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Reduced risk of non-A, non-B hepatitis after a first exposure to 'wet heated' factor VIII concentrate

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Summary. The risk of post-infusion non-A, non-B hepatitis (NANBH) in patients receiving a first exposure to unheated or conventionally 'dry heated' factor VIII concentrates approaches 100%, implying invariable contamination of these products. Amongst 18 patients who received a first treatment with a 'wet heated' commercial concentrate, five (28%) developed asymptomatic NANBH, suggesting a more efficient inactivation of NANB agent(s) by this process. 2/9 (22%) of the batches of concentrate used in the study were implicated in NANBH transmission. One of these two batches, responsible for NANBH in four patients, had been prepared from a plasma pool containing an unusually large proportion of donations with high alanine aminotransferase (ALT) levels. A resulting high level of viral contamination in this batch may have been sufficient to override the effects of the sterilization process. All patients remained anti-HIV sero-negative at 17–28 months of follow-up.

Most regularly treated haemophiliacs have abnormal biochemical liver function tests, and some 20% of biopsied patients have histopathological evidence of chronic active hepatitis or cirrhosis (Aledort *et al*, 1985). Progression from apparently benign to serious liver disease may be rapid (Hay *et al*, 1985), and recent reports suggest that liver disease is becoming an increasingly common cause of death (Eyster *et al*, 1985).

An important cause of liver disease in haemophiliacs is thought to be the transmission, by plasma products, of the agent(s) responsible for non-A, non-B hepatitis (NANBH). In patients receiving a first exposure to conventional factor VIII concentrates, acute NANBH is a virtual certainty, implying the invariable contamination of these products (Fletcher *et al*, 1983; Kernoff *et al*, 1985). Because there are no reliable serological tests for NANBH, attempts to eliminate this contamination have largely focussed on the possibility of sterilizing concentrates by chemical or physical means, usually by heating. Heating protocols used by different manufacturers vary considerably. Most have used 'dry heating' processes, in which heat is applied to the finished, lyophilized product. Although most 'dry heating' processes probably inactivate human immunodeficiency virus (HIV), those which have been adequately evaluated in patients have shown little or no effect on reduction of risk of NANBH transmission (Colombo *et al*, 1985; Preston *et al*, 1985).

A major problem of evaluation has been that the results of both *in vitro* studies using marker viruses, and inoculation experiments in chimpanzees (Hollinger *et al*, 1984), have been found to be poor predictors of outcome in humans (Colombo *et al*, 1985). Thus, it has become widely accepted that reliable evidence of product safety can only be obtained by prospective evaluation of 'first exposure' patients, who would be unlikely to have acquired any resistance or immunity to infection.

The purpose of this study was to assess the incidence of acute post-infusion NANBH and other viral transmission in a group of 18 patients who received a first exposure to a commercial factor VIII concentrate (Profilate Heat-Treated, Alpha Therapeutic UK Ltd), prepared by a process in which heat is applied before final lyophilization ('wet heating') in the presence of an organic solvent, n-heptane (U.S. patent, 1984).

PATIENTS AND METHODS

Patients were admitted to the study between September 1984 and August 1985, and followed for at least 40 weeks after a first infusion of concentrate. All were male, and all except patient 14, who had acquired haemophilia with a circulating anticoagulant, had a congenital deficiency of factor VIII. All

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except patient 6, who was Italian, were British. None had been treated with concentrate before, but seven had received other blood products up to a maximum of 56 donor units (Table I), equivalent to a maximum chance of previous NANBH of approximately 20% (Kernoff *et al*, 1985; Collins *et al*, 1983). In a previous study using unheated commercial concentrates, prior exposure to even large quantities of other blood products did not reduce the risk of NANBH after a first exposure to concentrate (Kernoff *et al*, 1985). Before treatment with concentrate, all patients had normal biochemical liver function tests, and none had other evidence of liver disease. Patient 14 was anti-HBs/anti-HBc seropositive before treatment, although he had no history of hepatitis or blood product exposure, and three patients had started a course of hepatitis B (HBV) vaccine before treatment. Otherwise, pre-infusion serological markers for HBV were negative in all patients, and all were anti-HIV negative.

Factor VIII concentrate was bought from Alpha Therapeutic UK Ltd, and had been manufactured from U.S.-derived commercial plasma pools obtained from approximately 5000–32 000 donations, none of which had been screened for ALT or anti-HIV. Nine different batches of concentrate were used. With one exception (patient 14), each patient was only exposed to a single batch (Table I, Fig 1). Treatment was given either to stop bleeding or as prophylaxis before surgery. The total amount of factor VIII received by each patient during the 40-week observation period varied greatly (Table I). In most cases the majority of this total was given at the beginning of the period. The protocol demanded that blood samples should be taken, and patients clinically assessed, immediately before their first exposure infusions, at least every 2 weeks for the first 12 weeks, and then at least every 4 weeks until 40 weeks. Particularly in children, it proved impossible to adhere to this regime in all patients. However, sampling frequency was considered sufficient to allow full analysis (Fig 1). The diagnosis of post-transfusion NANBH was based on a rise in serum aspartate transaminase (AST) and/or alanine aminotransferase (ALT) to exceed 2·5 times the upper limit of normal in at least two post-infusion samples taken within 4 weeks of each other. Other diagnostic criteria have been previously described (Kernoff *et al*, 1985). Pre- and post-infusion samples were examined by conventional techniques for serological evidence of transmission of HBV and HIV (all patients); and hepatitis A virus, cytomegalovirus, and Epstein Barr virus (all except patient 6).

The nature of the study and the reasons for wishing to use the concentrate were explained to all patients or their parents, and consent to follow-up obtained.

RESULTS

Five patients (28%) developed acute NANBH, which in all cases was asymptomatic. Incubation periods, defined as the intervals between the first infusions of the product and the first abnormal AST/ALT result, were 14, 43, 28, 56 and 41 d, respectively. These periods are similar to those previously reported using unheated and 'dry heated' concentrates (Fig 1). In three of the five patients who developed hepatitis, transaminase abnormalities resolved within the 40-week follow-up period. In the other two, abnormalities persisted beyond 40 weeks, indicating the development of chronic hepatitis. Two additional patients (3 and 5) developed mild, transient, ALT abnormalities which did not fulfil the criteria for diagnosis of hepatitis.

Two of the nine batches of concentrate (22%) were

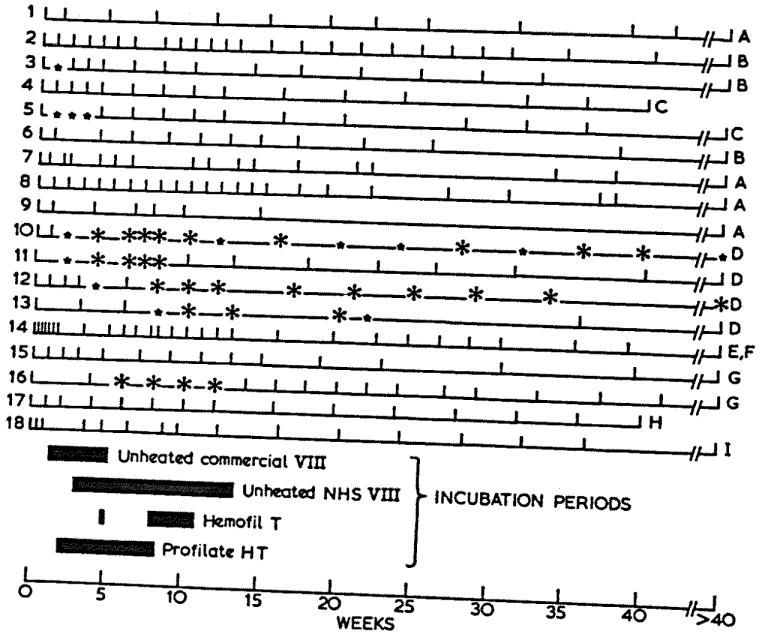

Fig 1. Patient follow-up. Vertical bars indicate sampling times and normal AST/ALT values. Small stars indicate abnormal AST/ALT less than 2·5 times upper limit of normal. Large stars indicate abnormal AST/ALT greater than 2·5 times upper limit of normal. Letters designate batches of concentrate used. Comparative NANBH incubation periods for unheated and dry heated products are taken from published data (Fletcher *et al*, 1983; Kernoff *et al*, 1985; Colombo *et al*, 1985).

Table I. Patient characteristics

Patient	VIII:C (u/dl)	Age (yr)	Total dose (units)	Batch	Reason for first exposure to Profilate	Previous exposure (donor units)	Time since last exposure	Hepatitis B vaccine	Outcome
1	<1	2	810	A	Spontaneous haemarthrosis	5 d.u. cryo	6 months		
2	<1	17	4600	B	Spontaneous haemarthrosis	10 d.u. blood/FFP	4 yrs	Yes	
3	10	24	2760	B	Dental extraction				
4	10	86	6120	C	Hand laceration				
5	12	35	2040	C	Dental extraction				
6	4	16	14100	B	Traumatic haemarthrosis			Yes	
7	<1	15 mo	6820	A	Spontaneous haemarthrosis				
8	5	22 mo	510	A	Prophylaxis for i/m vaccine				
9	<1	17 mo	270	A	Spontaneous haemarthrosis				
10	19	21	33800	D	Surgery	2 d.u. blood	11 yr		NANBH
11	12	28	7800	D	Trauma: multiple injuries				NANBH
12	35	24	27560	D	Surgery				NANBH
13	20	3	1560	D	Trauma: mouth injury				NANBH
14	<1	63	272600	E, F	Surgery	8 d.u.* blood/FFP	1 d		
15	14	67	14850	G	Surgery	1 d.u. blood	33 yr	Yes	
16	33	53	40500	G	Surgery	4 d.u. blood	20 yr		NANBH
17	10	14	22440	H	Post-arthroscopy bleed				
18	20	23	11180	I	Hand laceration	56 d.u. cryo	11 yr		

* Patient 14 was also treated with porcine factor VIII immediately after first exposure to Profilate.

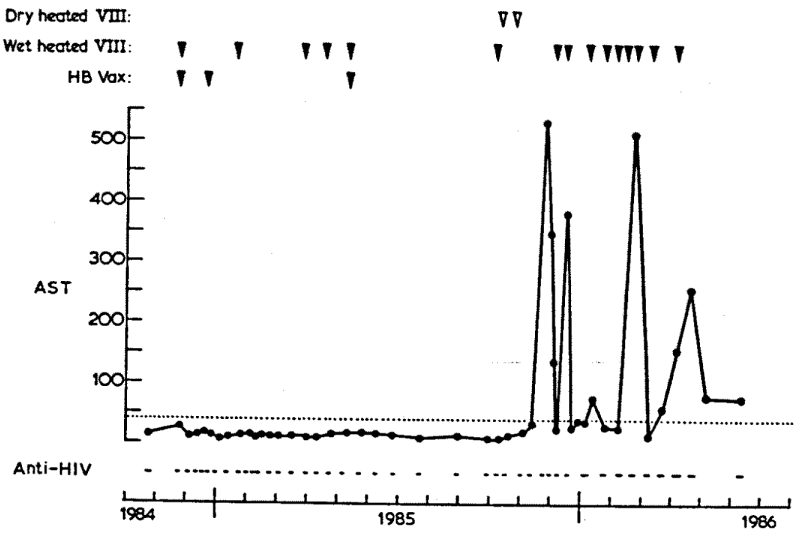

Fig 2. Course of patient 2 (see text).

implicated in transmission of NANBH. One of these batches (D) caused hepatitis in all four recipients, suggesting a batch-related rather than process-related problem. The second implicated batch (G) was given to two patients, only one of whom developed NANBH. These two patients were comparable in most respects (Table I), except that hepatitis B vaccine had only been given to the patient who did *not* develop NANBH. Although neither of the other two vaccinated patients developed hepatitis, neither was exposed to an implicated batch. Other variables, including age, previous blood product exposure, and total dosage of factor VIII infused, did not appear to influence the risk of NANBH.

The course of patient 2 (Fig 2) was of particular interest. He was treated with a total of 5060 units (110 u/kg) of Profilate over an 11-month period of uneventful follow-up. 4600 units were given in the first 40 weeks. At 11 months he was treated with 1075 units (23 u/kg) of a 'dry heated' commercial concentrate, and received a similar dose of the same product 2 weeks later. 27 d after initial exposure to this 'dry heated' concentrate he developed grossly abnormal liver function tests and symptomatic NANBH with jaundice. Transaminase abnormalities have persisted for 15 months, although anti-HIV remains negative.

Serological studies yielded uniformly negative results. In particular, there was no evidence of transmission of HBV, and all patients remained anti-HIV seronegative after 17–28 months of follow-up.

DISCUSSION

Factors influencing the efficiency of heat treatment in the inactivation of viruses contaminating factor VIII concentrate include the duration and temperature of heating, and whether the product is in a liquid or lyophilized state. 'Wet heating' is known to be much more efficient (McDougal *et al*, 1985). Nevertheless, and although recent reports suggest that the least aggressive 'dry heating' protocols (e.g. 60°C for 30 h) may not remove the risk of HIV transmission completely (van Den Berg *et al*, 1986; White *et al*, 1986), there is little doubt that all methods of heat treatment currently in use reduce this risk considerably. Particularly when heat treatment of the final product is combined with anti-HIV donor screening, the risk of HIV transmission appears negligible (MMWR, 1987). The same is not so for NANB agent(s), which seem more resistant to inactivation procedures than HIV. Commercial factor VIII concentrates subjected to 'dry heating' at 60°C for 30–72 h have been found to transmit NANBH to 84–100% of recipients (Colombo *et al*, 1985; Preston *et al*, 1985), attack rates which are similar to those associated with unheated concentrates, whether derived from commercial or volunteer plasma (Fletcher *et al*, 1983; Kernoff *et al*, 1985). The lower transmission rate found in this study suggests that the method used to prepare the concentrate, which included heating at 60°C for 20 h while the material was in a slurry with n-heptane, was more effective than conventional 'dry heating' and resulted in a lesser degree of viral contamination of the final product. This conclusion is reinforced by the course of patient 2, whose lack of evidence of NANBH during the first 11 months of follow-up is clearly unlikely to be attributable to host resistance factors. Reduced transmission rates have been found by other investigators using both the same and another 'wet heated' concentrate (Carnelli *et al*, 1986; Schimpf *et al*, 1987). A prototype 'dry heated' (60°C for 72 h) factor VIII concentrate produced by the National Health Service (NHS), now no longer available, was reported not to cause NANBH in four 'first exposure' recipients (Preston *et al*, 1985; Colvin *et al*, 1986). However, this concentrate was prepared from plasma pools obtained from only several hundred specially selected donors. Thus, firm conclusions cannot be drawn about the efficacy of heat-treatment. The current NHS product, designated '8Y', is fractionated by a different method, and more intensively 'dry heated' (80°C for 72 h). Initial clinical results appear encouraging but a full evaluation, carried out according to currently accepted criteria, has yet to be undertaken.

The risk of transmission of NANBH by factor VIII concentrate depends not only on the efficiency of any sterilization process, but also on characteristics of the source plasma. In the absence of any reliable serological markers for NANBH, interest in donor screening as a means of reducing viral contamination of concentrates has centred on the possibility of using 'surrogate' tests, including anti-HBc and ALT. There is good evidence that ALT screening, in particular, is likely to result in a reduced risk of NANBH in non-pooled products (Silverstein *et al*, 1984), and plasma used as source material for U.S.-derived commercial clotting factor concentrates is now invariably derived from ALT screened donors. This was not the case in 1984/85, when the concentrate used in the present study was manufactured, and examination of the production history of one of our two implicated batches (D) suggested that its propensity to cause NANBH might have been at least partially related to an unusually high level of NANB viral contamination in the plasma pool from which it was derived.

The plasma used to prepare batch D was collected in the U.S.A. in early 1985, and some was provided by independent contract plasma suppliers. In 1985 the West German Health Authorities ruled that all plasma products destined for use in that country should be derived from donor plasmas which had been individually screened for elevated ALT levels. This was not, and still is not, a requirement in the U.K. Without the knowledge of the manufacturers, one of their contract suppliers diverted plasma which had failed to meet the German requirements, and this was used to prepare batch D. The source plasma pool is now known to have contained a much higher than usual proportion of high ALT plasma, and it seems possible that this resulted in a high level of viral contamination which was sufficient to override the effects of the sterilization process. The asymptomatic course of the patients who developed NANBH may be indicative of a partial neutralization of NANB agent(s), since hepatitis associated with unheated commercial concentrates is usually symptomatic (Kernoff *et al*, 1985). All concentrates now manufactured by the company are derived from plasma donations which have ALT levels less than twice the upper limit of normal, and have been screened for anti-HIV. Whether or not such screening will eliminate or reduce the risk of post-infusion NANBH can only be assessed by a second clinical study, which is currently in progress.

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