ORIGINAL ARTICLE

Effect of β propiolactone viral inactivation on $\alpha 1$ antitrypsin values

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Aims: $\alpha 1$ Antitrypsin was undetectable in several patient samples treated with 0.5% β propiolactone, which was used as a virucidal agent. This study was designed to confirm β propiolactone as the cause and determine why it might have such an effect.

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

Results: The addition of β propiolactone to a volunteer's serum showed a fall in both $\alpha 1$ antitrypsin values and pH with increasing time and concentration of β 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 β 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 β propiolactone used and the length of time before the test was performed. The effect of β propiolactone on laboratory tests should be re-evaluated, with attention being paid to sample pH, storage time, and storage temperature.

Most of the $\alpha 1$ globulin fraction on serum protein electrophoresis is accounted for by $\alpha 1$ antitrypsin. The measurement of $\alpha 1$ antitrypsin is indicated in the evaluation of chronic obstructive airway disease, and in neonatal and adult liver disease. Abnormality, absence, or reduplication of the $\alpha 1$ globulin band are indicative of genetic variants of $\alpha 1$ antitrypsin. Under certain conditions, $\alpha 1$ antitrypsin has been seen to self assemble into amyloid fibrils,¹ helped by denaturing agents such as 8M urea and 6M guanidine, sodium citrate,² and lithocholic acid.³ Human $\alpha 1$ antitrypsin consists of 418 amino acids and has a theoretical isoelectric point of 5.37.

" β Propiolactone is widely used to treat high risk laboratory samples, blood products, and vaccines, with acceptable safety and efficacy"

LoGrip⁴ selected β propiolactone from a group of 23 potential virucides and demonstrated its efficacy at inactivating a wide range of viruses. He measured the half life of β pro-

piolactone as 24–32 minutes at 37°C, and 16–20 hours at 4°C. β Propiolactone has a highly reactive ring structure that reacts with nucleic acids and proteins as an alkylating agent. It is rapidly broken down to a non-toxic form, 3-hydroxypropionic acid.

No reports of oncogenicity or mutagenicity have been associated with the aqueous phase of β propiolactone. It is widely used to treat high risk laboratory samples, blood products, and vaccines,⁵ with acceptable safety and efficacy. However, Norley *et al* showed poor killing of human immunodeficiency virus (HIV) in vitro,⁶ and transmission of HIV has occurred.⁷ β Propiolactone is particularly poor at inactivating intracellular viruses.⁸

Ball *et al* showed that a concentration of β propiolactone of 0.25% had no effect on a wide range of laboratory tests.⁹ Recently, we have started to add β propiolactone at a concentration of 0.5% (Sigma, Poole, Dorset, UK; product code P5648; formula $C_3H_4O_2$) to high risk sera for protein electrophoresis and nephelometry. A laboratory error was suspected when three consecutive high risk sera showed a complete absence of

Table 1 An investigation into the effect of pH on serum $\alpha 1$ antitrypsin (AAT) concentrations

BPL concentration (%)	Base, acid, or buffer added to 2 ml serum aliquots	AAT concentration on day 4 (g/l)	pH on day 5
0	Nil	1.27	7.59
0	10 μ l 6M HCl	0.99	5.67
0	20 μ l 6M HCl	0.34	4.38
0.25	Nil	0.8	5.46
0.5	Nil	<0.29	4.74
0.5	Buffer*	0.81	6.33
1	Nil	<0.29	4.47
1	Buffer*	0.7	5.72
1	220 μ l 1M NaOH	0.62	7.7

Buffer was added before and NaOH was added after β propiolactone (BPL).

*46 mg Bis-tris+17.9 mg NaCl.

Figure 1 Absent $\alpha 1$ bands 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.

the $\alpha 1$ 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 $\alpha 1$ 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 $\alpha 1$ antitrypsin values.

METHODS

Volumes of 0, 5, 10, and 20 μ l 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 $\alpha 1$ 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 $\alpha 1$ 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.

Figure 2 The effect of β propiolactone (BPL) concentration and incubation time on serum $\alpha 1$ 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, $\alpha 1$ antitrypsin was undetectable (< 0.29 g/litre) at 21.5 and 42 hours.

RESULTS

A time and β propiolactone concentration dependent reduction in $\alpha 1$ antitrypsin concentration occurred (fig 2).

The fall in $\alpha 1$ 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 $\alpha 1$ antitrypsin concentration.

DISCUSSION

We have shown that β propiolactone causes a fall in $\alpha 1$ antitrypsin values with increasing time and dose, owing to a reduction in sample pH. This is consistent with Ball *et al.*,⁹ who showed a non-significant fall from 2870 to 2770 mg/litre after approximately three hours at a β propiolactone concentration of 0.25%.

LoGrip⁴ measured the $\alpha 1$ 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 $\alpha 1$ regions was given. It remains to be seen whether various $\alpha 1$ antitrypsin phenotypes are affected differently.

It has recently been reported that 50% of $\alpha 1$ antitrypsin is in a latent or polymerised form in solvent/detergent treated plasma produced by the American Red Cross.¹⁰

"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 electrophoresis¹¹ may also be the result of its low pH. The altered electrophoretic mobility of albumin seen in fig 1 may also be explained by LoGrip⁴, who demonstrated uptake of 42 moles of β propiolactone for each mole of albumin.⁴

The use of β propiolactone will cause artificially low $\alpha 1$ antitrypsin values when high concentrations are used, or there is a significant delay between the addition of β propiolactone and $\alpha 1$ 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.

LoGrip⁴ showed no effect of pH on virus inactivation and Groseil¹² 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

Take home messages

- The addition of β propiolactone to serum resulted in a fall in α 1 antitrypsin values, which was dependent on the concentration of β propiolactone used and length of time before the test was performed
- The acidity of the degradation products of β propiolactone may be responsible for this fall
- The effect of β propiolactone on laboratory tests should be re-evaluated, with attention being paid to sample pH, storage time, and storage temperature

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.

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