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

H. REZVAN, S. NASIRI, K. MOUSAVI, AND M. GOLABI*

From the Research and Development Department, Blood Research and Fractionation Co., Tehran, and the *Virology Dept., Iran University of Medical Sciences, Tehran, I.R. Iran.

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.

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 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,1,2 solvent-detergent (S/D) treatment,6,7 irradiation,5,8 pressure-cycling9 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\(^1\) 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.\(^2\) Coagulation activities of FVII, FVIIa, FII, FIX, FX, protein C, protein S and antithrombin III were determined before and after SD treatments of prothrombin according to Diagnostica, Stago, France Kits. Tween 80 was measured colorimetrically and finally the absorbance was determined spectrophotometrically at 535 nm.\(^2\) TNBP was assayed by gas chromatography using a Perkin-Elmer Sigma 1 equipped with a 10% SP-1000 column.\(^2\)

**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-S/D-treated virus control held at 24°C showed no significant virus inactivation. SD treatment could not inactivate poliovirus type I (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 \(\mu g/mL\) 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 10\(\mu g/mL\) and 5 \(\mu g/mL\) respectively.\(^2\)

**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 inactivating 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.\(^2\)

The procedure also successfully removed chemical
Table I. The effects of solvent-detergent treatments on coagulation factors and regulatory proteins in prothromin complex concentrate (PPSB).

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

*TNBP= Tri(n-butyl) phosphate
**FII= human coagulation factor II
***FVIIa=activated factor VII
****ATIII=Antithrombin III

8. Horowitz B, Lazo A, Grossberg H, Page G: Virus inactivation by solvent/detergent treatment and the manufacture of...
S/D Treatment for Factor VII Purification