RECOMBINANT FACTOR VIII AND VAT

Expert Witness Statement by Dr Trevor W Barrowcliffe, 14.10.96

Qualifications and Experience of Expert Witness

Qualifications: MA (Chemistry), University of Oxford
PhD (Medicine), University of London

Career:

1969-1974;	Research Scientist, Royal Free Hospital
1974-present;	Scientist, then Senior Scientist, then Head of Division of Haematology, National Institute for Biological Standards and Control

Experience: 27 years' experience in field of blood coagulation. Since 1974, responsible for establishment of National and International Standards for FVIII, and for batch release of all FVIII concentrates used in UK. Over 120 papers published, including many on FVIII. Knowledge of production processes used for FVIII concentrate.

Clinical Use of Factor VIII Concentrates

Factor VIII concentrates are used to treat haemophilia A, a congenital bleeding disorder affecting approximately 1 in 10,000 of the population. The cause of the disease is a lack of the clotting protein Factor VIII, an essential component of the complex cascade of blood coagulation. In the absence of FVIII, clot formation in response to vascular injury is either weak or non-existent and haemophilia patients suffer from a succession of bleeding episodes requiring replacement therapy with concentrates of FVIII. Many of the bleeds occur in muscles and joints and insufficient treatment with concentrates can lead to crippling and reduced life expectancy.

Treatment with FVIII concentrates is given in three ways:

1 on demand, to treat a bleed when it occurs;
2 as a planned intensive course when surgery is required;
3 as prophylaxis, ie regular injections 2-3 times per week to prevent the occurrence of bleeds.

The latter form of treatment is being increasingly used in several countries, including the UK, though cost is a major limitation.

The amount of FVIII concentrate used varies considerably among different patients and from country to country. In European countries, the average annual usage ranges from 0.5 to 5.0 International Units (IU) per head of the population. In the UK, the figure is approximately 3 IU per head, ie a total annual use of

approximately 160,000,000 IU, at a cost of over £60,000,000.

The FVIII concentrates currently in clinical use are prepared in two ways: by fractionation and purification from human plasma and by recombinant DNA technology from mammalian cells.

I want about no. of donations + regular donors

Plasma-derived FVIII Concentrates

The source plasma for manufacture of Factor VIII concentrates is collected from Blood Transfusion Centres as whole blood and plasmapheresis centres as plasma, and stored frozen until required. Manufacture takes place in large pharmaceutical units under stringent conditions and plasma from many thousands of donors is pooled to make a single batch. A variety of different manufacturing processes are used, involving various combinations of precipitation, conventional chromatography and immuno-affinity chromatography and the current products fall into 3 groups:

1 intermediate-purity concentrates, produced by conventional fractionation, specific activity less than 10 IU/mg;
2 ion-exchange purified concentrates, specific activity 50-200 IU/mg;
3 monoclonal antibody purified concentrates, specific activity more than 2,000 IU/mg, reduced to around 10 IU/mg after addition of albumin as an excipient. Considering that biochemically pure FVIII has a specific activity of over 4,000 IU/mg, it can be seen that in virtually all plasma derived concentrates, FVIII accounts for less than 10% of the protein in the final product.

A major problem with plasma derived concentrates has been their propensity to transmit blood-borne viruses. It was recognised in the early to mid-1980's that hepatitis B, non-A non-B hepatitis (now called hepatitis C) and HIV were all extensively transmitted. From 1985 all production processes were altered to incorporate a virus inactivation step, either solvent/detergent, wet heat or dry heat and in the last 10 years there have been no recorded instances of HIV transmission from FVIII concentrates and only a few possible instances of hepatitis B and C transmission. Recently, however, hepatitis A transmission has occurred with two products and parvovirus transmission remains a potential concern - the issue of CJD transmissibility has also been raised. Because of these concerns there has been a great deal of interest in the development and clinical use of recombinant FVIII.

Production of Recombinant FVIII

The publication in 1984 of a series of papers describing the biochemical structure of FVIII and its gene sequence allowed the possibility of isolation and preparation of DNA which coded specifically for the FVIII protein. Subsequently several companies developed methods for incorporation of the FVIII gene into suitable cell lines and thus it became possible for the first time to produce FVIII without the use of human plasma as a source material. A considerable number of other biological products are now produced by recombinant techniques, but unlike FVIII most of

these were not previously readily available from human blood or tissues.

Currently two products are licensed for clinical use, one being Recombinate from Baxter. The exact production methods are proprietary to the companies, but a summary of the process for Recombinate, as far as is known to the author, is as follows:

Expression System

The human FVIII gene is incorporated into Chinese Hamster Ovary (CHO) cells via a suitable expression vector, together with the gene for human von Willebrand Factor (vWF). The vWF gene is included because vWF is the natural carrier protein for FVIII in plasma; it protects it from degradation and enhances expression and secretion of the FVIII. It also allows the use of serum-free media for cell culture.

Cell Culture

From the master cell bank containing the appropriate genes, working cell banks are prepared, one of which forms the starting material for each batch. Cell culture takes place in 2,500 litre bioreactors for 3 days, after which the bulk of the contents is removed for purification. The culture medium does not contain human or bovine serum, but a number of animal derived proteins are used during production.

Purification

The main processes used are:

1 filtration, to remove CHO cells and cell debris;
2 immuno-affinity chromatography using a monoclonal antibody specific for FVIII - this eliminates the majority of contaminating proteins;
3 two successive ion-exchange chromatography procedures to remove residual contaminants, including vWF. From a typical harvest of about 7,000 litres culture medium, one litre of highly purified and concentrated FVIII is produced.

Formulation

Following transfer of the bulk purified FVIII from the manufacturing plant at Genetics Institute, the product is formulated, sterile filtered and freeze-dried at Baxter Healthcare Corporation. The formulation buffer contains sodium and calcium salts, histidine, polyethylene glycol and clinical grade albumin, derived from human plasma. The albumin is an important constituent which stabilises the FVIII during freeze-drying and provides the bulk protein necessary to form a satisfactory cake. Because of the high specific activity of FVIII, the amount of FVIII protein in a typical 1,000 IU vial is less than 1 mg, whereas the amount of albumin added is approximately 100 mg. In the

final product, therefore, more than 99% of the total protein is albumin and less than 1% FVIII.

The product is supplied as a dried powder to be reconstituted with water for clinical use, and in the solution for injection the bulk of the material (>95%) is actually water; the albumin constitutes approximately 1% and the FVIII only 0.01%, on a weight for weight basis.

Is Recombinate a Human Blood Product?

The active ingredient of Recombinate, ie recombinant FVIII, is clearly not derived from human blood, since the starting material is Chinese Hamster Ovary cells containing the FVIII gene. Indeed, one of the main purported clinical advantages of recombinant FVIII is its freedom from blood borne viruses; although this supposed advantage is tempered by the inclusion of plasma derived albumin in the final formulation, albumin has never been known to transmit HIV or hepatitis virus during 30 years of clinical use.

From another point of view, Recombinate, the final product, could be considered as a human blood product, albumin, containing a small amount (less than 1%) of a non-blood derived component, FVIII. However in terms of the clinical use of the material and its value, the non-blood part is the essential component; if the non-blood material were omitted the product would have no value for treatment of haemophilia A. If the albumin were omitted from the current formulation the FVIII would probably have very little biological activity because of its very low protein content which would lead to major losses on freeze-drying and storage.

However, Baxter and other Companies are developing alternative formulations not involving use of albumin or other human proteins, though these are not yet licensed for clinical use. Thus although plasma-derived albumin is an essential component in the current formulation of Recombinate, it is not an absolute requirement for the production of recombinant FVIII products.

From the regulatory point of view in Europe, Recombinate is not regarded as a blood product. Biological products such as those derived from human blood are recognised as being subject to greater variability between batches (ie different production runs) than conventional pharmaceuticals. Batch release ie independent testing of every batch for safety and efficacy, has been carried out at NIBSC for all human blood products used in the UK for over 10 years and since 1989 it has been recognised by EU directive number 89/381/EEC that blood products may be subjected to batch release in those member states (now the majority) where this is a requirement. Recombinant products in general are treated in the same way as conventional pharmaceuticals, ie they are not subjected to batch release. The licence for Recombinate in Europe does not include a requirement for batch release, despite the inclusion of human albumin in the product.

Overall, although the majority of the protein in Recombinate is derived from human blood, the active ingredient, recombinant FVIII, which gives the product its clinical

and financial value, is not derived from human blood.

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Prepared by Dr Barrowcliffe and signed in his absence