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REVIEW

Bacterial resistance to silver in wound care

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Summary Ionic silver exhibits antimicrobial activity against a broad range of micro-organisms. As a consequence, silver is included in many commercially available healthcare products. The use of silver is increasing rapidly in the field of wound care, and a wide variety of silver-containing dressings are now commonplace (e.g. Hydrofiber® dressing, polyurethane foams and gauzes). However, concerns associated with the overuse of silver and the consequent emergence of bacterial resistance are being raised. The current understanding of the biochemical and molecular basis behind silver resistance has been documented since 1998. Despite the sporadic evidence of bacterial resistance to silver, there have been very few studies undertaken and documented to ascertain its prevalence. The risks of antibacterial resistance developing from the use of biocides may well have been overstated. It is proposed that hygiene should be emphasized and targeted towards those applications that have demonstrable benefits in wound care. It is the purpose of this review to assess the likelihood of widespread resistance to silver and the potential for silver to induce cross-resistance to antibiotics, in light of its increasing usage within the healthcare setting.

Introduction

Ionic silver (Ag^+) is considered to be effective against a broad range of micro-organisms, with low concentrations documented to have therapeutic activity.[1,2] Silver has been described as being 'oligodynamic' because of its ability to exert a bactericidal effect at minute concentrations.[3] Consequently, a large number of healthcare products now contain silver, principally due to its antimicrobial activities and low toxicity to human cells. Such products include silver-coated catheters,[4,5] municipal water systems[6,7] and wound dressings.[8]

Wounds often provide a favourable environment for the colonization of micro-organisms.[6,8–10] In order to improve the opportunity for wound healing, it is important to create conditions that are unfavourable to micro-organisms and

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favourable for the host repair mechanisms, and topical antimicrobial agents are believed to facilitate this process. Antiseptic agents are now considered for the treatment of localized skin and wound infections because they have a lower propensity to induce bacterial resistance than antibiotics. One example of the early use of silver in wound care is silver sulphadiazine (AgSD) cream, developed in the 1960s, for the treatment of burns. Recently, a trend towards the use of wound cover dressings that contain silver has been evident, and today, a selection of foam, film, hydrocolloid, gauze and dressings with Hydrofiber® technology impregnated with silver are commercially available. However, concerns are being expressed regarding the overuse of silver and the possible emergence of bacterial resistance to silver, particularly within the clinical environment.[11,12] Silver-resistant bacteria have been reported since 1975[13-22] and research within this area is clearly increasing.[23] A preliminary understanding of the genetics underlying silver resistance has been known since 1998,[24,25] with a greater understanding of the biochemistry documented a year later.[26] Clinical evidence of silver-resistant bacteria has been principally in hospitals, specifically in burns wards, where silver salts (in the form of silver nitrate) are used as antiseptic agents.[13,27]

Many clinicians and researchers have questioned whether the widespread usage of silver could lead to cross-resistance to antibiotics, as has been suggested with a number of biocides, specifically triclosan, chlorhexidine and quaternary ammonium compounds (QACs).[28,29] However, in reference to the available evidence to date, this appears to represent an unjustifiable concern.

It is the purpose of this review to assess the likelihood of widespread resistance to silver and the potential for silver to induce cross-resistance to antibiotics, in light of its increasing usage within the healthcare setting.

Wound microbiology and antimicrobial agents

Wounds often provide a favourable environment for the colonization of micro-organisms which may both delay healing and cause infection. Bacteria found in wounds originate primarily from the mouth and colon, and constitute a unique collection of organisms that are potentially pathogenic. Consequently, broad-spectrum antimicrobial agents are required to control these mixed species populations to minimize the opportunity for infection. This has been reflected in the increased usage of silver in wounds, principally due to the fact that silver is relatively safe and exhibits broad-spectrum antimicrobial activity.[30-34]

Mode of action of Ag^+

In bacteria, silver ions are known to react with nucleophilic amino acid residues in proteins, and attach to sulphydryl, amino, imidazole, phosphate and carboxyl groups of membrane or enzyme proteins that leads to protein denaturation.[1,19,35] Silver is also known to inhibit a number of oxidative enzymes such as yeast alcohol dehydrogenase,[36] the uptake of succinate by membrane vesicles[37] and the respiratory chain of *Escherichia coli*, as well as causing metabolite efflux[38] and interfering with DNA replication.[1] One of the primary targets of Ag^+, specifically at low concentrations, appears to be the Na^+-translocating NADH:ubiquinone oxidoreductase system.[39,40] Silver has also been shown to be associated with the cell wall,[41] cytoplasm and the cell envelope.[42] Chappell and Greville[43] acknowledged that low levels of Ag^+ collapsed the proton motive force on the membrane of bacteria, and this was reinforced by Mitchell's work in 1961[44] and 1966.[45] Later work by Dibrov *et al.*[46] showed that low concentrations of Ag^+ induced a massive proton leakage through the bacterial membrane, resulting in complete de-energization and, ultimately, cell death. Overall, there is consensus that surface binding and damage to membrane function are the most important mechanisms for the killing of bacteria by Ag^+.[3] The reader is directed to a review paper which addresses the antimicrobial actions of silver compounds by Clement and Jarrett.[3]

In a complex biological system such as wound fluid, the maximum level of free (available) Ag^+ is approximately 1 μg/mL (above this level, Ag^+ complexes with anions such as chloride to form minimally soluble and inactive salt). Chloride ions present in the exudate of acute and chronic wounds affect the availability of ionic silver. The solubility of silver chloride is 14 μM[47] and AgSD is 3 mM.[48] In the presence of excess amounts of sodium chloride, the concentration of free Ag^+ is lowered to approximately 2 nM, regardless of the concentrations of silver nitrate added.[49] Experiments by Gupta *et al.*[50] have shown chlorides to have three levels of effect on the availability of Ag^+. Within external environments, Ag^+ binds strongly to the bacterial cell surface with toxic effects, often inhibiting the bacterial respiratory transport

chain.[51] Ag^+ is active against bacteria, fungi and viral pathogens at concentrations of 10^{-9} to 10^{-6} M.[1]

Bacterial resistance

General resistance mechanisms

Resistance to an antimicrobial agent can occur either by 'intrinsic' or 'acquired' mechanisms. Acquired resistance can arise by either mutation or the acquisition of various types of genetic material in the form of plasmids, transposons and self-replicating extra-chromosomal DNA.[52] Acquired resistance to a wide range of antibiotics has been observed in a variety of micro-organisms.[53] Intrinsic resistance is a phenotype demonstrated by micro-organisms before the use of an antimicrobial agent, i.e. a natural resistance property of an organism. Intrinsic resistance to antimicrobial agents can be provided by a number of mechanisms including the nature and composition of the bacterial cell wall that may act as a permeability barrier, thus reducing uptake of the compound, and also by constitutively synthesized enzymes that may bring about degradation of a compound.[52]

Biocides (such as silver) and antibiotics have differing modes of action. Biocides tend to target multiple sites on or within bacterial cells and hence have broad-spectrum activity. Antibiotics tend to target specific sites on or within a bacterial cell and have a narrower spectrum of activity. Antiseptic or biocide resistance can be acquired via mutations in normal cellular genes, plasmids or transposons.[54] Plasmid-mediated biocide resistance has been documented[55,56] as occurring in *Staphylococcus aureus*, coagulase-negative staphylococci, members of the Enterobacteriaceae and *Pseudomonas* spp.[57-61] The vast majority of biocides act on cell-surface components of the bacteria and/or the cytoplasmic membrane. Therefore, intrinsic resistance would involve natural resistance via the structure of the cell surface and its chemical composition.[62]

In comparison with bacteria, very little is known about the ways in which fungi can circumvent the action of biocides.[52] Resistance has been observed to arise from either inherent or acquired resistance, and chlorhexidine-resistance has been found in strains of *Saccharomyces cerevisiae*.[52] Similarly, biocide resistance to anaerobic bacteria has been poorly researched, indicating that resistance to these compounds is not widespread.

Transposons and integrons significantly enhance the development of transferrable resistance due to their ability to move from plasmid to chromosome and subsequently pass resistance on to daughter cells. Transposons and integrons have also been found to possess a number of genetic determinants encoding resistance to QACs and heavy metals. Selective pressure, which is exerted by one antimicrobial agent, is likely to maintain co-resistance phenotypes mediated by adjacent genes. Therefore, it is possible that exposing integron-carrying bacteria to residual concentrations of biocides may encourage antibiotic resistance.[63] However, further investigations are warranted in this area and at present it remains speculation.

Chromosomal mutations to antibiotics have been recognized for decades. However, fewer studies have been performed to determine whether mutation confers resistance to biocides.[52] An Ag^+ resistance determinant in Enterobacteriaceae has recently been cloned and sequenced, but none has yet been identified in Gram-positive bacteria despite staphylococci and other Gram-positive bacteria being exposed to silver compounds in clinical use.[1] In the hospital environment, it has been suggested that rather than plasmid-mediated resistance, continual exposure to sub-inhibitory antibiotic concentrations may cause subtle changes to the bacterial outer structure stimulating cell-to-cell contact.[52] However, it remains to be determined if residual concentrations of antiseptics in clinical situations could produce the same effect.[52,64]

Silver[23] suggested that it is possible that the widespread and uncontrolled use of Ag^+ may result in more bacteria developing resistance. However, the probability of transfer of silver resistance genes is considered to be low,[13,14] unstable and difficult to maintain[15,22] and transfer.[13,14] The frequency of occurrence of Ag^+ resistance has been shown to be variable,[65] with the conditions for distinguishing between Ag^+ resistance and Ag^+ sensitivity being poorly understood.[3,23] Examples of silver-resistant bacterial strains that have been isolated include *E. coli*,[15] *Enterobacter cloacae*,[15] *Klebsiella pneumoniae*,[15] *Acinetobacter baumannii*,[22] *Salmonella typhimurium*[13] and *Pseudomonas stutzeri*.[18]

Bacterial resistance to heavy metal ions can result from energy-dependent ion efflux systems rather than chemical detoxification. In Gram-negative bacteria, biocides are blocked from reaching targets in the cell by the outer membrane (OM) and active efflux mechanisms.[66,67] With reference to OMs, Pugsley and Schnaitman[68] documented that *E. coli* mutants that lacked the OM porins were more resistant to Ag^+. Li *et al.*[49] also suggested

that active efflux may play a major role in Ag^+ resistance, which was likely to be enhanced synergistically by decreases in OM permeability. Efflux pumps are composed of proteins either as an ATPase or chemi-osmotic cation/proton antiporter,[69-71] and Ag^+ has been associated with both of these mechanisms.[72]

Plasmid-mediated Ag^+ resistance has been identified in *P. stutzeri*, members of the Enterobacteriaceae and *Citrobacter* spp., although the mechanism of resistance has yet to be elucidated.[52] It has been documented that bacteria with silver-resistance plasmids accumulate less Ag^+ than the susceptible strains.[22] This observation was determined to be a non-active efflux process. A plasmid conferring resistance to a number of antibiotics and heavy metals including Ag^+ was obtained from a *Salmonella* species isolated from a burns unit after causing septicaemia, death in three patients and resulting in the closure of the burns unit at Massachusetts General Hospital.[72] This was the first report of the genetic and molecular basis for Ag^+ resistance. The plasmid, named pMG101, was 180 kb in size and conferred resistance to Hg^{2+}, tellurite and several antibiotics, together with Ag^+.[72] The region of pMG101 responsible for increased resistance to silver was cloned and sequenced. The gene cluster for Ag^+ resistance was found to contain nine genes,[23] seven of these were named and two were classified as open reading frames (*silP*, *ORF105*, *sil AB*, *ORF96*, *silC*, *silSR* and *silE*). For a more detailed description of how these genes function, the reader is directed to the paper by Silver.[23]

Laboratory studies have provided evidence of plasmid pMG101 transfer to *E. coli*. In fact, Ag^+ resistance conferred by plasmid pMG101 enabled the growth of *E. coli* in more than 0.6 mM Ag^+, which is in excess of six times the tolerable concentration for sensitive *E. coli*.[73] Plasmid pMG101 is known to encode a number of periplasmic and Ag^+-specific binding proteins.

Link between Ag^+ usage, resistance and antibiotics

Ag^+ resistance is most likely to be found in environments where greatest Ag^+ usage of silver-containing products might be expected, such as in the dental setting where amalgams are known to contain 35% silver,[74] burns units in hospitals[75] or the use of silver-coated catheters.[4]

Some biocides disrupt cellular targets, and subsequent mutations in these targets may confer low-level cross-resistance to certain antibiotics used in humans. Whilst a number of laboratory-based studies have indicated a possible association between bacterial resistance to biocides and cross-resistance to antibiotics, solid evidence is lacking. As such, this area remains contentious.

There does, however, appear to be some similarity between bacterial resistance to antibiotics and antiseptics. A study by Akimitsu *et al.*[76] reported methicillin-resistant *Staphylococcus aureus* mutants resistant to benzalkonium chloride that exhibited increased resistance to various beta-lactam antibiotics compared with the parent strain. Yamamoto *et al.*[77] characterized a multi-resistant plasmid of *S. aureus* that conferred resistance to kanamycin, gentamicin, tobramycin, amikacin, acriflavin and ethidium bromide, but also to benzalkonium chloride and chlorhexidine. Suller and Russell[78] investigated possible links between triclosan and antibiotic resistance in *S. aureus*. They isolated a *S. aureus* mutant that exhibited increased resistance to triclosan, but it did not display increased resistance to any antibiotic tested. Chuanchen *et al.*[79] have shown that triclosan is a substrate for three distinct efflux pumps in *Pseudomonas aeruginosa*. Research has shown that exposure to triclosan can select for multi-resistant mutants via upregulation of these same efflux pumps. *P. aeruginosa* deletion mutants defective in the efflux pumps became susceptible to triclosan. From this research, it is apparent that exposure to antibiotics and triclosan can select for multi-resistant bacterial pathogens via overexpression of identical multi-drug efflux systems. Studies by Al-Masaudi *et al.*[80] and Rutala *et al.*[81] have shown that hospital strains of antibiotic-resistant bacteria do not display increased resistance to biocides, and that *S. aureus*, after exposure to biocides, does not increase the transfer of antibiotic-resistant plasmids.

Efflux pumps which underlie intrinsic antibiotic resistance can also contribute to biocide resistance. One of these efflux systems is found in *E. coli*, *acr*AB locus[82,83] and the *P. aeruginosa mex*AB, *mex*CD and *mex*EF efflux systems.[84] Moken *et al.*[82] demonstrated that deletion of the *acr*AB efflux locus in pine-oil-resistant *E. coli* and in *E. coli mar* (multi-antibiotic-resistant) mutants resulted in a 10-fold increase in susceptibility to dimethyl benzyl ammonium chloride (quaternary amine) and chloroxylenol (phenol). It was found from this study and others that broad-spectrum efflux pumps can mediate resistance for both biocides and antibiotics.[83]

It is evident that bacteria tolerate biocides and antibiotics by employing the same types of cellular

mechanism. Activation of the same efflux pumps can result in decreased bacterial susceptibility to both antibiotics and biocides. Enzymatic modification and destruction are commonly used by bacterial resistance mechanisms.

Levy[85] stated that it is probable that the increasing use of biocides will eventually result in the selection of bacteria that are less susceptible. In fact, bacterial adaptation and resistance to biocides is certainly not a new phenomenon.[86] Furthermore, the contribution of biocides to the development of bacterial antibiotic resistance has yet to be fully elucidated. Additional research is required to examine the modes of action of biocides[87,88] and bacterial biocide resistance mechanisms, as well as to better characterize potential cross-resistance with antibiotics.

It has been shown that most interactions between chemotherapeutic agents and microbial populations occur at very low concentrations,[73] and that low concentrations produce a substantial stress in bacterial populations that eventually influences the rate of variation and the diversity of adaptive responses leading to high levels of resistance.[89] To date, it is unclear whether the use of heavy metals, such as Ag^+, is contributing to the emergence and spread of antibiotic-resistant bacteria; however, this is unlikely.[89] A recent paper by Cole *et al*.[91] concluded that there was a lack of antibiotic and antiseptic agent cross-resistance in target bacteria from the homes of antibacterial product users and non-users, as well as increased prevalence of potential pathogens in non-user homes. This study 'refutes widely, yet unsupported, hypotheses that use of antibacterial products facilitates the development of antibiotic resistance in bacteria'.[90] Gilbert and McBain's paper in 2004[91] concluded that the risks associated with the overuse of biocides has been overstated, and that it is now imperative that confidence is restored in products that form an essential part of domestic and hospital hygiene.

Use of Ag^+ in wound care

Silver has been used extensively for the treatment of burns,[92,93] with AgSD incorporated into bandages for use in large open wounds.[94,95] Many silver-coated and silver-containing dressings are now available for the treatment of wounds.

Although resistance to heavy metals, such as Ag^+, has been studied and reported, exact mechanisms are not known and there is little current evidence of emerging microbial resistance to silver. Increased use of Ag^+ in wound care has created some concern regarding the development of bacterial resistance but, unlike antibiotics, resistance to antiseptics such as Ag^+ is rare and sporadic. Certainly, with widespread use of Ag^+ in wound care, more potential pathogens are going to be exposed to this agent. However, it remains to be seen whether resistance will increase. With the knowledge that silver-resistance genes exist sporadically in certain types of bacteria, it would be appropriate for future studies to determine the actual prevalence of these genes within clinical and environmental settings. However, it is important to note that bacteria have been exposed to sub-inhibitory levels of Ag^+ for over four billion years and no widespread resistance has been evident to date, whereas widespread antibiotic resistance has developed within the last 60 years. A recent paper by Gilbert and McBains[91] suggested that in wound care, hygiene should be emphasized and targeted towards those applications which have demonstrable benefits.

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