

Silver sulphadiazine: A review of the evidence

This review provides a summary of the literature on the antimicrobial and clinical effects of silver sulphadiazine, and, on emerging patterns of resistance. This will, by nature of the two components, require separate focus on both silver and on the sulphonamide sulphadiazine. Silver has become a popular ingredient in wound dressings. Silver sulphadiazine has a novel use as a coating for various catheters and endotracheal tubes. The evidence points to silver sulphadiazine being a valuable topical agent in the treatment of burns and of lesser value in numerous other applications. It is silver that has emerged as the antimicrobial of greatest current interest.

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KEY WORDS

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Silver and silver compounds have been routinely used as general antimicrobial agents for over a century (Klasen, 2002; Lansdown, 2002; White, 2002). Silver, as the common ionic (active) form (Ag^+), is generally recognised as a safe, broad-spectrum antimicrobial agent. It is only the ionic form that has the antimicrobial activity (Russell and Hugo, 1994). Silver ions are made bioavailable through the interaction of aqueous fluid, typically wound exudate, with metallic (elemental) silver, or directly from silver salts (compound). This latter mechanism is the basis for the antimicrobial activity of silver nitrate, chloride and sulphadiazine, and may

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avoid the need for chemical oxidation in order to provide the 'active' species as is the case with metallic silver (Gibbins, 2003). Thus, the availability of silver ions is dependent upon dissociation of silver salts, or, on their solubility in wound fluid; this is pH dependent. However, wound fluid is a complex mixture which can vary considerably in composition; it is broadly similar in composition to serum as it contains proteins, lactate and electrolytes (e.g. Na^+ , K^+ , Cl^-), urea and glucose. Ionic silver Ag^+ is a highly reactive chemical species, which interacts with functional organic groups such as thiols. As integral protein side-groups, thiols and chemically similar species are key components of most proteins – including enzymes, and prokaryotic (bacterial) cell wall structures, and also nucleic acids. It is this binding that forms the basis of antimicrobial activity.

Silver was formulated as the salt of the sulphonamide antibiotic, sulphadiazine, in the 1960s by Fox (Fox, 1968). Silver sulphadiazine (SSD) is a non-ionised, water insoluble, fluffy white powder that is formed when sulphadiazine, a weak acid, reacts with silver nitrate to form the complex silver salt. A polymeric structure for SSD has been proposed, where six Ag^+ ions bind to six sulphadiazines via the nitrogen atoms of the sulphadiazine pyrimidine rings (Fox, 1983).

Since the introduction of SSD into clinical use, it has been used extensively in the

topical treatment of infected burns. More recently, it has been utilized in chronic wounds and the latest application is its incorporation into medical devices, such as catheters and dressings, for the prevention and treatment of infections.

SSD has largely been marketed as a 1% SSD cream as Flamazine™ (Smith and Nephew, Hull), and as a cream with 1% SSD in combination with 0.2% chlorhexidine digluconate as Silvadene™ (Monarch Pharmaceuticals, Tennessee, USA). This review will examine the laboratory evidence of its antimicrobial activity and cytotoxicity, and the clinical evidence of its efficacy. The advantages and limitations of SSD will also be explored.

Human pharmacology

Sulphadiazine is one member of four sulphonamide groups (others include sulfacetamide, mafenide, sulfasalazine and sulfisoxazole); it is characteristically rapidly absorbed and excreted from the gastrointestinal tract (Goodman and Gilman, 1990). From the topical route, Ag^{110} (radiolabelled silver) clearance has been measured in rats, and in ex-vivo, burned human skin (Harrison, 1979). Peak attachment of radiolabelled silver was observed to be 1% of the administered dose in 24 hours, leading to the deduction that SSD functions via the slow release of silver. Whereas low concentrations of silver may be distributed into the tissues, the plasma concentration of sulphadiazine may

approach therapeutic concentrations, depending on the surface area treated. Hence, silver is largely confined to cutaneous tissues and the sulphadiazine penetrates into the systemic circulation (Lockhart et al, 1983). This differential distribution pattern is reflected in varying therapeutic- and side-effects, which will be discussed later. The human pharmacology of silver sulfadiazine was reviewed in depth by Fox (1985).

Antimicrobial mode of action of silver sulphadiazine

The mode of action of SSD is not clearly elucidated, and whether the broad-spectrum antimicrobial activity is attributable to either the silver or the sulphadiazine moieties, or a synergistic combination of both, has been debated (McDonnell and Russell, 1999). In general, sulphonamides are broad-spectrum antibiotics that are predominantly bacteriostatic by inhibiting the formation of dihydropteroic acid, an intermediate of the folate pathway. SSD has been seen to cause surface and membrane blebs in susceptible (but not SSD-resistant) bacteria (Coward et al, 1973). Fox and Modak (1974) showed that silver, rather than sulphadiazine, binds to bacteria, and suggested that only a relatively small amount of available sulphadiazine appears to be active in this context.

There is evidence of antifungal (Wright et al, 1999) and antiviral activity (Thurman and Gerba, 1989), attributable to the silver moiety.

In wound treatment, it is probable that SSD functions by delivering sustained, low concentrations of silver (approx 1–2 ppm) into the wound environment, and that this interferes with, or modulates, multiple cellular processes. There are multiple deleterious effects in micro-organisms rather than a single, specific inhibitory mechanism (Fox and Modak, 1974). Given the anionic content of wound fluid, it is interesting to consider how silver solubility and available ions might exert a bactericidal effect. The chloride anion (Cl^-) is found in serum, and wound exudate. It will influence the availability of Ag^+ in solution. At low chloride concentrations (around a 100mM) as present in wound fluid, soluble silver will bind to the bacterial

cell surface, inhibiting respiration (Bragg et al, 1974). Moderate concentrations of chloride remove the silver as insoluble silver chloride precipitate, and, at higher chloride concentrations (such as might be found in exudate) silver is brought back into solution as the bioavailable anion AgCl_2^- (Gupta et al, 1998). The ability of highly diluted heavy metals (including silver in water) to inhibit micro-organisms was termed 'oligodynamic' by von Nägeli in 1893; this term has often been applied to silver. Silver products are thought to interact with microbial thiol groups, carboxylates, phosphates, hydroxyls, amines, imidazoles, and indoles, either singly, or in various combinations (Grier, 1983).

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Trevors (1987) reviewed the possible cellular effects of silver toxicity. These include:

- ▶▶ Binding of silver to base pairs in DNA, and thereby blocking transcription
- ▶▶ Binding to cell surface components, interfering with bacterial respiration and uncoupling ATP synthesis
- ▶▶ Preventing uptake of phosphate, and causing the release of components (such as glutamine, proline, succinate and phosphate from *Escherichia coli*).

Certain effects (such as phosphate release) may be reversed by the presence of thiol groups; such thiolated compounds as dithiothreitol and mercaptoethanol can combine with silver to form a stable, inactive complex that reduces toxicity to bacteria. Fox (1968) suggested that prolonged contact of SSD with biological fluids containing chloride and sulphhydryl groups, leads to the solubilisation of sulphadiazine, and thus enhances the oligodynamic action of silver with the additional antibacterial activity of the released sulphonamide.

The effect of halides, e.g. chloride on susceptibility to silver has been studied in two strains of *E. coli* by Gupta et al (1998). High concentrations of halides increased sensitivity of both a silver sensitive strain and a silver resistant strain to silver in in vitro tests, whereas low concentrations of chloride emphasised the differences between the sensitivities of the strains.

Most published studies on the mode of action of SSD concern bacterial cells, but irreversible inactivation of phosphomannose isomerase by silver sulphadiazine was demonstrated in *Candida albicans* (Wells et al, 1995). Phosphomannose isomerase is a zinc metalloenzyme essential for the biosynthesis of cell walls in fungi; and a cysteine residue, at position 150, has been shown to be the site of action of SSD. The same enzyme in *E. coli* was unaffected by SSD (Wells et al, 1995).

In addition to the antimicrobial effects of silver, positive benefits to the wound healing process have been reported (Lansdown et al, 1997; Demling and DeSanti, 2001; Kirsner et al, 2002). These focus on theoretical mechanisms involving:

- ▶▶ The displacement of zinc from metallothionines
- ▶▶ Changes in metalloprotein levels within the wound
- ▶▶ Effects on inflammatory cytokines.

Research is required to confirm and to clarify these mechanisms.

Antimicrobial spectrum of activity of SSD

When SSD was introduced in 1968 it was recommended as a topical prophylactic therapy for *Pseudomonas* spp. (Fox, 1968); since then it has been shown to possess inhibitory activity against a wide range of microbial species. Initially, bacteria were tested and both gram positive and gram negative species were found to be susceptible with minimum inhibitory concentrations (MICs) determined by serial doubling dilutions in trypticase soy broth ranging between <0.78 and 100 μg SSD/ml (Carr et al, 1973). Antifungal activity was determined in 50 clinical isolates of *C. albicans* by incorporating SSD into agar and inoculating with a multipoint inoculator; and MICs similar

to those quoted above were reported (Wlodkowski and Rosenkranz, 1973). Activity against dermatophytes was established using both liquid and agar dilution techniques described in the previous two studies, and all of the strains tested were inhibited at 100 µg SSD/ml or less (Speck and Rosenkranz, 1974). Binding of silver to phage DNA is the likely basis of antiviral activity (Rahn et al, 1973).

Hamilton-Miller et al (1993) utilised 409 clinical isolates from 12 different genera to provide reliable data of in vitro susceptibility to SSD. Precise details were provided of the methodology used to determine MICs, minimum bactericidal concentrations (MBC) and time-kill studies, including how stable suspensions of SSD were prepared. Strains tested were 20 each of coagulase negative staphylococci, *Streptococcus pyogenes*, *Enterococci*, *C. albicans*, *E. coli*, *Klebsiella pneumoniae*, *Enterobacter* spp., *Proteus mirabilis*, indole-positive *Proteus* spp., *Providencia stuartii* and *Pseudomonas aeruginosa*. Also, 97 strains of methicillin-resistant *Staphylococcus aureus* (MRSA) and 92 strains of *Acinetobacter* spp. were included. Furthermore, for all isolates, sulphonamide sensitivity was tested using diffusion from a disk impregnated with 50µg sulphamethoxazole. All organisms, including the multi-resistant species such as MRSA and *Acinetobacter* spp. were sensitive to SSD, irrespective of their sensitivity or resistance to sulphonamide.

Most MICs were between 16 and 64 µg SSD/ml, and SSD was bactericidal close to MICs. The figures reported in this study tended to be higher than those of previous studies, but the authors criticised 12 cited publications of in vitro studies completed between 1973 and 1991 (of which 3 are cited above) for incomplete descriptions of methodology.

As SSD is insoluble and unstable at alkaline pH, they commented that previous studies could not be accurately reproduced without details of how suspensions of SSD were prepared. Time-kill studies with selected strains of staphylococci showed that sulphonamide-resistant *S. aureus* (but not *Staphylococcus epidermidis*) were killed significantly more slowly than sulphonamide-sensitive strains. In a similar

study, Maple et al (1992) compared in vitro activities of 4 topical agents against 80 strains of MRSA collected from clinical specimens from sources throughout the world. SSD gave MIC₅₀ of 85 µg/ml and was recommended for clearance of MRSA carriage. SSD killed sulphonamide-sensitive and sulphonamide-resistant strains equally rapidly, and no SSD resistant mutants were recovered from inocula of 10⁹ cfu.

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The effect of sub-lethal concentrations of SSD on bacteria

Two studies have investigated the effects of sub-lethal concentrations of SSD on staphylococci (Edwards-Jones and Foster, 1994; 2002). In the first report, 300 cultures of *S. aureus* isolated from wound swabs were tested for the production of toxic shock syndrome toxin 1 (TSST-1). The isolates were recovered from surgical in-patients, burns patients and from community patients. All cultures were screened for the production of TSST-1, and 17% were found to be positive. TSST-1 producing strains were grown in vitro and incubated in sub-lethal concentrations of five topical antimicrobial agents. Levels of TSST-1 produced in the presence of antimicrobials were compared to cultures without additions. With SSD, effects were strain dependent, but in 47% of cultures tested, TSST-1 production was stimulated at least four times the level observed in control cultures and, in some cases, 16 times higher.

Preliminary data also suggested that SSD induced TSST-1 synthesis earlier during the growth cycle. Although TSST-1 is primarily associated with use of tampons, it has been detected in other infections, including burns. Since SSD is often used in the prophylactic treatment of burns, and *S. aureus* is a common cause of burn infection, this study indicates the need to employ concentrations of SSD in vivo that are microbicidal.

Using two selected strains of staphylococci, the effect of SSD on the production of further virulence factors was tested in vitro. Again, strain differences were seen, but sub-lethal concentrations of SSD were able to stimulate the release of coagulase, proteases and enterotoxin A and C (Edwards-Jones and Foster, 2002). This study provides further evidence that inappropriate concentrations of topical agents such as SSD may enhance the virulence of wound pathogens.

The clinical evidence for the efficacy of SSD

Early evidence that Silvadene™ had a beneficial effect on wounds, additional to its antimicrobial activity, came from an animal model. In clean wounds in pigs, rates of epithelialisation were increased by 28% by topical application of SSD (Geronemus et al, 1979). Best known as the Flamazine™ 1% cream (Smith & Nephew, Hull), SSD has been a mainstay of topical burns therapy (Pruitt, 1987), and has been used successfully in acute (Buckley et al, 2000) and chronic wounds (Bishop et al, 1992) to treat infection. A variety of topical silver preparations have been evaluated on chronic wounds (systematic review by O'Meara et al, 2000; 2001) in clinical trials with generally favourable results. Clinical evidence on SSD formulations is largely in the area of burns treatment (Hoffmann, 1984; Stern, 1989; Fakhry et al, 1995; Klases, 2002) although there are reports on use in chronic leg ulcers (Blair et al, 1988; Bishop et al, 1992), and acute wounds (Buckley et al, 2000).

In a study on non-healing leg ulcers treated with split-thickness skin grafts, van den Hoogenband (1984) demonstrated that pre-treatment with SSD cream for five days before grafting resulted in better healing. This was attributed to marked reduction in pre-operative levels of pathogens such as *S. aureus*, *Pseudomonas* spp., *Enterobacter* and β-haemolytic streptococci in the SSD-treated group.

In a study on 71 patients with venous leg ulcers, Ouvry (1989) found that the use of SSD cream was effective in wound cleansing and subsequent granulation tissue formation. This is supported by evidence that super-infections responded

well to the cream. Further supportive, but not conclusive, evidence on the use of SSD on leg ulcers is provided in the systematic review by O'Meara et al (2001). The authors cite clinical trial reports by Bishop et al (1992) and Blair et al (1988), drawing the conclusion that 'silver-based products may be useful for leg ulcers'. The systematic review highlighted trial design as the single most important reason why conclusive evidence was not available; most studies were flawed in their design resulting in type 2 errors.

SSD cream has also been evaluated in pressure ulcer treatment. In a comparison of 1% SSD (Silvadene™ cream) with povidone-iodine solution (PVP-I Betadine™) or sterile saline, Kucan et al (1981) found SSD alone to reduce wound bioburden below 10^5 per gramme of tissue. The difference between the mean bacterial levels reached after treatment was highly significant for SSD vs PVP-I ($P < 0.01$), but less so for SSD vs saline ($P < 0.10$). This translated into an improved clinical response at three weeks for the SSD group ($P \leq 0.022$).

The neuropathic foot ulcer, associated with diabetes, is another chronic wound that is compromised by microbial colonisation and frequent infection. SSD has been used on these wounds with good effect (Sturm et al, 1993), although there are no reports of controlled clinical trials.

The published data on SSD in chronic wounds such as leg ulcers, pressure ulcers and diabetic foot ulcers, support the control of wound bioburden as a pre-requisite to healing, and, for the active management of existing wound infection by the topical route. In terms of bioburden control, this may be at the colonisation stage, or, in the pre-infection or 'critical colonisation' stage described for chronic wounds (Kingsley, 2001; Fumal et al, 2002). The treatment of critical colonisation is, as yet, controversial until there is clear evidence that such treatment can preclude the development of infection, facilitate healing, and reduce morbidity. There is accumulating evidence that bacterial activity may impair healing (Wall et al, 2002; Stephens et al, 2003).

The role of SSD in medical products

Micro-organisms rarely exist in natural environments as single cells or pure cultures of an individual species, but usually as relatively stable communities of mixed species embedded in slime layers, called biofilms. Cells in biofilms exhibit decreased sensitivity to antimicrobial agents and also to host immunological responses, and have therefore been implicated in persistent infections (Costerton et al, 1999). Biofilms have been shown to form in wounds created in two animal models (Akiyama et al, 1993; Serralta et al, 2001; Akiyama et al, 2002), and are thought to be important in chronic wounds (Mertz, 2003). Biofilms have also been implicated in infections with coagulase negative staphylococci associated with medical devices (Tunney et al, 1996). Hence, factors that limit the development of biofilms are pertinent to the management of wounds and the clinical use of certain medical devices.

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The effects of antimicrobial agents on a biofilm model of clinical isolates of *S. aureus* were studied by Akiyama et al (1998). Immature biofilms of *S. aureus* were formed on plastic coverslips in plasma after incubation for 24 hours, and levofloxacin or 10% povidone-iodine, in combination with 70% sucrose and silver sulphadiazine or silver nitrate, was effective in eliminating *S. aureus* cells in the biofilm model. Because silver was more effective in eliminating cells in biofilm than floating cells, it was suggested that silver might be effective in treating wounds with *S. aureus* colonisation as well as infections (Akiyama et al, 1998). Recently, cadexomer iodine has been found to be effective in killing *S. aureus* in biofilms (Akiyama et al, 2004).

Another application for silver in the control of biofilms is in medical devices. Catheters have been used in hospitals since 1945, and a wide range of indwelling medical devices is now available. Infections associated with these devices are linked to biofilm formation, especially with coagulase negative staphylococci. One strategy to control such infections has been to incorporate antimicrobial agents — and silver products are candidates.

Bach et al (1994) compared colonisation of catheters impregnated with silver sulphadiazine and chlorhexidine (SSC) against control (C) catheters implanted into the jugular veins of rats, and demonstrated that impregnated catheters reduced the incidence and extent of colonisation. Despite the retention of antiseptic activity in SSC-impregnated catheters in vivo, incidence of bacteraemia was not affected and efficacy has been questioned (Bach et al, 1996). However, a review supports the use of antiseptic-coated catheters in reducing the incidence of both catheter colonization and catheter-related bloodstream infection (Veenstra et al, 1999). A recent study on 184 patients with haemato-oncological disease demonstrated that SSC impregnation reduces the overall risk of catheter colonisation and catheter-related infection (Ostendorf et al, 2005), and these findings have been recently corroborated in a clinical trial on neutropenic patients (Jaeger et al, 2005). Susceptibility of coagulase negative staphylococci isolates from bloodstream infections to SSC-impregnated central catheter has been demonstrated (Rosato et al, 2004). Cultures collected before and after the introduction of SSC catheters did not differ in their sensitivity to SSC in vitro, but the proportion of *S. epidermidis* isolated after their introduction diminished.

Methods of incorporating antimicrobial agents into catheters have not been completely optimised to date but the incorporation of antimicrobial agents between inner- and outer-sheath materials seems to be more effective than coating catheters (Tcholakian and Raad, 2001). The advantages of antibiotic- or antiseptic-coated catheters have also been debated, and central venous catheters coated with minocycline/rifampicin have been judged

to be more cost-effective than SSC catheters for patients catheterised for at least 7 days (Marciante et al, 2003). The prospect of bacteria developing resistance to antibiotics and antiseptics incorporated into medical devices is causing concern, although the selection of antibiotic-resistant bacteria is more probable than the development of antiseptic-resistant strains (Tambe et al, 2001).

In 1971 an outbreak of resistant *S. aureus* in the burns unit in the Royal Melbourne hospital led to the development of a dressing containing SSD and 0.2% chlorhexidine digluconate as Silvazine™ (Sigma Pharmaceuticals, Melbourne). Until recently, no in vitro study had compared the antimicrobial efficacy of dressings such as Flamazine™, Silvazine™ and Acticoat™ (Smith and Nephew, Hull). Survival curves of eight common burn wound pathogens were obtained by inoculating 1 cm² samples of dressings into bacterial suspensions and monitoring microbial viability with time (Fraser et al, 2004). No viable bacteria of any cultures were detected after 30 minutes exposure to Silvazine™, whereas SSD and Acticoat™ were less effective. Although SSD and Acticoat™ had similar activities, Acticoat™ was reported to be only bacteriostatic against *Enterococcus faecalis* and MRSA, and viable cells of

Enterobacter cloacae and *Pr. mirabilis* were recovered after 24 hours exposure. One limitation in testing dressings rather than active components alone is that the contribution of excipients to observed activity is not tested.

Another formulation of SSD is in the Urgotul® SSD dressing, (Urgo-Parema, Loughborough). This comprises a polyester mesh impregnated with carboxymethylcellulose, white soft paraffin and 3.75% SSD. Preliminary clinical data on burns patients showed good clinical and microbiological outcomes (Carsin et al, 2004).

Limitations of SSD

Undesirable characteristics associated with the clinical use of SSD are the emergence of resistant strains of microbial species, the claimed retardation of wound healing and the development of systemic side-effects. Delayed wound healing is claimed to be the clinical manifestation of mild toxicity, as evidenced from in vitro studies involving various skin cells lines (Hidalgo and Dominguez, 1998; Poon and Burd, 2004).

Resistance to silver sulphadiazine

The mechanisms of bacterial resistance to silver; encoded by plasmid-based genes, have been described in detail (Silver, 2003). Silver resistance results from an energy-dependent ion efflux from the cell (Gupta et al, 1999). Silver bioaccumulation by bacteria has been reported (Starodub, 1990) but the relationship between accumulation and resistance is not clear (Slawson et al, 1992). Silver resistant organisms have frequently been found in environments where silver toxicity may be expected to select for resistance, for example hospital burn wards (Klasen, 2000). A bacterial resistance pattern to silver; as used in a variety of topical wound treatments, has been reviewed (Percival et al, 2005). The authors concluded that, although no widespread resistance had been developed, increased usage of silver gives cause for some concern.

The emergence of SSD-resistant bacteria in a Burns Unit in Birmingham was recorded by Lowbury et al (1976). When the prophylactic effects of SSD were compared to silver nitrate (SN) in

severe burns patients, *S. aureus* was more frequently isolated from wounds treated with SSD, and coliforms were more frequently isolated from SN patients. When the efficacy of SSD was compared to a cream containing SN and chlorhexidine (SNC) in patients with extensive burns, sulphonamide-resistant gram negative bacteria became predominant.

During the clinical evaluation of antimicrobial agents in the treatment of burns patients in Birmingham between 1965 and 1974, resistance to sulphonamide was monitored in clinical isolates using the ditch plate method where 100mg sulphadiazine/litre was incorporated into the ditch. SSD was first utilised there in 1969, but until 1972 it was restricted to patients with small burns, after which time it was applied to extensive burns.

In 1974, increased recovery of sulphonamide-resistant strains was observed in *Acinetobacter anitratus*, *Klebsiella* spp., *E. coli*, *Enterobacter* spp., *Pr. mirabilis* and other *Proteus* species. For a selection of these organisms, MICs determined by the agar dilution method exceeded 1000 mg sulphadiazine/litre. Sulphadiazine resistance was detected in more strains recovered from SSD-treated patients (41/59, 70%) than from SN treated patients (23/50, 44%). The emergence of resistant gram negative bacteria reduced the effectiveness of SSD in the prophylactic treatment of extensive burns, and caused the suspension of SSD cream, and limited use of systemic sulphonamides and co-trimoxazole until proportions of sulphonamide-resistant isolates diminished.

Bridges and Lowbury (1977) demonstrated that cessation of all sulphonamide treatment in the ward resulted in a reduction in incidence of sulphadiazine-resistant strains. Conjugation experiments with *E. coli* K12, and resistant strains, showed that sulphadiazine resistance was transferable in *Klebsiella* and some other (but not all) strains. Heggars and Robson (1978) and Modak and Fox (1981) have also recovered SSD-resistant strains of bacteria. The validity of sensitivity testing has been questioned by *P. aeruginosa* cultures isolated from burns patients in

Key Points

- ▶▶ Silver sulphadiazine is a combination of two antimicrobial agents; silver and a sulphonamide antibiotic.
- ▶▶ SSD is best used in topical delivery; most evidence is based on the cream, increasingly it has been used to coat medical devices such as catheters and endotracheal tubes to reduce bacterial growth.
- ▶▶ SSD has a long history of effective clinical use, notably in burns.
- ▶▶ Much of the evidence acquired on SSD has been used to support the use of silver as a topical antimicrobial.

several countries that were observed to be resistant to SSD in vitro, but not in vivo when tested in an animal model (Modak and Fox, 1981). Sensitivity to sulphadiazine in this study was measured in a tube dilution assay in nutrient broth.

Another method for assessing the effectiveness of topical antimicrobial agents is the agar well diffusion test (AWD), where agar is seeded with the culture being screened, and agents being tested are introduced into wells cut into the plate. Although differential diffusion rates of various agents might influence the size of resultant zones of inhibition, Thomson et al (1989) found good correlation between clinical efficacy and in vitro inhibition of SSD.

In another similar study (Hendy and Smith, 1979), hospital isolates collected between 1975 and 1979 were routinely screened for sensitivity to silver by plating onto media containing 0.5mM silver nitrate. Silver-resistant strains of *Enterobacteriaceae* that were detected included *E. coli*, *Ent. cloacae*, *Kleb. pneumoniae*, *Pr. mirabilis* and *Citrobacter freundii*. Eleven in-patients whose burns had been treated prophylactically with SSD yielded silver-resistant bacteria. Of the 230 cultures collected during the study period, 211 were resistant to sulphamide, 97 of which were also silver-resistant, but 38 were not tested for silver sensitivity. Coincidentally, seven silver-resistant (but sulphamide-sensitive) strains were isolated from four non-burns patients with silver tracheotomy tubes, and one similar isolate came from a burns patient in A&E, whose previous treatment history was not recorded. At a later date (Trevors, 1987), these cultures were reported to contain one or more plasmids. Resistance of *Pseudomonas* spp. to silver has been reviewed (Cervantes and Silver, 1996).

The epidemiology of *P. aeruginosa* in a burn centre has been studied (Pimay et al, 2003). Essentially, two predominant genotypes were responsible for recurrent outbreaks and the colonisation of patients. One endemic strain developed multiple drug resistance at the end of the study period, and the other was antibiotic sensitive, but resistant to SSD. Whereas the former strain was more prevalent in

sputum samples, the latter was more frequently recovered from burn wound swabs. Primary reservoirs were not detected. In this study, the AWD was used to test for SSD resistance, and the authors suggested that this test might be of value in eliminating inappropriate use of SSD for burns patients colonised

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with SSD-resistant strains. McManus et al (1983) described a transferable multiple-antibiotic resistance plasmid that carried selectable sulphonamide resistance determinants, and warned that use of SSD could select not only for SSD-resistant strains, but antibiotic-resistant strains. Plasmid-mediated resistance to silver has been noted in a number of gram negative bacteria, such as *E. coli*, *Pseudomonas*, *Acinetobacter* and *Salmonella*. Occasionally, resistance to silver is lost on sub-culture in the laboratory (Bridges et al, 1979). Detailed analysis of *Salmonella* identified a cluster of seven genes that were organised into three divergently transcribed units. The gene products are thought to function as a periplasmic protein that binds silver; and two efflux pumps that export silver (Gupta et al, 1999).

Cytotoxicity of SSD

The systemic toxicity of the sulphonamide antibiotics is dependent upon their individual chemistry and metabolism (Shear et al, 1986). In general, the sulphonamides are known to induce agranulocytosis (Willoughby, 1977). Certainly, neutropaenia has been reported in burns treated with topical SSD, but this cannot be directly attributed to the sulphadiazine. It would appear to be the case that systemic or local toxicity from sulphadiazine, as delivered from 1% topical emulsion formulations dosed according to manufacturer's instructions,

is most unlikely. However, in a study on severely burned patients, where a large body surface area was treated with a topical SSD emulsion, measurable toxicity, including allergic contact reactions to the formulation excipients, eg. propylene glycol and cetyl alcohol has been reported (Degreef and Dooms-Goossens, 1985). Similar responses to the sulfadiazine were reported (Kulick et al, 1985) although this is contradicted by Degreef and Dooms-Goossens (1985) who maintain that, 'there is no evidence that SSD is contraindicated for patients with a history of sulfonamide hypersensitivity'.

Toxic effects of silver include skin irritation and discolouration (argyria) which is widely reported for silver nitrate solutions and colloidal silver but rarely associated with topical SSD (Gettler et al, 1927; Buckley, 1963; Marshall and Schneider, 1977; Wright et al, 1998). Allergic contact dermatitis to silver as SSD and as the nitrate has been reported, although with SSD most such reactions are to the excipients (Fisher, 2003; Agarwal and Gawkrödger, 2002; Iliev and Elsner, 1998).

Cytotoxicity in vitro has been described and postulated as a cause of delayed wound healing as reported anecdotally (Poon and Burd, 2004). In wound treatment, any potential for cytotoxicity arising from silver-releasing wound dressings or SSD creams, can be avoided through the prudent use of such products – particularly avoidance of unnecessary or prolonged use.

Serum silver levels have been found to rise after topical SSD treatment (Maitre et al, 2002). Upon dissociation, the sulphonamide clears from the body more rapidly than does silver (Boosalis et al, 1987). Elevated serum silver (over 20mg L⁻¹) is reported to cause renal dysfunction, liver and nerve toxicity; this occurs after prolonged exposure of leg ulcers and acute burns to 1% SSD (Maitre et al, 2002; Tsipouras et al, 1997), and, agranulocytosis in neonates (Viala et al, 1997).

Historically, silver nitrate was probably the first silver compound used on wounds, where it has an astringent and irritating effect.

Due to these problems, and lack of evidence, it is rarely used today except for the occasional reduction of hypergranulation.

Conclusions

Since its first appearance as a cream formulation for topical use some thirty years ago, SSD has become a very valuable antimicrobial agent, especially in burns therapy. It has, through widespread clinical usage, been proven to be remarkably safe with relatively low resistance potential. The advent of a new generation of silver-containing dressings and of SSD impregnation of medical devices has served to confirm the clinical utility of silver and of SSD in modern medicine.

References

Agarwal S, Gawkrödger DJ (2002) Occupational allergic contact dermatitis to silver and colophonium. *Am J Contact Derm* **13**(2): 74

Akiyama H, Kanzaki H, Tadi J, Arata J (1993) *Staphylococcus aureus* infection on cut wounds in the mouse skin: experimental staphylococcal botryomycosis. *J Dermatol Sci* **11**: 2334–8

Akiyama H, Yamasaki O, Kanzaki H, Tada J, Arata J (1998) Effects of sucrose and silver on *Staphylococcus aureus* biofilms. *J Antimicrob Chemother* **42**: 629–34

Akiyama H, Huh W-K, Yamasaki O et al (2002) Confocal laser scanning microscopic examination of glycocalyx production by *Staphylococcus aureus* in mouse skin: does *S. aureus* generally produce a biofilm on damaged skin? *Br J Dermatol* **147**: 879–85

Akiyama H, Oono T, Saito M, Iwatsuki K (2004) Assessment of cadexomer iodine against *Staphylococcus aureus* biofilm in vivo and in vitro using confocal laser scanning microscopy. *J Dermatol* **31**: 529–34

Bishop J, Phillips LG, Mustoe TA et al (1992) A prospective randomised evaluator blinded trial of two potential wound healing agents for the treatment of venous stasis ulcers. *J Vasc Surg* **16**: 251–7

Blair SD, Backhouse CM, Wright DD et al (1988) Do dressings influence the healing of chronic leg ulcers? *Phlebology* **3**: 129–34

Boosalis MG, McCall JT, Ahrenholtz DH et al (1987) Serum and urinary silver levels in thermal injury patients. *Surgery* **101**: 40–3

Bowler PG, Duerden BI, Armstrong DG (2001) Wound microbiology and associated approaches to wound management. *Clin Micro Rev* **14**(2): 244–69

Bragg PD, Rainnie DG (1974) The effect of silver ions on the respiratory chain of *E. coli*. *Can J Microbiol* **20**(6): 883–9

Bridges K, Lowbury EJJ (1977) Drug resistance in relation to use of silver sulphadiazine cream

in a burns ward. *J Clin Path* **30**: 160–4

Bridges K, Kidson A, Lowbury EJJ, Wilkins MD (1979) Gentamicin- and silver-resistant *Pseudomonas* in a burns unit. *Br Med J* (i): 446–50

Buckley WR (1963) Localised argyria. *Arch Dermatol* **88**: 531–9

Buckley SC, Scott K, Das K (2000) Late review of the use of silver sulphadiazine dressing for the treatment of fingertip injuries. *Injury Int J Care Injured* **31**: 301–4

Carr HS, Wlodkowski TJ, Rosenkranz HS (1973) Silver sulphadiazine: in vitro antibacterial activity. *Antimicrob Agents Chemother* **4**: 585–7

Carsin H, Wasserman D, Pannier M et al (2004) A silver sulphadiazine-impregnated lipidocolloid wound dressing to treat second-degree burns. *J Wound Care* **13**(4): 145–8

Cervantes C, Silver S (1996) Metal resistance in *Pseudomonas*; genes and mechanisms. In: Nakazawa T, Furakawa K, Haas D, eds. *Molecular biology of Pseudomonads. Silver S*. American Society for Microbiology, Washington DC, USA

Costerton JW, Stewart PS, Greenberg EP (1999) Bacterial biofilms: a cause of persistent infections. *Science* **284**: 1318–22

Coward JS, Carr HS, Rosenkranz HS (1973) Silver sulphadiazine effect on the ultrastructure of *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* **3**: 621–4

Degreef H, Dooms-Goossens A (1985) Patch testing with silver sulfadiazine cream. *Contact Dermatitis* **12**(1): 33–7

Demling RH, DeSanti L (2001) The role of silver in wound healing. Part 1: Effects of Silver on Wound Management. *Wounds* **13**(1): SupplA3–A15

Edwards-Jones V, Foster HA (1994) The effects of topical antimicrobial agents on the production of toxic shock syndrome toxin – 1. *J Med Micro* **41**: 408–13

Edwards-Jones V, Foster HA (2002) Effects of silver sulphadiazine on the production of exoproteins by *Staphylococcus aureus*. *J Med Micro* **51**: 50–5

Fakhry SM, Alexander J, Smith D (1995) Regional and institutional variations in burn care. *J Burn Care Rehab* **16**: 86–90

Fisher NM, Marsh E, Lazova R (2003) Scar localized argyria secondary to silver sulfadiazine cream. *J Am Acad Dermatol* **49**(4): 730–2

Fox CL, Modak SM (1974) Mechanisms of silver sulphadiazine action on burn wound infections. *Antimicrob. Agents Chemother* **5**(6): 582–8

Fox CL (1968) Silver sulphadiazine — a new topical therapy for *Pseudomonas* in burns. *Arch Surg* **96**: 184–8

Fox CL (1983) Topical therapy and the development of silver sulphadiazine. *Surg Gynecol Obstet* **157**: 82–8

Fox CL (1985) The human pharmacology of silver sulphadiazine. *Burns Inc Therm Inj* **11**(4): 306–7

Fraser JF, Bodman J, Sturgess R, et al (2004) An in vitro study of the anti-microbial efficacy of a 1% silver sulphadiazine and 0.2% chlorhexidine digluconate cream, 1% silver sulphadiazine cream and a silver coated dressing. *Burns* **30**: 35–41

Fumal I, Braham C, Paquet P, Pierard-Franchimont C, Pierard GE (2002) The beneficial toxicity paradox of antimicrobials in leg ulcer healing impaired by a polymicrobial flora: a proof-of-concept study. *Dermatology* **204**(suppl1): 70–4

Geronemus RG, Mertz PM, Eaglestein WH (1979) Wound healing. The effects of topical agents. *Arch Dermatol* **115**(1): 1311–14

Gettler AO, Rhoads CP, Weiss S (1927) A contribution to the pathology of generalised argyria with a discussion of the fate of silver in the human body. *Am J Pathol* **3**: 631–51

Gibbins B (2003) The antimicrobial benefits of silver and the relevance of micro-lattice technology. *Ostomy/Wound Management* **49**: Suppl 4–7

Goodman LS, Gilman A (1990) *Goodman and Gilman's The Pharmacological Basis of Therapeutics*. 8th edn. McGraw Hill, New York: chap 45, 'Antimicrobials'

Grier N (1983) Silver and its compounds. In: Block SS, ed. *Disinfection, Sterilization and Preservation*. 3rd edn. Lea and Febiger, Philadelphia

Gupta A, Matsui K, Lo JF, Silver S (1999) Molecular basis for resistance to silver cations in *Salmonella*. *Nat Med* **5**: 183–8

Gupta A, Maynes M, Silver S (1998) Effects of halides on plasmid-mediated silver resistance in *Escherichia coli*. *Appl Environ Micro* **64**(12): 5042–45

Hamilton-Miller JMT, Shah S, Smith C (1993) Silver sulphadiazine: a comprehensive in vitro reassessment. *Chemother* **39**: 405–9

Harrison N (1979) Pharmacology of sulfadiazine silver. *Arch Surg* **114**(3): 281–5

Heggars JP, Robson MC (1978). The emergence of silver sulphadiazine resistance in *Pseudomonas aeruginosa*. *Burns* **5**: 184–7

Hendy AT, Smith IO (1979) Silver-resistant *Enterobacteriaceae* from hospital patients. *Can J Microbiol* **25**: 915–21

Hidalgo E, Dominguez C (1998) Study of cytotoxicity mechanisms of silver nitrate in human dermal fibroblasts. *Tox Letts* **98**: 169–79

Hoffmann S (1984) Silver sulphadiazine: an antibacterial agent for topical use in burns. *Scand J Plast Reconstr Surg* **18**: 119–26

Iliev D, Elsner P (1998) Unusual edge effect in patch testing with silver nitrate. *Am J Contact Dermat* **9**(1): 57–9

Jaeger K, Zenz S, Juttner B et al (2005) Reduction of catheter-related infections in neutropenic patients: a prospective controlled randomized trial using a chlorhexidine and silver sulfadiazine-impregnated central venous catheter. *Ann Haematol* **84**(4): 258–62

Kingsley A (2001) A pro-active approach to wound infection. *Nurs Standard* **15**(30): 50–5

Kirsner RS, Orsted H, Wright JB (2002) Matrix metalloproteinases in normal and impaired

- wound healing: a potential role of nanocrystalline silver. *Wounds* **13**(3): Suppl C, 5–12
- Klasen HJ (2002) A historical review of the use of silver in burns. *Burns* **26**: 117–30
- Kucan JO, Robson MC, Heggers JP, Ko F (1981) Comparison of silver sulfadiazine, povidone-iodine and physiologic saline in the treatment of chronic pressure ulcers. *J Am Geriatr Soc* **29**: 232–5
- Kulick MI, Wong R, Okarma TB, *et al* (1985) Prospective study of side-effects associated with the use of silver sulfadiazine in severely burned patients. *Ann Plast Surg* **14**(5): 407–18
- Lansdown A B G, Sampson B, *et al* (1997). Silver aids healing in the sterile skin wound: experimental studies in the laboratory rat. *Br J Dermatol* **137**: 728–35
- Lansdown ABG (2002) Silver 1: its antibacterial properties and mechanism of action. *J Wound Care* **11**: 125–30
- Lockhart SP, Rushworth A, Azmy AAF, Raine P (1983) Topical silver sulphadiazine: side-effects and urinary excretion. *Burns* **10**(1): 9–12
- Lowbury EJJ, Babb JR, Bridges K, Jackson DM (1976) Topical chemoprophylaxis with silver sulphadiazine and silver nitrate chlorhexidine creams: emergence of sulphonamide-resistant Gram-negative bacilli. *Br Med J* **1**: 493–6
- Maitre S, Jaber K, Perrot JL, *et al* (2002) Increased serum and urinary levels of silver during treatment with topical silver sulfadiazine. *Ann Dermatol Venereol* **129**(2): 217–19
- Maple PAC, Hamilton-Miller JMT, Brumfitt W (1992) Comparison of the in-vitro activities of the topical antimicrobials azelaic acid, nitrofurazone, silver sulphadiazine and mupirocin against methicillin-resistant *Staphylococcus aureus*. *J Antimicrob Chemother* **29**: 661–8
- Marcianti KD, Veenstra DL, Lipsky BA, Saint S (2003) Which antimicrobial impregnated central venous catheter should we use? Modeling the costs and outcomes of antimicrobial catheter use. *Am J Infect Control* **31**(1): 1–6
- Marshall J P, Schneider R P (1977) Systemic argyria secondary to topical silver nitrate. *Arch Dermatol* **113**: 1077–78
- McDonnell G, Russell AD (1999) Antiseptics and disinfectants: activity, action and resistance. *Clin Microbiol Rev* **12**(1): 147–79
- McManus AT, Denton CL, Mason AD (1983) Mechanisms of in vitro sensitivity to sulfadiazine silver. *Arch Surg* **118**: 161–6
- Mertz P (2003) Cutaneous biofilms; friend or foe? *Wounds* **15**(5): 129–32
- Modak SM, Fox CL (1981) Sulfadiazine silver-resistant *Pseudomonas* in burns. *Arch Surg* **116**: 854–7
- O'Meara SO, Cullum N, Majid M, Sheldon T (2000) Systematic reviews of wound care management: (3) Antimicrobial agents. *Health Technol Assess* **4**(21): 1–237
- O'Meara S O, Cullum N, Majid M, Sheldon T (2001) Systematic review of antimicrobial agents used for chronic wounds. *Br J Surg* **88**: 4–21
- Ostendorf T, Meinhold A, Harter C, *et al* (2005) Chlorhexidine and silver sulfadiazine coated central venous catheters in haematological patients — a double-blind, randomised, prospective, controlled trial. *Supp Care Cancer*: 16 April, E-pub ahead of print
- Ouvry PA (1989) Essai de la sulfadiazine argentique dans le traitement local de l'ulcere veineux. *Phlebologie* **42**(4): 673–9
- Percival SL, Bowler PG, Russell AD (2005) Bacterial resistance to silver in wound care. *J Hosp Infect* **60**: 1–7
- Pirnay J-P, de Vos D, Cochez C, *et al* (2003). Molecular epidemiology of *Pseudomonas aeruginosa* colonisation in a burn unit: persistence of a multidrug-resistant clone and a silver sulfadiazine-resistant clone. *J Clin Microbiol* **41**(3): 1192–1202
- Poon VKM, Burd A (2004) In vitro cytotoxicity of silver: implication for clinical wound care. *Burns* **30**: 140–7
- Pruitt BA (1987) Opportunistic infections in burn patients: diagnosis and treatment. In: Root R, Trunkey R, Sande MA, eds. *New Surgical and Medical Approaches to Infectious Diseases*. Churchill Livingstone, New York: 245–61
- Rahn RO, Setlow JK, Landry LC (1973) Ultraviolet irradiation of nucleic acids complexed with heavy metals. III. Influence of Ag⁺ and Hg²⁺ on the sensitivity of phage and of transforming DNA to ultraviolet radiation. *Photochem Photobiol* **18**: 39–41
- Rosato AE, Tallent SM, Edmonds MB, Bearman GML (2004) Susceptibility of coagulase-negative staphylococcal nosocomial bloodstream isolates to the chlorhexidine/silver sulfadiazine-impregnated central venous catheter. *Am J Infect Control* **32**(8): 486–8
- Russell AD, Hugo WB (1994) Antimicrobial activity and action of silver. *Prog Medicinal Chemistry* **31**: 351–70
- Saffle JR, Schnebly WA (1994) Burn wound care. In: Richard RL, Stanley MJ, eds. *Burn Care and Rehabilitation: Principles and Practice*. Davis Co, Philadelphia, USA: 137–9
- Scott Ward R, Saffle JR (1995) Topical agents in burn wound care. *Phys Ther* **75**(6): 526–38
- Serralta AW, Harrison-Balestra C, Cazzaniga AL, Davis S, Mertz PM (2001) Lifestyles of bacteria in wounds: presence of biofilms? *Wounds* **13**(1): 29–34
- Speck WT, Rosenkranz HS (1974) Activity of silver sulphadiazine against dermatophytes. *Lancet* (ii): 895–6
- Shear NH, Spielberg SP, Grant DM, *et al* (1986). Differences in metabolism of sulfonamides predisposing to idiosyncratic toxicity. *Ann Int Med* **105**: 179–84
- Silver S (2003) Bacterial silver resistance: molecular biology and uses and misuses of silver compounds. *FEMS Micro Rev* **27**: 341–53
- Slawson RM, Van Dyke M, Lee H (1992) Germanium and silver resistance, accumulation and toxicity in MOs. *Plasmid* **27**(1): 72–9
- Stern HS (1989) Silver sulphadiazine and the healing of partial thickness burns: a prospective clinical trial. *Br J Plast Surg* **42**(5): 581–5
- Starodub ME, Trevors JT (1990) Silver accumulation and resistance in *E. coli*. *J Inorganic Biochem* **39**(4): 3017–25
- Stephens P, Wall IB, Wilson MJ, *et al* (2003) Anaerobic cocci populating the deep tissues of chronic wounds impair cellular wound healing responses in vitro. *Br J Dermatol* **148**(3): 456–66
- Sturm LJ, Rimler RJ, Fendell HS (1993) Alternative treatment for diabetic neuropathic ulcerations. *JAMA* **83**: 426
- Tambe SM, Sampath L, Modak SM (2001) In vitro evaluation of the risk of developing bacterial resistance to antiseptics and antibiotics used in medical devices. *J Antimicrob Chemother* **47**: 589–98
- Tcholakian RK, Raad II (2001) Durability of anti-infective effect of long-term silicone sheath catheters impregnated with antimicrobial agents. *Antimicrob Agents Chemother* **45**(7): 1990–93
- Thomson PD, Taddonio TE, Tait MJ, Prasad JK (1989) Susceptibility of *pseudomonas* and *staphylococcus* wound isolates to topical antimicrobial agents: a ten-year review and clinical evaluation. *Burns* **15**: 190–2
- Thurman RB, Gerba CP (1989) The molecular mechanisms of copper and silver ion disinfection of bacteria and viruses. *CRC Critical Reviews in Environmental Control* **18**(4): 295–315
- Trevors JT (1987) Silver resistance and accumulation in bacteria. *Enzyme Microb Technol* **9**: 331–3
- Tsipouras N, Rix CJ, Brady PH (1997) Passage of silver ions through membrane-mimetic materials, its relevance to treatment of burn wounds with silver sulfadiazine cream. *Clin Chem* **43**(2): 290–301
- Tunney MM Gorman S, Patrick S (1996) Infection associated with medical devices. *Rev Med Micro* **7**(4): 195–205
- Veenstra DL, Saint S, Saha S, Lumley T, Sullivan SD (1999) Efficacy of antiseptic-impregnated central catheters in preventing catheter-related bloodstream infection: a meta-analysis. *JAMA* **281**: 261–7
- Viala J, Simon L, Le Pommelet C, *et al* (1997) Agranulocytosis after application of silver sulfadiazine in a 2-month old infant. *Arch Pediatr* **4**(11): 1103–06
- Wall IB, Davies CE, Hill KE, *et al* (2002) Potential role of anaerobic cocci in impaired human wound healing. *Wound Repair Regen* **10**(6): 346–53
- Wells TNC, Scully P, Paravincini G, Proudfoot AEI, Payton MA (1995) Mechanism of irreversible inactivation of phosphomannose isomerase by silver ions and Flamazine. *Biochem* **34**: 7896–7903
- White RJ (2002) A historical overview of the use of silver in wound management. *Br J Nurs*: S3–S8
- Willoughby ML (1977) Disorder of granulocytes, monocytes and lymphocytes. In: *Paediatric Haematology*. Churchill Livingstone, Edinburgh
- Wlodkowski TJ, Rosenkranz HS (1973) Antifungal activity of silver sulphadiazine. *Lancet* (i): 739–40
- Wright JB, Lam K, Burrell RE (1998) Wound management in an era of increasing bacterial antibiotic resistance: A role for topical silver treatment. *Am J Infect Control* **26**(6): 572–7
- Wright JB, Lam K, Hansen DL, Burrell RE (1999) Efficacy of topical silver against fungal burn wound pathogens. *Am J Infect Control* **27**(4): 344–50