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ORIGINAL ARTICLE

INACTIVATION OF POLIOVIRUS TYPE-1 AND HSV-1 IN HUMAN COAGULATION FACTOR VII CONCENTRATE BY PASTEURIZATION

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Abstract

Background-Pasteurization is now available to treat products derived from plasma to inactivate transfusion-transmitted enveloped and non-enveloped viruses.

Objective-This study was performed to assess the capacity and efficacy of virus inactivation by pasteurization in human coagulation factor VII concentrate with two model viruses including poliovirus type 1 (non-enveloped) and herpes simplex virus type 1 (enveloped).

Methods-The viruses were added to the stabilized factor VII (70% sucrose, 20% glycine) and pasteurized for 10 h at 60° C. Viral infectivity was tested *in vitro* on cell culture (Vero) using standard microtitration assays and the virus titer was expressed as log_{10} $TCID_{50}$/ml.

Results-On completion of the pasteurization procedure, 6.3 log of poliovirus type 1 and 5.8 log of HSV-1 were inactivated. Recovery of factor VII activity using sucrose/glycine as stabilizer was 80%. Other stabilizers showed lower recovery of factor VII and were less suitable.

Conclusion-Viral inactivation by pasteurization procedures destroys both enveloped and non-enveloped viruses and can be applied to production of factor VII from plasma with relatively good recovery and can improve the safety of replacement therapy in hemophilia patients with inhibitors.

Keywords • Factor VII • viral inactivation • pasteurization

Introduction

The risks of transmitting viral infections such as AIDS, hepatitis B and C by blood and plasma-derived products, e.g., clotting factors, immunoglobulins and albumin, have long been known and still remain a topic of concern.[1-4] In the case of albumin, pasteurization was introduced as early as the 1940s in order to eliminate the risk of hepatitis associated with this product.[5] At the end of the 1970s this method was further developed for the heat treatment of coagulation concentrates containing factors II, VII, IX and X by the selection of appropriate stabilizers which protected the biological activity of plasma proteins.[6] Since the mid-1980s the viral safety of clotting factor concentrates has depended on the triple safeguards of i) donor selection, ii) viral screening and iii) one or more specific virucidal procedures.[7] During the last decade, general methods have been developed and validated for their application in the production of clotting factor concentrates namely heat treatment, solvent-detergent treatment, nanofiltration, etc.[8] The ability of pasteurization to inactivate both enveloped and non-enveloped viruses in some human plasma derivatives has been demonstrated.[9-11] A variety of compounds have been shown to affect the stability of proteins in solution. Sugars, polyols, and certain amino acids and salts are known to be protein stabilizers.[12-16]

In this paper we have focused on virus inactivation of human factor VII concentrates by the pasteurization method, evaluation and selection

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of appropriate stabilizers and validation studies according to CPMP guidelines.[17] Due to limitations in the availability of model viruses, we had to rely on two models for enveloped (HSV-1) and non-enveloped viruses (poliovirus type 1). Obviously if more viral lines such as the possibility of obtaining direct HIV cultures become available, further and more accurate information would be attained.

Materials and Methods

For the selection of appropriate stabilizers to preserve coagulation factor VII (FVII) activity in FVII concentrates during pasteurization (10 h, 60° C), various stabilizers were tested and FVII activity was measured by one-stage clotting assay.[18] To study the efficacy of virus inactivation by pasteurization, an aliquot of the stabilized aqueous solution of the product was spiked with a specific virus. Model viruses used were poliovirus type 1 and herpes simplex virus type 1. The resulting virus-spiked protein preparation were subjected to heat (10 h, 60° C). Viral infectivity was tested *in vitro* on cell culture (Vero) using standard microtitration assays.[19] The infectivity titres were calculated according to Reed and Muench method and expressed as $\log_{10}$ $TCID_{50}$/ml (tissue culture infectious dose 50% per ml).

Results

Different concentration of stabilizers i.e., sugars, amino acids, salts and combined (sugar and amino acid) were tested during pasteurization with the appropriate controls held at room temperature. The controls and pasteurized samples were assayed for FVII activity. Residual activity was expressed as a percentage of the activity of the control. The results are summarized in Tables 1. It was observed that recovery was significantly improved at higher levels of sucrose which served to solubilize as well as stabilize the concentrate. Other stabilizers were not suitable due to low recovery and solubility. The solution containing inappropriate stabilizer became opalescent indicating the onset of denaturation. Gelation results directly from protein denaturation. Highest recovery of FVII (80%) was observed using 70% sucrose and 20% glycine. Factor VII concentrate was stabilized in this way.

The inactivation kinetics of HSV-1 and poliovirus by heat treatment in either isotonic buffer solution or in a stabilized FVII preparation are shown in Figures 1 and 2. It was observed that HSV-1 and poliovirus were completely inactivated within six and eight hours respectively. The addition of sucrose and glycine protects against the inactivation of HSV-1 and poliovirus, a finding that was not unexpected. Sucrose has been used in the stabilized formulation for preservation of rickettsiae and viruses following lyophilization.[20-22] After pasteurization, reduction factor of HSV-1 and poliovirus were 5.8 log and 6.3 log respectively. The non-heated virus control held at room temperature showed no significant virus inactivation.

Table 1. Effects of different single and combined stabilizers on factor activity after pasteurization (10h at 60°C) of factor VII concentrate.

Stabilizer	Concentration [%(w/v)]or M	Recovery (%)	Appearance
Sucrose	20	<1	Gelation
	40	4	Gelation
	60	23	Opalescent
	70	28	Opalescent
	90	76	Clear
Trehalose	20	<1	Gelation
	40	5	Opalescent
	60	15	Clear
	70	26	Clear
Glucose	20	<1	Gelation
	40	3	Gelation
	60	15	Opalescent
	70	20	Clear
Glycine	20	23	Opalescent
	40	7	Gelation
	60	5	Gelation
DL-tryp-	0.06M	7	Opalescent
tophane	0.16M	10	Opalescent
	0.32M	15	Opalescent
	0.50M	15	Opalescent
Sucrose	50+20	45	Opalescent
+Glycine	70+20	80	Clear
Sodium	0.5M	<1	Gelation
Citrate	1.0M	<1	Gelation
	1.5M	<1	Gelation
	2.0M	<1	Gelation
Sodium	0.5M	<1	Gelation
Chloride	1.0M	<1	Gelation
	1.5M	<1	Gelation
	2.0M	<1	Gelation

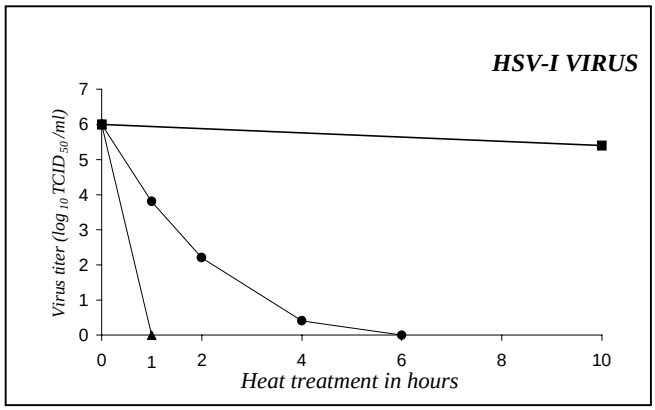

Fig 1. Inactivation of HSV-1 by heat treatment for 10 h at 60 C in an isotonic buffer solution (triangles), in a stabilized FVII solution (open circles) and in non-heated control held at room temperature (squares).

Discussion

The use of physical methods for the inactivation of viruses and other microorganisms has gained widespread application. However, in the case of its application to protein solutions it is a delicate matter which does not follow a universal rule and one that needs careful evaluation and validation under particular conditions. For instance pasteurization (heating at 60° C for 10 h) of human albumin in addition to its purification scheme employing Cohn-ethanol fractionation results in successful inactivation of blood borne viruses such as HBV[23] which has been reconfirmed recently.[24] Whereas in the case of FVII, inactivation of viruses by pasteurization is retarded significantly in the presence of certain stabilizers.[9]

The results of the present study demonstrates that pasteurization in the presence of high concentration of sucrose (70%) and glycine (20%) causes reduction in the infectivity of HSV-1 and poliovirus by a factor of 5.8 log and 6.3 log respectively, while 80% of the biological activity of FVII is retained. We can therefore conclude that, this procedure may be used to reduce infectivity of both enveloped and non-enveloped viruses in the process of producing FVII concentrate. However, inactivation is somewhat slower for the non-enveloped poliovirus (Fig. 2). In this respect, a stabilized FVIII concentrate heated in solution (60° C, 10 h) has good safety records both in chimpanzee trials and clinical uses; HIV, HCV and HBV transmissions have not been recorded.[9] The stabilizers, although non-toxic, due to their high concentrations have to be removed before injection. This can be easily achieved by adding a diafiltration step prior to filling of the product. The commercial products available on the market, use heat treatment (vapor heat and severe heat) as well as S/D methods[25], which is under investigation in our group and will be reported in due time. These experimental bench-scale results indicate that the manufacturing procedures for FVII concentrates which include pasteurization as a virus inactivation step provide a relatively high margin of safety under appropriate conditions. However, in order to achieve the required margin of safety, as requested recently by some regulatory authorities such as Paul Ehrlich, Germany which mentions the use of two inactivation steps with a combined inactivation factor of 10 log. A second step such as S/D should also be considered and validated.[7]

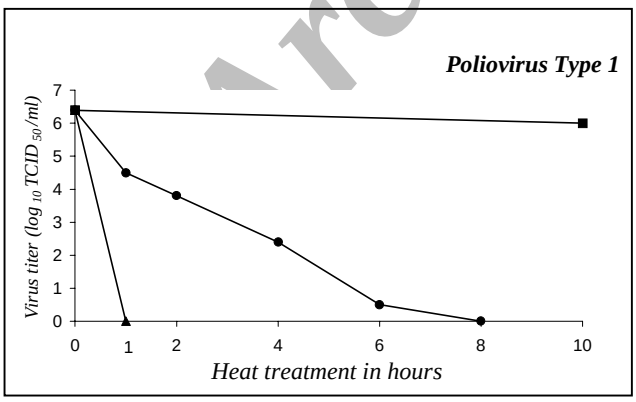

Fig 2. Inactivation of poliovirus type 1by heat treatment for 10 h at 60 C in an isotonic buffer solution (triangles), in a stabilized FVII solution (open circles) and in non-heated control held at room temperature (squares).

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