INFECTED BLOOD INQUIRY

BRENDON GRAY WITNESS STATEMENT

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Factor VIII concentrate which is safe with respect to hepatitis

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Introduction

Highly purified factor VIII (F-VIII) concentrates for the therapy of hemophilia have been available for several years [1, 2]; their favorable tolerance permits in-home therapy. The quality of life of hemophilia patients could be significantly increased with these preparations. In spite of their highly purified form, the risk for transmission of hepatitis has remained unchangeably high. There existed therefore an urgent need, to either eliminate and/or inactivate hepatitis viruses (HV) from/in F-VIII concentrates. There are two methods for killing HV in plasma or fractions: cold-sterilization through treatment with β-propiolactone [3] and ultraviolet irradiation and heat-sterilization in solution (10 hours at 60°C) in the presence of protein-specific stabilizers, which is, for example, already used for albumin [4]. For the production of a hepatitis-proof F-VIII

preparation, we chose the heat-stenlization analogous to the albumin process. The description of the process and the demonstration of the HV-inactivation are the subject of this study.

Materials and Methods

Human cryoprecipitate, prepared from the citrate plasma of 150 selected donors, whose plasma was free of hepatitis B (HB) surface antigen (HBsAg) and HBs-/HB-core-antibody (HBsAb/HBcAb).

Infectious hepatitis B-virus (HBV)-containing serum with an infectious titer of $10^{7.5}$ $CHID^{1)}_{50}$/ml was obtained from the Bureau of Biologicals, Bethesda, USA.

Reagents and analytical methods for the determination of F-VIII, F-VIII contaminating proteins and hepatitis-specific parameters have been described previously [5].

Results

a) Procedure

Highly purified and in solution heated F-VIII concentrate is prepared from pooled cryoprecipitate in 5 steps:

1) $Al(OH)_3$-adsorption,
2) fractionated precipitation with glycine,
3) precipitation of F-VIII from the glycine supernatant with NaCl,
4) heating of the dissolved F-VIII preparation after addition of glycine and saccharose as stabilizers (10 hours at 60°C), and
5) Reprecipitation of F-VIII from the stabilizer solution with NaCl.

The resulting F-VIII concentrate contains approximately 6 I.U. F-VIII:C/mg protein and is free of coagulable proteins and immunoglobulins.

b) Animal Experiments

In order to test the safety of the process with respect to hepatitis, F-VIII concentrates were prepared from a cryoprecipitate free of hepatitis marker, which was subsequently infected with HBV-containing serum. The infectious titer of this solution of cryoprecipitate was $10^{4.5}$

1) 1 chimpanzee infectious dose is that amount of HBV, which produces an infectious hepatitis in 50% of all inoculated animals

$CHID_{50}$/ml. The infected cryoprecipitate was processed into F-VIII concentrate according to the above procedure and an aliquot of the concentrate was heated. Four chimpanzees each were inoculated with the final product from both procedures; each of the animals received 1 ml F-VIII concentrate, heated or unheated, in an applied concentration of 25 I.U. F-VIII/ml. Only those animals were included in the experiment, which remained free of HBV-markers during a 6-months quarantine and showed no symptoms of a hepatitis upon liver biopsies. The animals were monitored for 9 months after inoculation of the preparations. Blood samples were removed weekly and examined for HBV-markers; liver biopsies were performed every 2 weeks. The results are summarized in Table 1: in all control animals, which had received unheated F-VIII concentrate, the HBsAg-, HBsAb- and HBcAb-titer increased and reached a maximum after 12 to 16 weeks; transaminases were significantly increased in 2 chimpanzees. The experimental animals, which received heated F-VIII concentrate, remained free of these symptoms. In agreement with this finding, the liver biopsies showed hepatitis-typical tissue alterations only in the control animals which had been treated with unheated concentrate. None of the animals treated with heated F-VIII showed any symptoms typical of hepatitis B or non-A/non-B hepatitis.

Table 1. Monitoring of the chimpanzees after inoculation with heated or unheated F-VIII concentrate: serological findings after incubation for 9 months

animal No:	sex	weight	maximal titer			transaminases
			HBsAg	HBsAb	HBcAb	
		kg	mg/ml	index*	index*	
		not heated (control group)				
1	M	60	880	4.1	5.5	increased
4	F	50	80	4.5	3.0	slightly increased
9	M	50	190	10^5	5.5	increased
17	M	60	110	10^3	3.5	not increased
		heated (experimental animals)				
3	M	50	-	1.1	1.4	not increased
8	F	35	-	1.2	1.1	not increased
10	M	45	-	1.0	1.3	not increased
18	M	50	-	1.2	1.1	not increased

*) positive finding: index > 2.1

c) Clinical Examination

44 patients received 232,000 I.U. F-VIII over a period of 18 months, the concentrate had been obtained from a total of 33,000 individual donations. The anamnesis of 12 of the 44 patients, among them 4 nursing infants, revealed no hepatitis at the beginning of the therapy. They were treated for up to 1 year with heated F-VIII concentrate and serologically monitored. During this period, a total of 172,000 I.U. F-VIII was administered to the patients, who were free of hepatitis, from 15 production lots, which were prepared from 25,000 individual donations. None of the 12 patients developed a hepatitis B. Liver biopsies were not performed and the transmission of a non-A/non-B hepatitis can therefore not be excluded with the same certainty as in the animal experiments; corresponding symptoms did not occur, however.

Discussion

The results show that the described procedure of production of F-VIII yields a concentrate of high purity and that, at the same time, the HV-infectivity is reduced by a factor of $>10^{4.5}$. The process therefore guarantees the production of a F-VIII concentrate that is safe with respect to hepatitis, if it is applied to a RIA-negative starting plasma, because the infectious titer of these plasmas is $\leq 10^{2.5}$ $CHID_{50}$/ml, based on experience [6]. The results of the clinical examination agree with these considerations: the F-VIII concentrates, which were obtained from approximately 25,000 RIA-negative, but possibly infectious individual donations, were safe with respect to hepatitis B after testing in 12 patients.

Summary

A process is described for the production of a F-VIII concentrate, which is safe with respect to hepatitis: a 2.5% solution of pooled cryoprecipitate from human citrate plasma is adsorbed onto $Al(OH)_3$, contaminating proteins are precipitated with glycine, and F-VIII is precipitated with NaCl. The precipitate is heated in aqueous solution with glycine and saccharose as stabilizers (10 hours at 60°C). After removal of the stabilizers, a F-VIII concentrate with a specific activity of approximately 6 units F-VIII:C/mg protein is obtained; it is free of coagulable proteins and immunoglobulins. The separation and inactivation of HBV during the purification and the subsequent heating was determined as follows: 1) dilution of HB-specific antigens after experimental addition of an infectious dose of HBV to a cryoprecipitate, which was free of HBsAg, HBsAb, and HBcAb; 2) inoculation of 4 chimpanzees each with heated and unheated F-VIII concentrate, prepared from infected cryoprecipitate; 3) treatment of 44 patients with heated F-

VIII concentrate from 15 production lots. Unlike the control animals, which all fell ill, the 4 chimpanzees, which had received the heated F-VIII concentrate, remained healthy. Based on laboratory data and the results of biopsies, hepatitis B and non-A/non-B hepatitis could be excluded. None of the 44 patients acquired a hepatitis B. 12 control subjects showed no increase of HBsAg or of the antibodies HBsAb and HBcAb or of transaminases. The process is therefore suitable, to prepare F-VIII concentrate free of hepatitis B and probably also free of non-A/non-B from RIA-negative, but possibly still infectious cryoprecipitate.

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