Robust Inactivation of Duck Hepatitis B Virus With Amustaline/GSH In Whole Blood

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Background
A chemical treatment process utilizing amustaline (S-303) and glutathione (GSH) has been developed to inactivate leukocytes and contaminating pathogens in RBC components (Figure 1). The treatment of whole blood (WB) using a variation of this system is being further explored as a solution for the developing world where component based transfusion products are not readily available. The process requires that the treated whole blood be held at ambient temperature for 24 hours after treatment, at which point the whole blood is ready for transfusion.

Duck Hepatitis B virus (DHBV) is a small, enveloped dsDNA virus in the Hepadnaviridae genus, a group of viruses in the family Hepadnaviridae. DHBV is a model virus for human hepatitis B virus (HBV) for which there are few good in vitro or in vivo models. Human hepatitis B virus can lead to life-threatening liver disease leading to cirrhosis and liver cancer. Hepatitis B prevalence is highest in sub-Saharan Africa and East Asia, where between 5–10% of the adult population is chronically infected (WHO). In high endemic areas, hepatitis B is most commonly spread through horizontal transmission via exposure to infected bodily fluids, highlighting the risk associated with blood transfusion.

The objective of this study was to evaluate the inactivation of DHBV in collaboration with Swiss Red Cross Humanitarian Foundation for Whole Blood Pathogen Inactivation for Africa. The inability to consistently supply blood components makes WB transmission common, and since transfusion-transmitted diseases are prevalent in the developing world, the development of a robust WB pathogen inactivation system is desirable. The INTERCEPT system for WB uses 0.2 mM S-303 and 2 mM GSH and 24h incubation at room temperature (RT) and is designed for the application in resource poor countries, where instrumentation and refrigeration may not always be available. At the conclusion of the RT incubation, the treated WB unit is suitable for storage up to 7 days.

Methods

For each experiment, a single WB unit was spiked with DHBV to a final concentration of ~4.5 logTCID50/mL. A 60 mM GSH stock was created and the WB was dosed to achieve a final concentration of 2 mM GSH in each unit. A control sample was removed prior to the addition of S-303. Each unit was then dosed with S-303, to a final concentration of 0.2 mM, and allowed to incubate at ambient temperature for 24 hours. At the conclusion of the 24 hour incubation, a test sample was removed and frozen to determine the levels of inactivation. Control samples were serially diluted ten-fold up to 105 and inoculated onto duck hepatocytes monolayers in 12-well plates to determine the pre-treatment titer. Test samples were diluted 1:2 and 1:10 and inoculated onto duck hepatocytes monolayers to determine residual post-treatment DHBV titers. The plates were incubated for 10 days at 37°C, fixed with ethanol and the presence of viable DHBV determined by indirect IFA with a mAb to the DHBV envelope protein. Plates were scored appropriately, resulting in a control titer of 4.6 logTCID50/mL from the control samples. No residual DHBV was detected in the Test samples, yielding a post-treatment titer of <0.7 logTCID50/mL. Log reduction was calculated as the difference between the mean titer in pre-S-303 samples and the mean titer in the 24 hour post-S-303 samples.

Results

Initial mean DHBV titers of DHBV were determined to be 4.6 logTCID50/mL using the duck hepatocyte infectivity assay, with a post-treatment titer of <0.7 logTCID50/mL. Following treatment with 0.2 mM S-303 and 2 mM GSH, no viable infectious DHBV was present in culture, resulting in a mean inactivation of >5.3 log10 (p<0.001). These results are comparable with >5.1 log10 inactivation of DHBV in AS-5 RBC after treatment with 0.2 mM GSH and 24h incubation at room temperature. The INTERCEPT Blood system for WB or RBCs is not approved for commercial use. The INTERCEPT Blood system for WB uses 0.2 mM S-303 and glutathione (GSH) using a variation of this system is desirable. The INTERCEPT system is designed for the application in resource poor countries, where instrumentation and refrigeration may not always be available. At the conclusion of the RT incubation, the treated WB unit is suitable for storage up to 7 days.

Table 1: Inactivation of DHBV in Whole Blood

<table>
<thead>
<tr>
<th>Replicate</th>
<th>Log Titers (TCID50/mL)</th>
<th>Log Reductiona</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-Inactivation Control UT=0</td>
<td>Post-Inactivation Test T=24</td>
</tr>
<tr>
<td>1</td>
<td>4.6</td>
<td>&lt; -0.7</td>
</tr>
<tr>
<td>2</td>
<td>4.1</td>
<td>&lt; -0.7</td>
</tr>
<tr>
<td>3</td>
<td>4.9</td>
<td>&lt; -0.7</td>
</tr>
<tr>
<td>4</td>
<td>4.6</td>
<td>&lt; -0.7</td>
</tr>
<tr>
<td>Mean ±SDb</td>
<td>4.6 ± 0.3</td>
<td>&lt; -0.7 ± 0.0</td>
</tr>
</tbody>
</table>

a. Log reduction is calculated as Log (pre-treatment titer) – Log (post-treatment titer) where titers are expressed as 10x TCID50/mL. Log reductions were determined from numbers that were not rounded.

Conclusions

Treatment of WB with 0.2 mM S-303 and 2 mM GSH inactivated >5.3 log10 of Duck hepatitis B virus. These results indicate that the WB treatment system can inactivate DHBV to the limit of detection and therefore provide a solution to help mitigate the transmission of hepatitis B virus in the developing world, where blood components are not always readily available for transfusion, and transfusion-transmission of infectious agents pose a consistent threat to the blood supply.

The INTERCEPT Blood system for WB or RBCs is not approved for commercial use.