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Factors influencing the activity of taurolidine against spores of *Bacillus subtilis* (NCTC 10073)

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Abstract

The cidal activities of aqueous taurolidine (2.0% w/v containing 5.0% w/v polyvinylpyrrolidone as a solubilising agent) and alcoholic taurolidine (2.0% w/v dissolved in Isopropyl alcohol 50% v/v) against spores of *Bacillus subtilis* NCTC 10073 were evaluated at 20°C, 37°C, 45°C and 55°C. Increased temperature increased both the rate and extent of sporicidal activity of both solutions. Total spore kill was not observed in either solution type over the range of temperatures and contact times examined. There were no observed differences between the sporicidal activities of aqueous and alcoholic taurolidine solutions at all temperatures examined. Ultrasonic energy (50 Hz operating frequency in a 150 W ultrasonic bath in conjunction with increasing temperature allowed to rise naturally from ambient temperature to 41°C over 4 h) enhanced the sporicidal activities of both solution types. However, the difference in activity between the two solution types was not significant. Compared to normal spores, alteration of spore coat layers (hydrogen-form spores) did not alter spore susceptibility to aqueous taurolidine at elevated temperatures of 37°C and 55°C.

Keywords: Bacillus subtilis; Taurolidine; Cidal activity

1. Introduction

Traditionally, sterilisation of pharmaceutical preparations and medical devices may be performed by a variety of methods including moist/dry heat, irradiation, gaseous, filtration or chemical (Denyer, 1987; Russell, 1990). As bacterial spores offer the greatest resistance to any sterilisation procedure, the efficacy of sterilisation is assessed against the ability to destroy bacterial spores. For the sterilisation of heat sensitive equipment, e.g. endoscopes, chemical sterilising solutions are frequently employed (Russell, 1990). Glutaraldehyde is one of the agents of choice for the sterilisation of medical equipment that may be damaged by other methods as it is sporicidal, non-corrosive to metals, rubbers, lenses and other materials (Power and Russell, 1989). However, there is a concern about the potential toxicity associated with the use of glutaraldehyde (Scott and Gorman, 1983).

Taurolin™ is a solution containing 2.0% w/v taurolidine and 5.0% w/v polyvinylpyrrolidone (as

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a solubilising agent) which is available in Western Europe (Jones et al., 1990; Jones et al., 1992). Taurolidine is a broad spectrum, non-antibiotic antimicrobial agent which is also effective in the prevention of endotoxic shock (Browne et al., 1976; Brearley and George, 1980; Myers and Allwood, 1980) and is administered, both therapeutically and prophylactically, usually into the peritoneal cavity and bladder (Jones et al., 1990; Jones et al., 1992).

In solution, taurolidine (a dimer) undergoes hydrolysis to liberate the monomeric form taurultam and formaldehyde, the zero-order kinetics implying that taurolidine acts as a reservoir for the reaction (Woolfson et al., 1988; Jones et al., 1990; Jones et al., 1992). These reports substantiate earlier suggestions (Myers et al., 1980) that taurolidine acts as a 'formaldehyde-releasing agent'.

Taurolin™ exhibits a broad spectrum of antimicrobial activity which includes Gram-positive and Gram-negative bacteria and fungi (Reeves and Schweitzer, 1974; Browne et al., 1976, 1977; Brearley and George, 1980). However, whilst there have been several reports concerning the activity of Taurolin™ against vegetative cells, there have been few reports concerning the activity against bacterial spores (Brearley and George, 1980).

Therefore, the aim of this study was to examine the effects of temperature, solution formulation and/or indirect ultrasonication on the sporicidal activity of taurolidine against normal and hydrogen-form spores of *Bacillus subtilis* NCTC 10073. This organism has been used by us in previous studies (Gorman et al., 1987, 1990).

2. Materials and methods

2.1. Chemicals

Taurolidine was a gift from Geistlich and Sons Limited, Wolhusen, Switzerland. Polyvinylpyrrolidone and Glycine were obtained from Sigma Chemicals Limited, St. Louis, USA.

All other chemicals were AnalaR, or equivalent, grade.

2.2. Preparation of spores

Spores of *B. subtilis* NCTC 10073 were produced on the medium of Beeby and Whitehouse (1965) by incubation at 30°C for 5 days. Following this, the spores were harvested, washed four times with sterile distilled water and incubated in phosphate buffer (0.066 mol/l, pH 7) containing 200 μg/ml lysozyme for 60 min at 37°C. The spores were washed a further two times with sterile distilled water and stored as dense suspensions at 4°C prior to use.

2.3. Preparation of ion-exchange (hydrogen-form) spores

Spores were converted to the hydrogen (H-form) forms as previously described (Kovacs-Proszt and Farkas, 1976; Gorman et al., 1983). In brief, cleaned normal-form spores were incubated in a HCl solution (pH 3.5) at 60°C for 1 h and then at 25°C for 3 h. The hydrogen-form spores were initially washed three times with phosphate buffer (pH 7) and then three times with sterile water to remove any adherent acid.

2.4. Determination of sporicidal activity

To a known volume (9.9 ml) of a 2.0% w/v aqueous or alcoholic (containing 50% v/v isopropyl alcohol) taurolidine solution, which had previously been equilibrated at the required temperature (20, 37, 45 or 55°C), was added 0.1 ml of a standardised spore suspension (normal or hydrogen-form spores). At required time intervals, 1 ml volumes were removed and serially diluted in 1.0% w/v glycine to inactivate excess antimicrobial agent (Jones et al., 1992). The number of colony forming units was determined using the drop-plate method of Miles and Misra (1938) onto nutrient agar plates (Oxoid Ltd.) which were incubated for a minimum of 72 h at 32°C.

Ultrasonic energy was applied to the taurolidine-spore suspensions by immersion in an ultrasonic bath (150 W, nominal operating frequency 50 Hz). Temperature was allowed to rise naturally from ambient (20°C) to 41°C over a 4-h period. Samples were removed at pre-determined intervals

and the number of viable spores surviving determined as above (Gorman et al., 1990).

3. Results

The effects of temperature on the activities of aqueous and alcoholic taurolidine solutions are shown in Fig. 1 and Fig. 2, respectively. Although total spore kill was not observed following immersion in either solution type over the range of temperatures examined (20, 37, 45 and 55°C), kills of approximately 98% and 96% were achieved following 4 h contact at 55°C with aqueous and alcoholic taurolidine, respectively. In general, elevated solution temperature increased the observed sporicidal activity of both aqueous and alcoholic taurolidine solutions. There were no observed differences between the sporicidal activities of aqueous and alcoholic taurolidine solutions at all temperatures examined.

The effects of indirect ultrasonication on the cidal activities of aqueous and alcoholic solutions of taurolidine are shown in Fig. 3. Indirect ultrasonication resulted in a progressive increase in the temperature of bathing solution within the ultrasonic bath from 22–40°C over a 4-h period. Initially, the rate of kill of spores of *B. subtilis* was greater following exposure to the aqueous taurolidine solutions compared to its alcoholic counterpart, however, the extent of kill after 4 h exposure to both solutions was similar.

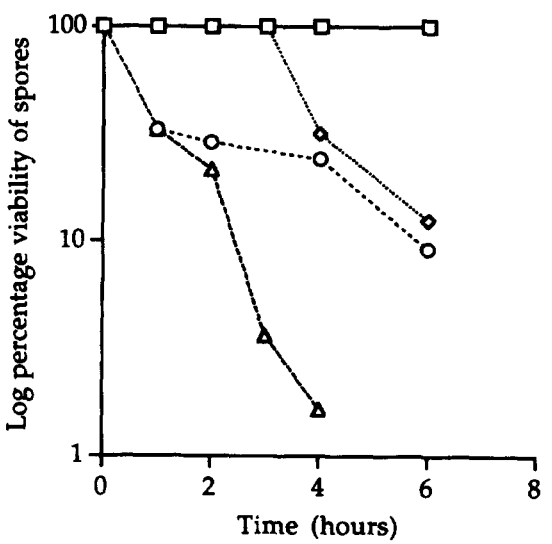

Fig. 1. The effect of temperature on the cidal activity of aqueous taurolidine (2.0% w/v, containing 5% w/v polyvinylpyrrolidone) against spores of *B. subtilis* NCTC 10073. Temperatures, (□) 20°C, (◇) 37°C, (○) 45°C and (△) 55°C.

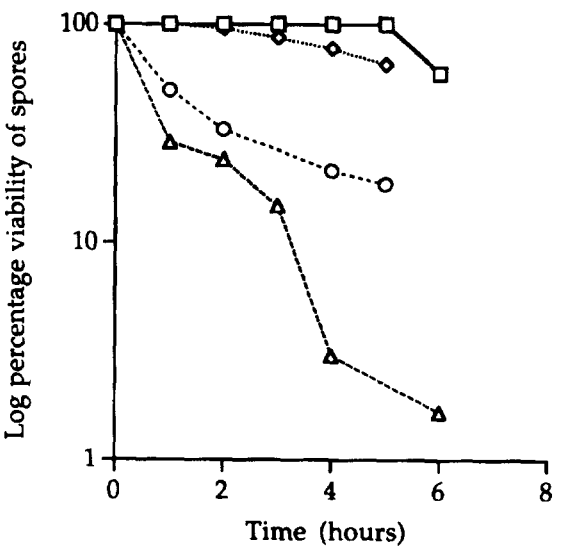

Fig. 2. The effect of temperature on the cidal activity of alcoholic taurolidine (2.0% w/v, dissolved in 50% v/v Isopropyl alcohol) against spores of *B. subtilis* NCTC 10073. Temperatures, (□) 20°C, (◇) 37°C, (○) 45°C and (△) 55°C.

The effect of aqueous taurolidine on the viability of normal and hydrogen-form spores of *B. subtilis* at 37°C and 55°C are shown in Fig. 4. Decreased viability of both spores forms was observed at the higher temperature, although complete spore kill, i.e. no detectable organisms, was not observed for either spore type. Interestingly, at each temperature, the viabilities of both normal and hydrogen-form spores were similar following treatment with taurolidine indicating that this agent did not exhibit preferential cidal activity against either spore form.

4. Discussion

We have previously shown that taurolidine in solution readily hydrolyses to liberate two

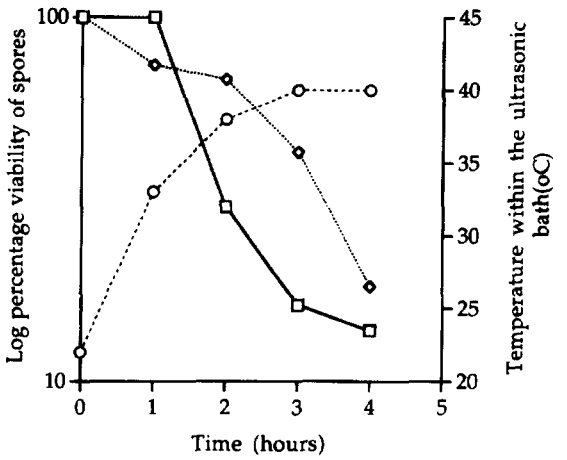

Fig. 3. The effect of indirect ultrasonication on the cidal ectivity of aqueous taurolidine (2.0% w/v, containing 5% w/v polyvinylpyrrolidone) and alcoholic taurolidine (2.0% w/v, dissolved in 50% v/v Isopropyl alcohol) solutions against spores of *B. subtilis* NCTC 10073. Aqueous taurolidine (□), alcoholic taurolidine (◇) and temperature within the ultrasonic bath (○).

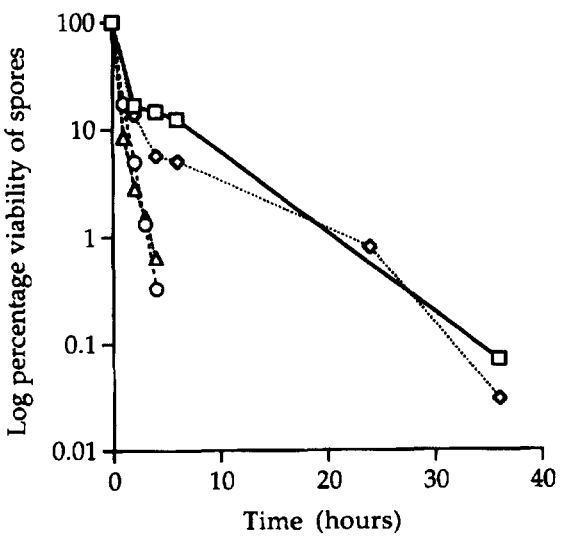

Fig. 4. The effect of temperature (37°C, 55°C) on the cidal activity of aqueous solutions of taurolidine (2% w/v containing 5% w/v polyvinylpyrrolidone) against normal-form and hydrogen-form spores of *B. subtilis* NCTC 10073; Normal-form spores at 37°C (□); hydrogen-form spores at 37°C (◇); normal-form spores at 55°C (○); hydrogen-form spores at 55°C (△).

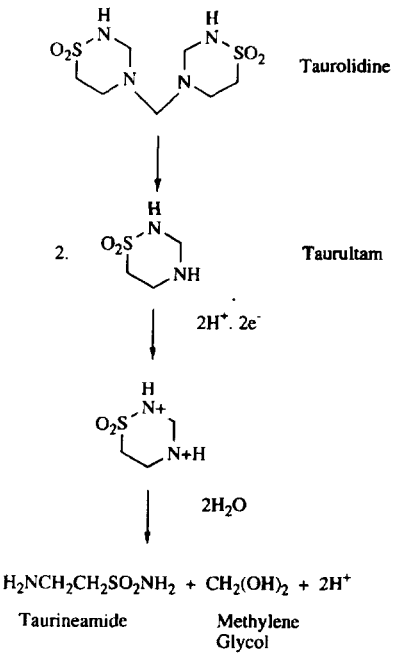

Fig. 5. The equilibria reactions of taurolidine and taurultam.

molecules of taurultam (Fig. 5; Jones et al., 1990; Jones et al., 1992). Under acidic conditions, or following uptake onto the bacterial cell and subsequent diffusion into the periplasmic space, taurultam undergoes further hydrolysis to liberate formaldehyde. Hence, taurolidine has been referred to as a 'formaldehyde-releasing agent', the controlled delivery of which to the bacterial cell may explain, in part, the low toxicity exhibited by Taurolin™ solutions.

Whilst several studies have reported the spectrum of activity of taurolidine against vegetative Gram-positive and Gram-negative bacteria and fungi (Reeves and Schweitzer, 1974; Brearley and George, 1980), there are few reports concerning the activity of this agent against bacterial spores. In one study, Brearley and George (1980) reported a 25% reduction in viability of spores of *Clostridium difficile* after 4 h exposure to Taurolin™ solution at 37°C and, consequently, reported that the sporicidal activity of taurolidine was negligible. In this current study, the viability of spores of *B. subtilis* following exposure to taurolidine (2.0% w/v) was examined over a range

of temperatures. As the solution temperature was increased, in general, there was an increased rate of spore kill. Increased temperature has been previously shown to increase the sporicidal activity of antimicrobial agents, e.g. chlorhexidine gluconate (Shaker et al., 1986; Gorman et al., 1987; Jones et al., 1995) and povidone-iodine (Gorman et al., 1985).

The formulation of antimicrobial agents, e.g. chlorhexidine gluconate and povidone-iodine, into an alcoholic vehicle has been reported to enhance their sporicidal activity (Gorman et al., 1985; Gorman et al., 1987; Jones, 1989). This has been suggested to be due to apparent 'softening' of the spore coat following contact with alcohol, thus allowing increased penetration of the antimicrobial agent to its proposed target site within the spore (Coates and Death, 1978; Gorman et al., 1987). We observed no such enhancement of activity with taurolidine, regardless of solution temperature and, consequently, the sporicidal activities of aqueous taurolidine solutions were similar to those of alcoholic taurolidine solutions. Therefore, the presence of isopropyl alcohol may induce coat softening and hence allow more rapid penetration of taurultam, formed after hydrolysis of taurolidine, into the bacterial spore. However, it is suggested that the rate-limiting step in the sporicidal process is the further hydrolysis of taurultam to liberate formaldehyde. Thus, despite the greater rate of penetration associated with alcoholic solutions of taurolidine, it is suggested that the rate of hydrolysis of taurultam within the spore structure is less than the corresponding reaction within the periplasmic space of *Escherichia coli*, the proposed site of hydrolysis of taurultam in Gram-negative bacteria (Jones et al., 1990; Jones et al., 1992).

There have been several reports concerning the synergy between ultrasonic energy and the sporicidal activity of antimicrobial agents. Sierra and Boucher (1971) reported that spore suspensions of *B. subtilis* were non-viable following immersion in 1% glutaraldehyde and sonication for 5 min whereas 60 min treatment with glutaraldehyde alone was required to exert the same effect. Ahmed and Russell (1975) observed synergy between ultrasonic energy and hydrogen peroxide in the killing of *B. cereus* spores. In a previous publication, we reported marked improvements in sporicidal activity of chlorhexidine gluconate when used in conjunction with either indirect or direct ultrasonic energy (Gorman et al., 1990). Taurolidine solutions, both aqueous and alcoholic, exhibited improved cidal activity against *B. subtilis* spores when used in conjunction with indirect ultrasonic energy, as compared to the sporicidal activities observed at lower temperatures (20°C and 37°C).

Acidic conditions have been reported to hydrolyse the monomer taurultam to formaldehyde and, thus, are of importance to the anti-microbial activity of taurolidine (Jones et al., 1990; Jones et al., 1992). Consequently, it was of interest to examine the sporicidal activity of taurolidine against hydrogen-form spores prepared by ion-exchange and providing hydrogen ions for reaction with taurultam molecules. Interestingly, for the normal and hydrogen spore-forms, there were no observed differences in the activities of aqueous and alcoholic taurolidine, at either 37°C or 55°C. This would suggest that either the rate of hydrolysis of taurultam at the surface of the hydrogen-form spores is relatively slow or, alternatively, the hydrogen-form spore is unable to deliver hydronium ions to the adsorbed molecules of taurultam in a form which will ensure enhanced hydrolysis when compared to the normal spores.

This study shows that taurolidine exhibits moderate sporicidal activity when used in conjunction with elevated temperatures and indirect ultrasonic energy. However, this activity was not enhanced by reformulation into an alcoholic solution. Further increases in the rate and extent of sporicidal activity are required before consideration of taurolidine for use in the 'cold' liquid chemosterilisation of delicate or thermolabile materials.

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