2010/00039

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Re: Plasma Protein Fraction lot 1194/1983

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I was instructed by the Procurator Fiscal at Glasgow to provide a report on "*the cause of death of a man, who died in 2004. He had 2 failed liver transplants, and cause of death was 1(a) Liver transplant graft failure, (b) Recurrent Hepatitis C. He had been a patient in the Royal Infirmary of Edinburgh, received his liver transplant there, and died there.*" and my terms of reference were :" *to ascertain whether or not there is any risk of error in the tests which have been applied to the blood transfusion and if so the size of that risk.*"

Methodology

I was given copy of the documentation of the release documentation of PPF lot 1194 from Scottish Protein Fractionation Centre, which was the product transfused to the patient according to the information received.

Examination and findings

General considerations

In 1983, Hepatitis B virus (HBV) was relatively well known and a blood donation screening assay was in use since 1971-1972. HIV was discovered that year (1983) and was characterised during the following two years. HCV was unknown since it was discovered in 1989 but, in 1983, clinicians were aware of the existence of non-A, non-B hepatitis suspected of being caused by a viral agent. The first evidence that various treatments of plasma derivatives could prevent non-A, non-B hepatitis became available in 1983-1987 (1).

Viral inactivation in 1983

In 1983, viral inactivation was not generally applied to plasma derivatives because it was considered that immunoglobulins were anti-viral and preparations of clotting factors (Factor VIII as cryoprecipitate, concentrates of

intermediate and higher purity as well as concentrates of Vit K-dependent factors II, VII, IX and X) were not submitted to such treatment because of evidence that clotting activity was decreased by heat and acidity. Only albumin-containing plasma fractions were submitted to heat-treatment to avoid infection with HBV. This was considered useful because industrial plasma fractionation required individual plasma pooling in lots of 100 to several 1000 of litres assembling 500 to 20,000 individual donations. As a result, despite the relatively low prevalence of HBV chronic infection among Scottish blood donors (<1%), it was considered justified to submit a stable protein preparation such as albumin (>95% of pure albumin) or PPF (85-90% pure albumin) to heat without damaging the protein content in quantity or in quality. It was known however that heat-treatment could cause protein aggregation and that aggregates could cause side effects in the recipients. This is why electrophoresis of the PPF after heat-treatment was performed in order to verify that the proportion of aggregates was minimal. This assay was also used to determine the proportion of monomeric (native) albumin.

As to heat treatment itself, it was known at that time that heating a liquid between 56°C and 60°C for 24 and 10 hours, respectively would inactivate HBV. In the case of the Scottish PPF, the schedule chosen was 56°C and 48h. In 1983, there was no report available to indicate the effect of this heat-treatment on the putative agent of non-A, non-B hepatitis. At that time, only injection of plasma derivatives to chimpanzees was available for this type of studies and such rare and highly expensive facilities were only available in the USA.

Examination of the document provided on lot 1194 prepared in May 1983.

The full copy of the lot release documentation is complete and in line with the manufacturing procedures utilised at that time. Close examination of each document reveals that lot 1194 passed all criteria for quality assurance and was therefore suitable for clinical use according to the procedures in use at the time in industrial plasma fractionation.

The document indicates that the rabbit pyrogen test used to avoid fever in reaction to the product was in conformity with the set criteria for this product. This control test was conducted by injecting 6.3 ml of PPF to three rabbits and by measuring the body temperature for 24 hours. In this case, the temperature remained stable (≤0.1°C) indicating a good tolerance of the product by these animals.

The rabbit pyrogen test was supplemented by another method to detect pyrogens called the limulus test. This test takes advantage of the ability of the lymph of the limulus crab to clot in the presence of bacterial pyrogen. This test was negative.

The toxicity tests were conducted in two guinea pigs and five mice injected respectively with 5 and 0.5ml of PPF lot 1194. Toxicity was monitored by weighing the animals daily for 4 days and on day 7; any loss of weight being

taken as an indication of toxicity. As, expected in the absence of toxicity, all animals tended to slightly increase weight.

Bacterial sterility of the product was insured by Millipore filtration that retains all bacteria. However such sterility was controlled by culture in two different media (thioglycolate and tryptone soya broth) for two weeks. In both media no bacterial colony was observed indicating sterility of the product.

Under the sheet entitled 'Biological indicators', it is mentioned that the heat-treatment of two lots (1194 and 1195) was conducted together which was not unusual considering that each lot was from a relatively small plasma pool; lot 1194 included 468 vials of finished product (plus 2 for library). It is indicated on page 4 of the documents that the HBV surface antigen (HBsAg) was negative. It is of note that this assay was performed outside the Protein Fractionation Centre at the Royal Infirmary in Edinburgh. The control of the heat-treatment is recorded as 'positive', which in my understanding indicates that the batch was effectively submitted to the 48 hours of incubation probably in a heated liquid cabinet. In three sheets, it is indicated that the probes of heat-treatment were positioned in different areas of the incubator and all were 'positive', suggesting that the temperature reached by each vial was consistent with the intended 56°C. The temperatures recorded by these probes ranged between 54 and 62°C.

Conclusions

As the result of the above observations made from copies of the lot release data provided, it can be concluded that the safety procedures applied to PPF lot 1194 were in line with the standards of the time. The safety procedure applied for inactivation of HBV (48h at 56°C) was considered effective. At the time, no evidence relative to the inactivation of the non-A, non-B hepatitis putative agent was available.

The available results of the safety procedures conducted on this PPF lot 1194: % of aggregated albumin, pyrogen tests in animals and limulus test, toxicity in two animal species, bacterial sterility and evidence that the heat treatment was indeed applied to the PPF lot, were provided and in conformity with set criteria for lot release.

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Date 23 October 2008

References

Mannucci PM. AIDS, hepatitis and haemophilia in the 1980s: memoirs of an insider. J Thromb Haemostasis 2003; 1: 2065.