

(14)

Study of viral safety of Scottish National Blood Transfusion Service factor VIII/IX concentrate

Haemophilia Directors for Scotland and Northern Ireland:

B. Bennett, A. A. Dawson, B. S. Gibson, A. Hepplestone, G. D. O. Lowe, C. A. Ludlam, E. E. Mayne and T. Taylor

Received 25 January 1993; accepted for publication 18 May 1993

SUMMARY. To assess the viral safety of the Scottish National Blood Transfusion Service (SNBTS) intermediate purity factor VIII and IX concentrates, the liver function and viral status were assessed prospectively in 13 recipients. None developed hepatitis or seroconverted to HIV or HCV. This study provides

additional evidence for the efficacy of dry heat treatment at 80°C for 72 h in preventing virus transmission by coagulation factor concentrates.

Key words: factor VIII concentrate, viral safety.

The availability of factor VIII and IX concentrates, during the past 20 years, has enabled many haemophiliacs to lead full and active lives. This dramatic improvement in life-style has been overshadowed by the potential for acquisition of transfusion-transmitted viral infections. Hepatitis viruses A, B, C and D, human immunodeficiency virus (HIV) and parvo virus can be transmitted by coagulation factor concentrates if not treated to inactivate viruses. Despite careful selection of blood donors and screening of individual donations for infectious viruses, blood product transfusion, in the past, has commonly resulted in infection of the recipient (Fletcher *et al.*, 1983; Kernoff *et al.*, 1985). Since the mid 1980s, viral inactivation procedures have been incorporated into the manufacturing process for all concentrates with a substantial reduction in the risk of viral infection of recipients. Despite apparently stringent viral inactivation procedures, however, viral transmission has been reported on several occasions (Brettler & Levine, 1989).

It is essential, therefore, for any new blood product to demonstrate its viral safety. To ensure rigorous and uniform testing of concentrates the Scientific and Standardisation Committee (SSC) of the International Society for Thrombosis and Haemostasis has issued recommendations on how products should be assessed (Mannucci & Colombo, 1989).

This study describes viral safety data on factor VIII

and IX concentrates manufactured by the SNBTS which have been used predominantly in Scotland and Northern Ireland for the past 7 years. In total 13 patients were studied prospectively, based on criteria based on the SSC recommendations; nine had never previously received a transfusion and four had received a few donations of cryoprecipitate or red cells. No case of hepatitis was observed and there was no serological evidence for HCV or HIV transmission.

PATIENTS

Twenty patients were enrolled in the study. Two were withdrawn by their parents after receiving factor VIII concentrate because the parents did not wish their children to undergo additional venepuncture. One patient was found to breach the entry criteria and was withdrawn with the approval of the Data Monitoring Committee. Four patients were enrolled but did not require therapy with coagulation factors during the period of the trial.

Details of the 13 remaining patients who attended the Haemophilia Centres of the Royal Infirmary in Edinburgh and Glasgow, Forresterhill Hospital in Aberdeen and Yorkhill Children's Hospital in Glasgow, are given in Table 1. Approval was obtained from each institutional Ethical Committee. The study protocol was based on that recommended by the SSC of the International Society for Thrombosis and Haemostasis (ISTH) (Mannucci & Colombo, 1989). In essence this consists of studying patients who have never previously received blood products and under-

Correspondence: Dr C. A. Ludlam, Department of Haematology, Royal Infirmary, Lauriston Place, Edinburgh EH3 9YW, Scotland, U.K.

Table 1. Details of blood products received by patients

Patient number	Diagnosis	Number of previous single-donor unit exposures	Product	Total dose in 6/12 (IU)
1	Haemophilia A	0	Z8	1060
2	Haemophilia A	0	Z8	5600
3	Haemophilia A	0	Z8	440
4	Haemophilia A	0	Z8	6710
5	vWD	0	Z8	1200
6	vWD	0	Z8	1150
7	Haemophilia A	3 (cryoppt)	Z8	1016
8	Haemophilia A	14 (cryoppt)	Z8	4840
9	Haemophilia A	4 (cryoppt)	Z8	480
10	vWD	45 (cryoppt) 7 RCC	Z8	7150
11	Haemophilia B	0	Defix	1500
12	Haemophilia B	0	Defix	1860
13	Factor X deficiency	0	Defix	400

Cryoppt = cryoprecipitate; RCC = red cell concentrate.

taking a fortnightly estimation of alanine aminotransferase (ALT, normal range 20–40 u/l) or aspartate aminotransferase (AST, normal range 10–35 u/l) for 16 weeks and then monthly for a further 3 months. The four patients who had received previous single donor products had normal ALT prior to receipt of concentrate and had no history of hepatitis. All patients were tested before the receipt of coagulation factor concentrate and again after 6 months for antibodies to HBV (Burrell *et al.*, 1974), HCV (Abbott, second generation test) and HIV (Abbott, HIV 1 and 2 recombinant). No patient had serological evidence of prior HBV, HCV or HIV infection and all were vaccinated against HBV prior to receipt of the concentrate.

An independent data monitoring committee was established (Professor Sir Richard Doll, Sir Patrick A. M. Forrest and Dr P. K. A. Kernoff) and the Communicable Disease (Scotland) Unit, Ruchill Hospital, Glasgow agreed to investigate any case of possible virus transmission.

COAGULATION FACTOR CONCENTRATES

The factor VIII concentrate (Z8) was of intermediate purity and the factor IX (Defix) also contained factors II and X. Both were manufactured by the Protein Fractionation Centre of the Scottish National Blood Transfusion Service, Edinburgh, from volunteer blood donations individually screened for HBsAg and anti-HIV (since October 1985). After freeze drying the vials were heated at 80°C for 72 h.

The total dose of product received by each patient during the 6-month study period is given in Table 1. A total of 25 different batches was used.

RESULTS

The results of individual determinations of ALT for each patient are set out in Table 2. No case of hepatitis (as defined by SCC protocol) was observed in any patient and in two patients insufficient samples were collected resulting in a breach of the protocol. All patients were seronegative for HBV, HCV and HIV on initial testing and for the latter two viruses at 6 months after infusion.

DISCUSSION

Prior to the introduction of a heat treatment regime it is likely that all recipients of factor VIII and IX concentrates became infected with HCV (Watson *et al.*, 1992) and some with HBV (Stirling *et al.*, 1983) and HIV (Ludlam *et al.*, 1985). None of the 13 patients in this study using heat-treated concentrates developed either hepatitis or infection with HCV or HIV.

A similar viral inactivation procedure is used for factor concentrates manufactured by Bio Products Laboratory, Elstree; and in a study of 32 patients no evidence of hepatitis or HIV transmission was observed (U.K. Haemophilia Centre Directors, 1988). However, as the extent of viral inactivation depends on many variables, and particularly the moisture content of the freeze-dried product, it is essential that each

Study of viral safety of factor VIII/IX concentrate 297

Table 2. Sequential ALT (or AST) data on patients after first infusion of factor VIII or IX concentrate

Patient	Preinfusion	Weeks												
		2	4	6	8	10	12	14	16	18	20	22	24	26
1	58	22	—	—	—	—	—	27	26	—	40	—	25	—
2	26	61	19	—	11	16	24	25	—	32	25	—	17	—
3	19	19	<5	18	15	—	16	12	20	17	10	—	7	—
4	21	—	27	24	25	23	24	21	22	26	—	—	46	62
5	28*	28*	47*	19*	—	29*	20*	—	27*	—	18*	—	18*	—
6	6	5	5	16	17	15	—	19	—	24	—	—	14	—
7	14	16	14	17	17	13	16	17	15	17	16	—	—	13
8	11	11	14	16	28	32	32	33	27	31	25	—	19	18
9	25	18	25	21	22	23	21	33	36	29	27	57	24	—
10	10	<10	17	11	10	<10	—	18	14	—	<10	—	16	<10
11	—	—	—	—	29	40	31	29	29	31	28	—	—	28
12	17	—	15	14	11	17	14	12	—	12	—	13	—	13
13	20	14	22	24	25	19	23	28	—	—	—	26	—	—

*AST data.

manufacturer provides evidence for the safety of their concentrate.

One of the difficulties of undertaking these studies is to find sufficient numbers of subjects in need of concentrate infusions and who have not previously received any blood product. Furthermore, it is sometimes difficult to obtain all the appropriate samples; in this study two patients did not fulfil the protocol criteria but the data were included. The SSC recommends the study of only previously untransfused patients. Some reservation has been expressed about including patients who have received a small number of single donations because of the possibility of prior infection with virus causing non-A non-B hepatitis which might be inapparent at the time of concentrate infusion. With the advent of reliable and sensitive serological and PCR-based tests for HCV, infection with this virus can be virtually excluded in those patients who have previously received single donor products. This study, therefore, has also demonstrated that it is possible to include pre-screened patients who have received minimal prior transfusion with single donor products as a way of increasing the number of subjects available for evaluation.

While this study has failed to demonstrate the transmission of blood source viruses with SNBTS coagulation factor concentrate, the confidence limits that can be put upon this are large. There is a 95% chance that true infectivity lies between 0 and 30% [rule of three (Hanley & Lippman Hand, 1983)] for the factor VIII concentrate which was given to 10 subjects.

It is not possible to provide a meaningful confidence limit for the factor IX concentrate because only three patients received it.

No evidence is available from this study to demonstrate non-infectivity to HBV, as all patients were vaccinated prior to the receipt of concentrate.

This study has not found any evidence of virus transmission by Z8 or Defix when assessed by a rigorous protocol. Furthermore, the study provides additional evidence that dry heating of coagulation factors concentrates at 80°C for 72 h is effective in preventing HCV transmission as previous studies have demonstrated that the use of the majority of unheated concentrates result in its transmission. During the past 7 years 60 million units of Z8 and 10 million units of Defix have been used to treat haemophiliacs in Scotland and Northern Ireland and, during routine surveillance of patients, none has seroconverted to HCV or HIV (data not shown).

ACKNOWLEDGEMENTS

The assistance of Dr R. R. C. Stewart was appreciated in the design and execution of the study which was supported by the SNBTS. Dr G. Stockdill kindly contributed a patient to the study. We thank Dr D. Goldberg, Ruckhill Hospital for carefully investigating a patient who was found after entry to the study to have breached the exclusion criteria. The assistance of the Data Monitoring Committee, Professor Sir

Richard Doll, Professor Sir Patrick A. M. Forrest and Dr P. B. A. Kernoff is gratefully acknowledged.

REFERENCES

- Brettler, D.B. & Levine, P.H. (1989) Factor concentrates for treatment of hemophilia: which one to choose? *Blood*, **73**, 2067-2073.
- Burrell, C.J., Parker, A.C., Ramsay, D.H. & Proudfoot, E. (1974) Antibody to hepatitis B antigen in haemophiliacs and their household contacts. *Journal of Clinical Pathology*, **27**, 323-325.
- Fletcher, M.L., Trowell, J.M., Craske, J., Pavier, K. & Rizza, C.R. (1983). Non-A non-B hepatitis after transfusion of factor VIII in infrequently treated patients. *British Medical Journal [Clinical Research]*, **287**, 1754-1757.
- Hanley, J.A. & Lippman Hand, A. (1983) If nothing goes wrong, is everything all right? Interpreting zero numerators. *Journal of the American Medical Association*, **249**, 1743-1745.
- Kernoff, P.B., Lee, C.A., Karayiannis, P. & Thomas, H.C. (1985) High risk of non-A non-B hepatitis after a first exposure to volunteer or commercial clotting factor concentrates: effects of prophylactic immune serum globulin. *British Journal of Haematology*, **60**, 469-479.
- Ludlam, C.A., Tucker, J., Steel, C.M., Tedder, R.S., Chingsong-Popov, R., Weiss, R.A., McClelland, D.B., Philip, I. & Prescott, R.J. (1985) Human T-lymphotropic virus type III (HTLV-III) infection in seronegative haemophiliacs after transfusion of factor VIII. *Lancet*, **ii**, 233-236.
- Mannucci, P.M. & Colombo, M. (1989) Revision of the protocol recommended for studies of safety from hepatitis of clotting factor concentrates. International Society for Thrombosis and Hemostasis. *Thrombosis and Haemostasis*, **61**, 532-534.
- Stirling, M.L., Murray, J.A., Mackay, P., Black, S.H., Peutherer, J.F. & Ludlam, C.A. (1983). Incidence of infection with hepatitis B virus in 56 patients with haemophilia A 1971-1979. *Journal of Clinical Pathology*, **36**, 577-580.
- UK Haemophilia Centre Directors (1988) Effect of dry-heating of coagulation factor concentrates at 80°C for 72 hours on transmission of non-A, non-B hepatitis: Study Group of the U.K. Haemophilia Centre Directors on Surveillance of Virus Transmission by Concentrates. *Lancet*, **ii**, 814-816.
- Watson, H.G., Ludlam, C.A., Rebus, S., Zhang, L.Q., Peutherer, J.F. & Simmonds, P. (1992) Use of several second generation serological assays to determine the true prevalence of hepatitis C virus infection in haemophiliacs treated with non-virus inactivated factor VIII and IX concentrates. *British Journal of Haematology*, **80**, 514-518.