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A STUDY ON THE APPLICATION AND EFFICACY OF SOLVENT-DETERGENT (S/D) TREATMENT IN THE PROCESS OF PURIFYING FACTOR VII FROM PROTHROMBIN COMPLEX

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ABSTRACT

The risks of transmitting viral infection by blood and plasma-derived products have long been known and still remain an area of concern. In this study, in the process of purifying human factor VII from prothrombin complex, S/D treatment using tri-n-butyl phosphate and Tween 80 was employed and its capability and efficacy was studied.

The results indicated that the process did not affect the biological function of the essential coagulation factors studied. In addition, the process was shown to be effective on enveloped viruses, where its inactivation factor for the model used, Herpes Simplex type I, was 5.5 logs. However, the procedure was not effective on poliovirus which was used as a model for non-enveloped viruses. It is therefore concluded that for increasing viral safety it is best to apply at least one more inactivating procedure which will be effective on non-enveloped viruses as well.
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Keywords: Human Factor VII concentrate, viral inactivation, solvent-detergent treatment.

INTRODUCTION

In the last few years blood transfusion in general and plasma fractionation in particular have developed into a discipline requiring skilled scientific expertise and developing technology to prevent transmission of blood-borne infections. The risk associated with the transmission of these infections through blood and blood components has been significantly reduced by donor selection[1] and screening for specific pathogens.[1,2,4]

There are, however, several limitations of screening:

a) Current tests may miss certain infectious units particularly during the early phase of infection;

b) Effective tests are not available for all pathogens;

c) Newly emerging pathogens can not be detected.

Thus, screening must be combined with inactivation procedures in order to obtain a lower-risk blood supply.[3,5]

A variety of inactivation procedures such as pasteurization,[3,5] solvent-detergent (S/D) treatment[6,7,8] irradiation,[5,9] pressure-cycling[10] and others, are currently used to lower the residual risk of infection.

The S/D methodology consists of incubating a plasma protein solution in the presence of a non-volatile organic solvent and detergents.[6,7] The treatment leads to a disruption of the structural integrity of lipid-enveloped viruses. The chemical additives, however, must be removed before treated blood components are used therapeutically. Therefore, ideally, the inactivation method should be highly effective against a broad range of pathogens, while still preserving the functional activity of a desired protein and be easily removable in the production process.

In this study application of S/D treatment in the process of a bench-scale purification of factor VII and IX from prothrombin complex and its effect on the activity of coagulation factors was investigated. Efficacy of the process was also studied by 'virus-spiking' studies followed by infectivity assays.

MATERIAL AND METHODS

To study the efficiency of virus inactivation by SD

treatment, an aliquot of the prothrombin complex concentrate (PPSB) was spiked with a herpes simplex virus type I and then treated with TNBP and Tween 80 to bring the concentration to 0.3% and 1% respectively, and held at 24°C for 6h with constant stirring. The virus titres of aliquots taken before and at various times during SD treatment were determined. The same work was performed for poliovirus type I. Viral infectivity was tested *in vitro* on cell culture (Vero) using standard microtitration assays[11] and the infectivity titres were calculated according to Reed and Muench's method and expressed as $\log_{10}$ $TCID_{50}$/mL (tissue culture infectious dose 50% per mL). The virus-sterilizing agents (TNBP and Tween 80) were removed by washing with buffer during the purification procedure using DEAE-Sepharose CL-6B chromatography which has been described in an earlier paper.[12] Coagulation activities of FVII, FVIIa, FII, FIX, FX, protein C, protein S and antithrombin III were determined before and after S/D treatments of prothrombin according to Diagnostica, Stago, France Kits. Tween 80 was measured colorimetrically and finally the absorbance was determined spectrophotometrically at 535 nm.[12] TNBP was assayed by gas chromatography using a Perkin-Elmer Sigma 1 equipped with a 10% SP-1000 column.[12]

RESULTS

The inactivation of HSV-I by 5.5 log using Tween 80 (1%) and TNBP (0.3%) within 6 hours at 24°C is demonstrated in Fig. 1. The non-SD-treated virus control held at 24°C showed no significant virus inactivation. SD treatment could not inactivate poliovirus type 1 (non-enveloped virus) due to the absence of lipoprotein structure (Fig. 2). We extensively analysed PPSB concentrate before and after SD treatment for biological activity of the relevant clotting factors (Table I). Interestingly, it was found that there was no significant change in the activity of factor VII and other coagulation factors (FII, FIX and FX) after SD treatment of PPSB. It is concluded that both S/D treatment methods, using Triton or Tween, have no major adverse effects on biological activity of the relevant protein. Although as can be observed in Table I, Triton X-100 had milder biological effects than Tween 80 during S/D treatment of PPSB.

The virus-sterilizing agents (Tween 80, TNBP) were removed by washing out buffer during chromatography readsorption of the PPSB to DEAE-Sepharose CL-6B. Elimination of Tween 80 and TNBP was within acceptable, non-toxic range (2.0 and 4.6 µg/mL respectively) in the purified factor VII.

The maximum acceptable amount of TNBP and Tween 80 according to NYBC specification and FDA approval has been reported to be 10µg/mL and 5 µg/mL respectively.[12]

DISCUSSION

In the last 15 years, plasma products derived from human blood have become significantly safer in developed countries.[3,4,5]

In the mid-eighties, S/D treatment undoubtedly represented a major breakthrough for the improved viral safety. However, since virus inactivation of protein solutions does not follow a universal rule, careful evaluation and validation under particular conditions should be carried out.

The results of the present study indicate that the use of TNBP and Tween to disrupt viral envelopes has no effect on the functional activity of the required coagulation factors, in the process of purification of factor VII and IX which has been described in an earlier paper.[13]

The procedure also successfully removed chemical

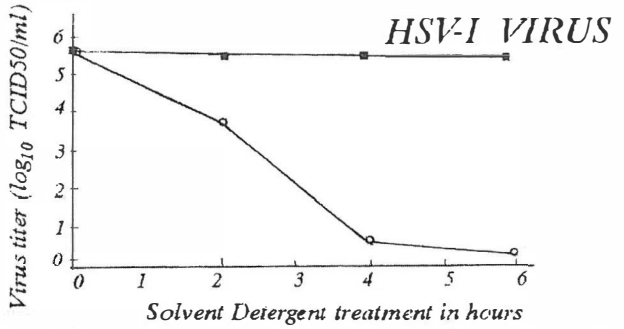

Fig. 1. Inactivation of HSV-I by TNBP (0.3%) and Tween 80 (1%) in prothrombin complex concentrate (PPSB) for 6h at 24°C (o), non-SD treated control at room temperature (■).

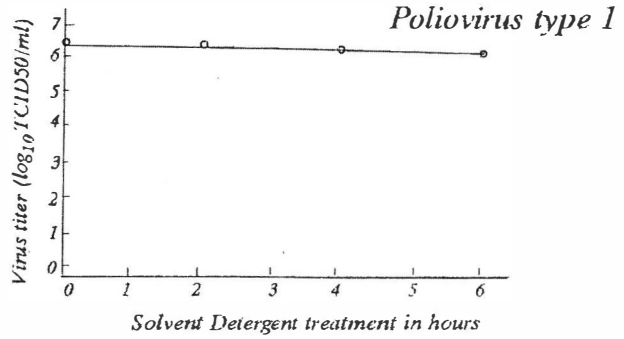

Fig. 2. No inactivation of poliovirus type 1 by TNBP (0.3%) and Tween 80 (1%) in prothrombin complex concentrate (PPSB) for 6h at 24°C.

Table 1. The effects of solvent-detergent treatments on coagulation factors and regulatory proteins in prothromin complex concentrate (PPSB).

	FII** u/dl	FVII u/dl	FIX u/dl	FX u/dl	FVIIa*** u/dl	Protein C u/dl	Protein S u/dl	ATIII**** u/dl	pH
PPSB [6h at room temp.]	1600	260	1440	1800	550	156	215	<10	7.32
PPSB+Tween 80 (1%) +TNBP* (0.3%) [6h at 24°C]	1700 (6%)[a]	280 (7%)[a]	1540 (7%)[a]	2000 (11%)[a]	600 (9%)[a]	149 (4%)[a]	270 (25%)[a]	<10	7.41
PPSB+Triton X-100 (1%)+TNBP (1%) [4h at 30°C]	1650 (3%)[a]	270 (4%)[a]	1460 (1%)[a]	1850 (3%)[a]	560 (2%)[a]	127 (18%)[a]	235 (9%)[a]	10	7.34

*TNBP= Tri(n-butyl)phosphate

**FII= human coagulation factor II

***FVIIa=activated factor VII

****ATIII=Antithrombin III

[a]Percent increase or decrease of activity.

additives leaving trace amounts which are considered safe.[12] However, as it was expected, this inactivation is only effective on lipid-enveloped viruses. In our study, HSV was employed as a model virus for this group of viruses, and was inactivated by 5.5 log. Therefore, since most of the viruses of concern, namely HIV, HCV and HBV are in fact lipid-enveloped, it can be deduced that this procedure within the reported purification scheme may result in safe products as far as these viruses are concerned. Indeed, complete safety of most blood components using this procedure for lipid-enveloped viruses has been reported.[3,5,6,10] Nevertheless, this procedure may not be effective on non-enveloped virus, and reports of transmission of HAV and B19 do exist.[14,15,16] Our study also confirmed that in the reported purification scheme,[13] S/D treatment is not effective on poliovirus which is in fact a model virus for non-enveloped viruses and was used in our study. Therefore it can be concluded that in order to achieve a high margin of safety, S/D treatment must be combined with a second virus inactivation procedure which may be capable of inactivation of non-enveloped viruses.[9] In fact, effectiveness of pasteurization, using suitable stabilisers in this procedure has been reported earlier.[17] In this respect, some international regulatory bodies such as Paul Ehrlich, Germany, require the use of two inactivation procedures with a combined inactivation factor of more than 10 logs.[18]

It should also be noted here that, to this date, there has been no report of protein neoantigen formation due to S/D treatment, which is a great advantage, since neoantigens are an important point to consider regarding the safety of inactivation procedures.[5,9]

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