Effect of $\beta$ propiolactone viral inactivation on $\alpha_1$ antitrypsin values

S J Katona, M Bowen, E R Kaminski

Aims: $\alpha_1$ Antitrypsin was undetectable in several patient samples treated with 0.5% $\beta$ propiolactone, which was used as a virucidal agent. This study was designed to confirm $\beta$ propiolactone as the cause and determine why it might have such an effect.

Methods: Volumes of 0, 5, 10, and 20 $\mu$l of $\beta$ propiolactone were added to 2 ml aliquots of serum to make final concentrations of 0%, 0.25%, 0.5%, and 1% of $\beta$ propiolactone. $\alpha_1$ Antitrypsin concentrations and the pH were measured at different time intervals. The effects of adding buffer before the addition of $\beta$ propiolactone, NaOH after $\beta$ propiolactone, and 6M HCl instead of $\beta$ propiolactone were also measured.

Results: The addition of $\beta$ propiolactone to a volunteer’s serum showed a fall in both $\alpha_1$ antitrypsin values and pH with increasing time and concentration of $\beta$ propiolactone. This effect was also seen when adding HCl, but was partially prevented by buffering the serum or adding NaOH.

Conclusions: These results suggest that it is the acidity of the degradation products of $\beta$ propiolactone that is responsible for the fall in $\alpha_1$ antitrypsin values. This fall in $\alpha_1$ antitrypsin values was dependent on the concentration of $\beta$ propiolactone used and the length of time before the test was performed. The effect of $\beta$ propiolactone on laboratory tests should be re-evaluated, with attention being paid to sample pH, storage time, and storage temperature.


table

<table>
<thead>
<tr>
<th>BPL concentration (%)</th>
<th>Base, acid, or buffer added to 2 ml serum aliquots</th>
<th>AAT concentration on day 4 (g/l)</th>
<th>pH on day 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Nil</td>
<td>1.27</td>
<td>7.59</td>
</tr>
<tr>
<td>0</td>
<td>10 $\mu$l 6M HCl</td>
<td>0.99</td>
<td>5.67</td>
</tr>
<tr>
<td>0</td>
<td>20 $\mu$l 6M HCl</td>
<td>0.34</td>
<td>4.38</td>
</tr>
<tr>
<td>0.25</td>
<td>Nil</td>
<td>0.8</td>
<td>5.46</td>
</tr>
<tr>
<td>0.5</td>
<td>Nil</td>
<td>&lt;0.29</td>
<td>4.74</td>
</tr>
<tr>
<td>0.5</td>
<td>Buffer*</td>
<td>0.81</td>
<td>6.33</td>
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<tr>
<td>1</td>
<td>Nil</td>
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</tr>
<tr>
<td>1</td>
<td>Buffer*</td>
<td>0.7</td>
<td>5.72</td>
</tr>
<tr>
<td>1</td>
<td>220 $\mu$l 1M NaOH</td>
<td>0.62</td>
<td>7.7</td>
</tr>
</tbody>
</table>

Buffer was added before and NaOH was added after $\beta$ propiolactone (BPL).

*46 mg Bis-tris+17.9 mg NaCl.
the α1 band on electrophoresis using Sebia’s automated Hydrasys system (fig 1). At the same time, two other high risk samples were noted to have an α1 antitrypsin concentration measured by nephelometry of less than 0.29 g/litre. Retesting of the two untreated original samples gave normal results of 1.77 and 1.35 g/litre.

The pH of a serum sample containing 0.25% β propiolactone was measured as 5.53 using a Radiometer ABL 700 series blood gas analyser, and raised the possibility of acidity being the cause of the low α1 antitrypsin values.

**METHODS**

Volumes of 0, 5, 10, and 20 µl of β propiolactone were added to 2 ml aliquots of serum from a normal volunteer in a class III biological safety cabinet, to make final concentrations of 0%, 0.25%, 0.5%, and 1% of β propiolactone. They were left for one hour at ambient temperature and then stored at 4°C. Measurements for α1 antitrypsin were then taken at different time intervals using a Beckman array nephelometer. A Hanna Instruments laboratory microprocessor pH metre was used to measure the pH of samples.

To determine whether the pH of the serum was responsible for the low α1 antitrypsin values, buffer was added before the β propiolactone, 1M NaOH was added after β propiolactone, and 6M HCl was added instead of β propiolactone.

**RESULTS**

A time and β propiolactone concentration dependent reduction in α1 antitrypsin concentration occurred (fig 2). The fall in α1 antitrypsin was reduced by adding buffer and correcting the pH with NaOH. The addition of 6M HCl instead of β propiolactone reproduced the fall in α1 antitrypsin concentration.

**DISCUSSION**

We have shown that β propiolactone causes a fall in α1 antitrypsin values with increasing time and dose, owing to a reduction in sample pH. This is consistent with Ball et al, who showed a non-significant fall from 2870 to 2770 mg/litre after approximately three hours at a β propiolactone concentration of 0.25%.

LoGrippo measured the α1 region on electrophoresis in three samples containing 0.35% β propiolactone, and found a decrease of greater than 10% compared with the untreated samples, but not for samples treated with both β propiolactone and ultraviolet irradiation. Crucially, no indication of the time delay in measuring the α1 regions was given. It remains to be seen whether various α1 antitrypsin phenotypes are affected differently.

It has recently been reported that 50% of α1 antitrypsin is in a latent or polymerised form in solvent/detergent treated plasma produced by the American Red Cross.

"The control of pH may preserve the validity of various tests without affecting viral killing"

The effects of β propiolactone on cell morphology, electrolytes, coagulation tests, and haemoglobin electrophoresis may also be the result of its low pH. The altered electrophoretic mobility of albumin seen in fig 1 may also be explained by LoGrippo, who demonstrated uptake of 42 moles of β propiolactone for each mole of albumin. The use of β propiolactone will cause artificially low α1 antitrypsin values when high concentrations are used, or there is a significant delay between the addition of β propiolactone and α1 antitrypsin analysis. The effect of β propiolactone on laboratory tests should be re-evaluated, with attention being paid to sample pH, incubation time, and temperature.

LoGrippo showed no effect of pH on virus inactivation and Grosell showed that the reactivity of β propiolactone with DNA is much greater between pH 7.4 and 8.0 than at pH 7.0 or

**Figure 1** Absent α1 bonds on the electrophoretic strip of three high risk samples, treated with β propiolactone at a concentration of 0.5% (samples 1–3). All the samples were from different patients and were analysed as part of routine laboratory work. Samples 4–11 were not treated with β propiolactone.

**Figure 2** The effect of β propiolactone (BPL) concentration and incubation time on serum α1 antitrypsin concentrations. Lower limit of detection 0.29 g/litre, adult normal range 1.1–2.1 g/litre. In serum at a 1% concentration of BPL, α1 antitrypsin was undetectable (< 0.29 g/litre) at 21.5 and 42 hours.
pH 6.5. Therefore, the control of pH may preserve the validity of various tests without affecting viral killing.

We are currently auditing the use of viral inactivation methods in different UK laboratories, with particular attention to whether these are being applied to all laboratory specimens.

ACKNOWLEDGEMENT
Special thanks to K Boswijk, who first observed the low α1 antitrypsin values in patients’ sera that had been treated with β-propiolactone, and retested the untreated samples.

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