HT – HIV

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U.K. NBTS/NIBSC TECHNICAL AND SCIENTIFIC WORKING GROUP ON VIRAL CONTAMINATION OF BLOOD PRODUCTS

Report of a meeting held at the National Institute for Biological Standards and Control, March 30th 1988

In opening the meeting, Dr. G.C. Schild, Director, NIBSC, welcomed the participants and noted the wide and varied group of attendees at the meeting. He stressed the importance of contamination of blood and blood products by retroviruses as a health issue. One of the main reasons for calling this meeting was the confusion that existed over the variety of different methods manufacturers were employing to remove or inactivate contaminating viruses. Dr. D.P. Thomas (NIBSC) then reviewed the background for the withdrawal of Factor VIII HT in the U.K. and Canada. There had now been anecdotal evidence about 18 patients who had seroconverted after receiving heat-treated Factor VIII, mostly at 60° for 30 hours. There was also growing concern over viruses other than HIV1 and hepatitis. The meeting had been organised at the request of the DHSS, and all manufacturers who sell or supply blood products in the U.K. had been invited to participate. Manufacturers had been asked to report on their laboratory studies and to outline the epidemiological evidence on the safety of their product.

Dr. C. Heldebrant (Alpha Therapeutic) reviewed the clinical studies performed using the so-called wet treatment (N-heptane slurry, heated 60°C for 24 hours). With this product they had found that, in 55 patients (33 naive), 13% developed non-A, non-B hepatitis with no patients developing anti-HIV. In vitro studies of viral inactivation with Profilate subjected to dry heat demonstrated that 4 logs of HIV were inactivated in 2 hours. The product treatment time is in fact 24 hours,

so the assumption is that all virus is inactivated. They nowadays use a P24 antigen assay using the Abbott kit, instead of reverse transcriptase assays. He noted that the virus appeared relatively fragile, but wondered whether laboratory staff should be asked to work with high titres of HIV when other safer model viruses are available. He pointed out that the emphasis for the demonstration of inactivation with high log titres creates problems - there were no standard inocula and many defective particles. During questions, the suggestion was put that fresh lymphocytes should be added to the culture to obtain high titres of HIV.

Dr. M. Rodell (Armour) reviewed the production process of Monoclate and presented data on HIV inactivation in vitro by heat treatment with two of their older products. He said that stabilisers or high protein levels (used in earlier products) can protect viruses from dry heat. In model virus experiments with Sindbis, VSV and HIV, all showed cumulative loss when a monoclonal antibody column was employed for purification of Factor VIII. In vaccinia virus spiking, a 4 log reduction was obtained on the monoclonal antibody column alone. Greater than 7 logs removal was obtained with Sindbis virus on two columns, followed by lyophilisation and heat treatment. He reported that over 700 patients had been treated with their product, Monoclate. In 26 naive patients treated with 9 lots of Monoclate for 6-19 months, none had developed NANB hepatitis, although two patients had developed antibodies to Factor VIII:C.

Dr. J.K. Smith (PFC, Oxford) reviewed the epidemiological data in patients receiving the BPL products 8Y and 9A, which were treated to 80° for 72 hours. In 32 patients, followed in the recommended fashion with liver function tests, none had developed NANB hepatitis. Using the rule of three, this meant that the 95% confidence limits allowed a possibility of a 0-9% incidence of NANB hepatitis. Whether the product is truly safe

will only be determined following the larger trial that is currently in progress. In relation to anti-HIV, no seroconversions had been reported after 8Y or 9A in some 1500 patients treated with 150 batches of product.

Dr. B. Cuthbertson (Edinburgh PFC) reviewed the effect of manufacturing processes on virus viability and reported that the cryoprecipitation step produces approximately a 1 log loss of HIV, adsorption produces 1 log loss, and freeze-drying a further 1.2 logs loss of the virus. Preliminary data from the Scottish National Blood Transfusion Service show that 2 hours' treatment at 68°C causes a 3 log loss of HIV, but 24 hours' are needed to lose 4 logs. In 2 batches known to contain HIV-infected donations, in one case the batch was treated for 2 hours at 68° and there were 0 out of 6 seroconversions. In the other batch, treated at 68°C for 24 hours, there were 0 out of 7 seroconversions. He described the proposed specification for validation of virus inactivation procedures that were being considered by the U.K. BTS/NIBSC Working Party. The following recommendations were likely to be made:- 1) One single step in the process should inactivate at least 5 logs of virus; 2) Data should be generated on two model viruses; 3) Virus inactivation experiments must simulate full-scale production; 4) Virus replication (culture tests) must be used; 5) Virus replication must be validated in the presence of coagulation factors; 6) Any process modification must be revalidated.

Professor N. Heimburger (Behringwerke) reviewed the production of Haemate HS, which is heated in the wet state for 10 hours with stabilisers. In this process, the total elimination of HBsAg during manufacture is 3.5×10^6. His data showed that, at 60° in solution 6 logs of HIV were inactivated in 10 minutes. However, HIV inactivation in Factor VIII products was greater than 5 logs in 1 hour. He discussed the study carried out by Schimpf et al., published last year in the New England

Journal of Medicine (316: 918-22), in which 26 naive patients with Factor VIII deficiency and von Willebrand's disease were treated with wet heat treated Factor VIII. In this prospective study, no patients developed NANB hepatitis, hepatitis B or seroconverted for HIV. In a large retrospective study of Haemate HS, no cases of anti-HIV seroconversion were observed. In response to a question, he said that the loss of Factor VIII on pasteurisation was less than 40%.

Dr. E. Greene (Cutter Labs.) reported studies on dry heat treatment of Factor VIII at 68° for 96 hours. In model virus studies, using a xenotrophic C mouse retrovirus at a titre of 10^8, no virus was detectable after 96 hours of heat treatment. In a study of HIV, at 60°C wet heat, there was rapid inactivation at the rate of 1 log of virus per 24 seconds. In contrast, at the same temperature by dry heat, the inactivation was 1 log per 32 minutes. The current Cutter product was wet heat treated at 60° for 10 hours, which was believed to inactivate any HIV present.

Dr. Piszkiewicz (Baxter/Travenol) commented that slowing off of inactivation with time may be due to heterogeneity of virus particles. He had studied the inactivation of HIV in Baxter/Travenol products and also mentioned the difficulty of using very high titre isolates. He questioned the validity of summating separate inactivation rates. When AT III was pasteurised at 60°, all HIV (10^6 logs) had been inactivated in 5 minutes, even in the presence of a citrate stabiliser. With their new product (Hemofil M) produced through monoclonal antibody-anti-Factor VIII:C purification, inactivation of HIV had been studied in the cryoprecipitate with added detergent (TNBP/Triton). They had found that all HIV (greater than 10^4) had been inactivated in 1 minute. They then loaded the cryoprecipitate on to an affinity column and used model viruses to

study column elution (Sindbis, EMC). On washing, the infectivity dropped by 4-5 logs. He showed that 0.3% TNBP plus Triton X-100 inactivated greater than 10^4 virus in seconds. Immunoaffinity chromatography also produced a reduction of virus titre by 10^4 logs. Their clinical results to date showed that no seroconversions had taken place and no patients had developed NANB hepatitis following treatment with their new product, Hemofil M.

Dr. J. Eibl (Immuno) described their process of steam inactivation of viruses and considered the theoretical and practical factors influencing a required total virus kill. Mrs. Kunshak (Immuno) presented clinical data using Factor VIII, prepared by Immuno, which had been vapour treated at 60°C for 10 hours at a pressure of 1190 mbar. She reported on 4 studies using the usual eligibility criteria and, overall, there had been 0/97 seroconversions for anti-HIV, 0/65 cases of NANB hepatitis (as determined by ALT) and 4/37 seroconversions for HBV. By using the rule of three, it had been calculated that the maximum risk of NANB was 5%, a maximum of 3% for HIV and a "significant reduction compared with historical controls" for HBV. On questioning, she expressed the view that the seroconversions for HBV had not been due to the product and were related to the fact that the study had been carried out in an area of Southern Italy where hepatitis B was endemic. When the product was put into chimpanzees, they did not show seroconversion for HBV.

Dr. B. Habibi (CNTS, Paris) commented that, for the past year, the CNTS had adopted the New York Blood Center method for viral inactivation in clotting factor concentrates. He mentioned that there had been 3073 cases of AIDS in France so far, including 34 haemophiliacs. In the latest French prospective trial, with solvent detergent treated Factor VIII in 17 patients, 10 of whom were naive and all had been HBV vaccina-

ted, there were no seroconversions for anti-HIV or NANB hepatitis. He reviewed four studies carried out world-wide with the solvent-detergent treated Factor VIII, in which some 2 million units had been administered to patients; there were no cases of seroconversion for anti-HIV in 54 patients, no cases of NANB hepatitis in 40 patients and no cases of hepatitis B in 10 patients.

Dr. T.W. Barrowcliffe (NIBSC) presented comparative data on Factor VIII concentrates in two systems. Polypeptide analysis according to the method of Weinstein showed that Factor VIII was more degraded in all concentrates than in plasma. Concentrates treated by 'wet heat', whether aqueous or solvent dispersion, tended to be more degraded than dry-heated products, but it was likely that the viral inactivation process was responsible for only part of the degradation. The immunosuppressive effects of concentrates was studied by in vitro incubation with a T lymphocyte cell line and measurement of IL-2, in collaboration with Dr. R. Thorpe (NIBSC). Concentrates differed considerably in their ability to inhibit IL-2 secretion. The most inhibitory products were the 'wet-heated' concentrates, whereas some dry-heated concentrates had virtually no inhibitory effect. Taken together, these results suggest that the use of wet heat for viral inactivation is associated with more degradation of both Factor VIII and non-Factor VIII proteins.

Professor A.L. Bloom (Cardiff) reviewed the factors which influenced the choice of a clinician in selecting a Factor VIII concentrate. This depended on many factors, including the charcteristics of the concentrate, the severity of the defect, the HIV/HBV status of the patient, whether the patient had inhibitors, age, geographical factors, etc. As far as NANB hepatitis transmission was concerned, there was a variety of treatments that had been employed which were unsuccessful, including 60°C

for 30 hours in the dry state and 68° for 72 hours in the dry state. He considered that steam pressure and the use of alpha-heptane slurry was partially successful. As far as the successful published studies were concerned, he considered the Biotest ultraviolet treated products and the Behringwerke Haemate HS wet treated at 60° for 10 hours as safe products. Still under study were the Armour product Monoclate, heated at 60° for 30 hours and prepared on a monoclonal antibody column; the Hemofil M, solvent treated and purified on a monoclonal antibody column; the New York Blood Center process, involving a solvent-detergent; and the NHS product heated at 80° for 72 hours. As far as HIV/HBV status and superinfection, clinicians usually considered it undesirable to continue exposing patients already positive for HIV and/or HBV to more virus, but thought that these patients could be given reasonably safe, reasonably priced products, even if they were not totally secure. If crude concentrates of Factor VIII are immunosuppressive, why was this not noticed during the period 1965-1980? Indeed, are new concentrates really pure? - they all have added albumin. He considered that there may be an advantage in giving Monoclate to HIV-positive patients if the immunological status is thereby improved. He pointed out that geographical factors were highly important. For example, the prevalence of HIV antibodies in blood donors is some 10 times lower in the U.K. than in the U.S. This meant that the 'window' for undetected virus in screened donors was approximately 1 in 40,000 in the U:S.A., but 1 in 400,000 in the U.K. If the average donor pool used for the preparation of concentrates in the U.S. was approximately 20,000, this meant that it was theoretically possible for 50% of lots to be infected. In contrast, in the U.K., where the donor pools were on average only 8,000, it meant that there only 2% of the lots were likely to be infected. This calculation is very relevant to the amount

of data required in the U.K., as opposed to the U.S.A., to establish safety in patients receiving the new treated products. He also reminded the audience that the use of high purity Factor VIII concentrates had very substantial cost implications. For example, in a medium-sized haemophilia centre, switching from existing products costing 16p per unit to a new generation one costing 45p per unit represented an additional £1m per year for that centre alone.

In the general discussion, several points were raised concerning the legal implications of producing a 'less safe' product for patients who were already HIV-positive. Dr. Thomas asked whether the seemingly-low NANB hepatitis incidence in the U.K. is valid. The usual figure quoted in the U.K. is 1 in 200, whereas in the U.S.A. and Canada it is 10% (even with volunteer donors), in Paris it is 10% (Dr. Habibi) and a recent Dutch study had reported an incidence of 2%. Professor Bloom commented that some NANB hepatitis may be overlooked, since patients may not all get noticeably ill. On the question of ALT testing of donors, Dr. Habibi commented that this is already taking place in France. High ALT (x 2 normal) is found in 1.8% of all donations in France; in the U.S., elevated ALT donations are used only in preparation of albumin. Dr. F. Rotblat (DHSS) commented that, on present evidence, no product can be called completely safe from the hazard of NANB hepatitis.

Dr. Thomas, in concluding the meeting, thanked the participants for coming. It had been a very useful and stimulating meeting and he felt sure that participants had become better informed on the problems facing clinicians, manufacturers and control authorities in this difficult field.