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INVESTIGATIVE REPORT

The *In vitro* Activity of Pentane-1,5-diol against Aerobic Bacteria. A New Antimicrobial Agent for Topical Usage?

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Multi-resistance to antibiotic therapy and to biocides is becoming increasingly common, which has led to mounting concern worldwide regarding the future use of traditional antimicrobials. Diols or glycols also have antimicrobial effects. Pentane-1,5-diol has low oral toxicity, is essentially non-irritating to the skin and has high antimicrobial activities against bacteria, fungi and viruses. The effect of pentane-1,5-diol against both sensitive and multi-resistant Gram-positive and Gram-negative bacteria was tested *in vitro* against 85 bacterial strains showing minimal inhibitory concentrations in the range of 2.5 to 15.0% (vol/vol) against both antibiotic-susceptible and multi-resistant aerobic bacteria. The exact mechanism of action is unknown but probably pentane-1,5-diol sucks water out of the bacterial cells which then collapse, a mechanism to which it is probably very difficult to develop resistance. The high activity against multi-resistant bacteria makes pentane-1,5-diol an interesting new compound for topical antimicrobial therapy in humans. *Key words: diols; antimicrobial agents; antibiotics; multi-resistance.*

Worldwide, there are increasing problems with multi-resistant bacteria. Such problems are particularly evident in hospitals, where they frequently present as nosocomial outbreaks. The most important nosocomial resistance today is caused by methicillin-resistant *Staphylococcus aureus* (MRSA) (1, 2), vancomycin-resistant enterococci (VRE) (3, 4) and Enterobacteriaceae with extended-spectrum beta-lactamases (5, 6). In addition, MRSA can also be resistant to aminoglycosides, often also to fluoroquinolones, and most other antibiotics (7, 8). Some strains are even becoming resistant to the glycopeptides (7). These multi-resistant clones of *S. aureus* often occur as epidemic strains. Moreover, the VRE are also appearing as multi-resistant strains which are often resistant to almost all antibiotics (3, 4). These bacteria are mainly spread by person-to-person contact (9, 10). Recently clonal spread of one strain of *S. aureus* resistant to fusidic acid has been observed in Sweden, Denmark and Norway,

mainly among patients with bullous impetigo (11, 12). There are also reports of fusidic acid-resistant *S. aureus* from the UK, Canada and Japan (13). Patients with regular impetigo, atopic dermatitis and other skin infections may also harbour *S. aureus* strains resistant to fusidic acid (13, 14). International spread of *S. aureus* strains resistant to mupirocin has also been reported (2).

The use of non-antibiotic antimicrobial agents or biocides might be an alternative to antibiotics but may pose problems (15–17). The development of bacterial resistance to anilides (e.g. triclocarban) (15–17), bis-phenols (e.g. triclosan) (15–19), quaternary ammonium compounds (primarily chlorhexidine chloride and cetrimide) (15–21), iodine and benzalkonium (15–18) has been described. However, biocides also act non-specifically and against broader targets than antibiotics. Agents such as alcohol and chlorhexidine produce a denaturation of cytoplasma proteins and coagulation of cell contents (15). The bactericidal action of biocidal agents that exhibit surface-active properties, such as the quaternary ammonium compounds, and phenols, results from a generalized disruption of the cell membrane (15). Bacteria that are resistant to both biocides and antibiotics have been found, for example, in patients with leg ulcers (15, 18).

Diols or glycols are used as solvents, as anti-freezing agents or as vehicles in pharmaceutical preparations and some of them have antimicrobial effects (22–24). So far propane-1,2-diol (propylene glycol) is the only diol widely used in clinical dermatology. It is used in the treatment of patients with pityriasis versicolor, *Pityrosporum* folliculitis and seborrhoeic dermatitis (25).

Pentane-1,5-diol is active against herpes virus (EP 0 479 850 B1 and US patent 5, 369, 129). The activity against various bacteria and fungi, compared with that of propane-1,2-diol, is also described in these patents. Pentane-1,5-diol was two to three times more active than propane-1,2-diol. An *in vitro* laboratory study of certain diols showed that the antimycotic activity of diols or glycols was increased with an increasing length of the carbon chain (24). There are a few old reports of the activity of diols, including pentane-1,5-diol against non-pathogenic bacteria (26). However, it is not possible to draw any clear conclusions from these reports.

The aim of the present investigation was to study the antibacterial activity *in vitro* of pentane-1,5-diol against antibiotic-susceptible and resistant aerobic bacteria.

MATERIALS AND METHODS

Bacterial strains

Seventy recent clinical isolates and 15 strains from the Culture Collection University of Gothenburg (CCUG), Sweden (www.ccug.gu.se) were tested. The strains belonged to 11 bacterial groups: methicillin-susceptible *S. aureus* (MSSA), MRSA, coagulase-negative staphylococci, alpha-haemolytic streptococci, beta-haemolytic streptococci, enterococci, *Escherichia coli*, *Enterobacter* spp., *Acinetobacter* spp., *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia*. The following strains from the CCUG were tested: MRSA 33115, 38266, 41787, 45007, 45008, 46463, 46618, 46740, 46870, 47019 and *Enterococcus faecium van A* 37832, 39128, 43324, *van B* 37593. One CCUG strain of *P. aeruginosa* was also included: 17619.

Minimum inhibitory concentrations

The minimal inhibitory concentrations (MICs) were determined using the agar dilution method according to the SRGA (www.srga.org). Pentane-1,5-diol with a purity of 98.5% was obtained from Merck Schuchardt, Hohenbrunn, Germany. Pentane-1,5-diol was added to Paper Dish Method agar medium (AB Biodisk, Solna, Sweden) giving final concentrations of 2.5, 5.0. 7.5, 10.0, 12.5, 15.0, 17.5 and 20.0% (vol/vol). For growth of streptococci the medium was supplemented with 5% defibrinated horse blood. The plates were inoculated using a multipoint inoculator A 400 (Denley, Sussex, UK). The inoculum effect was tested at 10^3 and 10^5 colony forming units (cfu)/spot. Incubations were done overnight at 35–37℃.

RESULTS

Pentane-1,5-diol was effective against all bacterial strains tested with MICs ranging from 2.5 to 15.0% using an inoculum of 10^3 cfu (Table I). The lowest value of 2.5% was found for two strains of *Acinetobacter* spp. and one strain of *S. maltophilia*. The highest value was seen in only one strain of a coagulase-negative *Staphylococcus*. With a higher inoculum of 10^5 cfu the MIC generally increased one step, e.g. from 7.5% to 10.0% (data not shown). MICs were higher for the staphylococci and lowest for the enterococci and Gram-negative rods. There was no difference in activity of pentane-1,5-diol against antibiotic-sensitive and -resistant bacteria (Table I).

DISCUSSION

Infections caused by multi-resistant bacteria have become a major problem in health care (1, 2, 9, 10, 27). In order to manage such bacterial infections in health care and to provide well tolerated alternatives for topical antimicrobial treatment in humans and other mammals, treatments and solutions for antiseptic use with compositions containing antimicrobial diols provide a new opportunity.

Pentane-1,5-diol ($C_5H_{12}O_2$) is a viscous oily liquid (22). Its molecular weight is 104.15. Freezing point is −18℃, boiling point is 238–240℃ and flash point is 125℃. The specific gravity is 0.9925. Pentane-1,5-diol

Table I. *Minimum inhibitory concentrations (% vol/vol) of pentane-1,5-diol to 85 bacterial strains tested by agar dilution technique at 10^3 cfu/spot.*

Bacterial species	n	Mean (range)	Median
Staphylococcus aureus, MSSA	11	12.0 (10.0–12.5)	12.5
Staphylococcus aureus, MRSA	10	11.0 (7.5–12.5)	12.5
Coagulase-negative staphylococci	10	8.8 (5.0–15.0)	7.5
Alpha-haemolytic streptococci	5	6.5 (5.0–7.5)	7.5
Beta-haemolytic streptococci			
group A	5	7.5 (7.5)	7.5
group C	3	9.2 (7.5–10.0)	10.0
group G	5	8.0 (7.5–10.0)	7.5
Enterococcus spp.	9	6.4 (5.0–7.5)	7.5
Escherichia coli	5	5.0 (5.0)	5.0
Enterobacter spp.	5	5.0 (5.0)	5.0
Acinetobacter spp.	6	4.2 (2.5–5.0)	5.0
Pseudomonas aeruginosa	5	6.0 (5.0–10.0)	5.0
Stenotrophomonas maltophilia	5	4.5 (2.5–5.0)	5.0
All strains	85	7.7 (2.5–15.0)	7.5

MSSA, methicillin-susceptible *S. aureus*; MRSA, methicillin-resistant *S. aureus*.

is miscible with water, methanol, ethanol, acetone, ethyl acetate and ether. There is limited solubility in benzene, trichloroethylene, methylene chloride, petroleum ether and heptane. Pentane-1,5-diol is used as plasticizer in cellulose products and adhesives, in dental composites and in brake fluid compositions (22, 23, 26). It is also used as a preservative for grain (28). Pentane-1,5-diol has a low oral toxicity; LD_{50} for rats is 5.89 g/kg and LD_{50} for rabbits is greater than 20 ml/kg (22, 23). It is essentially non-irritating to the skin and only very mildly irritating to the eyes (22, 23). The metabolism of pentane-1,5-diol was studied in four rabbits (23). The animals were given 8.5 g of oral pentane-1,5-diol. There was no presence of any unchanged pentane-1,5-diol in the urine. However, small amounts of glutaric acid as a metabolic product were found in the urine when the rabbits were given pentane-1,5-diol. Glutaric acid was quickly metabolized to carbon dioxide (29).

The results obtained in this study demonstrate that pentane-1,5-diol is effective *in vitro* against several different groups of bacteria including multi-resistant bacteria. The inoculum effect tested at 10^3 and 10^5 cfu/ spot is low.

In a cream formulation, successfully used in the treatment of patients with atopic dermatitis (unpublished data), we have incorporated 25% of pentane-1, 5-diol. The exact mechanism of action of pentane-1, 5-diol is unknown but like polyethylene glycol it may act by sucking water out of the cells, a mechanism to which it possibly is very difficult to develop resistance.

ACKNOWLEDGEMENT

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