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## Safety trial of heated factor VIII concentrate (8Y)

K J PASI AND F G H HILL

*Department of Haematology, The Children's Hospital, Birmingham*

**SUMMARY** Seventeen previously untreated boys with haemophilia A were treated with high purity heat treated factor VIII concentrate (8Y) for up to 36 months. Liver function tests were assessed monthly. No boy's serum has been shown to contain HIV antibodies and no increases in alanine transaminase activity have been detected. In only one patient was a single rise in aspartate transaminase activity noted, and this was without a corresponding rise in alanine transaminase. A second patient's serum contained hepatitis B core antibody transiently. It was thought likely in both cases that the abnormalities reflected intercurrent infections rather than disease associated with transfusion.

The physical treatments used in the production of 8Y seem to inactivate the agent(s) responsible for non-A, non-B hepatitis and HIV transmission by transfusion of factor VIII has been abolished. There are, however, problems associated with conducting safety trials in young haemophiliac patients.

Although the introduction of factor concentrates has revolutionised the treatment of bleeding episodes in haemophilia, transmission of viruses remains a serious concern. Until recently non-A, non-B hepatitis had nearly a 100% incidence in patients previously treated with large pool unheated concentrates, whether they were drawn from paid or volunteer donors, implying inevitable contamination of such products.<sup>1 2</sup> The introduction of dry heat treatment of freeze dried factor VIII concentrate to inactivate HIV, has shown little or no effect on reducing the risk of transmission of non-A, non-B hepatitis to those at greatest risk, namely previously untreated haemophiliacs.<sup>3 4</sup> Heating in solution—wet heat treatment of factor VIII concentrate—may reduce the risk of non-A, non-B hepatitis to about 30%,<sup>5</sup> or may even abolish it.<sup>6</sup> Other methods of viral inactivation may also reduce the incidence of non-A, non-B hepatitis including solvent/detergent treatment,<sup>7</sup> or heating in hot vapour,<sup>8</sup> though the latter process failed to inactivate hepatitis B completely.<sup>8</sup>

In 1985 the Blood Products Laboratory (Elstree) introduced a new factor VIII concentrate, type 8Y, which is heated at 80°C for 72 hours. Dry heat treatment at 60°C had little effect on the transmission of non-A, non-B hepatitis,<sup>3</sup> and 80°C is a higher temperature than previously reported in attempts to inactivate non-A, non-B hepatitis<sup>9</sup> and 'model' retroviruses.<sup>10</sup> Initial results using this

product were encouraging, and there were no cases of non-A, non-B hepatitis reported.<sup>11</sup>

We have therefore studied 17 previously untreated haemophiliac boys for up to 36 months after infusion of factor VIII concentrate 8Y to assess the risks and incidence of viral transmission. We will also address the problems of performing safety studies in young haemophiliac boys.

### Patients and methods

Seventeen boys were admitted to the study from a single paediatric haemophilia centre during the period July 1985 to April 1988. Patient selection complied with the criteria recommended by the International Committee on Thrombosis and Haemostasis (Miami, Florida 1984) for evaluating treated clotting factor concentrates. Accordingly no boy had been previously exposed to any blood nor blood products, had normal transaminase activities, and no markers of hepatitis B infection or clinical evidence of liver disease at the beginning of the study. All were negative for HIV antibody. All the boys had haemophilia A; 13 had severe haemophilia (VIII:C < 0.01 U/ml) and four had mild haemophilia (VIII:C 0.08–0.14 U/ml). Their ages ranged from 2 weeks to 16 weeks, and the mean age of the severely affected group at entry to the study was 14 months. All the boys were immunised against hepatitis B infection at or around the time of the first infusion

1464 *Pasi and Hill*

with 8Y. Treatment was given according to the clinical judgment of the attending physician. Informed parental consent was gained in all cases.

## CONCENTRATE

Dried factor VIII fraction, high purity, heat treated, type 8Y, became available in 1985. It is prepared from large pool fresh frozen plasma from unpaid donors of the National Blood Transfusion Service in England and Wales. Each unit is screened to make sure that it does not contain hepatitis B surface antigen (HBsAg) by third generation tests, but it is not screened for surrogate markers of non-A, non-B hepatitis (serum aminotransferase activities) or hepatitis B core antibody (HBcAb). Since 1986 plasma has also been screened to make sure that it does not contain HIV antibodies. After lyophilisation, the concentrate is heated in a dry state at 80°C for 72 hours in its final container. It is of high purity and high specific activity, in excess of 2 IU factor VIII/mg protein. Studies in our unit have indicated that the biological half life and recovery of VIII:C and its effectiveness in stopping bleeding episodes in patients with haemophilia are similar to those of unheated concentrates (unpublished observations). Twenty production batches of 8Y have been used in the study over a 36 month period during 1985-8.

## DIAGNOSTIC AND FOLLOW UP CRITERIA

Blood samples were collected before the first infusion of 8Y, and then at monthly intervals irrespective of whether additional infusions of concentrate were given. This protocol is in contrast to the International Committee on Thrombosis and

Haemostasis (Miami, Florida 1984) recommendations that testing should be done every two weeks for the first four months after the initial infusion. Monthly testing was adopted, as most parents would only consent to blood samples every four weeks unless treatment was required before the routine monthly sampling. Full compliance with this monthly regimen was sought. Samples were tested at each occasion for alanine transaminase, and alkaline phosphatase activities, bilirubin concentration, antibody to HIV hepatitis B markers, and a full blood count was also done.

Non-A, non-B hepatitis was defined as alanine transaminase activity more than 2.5 times the upper limit of normal for age on at least two separate occasions during the follow up period, other causes of hepatitis having been excluded.

## Results

Demographic data, baseline VIII:C concentrations, total dose of concentrate, number of batches, initial indications for infusion, and duration of follow up are shown in the table. All the boys have remained negative for HIV antibody.

Transaminase activities were measured on 348 separate occasions. No increases in alanine transaminase activity were detected but one child had an isolated increase in aspartate transaminase activity. This rose from 27 IU/l to 131 IU/l six weeks after treatment with a new batch of 8Y. The abnormal aspartate transaminase activity returned to normal within 12 days. Viral serology for hepatitis A, Epstein-Barr virus, and cytomegalovirus was

Table *Clinical details of the patients studied*

Case No	VIII:C (U/ml)	Age at entry	Indication for treatment	Total dose of 8Y (units)	No of treatments	No of batches	Period studied (months)
1	<0.01	13 Months	Injury to finger	66,050	127	13	36
2	<0.01	13 Months	Hepatitis B vaccination	33,255	93	17	31
3	<0.01	17 Months	Muscle bleed	62,525	127	14	30
4	<0.01	11 Months	Head injury	68,125	151	17	28
5	0.14	15 Years	Fractured femur	74,635	34	4	25
6	<0.01	9 Months	Haemarthrosis	31,850	64	9	25
7	<0.01	3 Months	Hand injury	27,205	97	10	21
8	<0.01	39 Months	Muscle bleed	28,515	40	9	20
9	0.09	9 Years	After operation	15,635	20	2	19
10	<0.01	15 Months	Head injury	24,610	43	7	18
11	<0.01	15 Months	Haemarthrosis	31,840	67	5	17
12	<0.01	5 Months	Ear injury	14,540	38	7	17
13	<0.01	21 Months	Haemarthrosis	10,440	24	6	17
14	0.10	6 Years	Head injury	1,920	2	1	16
15	<0.01	17 Months	Mouth bleed	27,550	69	7	~15
16	<0.01	2 Weeks	Umbilical bleed	1,370	5	4	9
17	0.14	5 Years	After operation	10,870	12	2	4

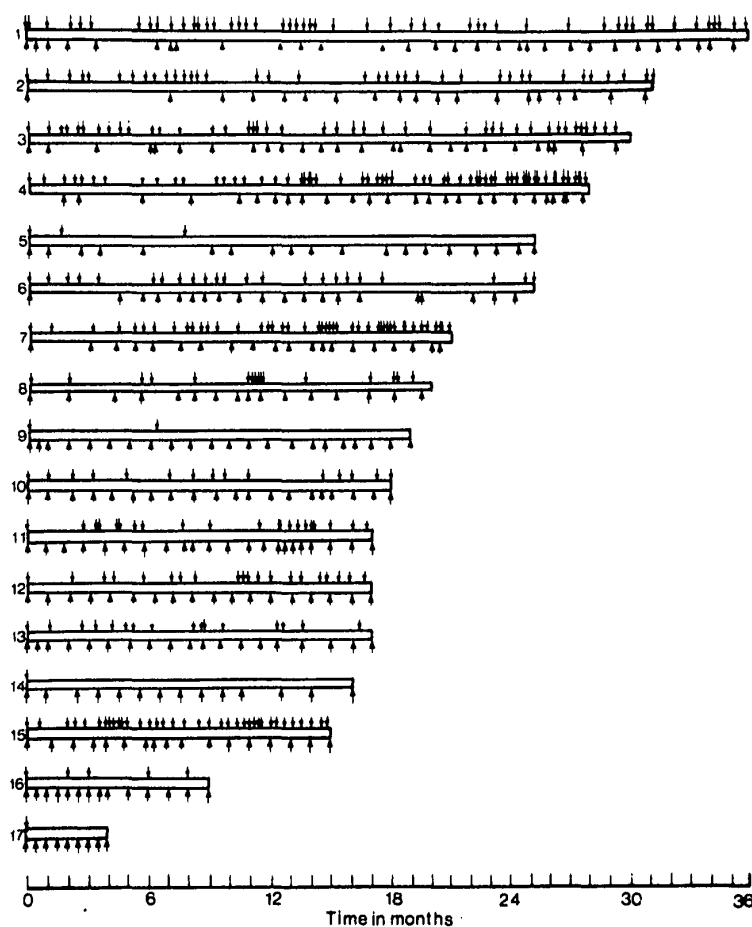
*Safety trial of heated factor VIII concentrate (8Y) 1465*

Fig 1 Timing of testing in relation to treatment episodes. Each bar represents the period of follow up of each boy. Arrows above the bar represent treatment episodes. Arrows below the bar represent points at which blood tests were carried out.

negative on both acute and convalescent sera. The child remained well throughout with no signs of liver disease or any other illness.

All the boys were immune to hepatitis B after immunisation. In one of the 17 a transient HBcAb was observed; the temporal pattern and equivocal or low concentration of this antibody suggested that it may have been passively acquired. He has had no clinical or biochemical evidence of liver disease, and

all other family members are non-immune to hepatitis B. He remains HBsAb positive and HBcAb negative.

Fig 1 shows the temporal relation of testing to treatment episodes. In some of the patients entered into the study early on it proved impossible to adhere strictly to a monthly regimen, particularly in the younger children. The mean monthly attendance for the total period of study was 85%, and 94% for

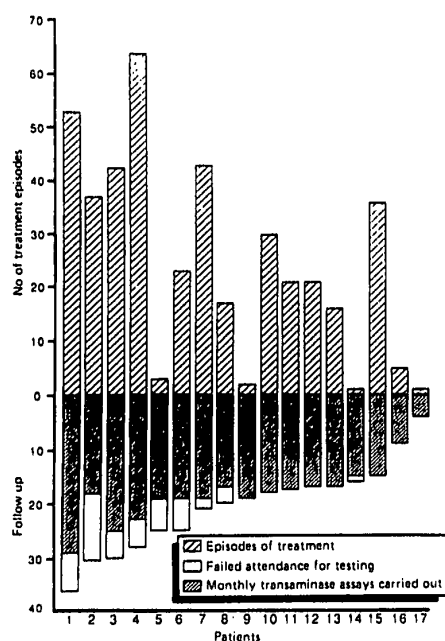


Fig 2 Number of treatment episodes shown in relation to number of monthly transaminase assays carried out and failed monthly attendances for testing. Total length of the downward bar is equivalent to the total period of follow up (months).

the last year of the study. Failure to attend was usually because the boys were well, and the parents were consequently reluctant to bring them to the hospital for blood testing (fig 2).

### Discussion

As experiments in chimpanzees do not provide a safe model for the infectivity of hepatitis in humans<sup>3 12</sup> and non-A, non-B hepatitis is commonly asymptomatic in its acute stages, the only way to show infectivity has been to carry out clinical trials. Patients most at risk of developing non-A, non-B hepatitis are those who have never previously been exposed to any blood or blood products. Such patients are usually young boys. Carrying out clinical trials in young children, particularly when frequent venepuncture is required, poses ethical problems as also noted by other investigators.<sup>5</sup>

Strict adherence to two weekly testing regimens (as recommended by the International Committee on Thrombosis and Haemostasis) in small children

requires considerable parental cooperation and altruism. A particular problem in this type of study is the lack of any immediate benefit to the patient from the regular blood testing. Parents are often unwilling to submit their children to frequent and sometimes difficult and traumatic venepuncture, especially if the child is well at the time. Collection of capillary blood offers an alternative and less invasive method of blood sampling. Capillary blood sampling is, however, potentially more hazardous to other personnel because of blood spillage and contamination. Because of this staff may be unwilling to collect capillary blood samples from these patients until a product's safety has been established. Venepuncture is therefore required, and even carried out by experienced paediatric staff may be difficult in young boys. Parental anxiety that veins may be damaged and that treatment of acute bleeds may not therefore be possible must be allayed. As liver disease is still a serious problem in haemophilia, obtaining information about the safety of products is of prognostic importance for their sons. Detailed information, encouragement of parents to take an active part in treatment, and regular encouragement by telephone or letter of defaulters to attend, have all improved compliance and achieved fairly strict monthly testing in most of the young boys.

It has been argued that monthly blood testing, until four months after the start of treatment will identify most patients who develop non-A, non-B hepatitis,<sup>11</sup> and so it is now becoming more widely acknowledged that in young boys strict monthly testing, if rigidly achieved, is acceptable to assess risk (Proceedings World Federation of Haemophilia, 1988).

We have found that in previously untreated boys receiving 8Y, alanine transaminase activities have remained normal throughout the three year study period; this suggests that 8Y does not transmit non-A, non-B hepatitis in such young children and that the heating process (80°C for 72 hours) seems to inactivate the agent or agents responsible for transmission of non-A, non-B hepatitis. In the United Kingdom the incidence of non-A, non-B hepatitis in the donor population was previously estimated as 0.3%.<sup>13</sup> It is likely that all production batches of 8Y will carry at least one donation capable of transmitting non-A, non-B hepatitis, even when diluted with thousands of non-infective donations. We presented further evidence for the efficacy of this heat treatment process when we showed that a reduced incidence of parvovirus B19 infection, a heat resistant virus, was observed in the boys treated with 8Y compared with a previous group of boys treated with unheated large pool

## Safety trial of heated factor VIII concentrate (8Y) 1467

concentrates (MD Williams, AC Bedall, K Al Rubei, *et al.* Transmission of parvovirus B19 by clotting factor concentrate. Abstract presented at 20th Congress of International Society of Blood Transfusion, London, 1988). There was also no apparent transmission of HIV as all boys remained negative for HIV antibody, thus providing circumstantial evidence that HIV transmission has been abolished. Despite the heat sensitivity of HIV being well established,<sup>10</sup> however, we cannot be sure that any batch was actually infected with HIV because the incidence of HIV seropositivity in the donor population is only 0.002% and the risks of HIV transmission are further reduced by the introduction of a national HIV antibody screening programme in the United Kingdom.

'Wet' heated products, whether heated in hot vapour or moistened, have been proposed as being safer than products heated when dry,<sup>5 6 8</sup> usually at 60°C, and that such products are to be preferred because 'dry' heating is ineffective in preventing hepatitis. Our data support the contention that 'dry' heating of factor VIII concentrate made from plasma from British volunteers (HIV antibody and hepatitis B antigen screened and negative) at 80°C for 72 hours is a safe and effective process for viral inactivation. In the future, however, safety studies in previously untreated patients will inevitably have to be undertaken in young newly diagnosed haemophiliacs. As haemophilia is a rare disorder such studies will inevitably be small, and consequently the statistical confidence limits of such studies will be limited. Further study on more untreated patients, adhering as closely as possible to International Committee on Thrombosis and Haemostasis recommendations, is required to improve statistical confirmation of product safety. More frequent assessment of alanine transaminase activity is now feasible using capillary blood samples, because the safety of 8Y shown by this study has allayed the anxieties of staff about performing capillary blood sampling.

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Correspondence and requests for reprints to Dr KJ Pasi, Department of Haematology, The Children's Hospital, Ladywood Middleway, Ladywood, Birmingham B16 8ET.

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