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Confirmation of viral safety of dry heated factor VIII concentrate (8Y) prepared by Bio Products Laboratory (BPL): a report on behalf of U.K. Haemophilia Centre Directors*

C. R. RIZZA, M. L. FLETCHER AND P. B. A. KERNOFF *Oxford Haemophilia Centre, and Royal Free Hospital, London*

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Summary. Twenty-seven factor VIII deficient patients who had previously not been treated with blood or blood products were studied after infusion of a total of 24 batches of NHS factor VIII (8Y) concentrate produced by Bio-Products Laboratory, Elstree. Follow-up was carried out according to guidelines laid down by the International Society for Thrombosis and Haemostasis.

Serial estimations of amino transferase level carried out

over a 26-week period revealed no elevation of these enzymes attributable to hepatitis. Studies of various virological markers found no evidence of infection with hepatitis C, hepatitis B or HIV following transfusion. This confirms a previous finding that severe dry heating of factor VIII at 80°C for 72 h seems to reduce the risk of transmitting hepatitis C from approximately 90% to a rate of 0-11%.

In the summer of 1985 the Blood Products (now Bio Products) Laboratory (BPL) at Elstree introduced a new concentrate of factor VIII (8Y) for the treatment of haemophilia A and von Willebrand's disease. This concentrate is heated in the dried state at 80°C for 72 h with the aim of inactivating HIV and other blood-borne pathogenic viruses, in particular those causing non-A, non-B hepatitis (hepatitis C) and hepatitis B.

An earlier study of 8Y in the U.K. by Haemophilia Centre Directors (Study Group of the U.K. Haemophilia Centre Directors, 1988) showed no evidence of transmission of HIV, hepatitis B or non-A, non-B hepatitis. During the course of that early study the International Society for Thrombosis and Haemostasis (ISTH) issued its first recommendations (Schimpf *et al.* 1987) for carrying out such studies. Our early study did not comply fully with these recommendations: in particular, some patients had been treated previously with blood or blood products, albeit very small amounts. It was

therefore decided to carry out a further study of 8Y adhering strictly to the ISTH guidelines, which were further revised in 1989 (Mannucci & Colombo, 1989).

Concentrate

Factor VIII concentrate (8Y) was used without selection from routine production lots, each prepared from 6000-12 000 donations of plasma from unpaid donors of the National Blood Transfusion Service in England and Wales. Individual donations had been screened for anti-HIV-1 and HBsAg. Donations were not screened for alanine aminotransferase (ALT) or aspartate aminotransferase (AST) or anti-HBc. Nor were they screened for anti-HCV as the study was conducted before donor testing was started in England and Wales in September 1991.

Protocol for study: entry criteria

(1) All patients entering the study were patients deficient in factor VIII requiring factor VIII to treat bleeding episodes or to prevent bleeding. Informed consent was obtained from all patients. Local Ethical Committee approval was obtained at each participating Centre.

(2) Patients had no previous exposure to blood or any blood product.

(3) ALT or AST or both were within the normal range before treatment with 8Y.

(4) There was no other evidence of liver disease before treatment with 8Y.

* The following participated in the study: Dr B. T. Colvin, Dr H. Daly, Dr D. J. K. Evans, Dr I. M. Franklin, Dr I. M. Hann, Dr F. G. H. Hill, Dr E. Miller, Dr A. Milne, Dr D. Mitchell, Dr I. A. Parapia, Professor F. E. Preston, Dr G. Savidge, Dr M. J. Strevens, Dr D. S. Thompson, Dr E. Thompson and Dr D. N. Whitmore.

Correspondence: Dr C. R. Rizza, Oxford Haemophilia Centre, Churchill Hospital, Oxford OX3 7LJ.

Table 1.

Patient	Sex	Age	Factor VIII (%)	Reason for treatment
1	M	1 yr	<1	Haemarthrosis
2	M	7 yr	4	Orchidopexy
3	F	30 yr	<1*	Haematuria, haemarthrosis
4	M	1 mth	0	Umbilical bleeding
5	M	6 yr	<1	Post-operative bleeding
6	M	3 mth	<1	Haemarthrosis
7	M	4 yr	25	Haemarthrosis
8	F	56 yr	26†	Suspected cerebral haemorrhage
9	F	49 yr	39†	Sterilization
10	M	65 yr	18	Surgery for bowel carcinoma
11	M	17 yr	16	Operation on toes
12	M	1 yr	<1	Cut tongue
13	M	3 yr	<1	Muscle haematoma*
14	M	3 yr	<1	Haematoma face
15	M	1 yr	2	Haematoma forehead
16	M	1 yr	1†	Cut tongue
17	M	62 yr	10	Coronary artery bypass
18	M	4 yr	3	Tongue haemorrhage
19	M	2 yr	11	Haemarthrosis
20	M	6 yr	10	Tonsillectomy
21	M	3 yr	2	Haemarthrosis
22	M	21 yr	9	GI bleeding
23	M	6 mth	1	Bleeding mouth
24	M	3 yr	12	Haemarthrosis
25	M	7 yr	<1	Haemarthrosis
26	M	1 yr	<1	Cut lip
27	M	52 yr	17	Cut rectum

* Acquired antibody to factor VIII.

† von Willebrand's disease.

(5) Test for anti HIV-1 was negative before treatment with 8Y.

(6) Test for HBsAg was negative before treatment. It was recommended that hepatitis B vaccination be carried out before entry to the study. Unvaccinated patients were negative for anti HBs before treatment.

Follow-up and sampling frequency post infusion

Patients were followed up for 26 weeks after first exposure to 8Y. Blood samples were obtained immediately before treatment, at least every 2 weeks for the first 16 weeks and thereafter at least every 4 weeks until 26 weeks. Appointments for follow-up could not always be kept on the specified day, and ISTH guidelines allow '4-5 days for adjusted appointments'; if there was any gap of more than 35 d between testing at any time up to 26 weeks the follow-up was classed as non-compliant. Blood samples were tested for ALT, AST, or both, and for virological markers (HBsAg, HBsAb, HIV-1 Ab) in the participants' local laboratories. Each local laboratory defined its own normal range.

End point: definition of hepatitis

Acute hepatitis was defined as a rise of serum ALT or AST to more than 2½ times the upper limit of normal on at least two samples taken within 2 weeks or less of each other.

Organization and liaison

This was a multi-centre trial co-ordinated by Dr C. R. Rizza (Oxford Haemophilia Centre) and Dr P. B. A. Kernoff (Royal Free Hospital Haemophilia Centre). All Haemophilia Centres in the U.K. were invited to participate. A data collection centre was established at Oxford where a nominated member of staff had responsibility for data collection and answering questions about the protocol.

The co-ordinators undertook to inform all participants immediately if there were reports of any adverse events attributable to the infused factor VIII. It was planned that the study should run for a minimum of 2 years and that at least 20 patients who met the strict follow-up criteria laid down in the protocol would be studied.

RESULTS

Eighteen centres participated in the study and registered 49 patients as possible entrants. Upon subsequent closer questioning 11 of these patients were found to be ineligible: four had previously received blood or blood products, six had no pre-infusion ALT or AST tests carried out and one had abnormal liver function tests before infusion of factor VIII. Thirty-eight patients were therefore entered into the study. In the course of follow-up one patient was withdrawn suffering from cancer with secondary deposits in the liver.

Safety of Heated Factor VIII 271

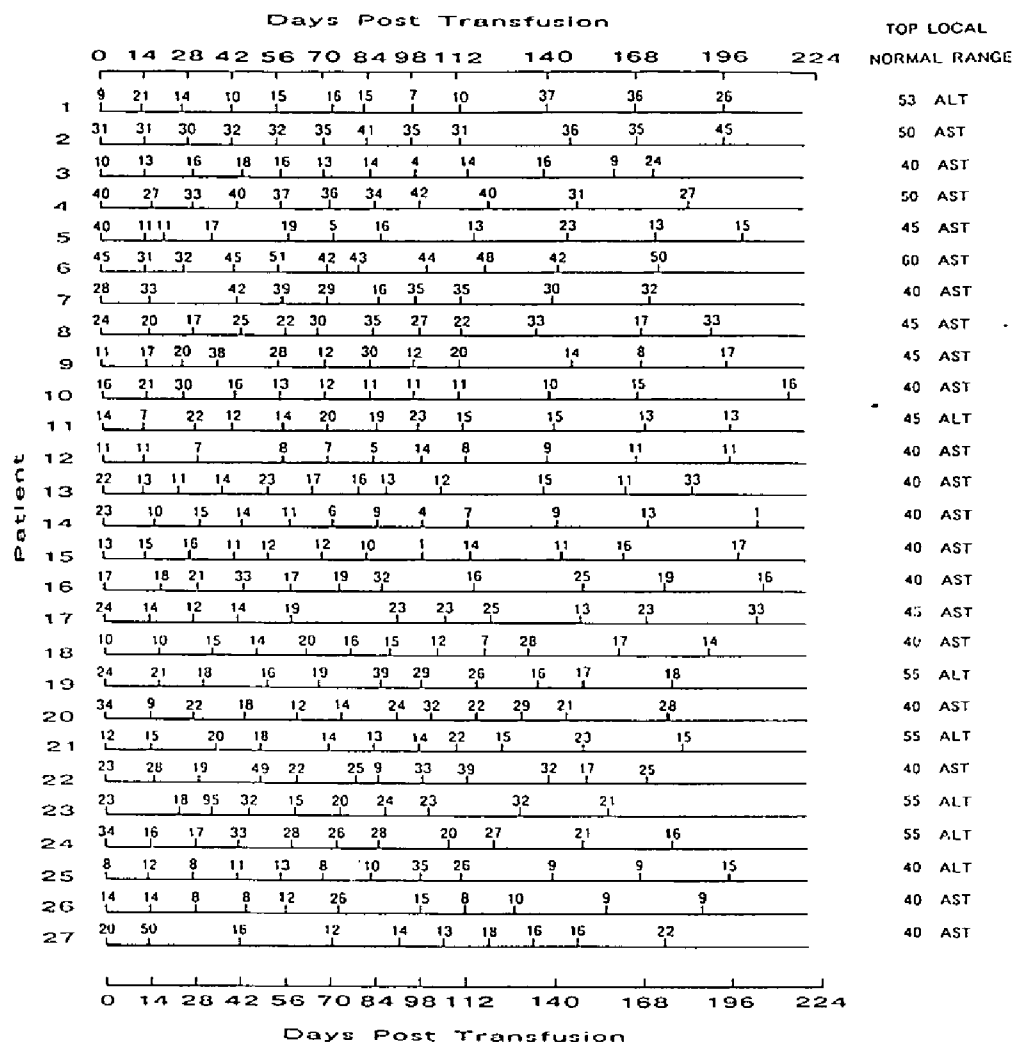


Fig 1. Serial serum transaminase levels in 27 patients after first infusion of factor VIII (8Y) concentrate. Figures down right-hand side represent upper limit of local normal ranges for AST or ALT in IU/L. Day 0 = Day of infusion. AST/ALT concentration given as IU/L.

Of the remaining 37 patients, 27 (Group I) met the protocol's strict follow-up criteria. The characteristics of these 27 patients and the reason for treatment with factor VIII are shown in Table 1. The serum ALT or AST levels in these patients and the times of testing are shown in Fig 1. None showed an increase of ALT or AST level $> 2.5 \times$ the upper limit of normal. Three (patients 7, 22 and 27) showed a marginal elevation (42, 50 and 49 IU respectively) in a single test which was not confirmed at subsequent testing. In one other case (patient 23) the sample taken on the 33rd day showed a rise in the ALT level to 95 IU/L; however, it was noted that this blood sample had been obtained by fingerprick and was grossly haemolysed; samples taken 10 d before and 12 d after the elevated value were in the normal range. A total of 24 batches of factor VIII was used in those 27 patients. The frequency of follow-up of the remaining

10 patients (Group II) did not comply fully with the study protocol. In the follow-up of eight patients there was only one gap of more than 35 d between tests, and in two cases there were two gaps. None of this non-compliant group had an elevation of ALT or AST $> 2.5 \times$ the upper level of normal.

HBV-1 and HBV transmission

Twenty-one of the 27 patients in Group I had been vaccinated against hepatitis B. No patient who had not been vaccinated became anti HBs positive and none became HBsAg positive.

HCV transmission

Tests for HCV antibody were not available when the study was set up and were not included in the protocol. However, as soon as tests for anti-HCV became available, all participant centres were asked to test for anti-HCV as an additional test.

No patient in Group I or Group II developed antibody to HCV as measured by first-generation ELISA methods following transfusions of 8Y. Twenty-two patients have been tested by second generation tests for anti HCV at the end of the study and all were negative. In the remaining five only first generation test results were available. Those were all negative at the end of the study. One patient was found to be anti-HCV positive in the course of the study but testing of earlier samples showed that he had been anti-HCV positive before receiving 8Y. Repeat testing using second generation tests showed him to be negative at the end of the study.

DISCUSSION

Twenty-seven patients meeting ISTH criteria for selection and follow-up showed no significant or sustained elevation of serum aminotransferase in a follow-up period of 6 months after injection with a total of 24 batches of severely dry heated factor VIII (8Y) concentrate. Anti-HCV testing supported the conclusion that no patient acquired HCV infection. The conventional statistical interpretation of this result using the 'rule of three' (Hanley & Lippman-Hand, 1983) is that the risk of transmitting HCV/NANBH with this concentrate lies between 0 and 11%; this is comparable with the conclusion of an earlier study (Study Group of the U.K. Haemophilia Centre Directors, 1988) of this concentrate using different criteria for patient selection but similar criteria for test frequency. All patients in the original study (Study Group of the U.K. Haemophilia Centre Directors, 1988) who have been tested and have received no other type of concentrate have remained negative for anti-HIV and anti-HCV (Colvin, 1990).

Since all the plasma used in the production of these lots of concentrate was screened for anti-HIV-1 it is not possible to say that non-transmission of HIV-1 by 8Y was due solely to the heating process. For similar reasons, neither this study nor our previous study (Study Group of the U.K. Haemophilia Centre Directors, 1988) proved that HBV was inactivated by heating at 80°C for 72 h.

The 24 lots of 8Y factor VIII to which the patients were exposed in the present study were produced from a total of approximately 270 000 donations of plasma unscreened for

markers of HCV. Since the incidence of non-A, non-hepatitis following transfusions of blood from a London donor population is approximately 0.26% (Contreras *et al.*, 1991) one can reasonably conclude that heating this large-pot concentrate at 80°C for 72 h in the final vial prevented transmission of HCV.

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