HEAT INACTIVATION OF VIRUSES IN ANTIHEMOPHILIC FACTOR CONCENTRATES

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Presentation before the XVI International Congress of the World Federation of Hemophilia, Rio de Janeiro, August 24-28, 1984

With the recognition that infectious viruses may be transmitted via blood and blood products, much effort has been expended toward their removal or inactivation. Experimental approaches have included heating, chemical inactivating agents, UV irradiation, solvent extraction of essential lipids, neutralization with specific antibodies, and removal by various fractionation techniques. In all cases, conditions must be selected wherein inactivation can occur without significantly affecting the safety or efficacy of the product in question. This has been accomplished with the fraction V products i.e., albumin and plasma protein fraction where heating at 60° for 10 hours with the inclusion of suitable protein protective agents renders the products apparently free of infectious viruses. Applying these same conditions to plasma components with highly labile biologic activities such as clotting factors causes total loss of activity.

During the past 5-6 years in our laboratories, we have extensively studied and described conditions and protecting agents whereby concentrates of labile plasma activities could

be heated without excessively destroying activity. For example, high concentrations of sugars in combination with various amino acids as stabilizers were found to be satisfactorily protective.

More recently, we defined time and temperature conditions for heating AHF and Factor IX concentrates in their final freeze dried containers which inactivated NANB hepatitis as well as a number of model viruses. After heating, the properties of these products after heating were essentially indistinguishable from those of the pre-heated materials as determined by a number of physical-chemical and biological criteria; and they exhibited comparable efficacy when administered to hemophilic recipients. In keeping with the theme of this symposium, I will discuss the virus inactivation data generated by these heating conditions. In all cases, heating was at 68°C for 72 hours. The first series of slides show the inactivation kinetics of several model viruses heated at 68°.

(SLIDE 1): Here is shown the inactivation of cytomegalovirus which had been added to a Factor VIII solution which was then lyophilized, heated at 68°C, and the virus remaining was titered. CMV, a member of the herpes virus family, is pathogenic for humans. As shown here, from a 0.time level of 2 logs, no virus were detected at the 10 hour sampling point and beyond.

(SLIDE 2): With the herpes simplex virus type 1 also a human pathogen, inactivation occurred relatively quickly albeit we were unable to obtain greater than a 1 log titer at 0 time.

With VSV, an RNA Rhabdovirus shown in the next slide (SLIDE 3), 3.5 logs were totally inactivated by 10 hours.

The next slide (SLIDE 4) shows our inactivation kinetics with Sindbis, an RNA alpha virus which has been a widely used model virus in these kinds of inactivation studies. It is a convenient virus to use because relatively high initial titers can be achieved. As seen here, from 6 logs of virus in the initial sample, there was no detectable virus found in the 72-hour sample. A similar experiment was carried out with feline leukemia virus, a retrovirus which as the name implies, causes cancer in cats as well as a profound immunosuppression.

The data in the next slide (SLIDE 5) show that this virus also is inactivated under the conditions described, although at 72 hours, virus were detectable in the undiluted titration sample. We are grateful to Drs. Bert Dorman and J. F. Hsu of Advanced Genetics Research Institute, Oakland, Calfornia, who kindly performed the FeLV titrations.

Further studies with retroviruses involved determining the distribution of the viruses during purification of our AHF concentrate from plasma and the kinetics of their heat inactivation similar to the experiments just described. Collaborative studies were carried out with Dr. Preston Marx of the University of California at Davis with a type D retrovirus he and his collaborators recently described called Simian AIDS virus, and also with Dr. Jay Levy, University of California, San Francisco with a xenotropic mouse C retrovirus which could be titrated with high precision. The recovery of SAIDS virus during fractionation is shown on the next slide (SLIDE 6). It

is evident that the addition of this virus to human plasma which is then frozen and thawed to prepare cryoprecipitate causes a profound loss of virus; only about 0.03% survives in the cryo. Cryoprecipitate was subsequently spiked to 1.5×10^6 virus particles and even less virus survived the processing to purified AHF; only about 0.013%. When the AHF liquid concentrate was spiked with SAIDS virus, lyophilized, and then subjected to our heating process, the results seen on the next slide (SLIDE 7) were obtained. As seen here, about 1/2 log of virus does not survive lyophilization but then much of the remaining virus appears to withstand the heating process. Nonetheless, if we calculate the total viral reduction from plasma to the final AHF concentrate, it turns out that the cumulative effects of plasma, processing, and heat causes between an 8-9 log reduction of simian AIDS virus. These results differ from those obtained by Dr. Levy using the mouse C retrovirus. As shown in the next slide (SLIDE 8), the mouse C retrovirus was much more resistant to the procedures used in the concentration of AHF. The retrovirus was not affected by mixing with cold plasma and there was only a 10-fold reduction in the titer found in the cryoprecipitate. Further processing to a lyophilized concentrate indicated a loss of about 1000-fold in the titer of infectious virus with about 100-fold loss occurring during lyophilization. Nevertheless, a substantial amount of infectious virus remained in the final pre-heated concentrate. The effect of heating on this retrovirus contained in our lyophilized AHF concentrate is shown in the next slide (SLIDE 9). The infectious virus titer

was reduced substantially in 1 hour although residual virus (about 2 IP/ml) was still present after 48 hours. Virus were detected in 2 out of 3 samples heated for 72 hours which when considering the total volumes, averages to less than 1 IP/ml. No infectious virus was detected in samples heated for 96 hours. These studies suggest that if infectious retrovirus are present in human plasma at titers over 1000 IP/ml, some could survive the process of fractionation to AHF concentrates. Prolonged heating at 68°C for at least 72 hours, would be expected to bring about their inactivation. The effect of this heating process on the infectivity of NANB hepatitis was evaluated in a 4-chimpanzee experimental study. Both our AHF and F. IX concentrates were evaluated. The experiment was designed to demonstrate inactivation of a known quantity of the Hutchinson strain of NANB and an unknown quantity of endogenous NANB. To achieve this, samples of AHF and Factor IX concentrates were heated in lyophilized form at 68° for 72 hours to inactivate endogenous NANB which was most likely present. They were then reconstituted in solution, spiked with Hutchinson NANB, lyophilized, heated again at 68°C for 72 hours, and then inoculated into susceptible chimpanzees at a dose equivalent to 2500 CID (if no inactivation had occurred). One control chimp received AHF concentrate containing this dose level without having been heated. A fourth chimp received the total volume of inoculum which had been heated at 68° 72 hours but not spiked with NANB. The following slides show the results of this experiment (SLIDE 10). This chimp received the spiked and heated AHF. As can be seen, there was no evidence of

hepatitis through 15 weeks as indicated by transaminase levels or abnormal liver histopathology. At week 15, the animal was rechallenged with the same AHF preparation which had not been heated. Positive liver histopathology was recorded 3 weeks post-inoculation and elevated ALT levels peaking at about 11 weeks; clearly, validating that this chimp was susceptible to NANB infection and that heating inactivated the NANB spike. Similar results were obtained with our Factor IX concentrate as seen in the next slide (SLIDE 11). Here again, there was no evidence of hepatitis through the first 15 weeks but after rechallenge with spiked and unheated product, symptoms occurred which were diagnostic for hepatitis.

The next slide (SLIDE 12) shows the results of infusing spiked and unheated AHF to a chimp serving as a separate positive control. The first evidence of hepatitis was observed in liver biopsy examination at 4 weeks post-inoculation. The peak in elevated ALT levels was at 11 weeks, a pattern very comparable to that seen on rechallenging the first animal. The last slide (SLIDE 13) shows the effect of heating on endogenous NANB hepatitis. There was no evidence of hepatitis through 15 weeks. The chimp was rechallenged with non-heated product to verify her susceptibility as well as the presence of an endogenous NANB infectivity.

From this chimpanzee experiment, we conclude that the heating process we have employed i.e., 68°, 72 hours has inactivated a known amount (2500 CID) of at least one type of NANB hepatitis as well as an unknown quantity of endogenous NANB hepatitis.

In conclusion, the data presented here have demonstrated that heating antihemophilic factor concentrates in their lyophilized state at 68°C for 72 hours effects significant viral inactivation. Model viruses such as CMV, HSV-1, VSV, and Sindbis were inactivated under these conditions. Retrovirus infectivity such as FeLv, mouse C retrovirus, and Simian AIDS virus were also found to be inactivated by these heating conditions or a combination of fractionation plus the heating. Furthermore, this heating process has demonstrated the inactivation of a known quantity of NANB hepatitis. The heating process described should effect safer antihemophilic factor concentrates.

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