The Penrose Inquiry

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Introduction of dry heat treatment of factor VIII concentrate

The SNBTS briefing paper (Dr P.Foster, November 2010) [PEN.013.1309] on the development of heat treatment of coagulation factors is a comprehensive and precise description and analysis of the developments in several European countries, in particular Scotland, and in North America concerning this topic during the period of 1981 till 2006. It shows that since 1944 a variety of methods has been developed and studied to destroy or remove blood borne viruses. However, before the 1980s none of such procedures were applied successfully for the manufacturing of plasma products with the exception of albumin. Thereafter, mainly due to the growing concern about the transmission of the agent responsible for non-A/non-B hepatitis (NANB), and later Human Immunodeficiency Virus (HIV), more intense research efforts about chemical and physical (heating/irradiation) inactivation of viruses were reported.

Of the methods using heat to inactivate viruses, pasteurization and dry heat treatment were considered to be very promising. The pasteurization of albumin is the classical example of successful virus inactivation of a plasma derived product. Several pharmaceutical companies tried to use one of these heating methods for virus inactivation of factor VIII concentrates. However, initially such products were not widely used for the treatment of haemophilia patients because the evidence of effectiveness and safety was very limited or not convincing and also because the yield was very low.

When PFC/SNBTS (hereafter called PFC) started to work on the pasteurization of factor VIII concentrate, it was not clear as to which virus inactivation method would be the most promising. Since the beginning of the eighties PFC has been actively involved in pursuing the technology of heat inactivation of several blood born viruses. As can be seen from table 3 of the Foster report, albumin pasteurisation, with which long experience existed within PFC, was used as a benchmark.

In 1981, following an oral report of investigations in Germany, PFC started to explore the pasteurisation of (liquid) factor VIII by a method which was subsequently described in the scientific literature (Heimburger et al (1982)(Reference: Blut 1982; 44(4); 249-251). At that time manufacturers of clotting factor concentrates (including PFC) which aimed to include virus inactivation in the manufacturing process, had to address various challenges: increasing the stability of factor VIII and thereby its yield, get access to marker viruses and experimental animals, establish the degree of virus inactivation, and avoid that structural abnormalities of factor VIII occur which could cause inhibitor formation. Some of these issues required the study of variables in the process of manufacturing and virus inactivation. Other problems like access to marker viruses could only be addressed when the nature and characteristics of the viruses were known.

Initially PFC performed a number of laboratory studies with the aim to increase the stability of factor VIII (the yield). Thereafter in 1983 the clinical efficacy and tolerability of a pasteurised factor VIII-concentrate were studied. In total 11 pilot preparations were produced. It appeared that one out of 3 haemophilia patients suffered from an adverse reaction (probably due to factor VIII antibodies) following the use of pasteurized factor VIII concentrate manufactured by PFC. This serious side effect was one of the reasons for the decision by PFC to stop in 1984 the distribution because the pasteurized product appeared not acceptable for clinical use.

During the period from 1981 till 1983, when PFC was preparing pilot batches of pasteurized factor VIII, the commercial industry started the marketing of heated (Baxter, Behring) or chemically (Biotest) virus inactivated factor VIII-concentrate. The Baxter product was said to carry less risk of transmitting non-A/non-B hepatitis. Details of the method of heating and the yield of factor VIII were however not made available by Baxter and the published clinical results and safety data of this product were very limited. It took till July 1983 before PFC was unofficially told by a representative from another industry (Armour) that dry heating was used for some commercial factor VIII concentrates but again further details about the method were not provided (Poster presentation from Armour on testing of heat treated factorVIII in chimpanzees, Congress of World Federation of Haemophilia, Stockholm 1983).

In October 1984, before starting routine production of pasteurized factor VIII concentrate several developments led PFC to change its strategy for virus inactivation. At the time it was known that AIDS is caused by a virus called HIV (Human Immunodeficiency Virus), that this virus (HIV) is heat sensitive and preliminary data indicated that dry heating may inactivate the virus. When it became known that a number of haemophilia patients exclusively treated with (non-heated) factor VIII concentrate from PFC had been exposed to HIV and showed antibodies to HIV, PFC decided to change from pasteurization to dry heat treatment of factor VIII assuming that HIV might be more susceptible for this virus inactivation method (than the agent responsible for NANB hepatitis). It was decided to use 68˚C as heating temperature, which was also used by other manufacturers, and 2 hours heating i.e. twice as long as the period necessary to inactivate HIV. Later it was shown that when sucrose was added to the final product, the heating process could be increased to 24 hours.

In December 1984, three months after the start of the manufacturing, the first dry-heated factor VIII concentrate prepared by PFC was issued for clinical evaluation and one week later distribution started for routine use in haemophilia treatment. All non-heated factor VIII concentrate was recalled.

In table 1 of the Foster report data are shown about the introduction of heat-treated factor VIII concentrate in different European countries, USA, Canada and Japan. This table can be completed using data from three neighbouring countries, Ireland, Belgium and the Netherlands.
Ireland changed in 1985 to imported heat-treated factor VIII (Pages 66 and 67 of the Report of the Tribunal of Inquiry into the infection with HIV and hepatitis C of persons with haemophilia and related matters. September 2002 – Lindsay Tribunal).
In Belgium dry heated lyophilized cryoprecipitate was introduced in 1986 (ref. J.Vermijlen & E.Briet, Lancet 342: 693 – 694, 1993). Only several years later dry heated factor VIII concentrate prepared from Belgian plasma became available.

In the Netherlands the national fractionation centre (CLB, now called Sanquin) signed a technology agreement and patent license agreement with Baxter Travenol (USA) in October 1984. In June 1985 CLB introduced dry-heat-treated factor VIII concentrate when the regulatory authorities had licensed the product. As glucose had not been added to batches of factor VIII concentrate since 1984, all stocks of factor VIII concentrate produced since that time and also distributed factor VIII concentrate could still be heated.

These data confirm, as is stated at page 8 of the PFC report, that this enabled " ....Scotland to be the first country in the world to provide all patients with factor VIII concentrate safe from transmission of HIV". It should be taken into account that in some countries commercial (dry) heat treated factor VIII concentrate was imported and used for the treatment of some (severe) patients before mid 1984 although the evidence that some of those concentrates were safe from transmission of HIV became available only later.

With regard to inactivation of NANB hepatitis (Hepatitis C), the first commercial heated clotting factor VIII concentrate (Baxter, Hemofil T) became licensed in the USA in March 1983 and subsequently in several European countries. Heat treatment was applied to prevent the transmission of NANB hepatitis and hepatitis B. The effectivity of virus inactivation (heating at 60°C for 72 hours) of freeze dried factor VIII concentrate had been studied in chimpanzees. Heated factor VIII concentrate to which known infectious material was added before the heating process did not cause NANB hepatitis following injection in the animals. Thereafter, 4 other dry or wet heated products, heated at 60 to 68 °C for 10 to 72 hours were licensed by the Food and Drug Administration (FDA) in the USA (see para 7.42 of the Penrose Inquiry Preliminary Report). However, the former mentioned positive results of animal testing were not confirmed when higher infectious doses were used. In addition a number of clinical studies in various countries did not substantiate the initial positive animal results. For instance in the study by Colombo et al ("Transmission of Non-,non-B hepatitis by heat-treated factor VIII concentrate, the Lancet, page 1-3, July 6,1985) [LIT.001.0369] 84% of 13 haemophilia patients given Hemofil T, developed NANB hepatitis during the 12 months following the administration of heat-treated factor VIII. Only when later dry heat treatment of factor VIII for 72 hours at 80°C (8Y HT; produced by BPL) was used no transmission of NANB hepatitis did occur, as was published in 1988.

PFC was not the first to introduce dry heating of factor VIII concentrate to prevent NANB hepatitis. If in 1984 PFC would have used the same heating conditions for factor VIII concentrate which it used to inactivate HIV, i.e. 68°C for 2 hours, for the inactivation of the agent responsible for NANB hepatitis (HCV) this would have been inadequate. As is clear from its briefing report (Dr.P.Foster), PFC was aware of this.

Should/could PFC have moved more quickly (e.g. in early 1984) to introduce the dry heat treatment of factor VIII?

According to the Guidelines of the World Health Organisation ("Guidelines on viral inactivation and removal procedures intended to assure the viral safety of human blood and plasma products". WHO Technical Report, Series No. 924, Annex 4 2004, http://www.who.int/bloodproducts/publications/WHO_TRS_924_A4.pdf) the ability of a process to inactivate/ remove viruses should take into account:
- the reduction of virus titre achieved;
- for inactivation processes, the rate of inactivation;

- the robustness of the inactivation step in response to changes in process conditions;
- the selectivity of the process for viruses of different classes;
- validation studies which need to be well documented.

Therefore, the safe introduction of a significant change (like virus inactivation) in the manufacturing process of plasma products such as factor VIII requires knowledge about:

- I. The nature and characteristics of virus (es) to be inactivated.

A. With regard to HIV.

The first indication about the cause of AIDS was presented in May 1984 when Gallo et al published that HTLV III virus was present in 18 out of 21 patients with so-called pre-AIDS and in 26 out of 72 patients with overt AIDS [LIT.001.3769]. Earlier, in May 1983, Barre-Sinoussi et al of the group of Montagnier published in Science the isolation of a new retrovirus from a lymph node of a homosexual patient with multiple lymphadenopathy (thought to be an early stage of AIDS) [LIT.001.0058]. The authors concluded that "the role of this virus on the aetiology of AIDS remains to be determined". The findings by Gallo and Barre-Sinoussi were discussed in an editorial article in the Lancet (12/05/84) [PEN.012.1529] in which it was stated that: "Of course the finding that a rather unusual virus is present more often in AIDS patients than in healthy controls is no proof of causality; it is possible that these viruses are simply passengers yet another opportunist infection to which those patients are susceptible. Nevertheless, their independent observation in two laboratories, the fact that they have been identified in many of the risk groups (and only rarely in controls), and longitudinal observations suggesting that sero-conversion may precede clinical illness will all, if confirmed, lend credence to our prejudice that viruses such as these are likely to be the guilty party".

Thus, in May 1984 it was likely, but not yet definitive, that AIDS is caused by a retrovirus. The characteristics of such a virus (such as heat sensitivity) were still unknown. Consequently, if heat treatment for inactivation of HIV would have been introduced in early or mid 1984 (or earlier) it would not have been based on evidence but rather on speculations about the origin of the virus.

B. With regard to HCV.

Post-transfusion non-A/non-B hepatitis (NANB hepatitis) has been recognized as a clinical entity long before the discovery of the hepatitis C virus (HCV) as its major causative agent. In the eighties, pooled plasma from chimpanzees with NANB hepatitis was used by Houghton et al to recover nucleic acids and to subsequently clone the genome of the etiologic agent into a bacteriophage vector. The cDNA clones derived from this plasma were expressed in bacteria (Escherichia coli). This cDNA library was then screened for clones expressing viral antigen. Eventually in 1989, a clone, named 5-1-1, was found that encoded for antigen which bound circulating antibodies present in serum from several NANB hepatitis patients. In subsequent years the complete sequence of the HCV RNA genome has been determined which made it possible to define that HCV belongs to the *Flaviviridae* family, an observation which is relevant to define which viruses can be used as model viruses for HCV (e.g. bovine diarrhoea virus).

Following the cloning of parts of the HCV genome, the first generation of anti-HCV screening tests allowed the routine screening of blood donations. Before screening tests for HCV became available the evidence of freedom of transmission by factor VIII (and other clotting factors) depended on animal (chimpanzees) experiments which were very

costly, time consuming, requiring infectious material with known viral potency, and because the number of animals was very limited the outcome was sometimes not reliable (see for instance Hemofil T). Clinical studies were therefore required before a definitive result (inactivation or no complete inactivation) could be presented (see 8 Y HT of BPL).

II. Access to sufficient quantities of HIV and HCV producing cell lines to perform so-called validation studies (spiking experiments) in the laboratory and studies in experimental animals.

The inactivation of viruses in products such as factorVIII concentrate can be investigated by adding a known quantity of the relevant virus to the product and submit this to the inactivation method (i.c. heating). This method is called virus spiking. Next, the residual amount of virus is measured and by comparing this with the originally added quantity the reduction of virus particles is calculated. The virus material used for the spiking experiments is produced (magnified) by cell lines which have been infected with it.

A. With regard to HIV.
When in May 1984 Gallo et al published their findings about HTLV III (later called HIV), it took some time before sufficient virus material was made available to other parties. In Amsterdam it took till July 1984 before spiking experiments with HIV derived from cell lines could start. It is possible that laboratories in some other countries began somewhat earlier (for instance at mid 1984) but much earlier is unlikely.
Using virus spiking it was possible to establish that HIV is sensitive to heat and that dry heating at 60°C for 2 hours completely destroys the added quantity of HIV.
As the virus material (HIV) for validation studies became only available in mid 1984, it was not possible for PFC to do such studies earlier.

B. With regard to HCV.
As is described above it took till 1989 before the first part of the genomic structure of HCV was published and during the following years the complete genomic structure of HCV was elucidated. Although HCV itself has not been not isolated (and thus not available for spiking experiments like described for HIV), it is possible to use so-called model viruses which resemble viruses that may be present (like HCV) in the starting material (plasma) and which share many properties including a lipid membrane, an RNA-genome and a particle size of 40 – 50 nm. The models for HCV include Bovine vial diarrhoea virus (BVDV), Sindbis virus and Yellow fever virus.
The information about the origin of HCV and its model viruses became only available after 1989 and before that time validation of HCV inactivation was dependent on animal experiments (studies in chimpanzees). As is described in the PFC report, there are several limitations (logistical, economic and ethical) to the use of chimpanzees for the validation of virus inactivation methods. In addition the poor reliability of HCV-inactivation testing using chimpanzee experiments was shown when some of the earlier heat treated products were apparently safe in chimpanzees but when such products subsequently were used in humans HCV-transmission was observed.
As with HIV, the virus material for validation studies was not available in 1984 (or before) and only when the genome of the virus was determined, it was possible to decide which viruses resembled and could be used as model viruses for HCV.

III. Yield of factor VIII and structural protein changes.

Manufacturing consistency and integrity of the final product with regard to protein function and structure must be demonstrated when viral inactivation is included in the process. This holds particularly for products like coagulation factors which are labile and can undergo denaturation with loss of biological activity. Several analytical tests are applied for process samples and final product. These tests include total protein, one or more functional assays for the protein of interest (coagulant activity) and the assessment of fragmentation and/or aggregation.
Neoimmunogenicity is a special case of structural changes which may result in an immune response in recipients. There are a few documented instances in the literature where (heat)treated products had unexpected immunogenicity and had to be withdrawn from the market. PFC reported that one recipient of its pasteurized factor VIII concentrate developed adverse reactions which were attributed to antibodies to factor VIII.
The detection of neoimmunigenicity before the product is used, is very difficult. Animal neoimmunogenicity studies are not generally required. The best proof of absence of neoantigens is derived from careful clinical studies.

Conclusion

The answer to the question "Should/could PFC have moved more quickly to introduce the dry heating of factor VIII concentrate?" is NO for the following reasons:
- the procedure (the proper conditions) to inactivate blood borne viruses, in particular those present in plasma, by dry heating were not known until the later part of 1984;
- the characteristics of the viruses to be inactivated (HIV and HCV) were not known until the beginning of 1984 (HIV) respectively 1989 (HCV);
- cell lines producing sufficient quantities of HIV and HCV to perform validation studies (virus spiking experiments) in the laboratory were not available until mid 1984;
- methods to improve the yield of factor VIII and to determine that the structure of factor VIII (or other clotting factors) after heating is still intact were not yet available.

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Date: 20 July 2011