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SAFETY OF BLOOD DERIVATIVES PASTEURIZED IN THE DRY STATE

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Recognition of the transmission of the etiologic agent of the acquired immune deficiency syndrome (AIDS) by blood derivatives such as F VIII and F IX (1-3), as well as the well known risk of transmission of non-A, non-B hepatitis by such products (4-5), has led regulatory agencies throughout the world to mandate the introduction of sterilization procedures for these products. In the United States the National Hemophilia Foundation Medical and Scientific Advisory Council has recommended that lyophilized heat treated products should be used for the treatment of hemophilia (6). Data on which this recommendation was based have recently been reported (7). The present report presents data which question the efficacy of this procedure.

METHODS

Commercial preparations of F VIII, F IX and anti-thrombin III were contaminated by addition of 1/10th volume of AIDS virus culture supernatant. In all except one experiment HTLV-III_b produced in H9/HTLV III_b cells (8) grown in RPMI 1640 20% fetal calf serum was used as the viral inoculum. One study utilized LAV grown in phytohemagglutinin stimulated lymphocytes grown in RPMI 1640, 10% fetal calf serum, 10% interleukin-2. The LAV inoculum was kindly provided by Dr. J.S. McDougal.

An aliquot of each virus contaminated mixture was rapidly frozen by swirling in alcohol and dry ice. The remainder was distributed into lyophilization vials using commercial fill volumes and vial sizes. The vials were shell frozen by swirling in alcohol/dry ice and lyophilized for 48 hours with shelf heating to 80°F for the last 24 hours. Vials were stoppered under vacuum with a stoppering device. The moisture content of samples varied from 0.83 to 1.5%.

Vials were heated for different periods of time at 60°C by complete immersion in a water bath.

For assay vials were rehydrated with sterile distilled water. Frozen samples were rapidly thawed by swirling in a 37°C water bath. Assays were carried out as described (9) except that titrations were carried out in 24 well plates or in 96 well microtiter plates in RPMI 1640 medium, 20% fetal calf serum, 2 μg/ml polybrene, but without anti- interferon. 30% of the medium used for setting up titrations was "conditioned" supernatant from H-9 cultures. After dilution of samples in this infection medium, H-9 cells were added to a concentration of 8×10^5/ml. Cultures were fed twice weekly and supernatants were harvested for reverse transcriptase assay (9) at 14 days. To increase the sensitivity of detection of small amounts of residual virus in heated samples macrocultures were set up with 5-10 ml of sample added to an equal volume of cells at 8×10^6/ml. After 1 hour for adsorption at 37°C the cultures were fed to bring the cell concentration to 8×10^5/ml. Macrocultures were followed with weekly tests for reverse transcriptase for 4 weeks since control titration experiments revealed that this duration of followup was required to achieve optimal titration endpoints.

RESULTS

Table 1 shows detailed results in a study designed to determine the effect of heating at 60°C on HTLV-III in a lyophilized low purity Factor VIII preparation. Lyophilization alone resulted in a 0.5 Log_{10} drop in titer. No further loss in titer was seen after 10 hours of heating. A 1 Log_{10} drop in titer was observed after 30 and 48 hours of heating. After 72 hours at 60°C 2 vials showed a 2.5 Log_{10} reduction in titer one vial showed >2.5 and <4.5 Log_{10}.

Table 2 summarizes the effect of heating at 60°C on HTLV-III, or LAV, suspended and lyophilized in four products. Little or no inactivation was seen with heating times up to 30 hours. The low purity F VIII preparation seemed to provide exceptional stabilization with little or no inactivation being seen up to 48 hours of heating and only 2.0 Log_{10} inactivation seen in 2 samples heated for 72 hours.

DISCUSSION

These findings indicate that in the present study pasteurization at 60°C in the dry state had only a modest process efficacy for inactivation of HTLV-III/LAV. Lyophilization itself inactivates 0.5-2.0 Log_{10} of infectivity, however as lyophilized products transmit AIDS, this is clearly not sufficient to yield sterile products.

Our findings are in marked contrast to those reported by McDougal et al who reported that HTLV-III/LAV titer was reduced 10 fold in 32 minutes when virus contaminated F VIII preparations were heated at 60°C in the lyophilized state (7). These authors extrapolated their estimate to a 37 Log_{10} Kill in 20 hours at 60°C. This is clearly inconsistent with our findings, and those reported by Levy et al who found a 2.5 Log_{10} inactivation of their AIDS virus isolate (ARV) resulting from 24 hour heating at 68°C (10).

It is difficult to explain the difference between the results reported by McDougal et al and the present findings. These differences may reflect the use of antigen assays by McDougal et al for detection of infected cultures instead of reverse transcriptase assays which were used in this study. Heated virus may infect more slowly and thus be less detectable by antigen assays at the times when cultures were harvested. Furthermore the use of macrocultures may have permitted the detection of small quantities of residual virus in the present study.

An additional important variable is the moisture content of the samples. Horowitz et al report that the rate of inactivation of model viruses was reduced 15-33% by a reduction of moisture content from 2.0 to 1.4 (11). The moisture content of the samples tested by McDougal was not specified.

Heating in the dry state has been shown to have only a modest sterilization effect on Hepatitis B virus (12). Furthermore heated Factor VIII products have transmitted non-A, non-B hepatitis to patients in two studies (13,14).

The present report of only a modest sterilization process efficacy for HTLV-III/LAV adds to our concern with the efficacy of this procedure.

It should however be stressed that our findings do not necessarily indicate that presently available dry heat treated products are unsafe with respect to transmission of AIDS. Indeed one study found no anti-HTLV-III seroconversion in 18 recipients of a dry heat treated Factor VIII preparation (15). Purification and processing steps prior to lyophilization can remove or inactivate virus, and lyophilization alone under commercial conditions probably inactivates more virus than we observed using shell freezing. Furthermore some products are heated at higher temperatures than the 60°C which we evaluated. Nevertheless, our findings indicate the need for caution in relying on the efficacy of dry heat sterilization. Careful long term surveillance of recipients of such products for seroconversion to anti-HTLV-III is needed.

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TABLE 1

EFFECT OF HEATING AT 60°C ON HTLV-III IN LYOPHILIZED FACTOR VIII

MATERIAL	REVERSE TRANSCRIPTASE, CPM X10^3 MACRO[1] 10^0	MICRO[2] 10^0	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	$TCID_{50}$[4] PER ML	LOG_{10} KILL
HTLV-3 Stock #8	-	-	-	55, 151	77, 521	11, 18	47[5], 6	$10^{4.5}$	-
" dil 1:10 in AHF I	-	-	383, 386	187, 171	5, 4	9, 8	5, 12	$10^{3.5}$	-
" " Lyophilized	295	508, 540	262, 314	340, 42	15, 7	6, 8	5, 7	$10^{3.0}$	0.5
" " " + 10 hrs 60°C	211	586, 459	172, 153	333, 263	ND	ND	ND	$>10^{3.0}$	0.5
" " " + 30 hrs 60°C	3	927, 504	68, 280	11, 24	ND	ND	ND	$10^{2.5}$	1.0
" " " + 48 hrs 60°C	404	614, 176	150, 361	138, 9	ND	ND	ND	$10^{2.5}$	1.0
" " " + 72 hrs 60°C	162	212, 1062	7, 16	8, 11	ND	ND	ND	$10^{1.0}$	2.5
" " " "	212	14, 19	7, 8	4, 6	ND	ND	ND	$<10^{1.0}$	$>2.5<4.5$
" " " "	243	41, 142	6, 19	10, 18	ND	ND	ND	$10^{1.0}$	2.5

[1] 10 ml undiluted sample in 100 ml cultures

[2] 0.1 ml undiluted sample in 1.5 ml cultures

[3] Culture lost due to contamination

[4] The mean of negative cultures was 7.6 X 10^3 CPM. Cultures were considered infected when the reverse transcriptase activity exceeded 3X the negative control mean. The results on these cultures are underlined. The titers for lyophilized samples were reduced by 0.5 Log_{10} since 30 ml lyophilized samples were reconstituted in 10 ml.

[5] Presumed contamination.

TABLE 2

INACTIVATION OF HTLV III/LAV BY HEATING IN THE DRY STATE IN DIFFERENT COAGULATION FACTORS

Log_{10} Inactivation [1]

Time at 60°C (Hours)	Factor VIII Low Purity [2]	Factor VIII High Purity [3]	Factor IX [4]	Anti-Thrombin-III
5	ND	ND	ND	<1.5
10	0	1.0	0	<1.5
30	0.5	<1.7 [5], 1.5	<1.9 [5], <2.5	ND
48	0.5	<3.5	<2.5	ND
72	2.0, 2.0, <4.0	<3.5, >3.5, >3.5	<2.5, <2.5, ≥2.5	ND

[1] Relative to titer of Lyophilized unheated preparation.

[2] Moisture content 1.49%

[3] Moisture content 1.06%

[4] Moisture content 0.83%

[5] Experiment carried out with LAV virus

REFERENCES

1. Pneumocystis carinii pneumonia among patients with hemophilia A. Morbid Mortal. Weekly Rep. 1982; 31:365-367.
2. Wilmer E, Barre-Sinoussi F, Rouzioux C, Gazengel C, Vezinet-Brun F, Dauguet C, Fischer A, Manigne P, Chermann JC, Griscelli C, Montagnier L. Isolation of new lymphotrophic retroviruses from two siblings with hemophilia B, one with AIDS. Lancet 1984; 1:753-757.
3. Evatt BL, Francis DP, McLane MFT et al. Antibodies to human T cell leukemia virus associated membrane antigens in hemophiliacs: Evidence for infection before 1980. Lancet 1983; 1:698-700.
4. Fletcher ML, Trowell JM, Craske J, Parier K, Rizza CR. Non-A, non-B hepatitis after transfusion of factor VIII in infrequently treated patients. Br. Med. J. 1983; 287:1754-1757.
5. Kernoff PBA, Lee CA, Karayiannias P, Thomas HC. High risk of non-A, non-B hepatitis after a first exposure to volunteer or commercial clotting factor concentrates: Effects of pooled human immunoglobulin. Br. J. Haematol. 1984; 58:174.
6. National Hemophilia Foundation Medical Scientific Advisory Council 1984. Recommendation concerning AIDS and therapy of hemophilia (Revised Oct. 13 1984) National Hemophilia Foundation, New York 1-2.
7. McDougal JS, Martin LS, Cort SP, Mozen M, Heldebrant CM, Evatt BL. Thermal inactivation of the acquired immunodeficiency syndrome virus, Human T Lymphotropic virus-III/Lymphadenopathy associated virus, with special reference to antihemophilic factor. J. Clin. Invest. 1985; 76:825-877.

8. Popovic M, Sarngadharan MG, Read E, et al: Detection, isolation, and continuous production of cytopathic retroviruses (HTLV-III) from patients with AIDS and pre-AIDS. Science 1984; 224:497-500.

9. Prince AM, Horowitz B, Dichtelmuller H, Stephan W, Gallo RC. Quantitative assays for evaluation of HTLV-III inactivation processes: Tri(n-butyl)phosphate/Sodium cholate and B-propiolactone. Cancer Res. (Suppl.) 1985; 45:4592S-4594S.

10. Levy JA, Mitra GA, Wong MR, Mozen MM. Inactivation by wet and dry heat of AIDS-associated retroviruses during Factor VIII purification from plasma. Lancet 1985; i:1456-1457.

11. Horowitz B, Wiebe ME, Lippin A, Vandersande J, Stryker MH. Inactivation of viruses in Labile blood derivatives 2. Physical Methods Transfusion 1985; In Press.

12. Hollinger FB, Dolana G, Thomas W. Gyorkey F. Reduction in risk of hepatitis transmission by heat-treatment of a human factor VIII concentrate. J. Inf. Dis. 1984; 150:250-262.

13. Colombo M, Manucci PM, Carnelli V, Savidge GF, Gazengel C, Schimpf K. and the European Study Group. Transmission of non-A, non-B hepatitis by heat treated factor VIII concentrate. Lancet 1985; ii:1-4.

14. Preston FE, Hay CRM, Dewar MS et al. Non-A, non-B hepatitis and heat-treated factor VIII concentrate. Lancet 1985, ii:213.

15. Rouzioux C, Chamaret S, Montagnier L, Carnell V, Rolland G, Mannucci PM. Absence of antibodies to AIDS virus in hemophilacs treated with heat treated Factor VIII concentrate. Lancet 1985; i:271-272.