Cationic compounds with activity against multidrug-resistant bacteria: interest of a new compound compared with two older antiseptics, hexamidine and chlorhexidine

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Abstract

Use of antiseptics and disinfectants is essential in infection control practices in hospital and other healthcare settings. In this study, the *in vitro* activity of a new promising compound, para-guanidinoethylcalix[4]arene (Cx1), has been evaluated in comparison with hexamidine (HX) and chlorhexidine (CHX), two older cationic antiseptics. The MICs for 69 clinical isolates comprising methicillin-resistant *Staphylococcus aureus*, methicillin-sensitive *S. aureus*, coagulase-negative staphylococci (CoNS) (with or without *mecA*), vancomycin-resistant enterococci, *Enterobacteriaceae* producing various β-lactamases and non-fermenting bacilli (*Pseudomonas aeruginosa, Acinetobacter baumanii, Stenotrophomonas maltophilia*) were determined. Cx1 showed similar activity against *S. aureus*, CoNS and *Enterococcus* spp., irrespective of the presence of *mecA* or *van* genes, or associated resistance genes, with very good activity against CoNS (MIC <1 mg/L). Variable activities were observed against *Enterobacteriaceae*; the MICs determined seemed to be dependent both on the genus (MICs of 2, 8 and 64 mg/L for *Escherichia coli*, *Klebsiella pneumoniae* and *Yersinia enterocolitica*, respectively) and on the resistance phenotype production of [Extended Spectrum β-Lactase (ESBLs) or other β-lactamases; overproduction of AmpC]. Poor activity was found against non-fermenting bacilli, irrespective of the resistance phenotype. CHX appeared to be the most active compound against all strains, with broad-spectrum and conserved activity against multidrug-resistant strains. HX showed a lower activity, essentially against Gram-positive strains. Consequently, the differences observed with respect to Cx1 suggest that they are certainly not the consequence of antibiotic resistance phenotypes, but rather the result of membrane composition modifications (e.g. of lipopolysaccharide), or of the presence of (activated) efflux-pumps. These results raise the possibility that Cx1 may be a potent new antibacterial agent of somewhat lower activity but significantly lower toxicity than CHX.

Keywords: Antiseptics, calixarene, cationic compounds, chlorhexidine, hexamidine, multidrug-resistant bacteria, nosocomial pathogens
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Introduction

Quaternary ammonium compounds (QAC) such as benzalkonium chloride, bisbiguanides such as chlorhexidine (CHX), polymeric biguanides such as polyhexamethylene biguanide (PHMB) and diamidines such as hexamidine (HX) have been widely used for over half a century [1]. Due to their intrinsic positive charge, these cationic compounds bind with high affinity to the negatively charged cell walls and membranes of bacteria, and disruption is brought about by perturbations of the binding sites [2]. Biocides are clearly different from antibiotics with respect to their (i) mode of action, (ii) condition of use, and (iii) acquired and intrinsic mechanisms by which bacteria resist their effects. However, intensive exposure of hospital pathogens to biocides, similar to that of antibiotics, may result in the emergence of—often associated—resistance to these agents [3]. For example, *qac* genes (which confer resistance to quaternary ammonium compounds) are often found in *Staphylococcus aureus* strains carrying *mecA* genes or the β-lactamase gene *blaZ*, on transposon Tn*552* [4,5]. The progressive reduction of the therapeutic effectiveness of the available antibiotics and antiseptics as a result of the spread of antimicrobial resistance underlines (i) the

necessity to evaluate the efficiency of available antiseptics, and (ii) the urgency of the development of new classes of drugs for the treatment of infectious diseases. A major challenge is to find drugs that act against multiple multidrug-resistant strains.

The antimicrobial activity of a new antibacterial drug, *para*-guanidinoethylcalix[4]arene (Cx1), has been tested and is presented here. This lead compound is a novel member of the family of cationic antibacterial compounds; it is a calixarene-based compound with four guanidinium functions, which may interact with the negatively charged bacterial cell wall. Cx1 shows high water solubility, with broad *in vitro* activity against Gram-positive and Gram-negative bacteria [6]. Moreover, it is devoid of cytotoxicity against two eukaryotic cell lines, HaCaT and MRC-5. By contrast HX and CHX show effects on cell viability after only 24 h exposure [6; M. Grare and R. E. Duval, unpublished data].

The purpose of this study was: (i) to extend knowledge about the *in vitro* activities of two widely used antiseptics, HX and CHX, by testing them against 39 multidrug-resistant Gram-positive bacteria [15 *S. aureus*, methicillin-resistant *S. aureus* (MRSA) or methicillin-sensitive *S. aureus* (MSSA), 12 coagulase-negative staphylococci (CoNS), resistant or susceptible to methicillin, 14 *Enterococcus* spp., with or without *van* genes] and 30 multidrug-resistant Gram-negative bacteria (20 *Enterobacteriaceae*, with or without ESBL, and ten non-fermenting bacilli); and (ii) to investigate the potential of a new antibacterial drug, named Cx1, against these pathogens.

Materials and Methods

Bacterial strains

Escherichia coli ATCC 25922, *S. aureus* ATCC 25923 and ATCC 29213, *E. faecalis* ATCC 29212 and *Pseudomonas aeruginosa* ATCC 27853 were used as reference strains following guidelines of the CLSI (formerly NCCLS) [7] and of the Comité de l'Antibiogramme de la Société Française de Microbiologie [8]. Other reference strains were chosen to represent susceptible strains corresponding to resistant clinical isolates tested: *Proteus mirabilis* ATCC 43071, *Klebsiella oxytoca* ATCC 700324, *Providencia stuartii* ATCC 33672, *Yersinia enterocolitica* ATCC 9610, *Acinetobacter baumanii* ATCC 19606, *S. epidermidis* ATCC 12228 and *Streptococcus pneumoniae* ATCC 49619. Also included were two VISA strains (Mu3 and Mu50) [9].

Sixty-nine clinical isolates were collected from University Hospital of Nancy: (i) 39 multidrug-resistant Gram-positive isolates including three MSSA, ten MRSA, 12 CoNS resistant (n = 10) or not resistant (n = 2) to methicillin and 14 *Enterococcus* spp., with or without *van* genes; (ii) 30 multidrug-resistant Gram-negative isolates including 20 ESBL-producing or -non-producing isolates of *Enterobacteriaceae* and 10 non-fermenting bacilli.

Each isolate was from a different patient, and each was judged to be clinically significant when it was first recovered. Isolates were selected on the basis of their antimicrobial susceptibility profile. Antimicrobial resistances were determined by the automated instrument VITEK2 (bioMerieux, Marcy L'Etoile, France). The presence of resistance genes was investigated by PCR multiplex analysis adapted from methods previously described by Dutka-Malen *et al.* [10] for *van* genes, or Del Vecchio *et al.* [11] for *mecA* genes. Strains were grown on Mueller Hinton agar (BD, 225250) or in Mueller Hinton broth (MHB) (BD, 275730), complemented with 5% lysed sheep blood for the streptococci.

Antimicrobial agents

Three drugs were tested: hexamidine diisethionate (FW = 668.22; compound 1), chlorhexidine digluconate (FW = 897.74; compound 2), and *para*-guanidinoethylcalix[4]arene (FW = 1221.1; compound 3) (Fig.1.)

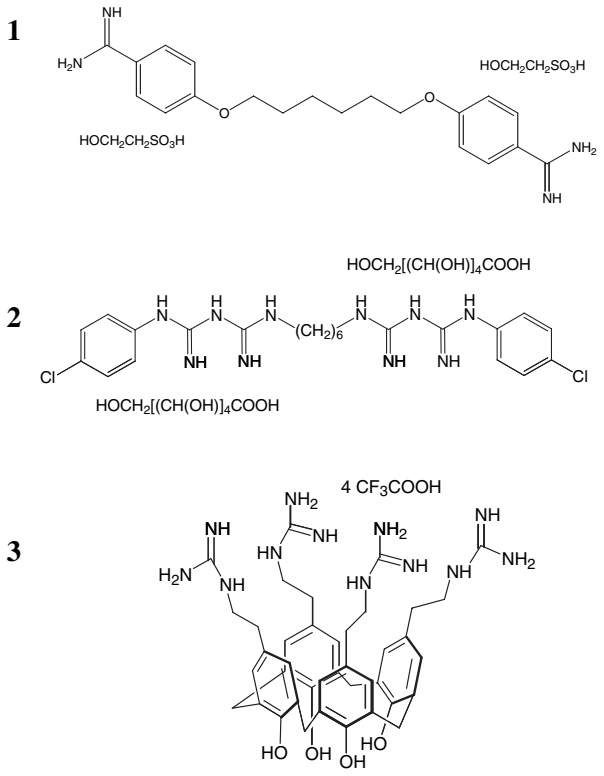

FIG. 1. Chemical structure of: (1) hexamidine diisethionate (HX); (2) chlorhexidine digluconate (CHX); and (3) para-guanidinoethylcalix[4]arene (Cx1).