, a chest X-ray at the completion of chemotherapy revealed a persistent right paratracheal mass. Surgical removal was not thought possible. Regular chest X-rays and computerised tomographic (CT) scans for the subsequent 5 years showed no change in this mass. Serial tumour marker levels remained normal. 5 years after receiving chemotherapy the patient was found on routine follow-up to have a raised AFP of 171 IU/l with a normal β-hCG. There were no symptoms or abnormal clinical findings. Chest X-ray and CT scan demonstrated enlargement of the previous paratracheal mass. Salvage chemotherapy was started.

A 5-year interval between treatment and relapse in a patient with non-seminomatous germ cell testicular tumour is to our knowledge the longest yet reported. This and a previous report of late relapse 4 years after chemotherapy[4] emphasise the danger of equating cure with 2 years of disease-free survival in this disease. The natural history of residual tumour after chemotherapy is uncertain. Non-progressive residual tumour cannot be assumed to be benign even after 5 years. The treatment of choice for residual tumours after chemotherapy must be surgical removal if possible.[5,6] Radiotherapy should be given if the tumour is inoperable or if surgical removal is incomplete. Prolonged frequent follow-up with serial tumour marker determination seems worthwhile in this small group of patients.

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LIVER DISEASE IN HAEMOPHILIA

SIR,—Dr Hay and colleagues (June 29, p 1495) found histological signs of chronic active hepatitis (CAH) or cirrhosis in 13 (38%) of 34 multitransfused haemophiliacs who were not carriers of hepatitis B virus (HBV) and who had persistently raised serum aminotransferase (ALT) levels. Moreover, since CAH or cirrhosis did develop during follow-up in some patients with an initial diagnosis of chronic persistent hepatitis, Hay et al concluded that non-A, non-B hepatitis in haemophiliacs often progresses to severe liver disease. These findings contrast with the results of a large retrospective study of 155 unselected liver biopsy or necropsy specimens collected from haemophilia centres worldwide.[1] A panel of pathologists found histological features of severe liver disease (CAH or cirrhosis) in only 22% of cases. Hay's findings also contrast with several reports (cited by Aledort et al[1]) indicating that liver disease is a numerically negligible cause of death in haemophiliacs, and with the results of our prospective study of 10 haemophiliacs with non-A, non-B hepatitis,[2] who have now been followed up for 10 years.

Our study includes only patients who had persistently increased ALT levels on three consecutive annual visits (1975–77). The baseline histological investigation in 1977 revealed chronic persistent hepatitis or chronic lobular hepatitis in 6 patients and CAH in 4. A second liver biopsy, done in 1980 and compared blindly with the first by an independent pathologist, showed persistence of chronic lobular or persistent hepatitis or improvement of CAH to chronic persistent hepatitis.[2] The non-progressive course of non-A, non-B chronic hepatitis in our patients is further confirmed by a third liver biopsy in 1983 (unpublished) which shows continuation of chronic persistent or lobular hepatitis in all cases. Thus, since our patients had similar ALT pattern and length of follow-up as those investigated by Hay et al, we think that other factors must be considered to explain the different courses of liver disease. The fact that our patients were considerably younger than those studied by Hay et al (mean age 12 years, range 3–44 *vs* 32 years, range 3–70) suggests that the degree of liver damage might be inversely related to the age at which patients become infected. Children with chronic hepatitis B tend to have high levels of virus replication in the liver without severe liver disease.[3] So, in view of the many epidemiological similarities between hepatitis B and non-A, non-B hepatitis, it is not surprising that children with non-A, non-B infection tend to have less progressive and more "tolerated" liver disease than adults with the same infection.

In contrast to the non-progressive disease found by us in patients with non-A, non-B hepatitis, we have data indicating that liver disease is progressive and severe in haemophiliacs infected with the hepatitis delta virus. Because our sole haemophiliac patient who died of cirrhosis had delta infection[2] we decided in 1980 to do liver biopsies in HBsAg positive haemophiliacs who had raised ALT levels for at least 2 years. Delta antigen and HBcAg were sought by immunoperoxidase techniques with specific antisera in formalin-fixed sections of the liver of 7 haemophiliacs (mean age 12 years, range 6–39) selected on the above criteria. The 3 delta-positive patients had liver cirrhosis or severe CAH, whereas the 4 delta-negative patients had chronic persistent hepatitis[3] or mild CAH. When children are infected with hepatitis delta agent, which injures the liver through non-immune mechanisms, the liver disease is much more progressive than that in age-matched delta-negative HBsAg carriers without.[4] Our data suggest that this is true for haemophiliacs also.

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FREE RADICALS AND ALCOHOLICS

SIR,—Professor Dormandy and his colleagues have made several contributions to *The Lancet* describing increased "free-radical activity" in various clinical conditions. The latest of these (Aug 10, p 291) recorded increased free-radical activity in alcoholics. The measure of free-radical activity used was the serum level of a diene conjugated non-peroxide isomer of linoleic acid (9,11-LA'). However, only circumstantial evidence indicates that measurement of this isomer is an assay of free-radical activity and our work[1] and theoretical considerations suggest the contrary. We have shown that small rodents (rats, mice, guineapigs) with high metabolic rates and, therefore, with presumably greater fluxes of oxygen radicals in their tissues have insignificant levels of serum 9,11-LA' in comparison with man and large ruminants. Furthermore, exposure of rats to high doses of the red-blood-cell peroxidising agent phenylhydrazine and the liver peroxidising agent bromotrichloromethane failed to increase 9,11-LA' levels in plasma phospholipids.[1] Induction of lipid peroxidation in human or rat blood with either ultraviolet irradiation or phenylhydrazine also fails to increase plasma 9,11-LA' levels. These results indicate that 9,11-LA' is not generated during free-radical-induced injury to liver or blood cells.

There are also several theoretical reasons why 9,11-LA' is unlikely to be derived from free-radical activity. For example,