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Silver in medicine: The basic science☆

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ABSTRACT

Silver compounds are increasingly used in medical applications and consumer products. Confusion exists over the benefits and hazards associated with silver compounds. In this article, the biochemistry and physiology of silver are reviewed with emphasis on the use of silver for wound care.

1. Introduction

Silver is a naturally occurring element with an atomic weight of 107.870 and an atomic number of 47 [1]. Silver may be found in nature as the pure element, but more commonly occurs in ores, including Argentite (Ag_2S), and horn silver (AgCl); and in combination with lead, lead–zinc, copper, gold, and copper–nickel [1]. Pure silver has a brilliant white metallic luster and is slightly harder than gold but highly malleable and ductile [1]. Silver has a melting point of 961.93 °C, a boiling point of 2212 °C and a specific gravity of 10.5 [1]. Silver has the highest electrical conductivity and lowest contact resistance of any element and the highest thermal conductivity of any metal [1].

There are 59 known isotopes of silver. Only 2 isotopes (Ag^{107} and Ag^{109}) are naturally occurring and stable. Silver exhibits three oxidation states Ag [+1], Ag [+2] and Ag [+3] (pure metallic silver is Ag [0]). Of these, only the Ag [+1] state is sufficiently stable for use as an antibiotic as the other cations are highly reactive and short-lived [2,3]. Silver compounds ionize in the presence of water and biologic fluids to release Ag (+1) [2].

Human experience with silver is ubiquitous, with the metal used for currency, jewelry, and food handling from the beginning of recorded history. There is evidence that humans learned to separate silver from lead as early as 3000 BC [1]. The use of silver for currency and for drinking cups is mentioned in the first book of the Old Testament [1,4]. In addition to jewelry and silverware, silver in contemporary life is used in dental fillings, photography, water disinfection, brazing and soldering, and electronic equipment [5].

2. Biochemistry and physiology

Silver is not a recognized trace metal in humans and appears to have no known physiological role or nutritional value [2,6]. Silver does occur in the body at low concentrations secondary to natural exposure via inhalation or ingestion [2,5,7]. Silver is released into the air and water through the natural weathering of rocks (rain and water exposure) and by human activities including cement manufacture and the burning of fossil fuels [5]. Unlike mercury, silver does not appear to concentrate in aquatic animals [5]. The US Environmental Protection Agency recommendation for daily intake limits of silver is 0.005 mg/kg/day [7]. The allowable levels of silver in drinking water are 0.1 mg/L [5] or 50 parts per billion [8]. Typical silver levels in normal individuals include a blood concentration of <2.3 μg/L

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and a urinary excretion of 2 μg/day [3,9]. This is largely from ingestion in food and drinking water. The daily oral intake of silver from dietary sources is estimated to be 27–88 μg/day [8,10]. Orally ingested silver is mostly absorbed through the small intestine [8]. The GI tract is the main avenue of excretion for ingested silver [8].

Silver in the non-ionized state has no biocidal action [2]. Pure (elemental) silver is generally considered nontoxic when used at clinical doses [11,12]. Silver jewelry (often used for body piercing), likewise is inert. White and Cutting note that "the interaction of metallic silver with intact skin does not cause any detectable increase in blood levels and is not of great toxicologic interest" [3].

Silver is not an eye or skin irritant (US EPA Toxicity Category IV), and is not a skin sensitizer [7]. Silver is not known to have human carcinogenic potential, and does not appear to be a mutagen [7].

In the presence of sweat, sebum or moisture, silver ions placed on intact skin will accumulate on the skin surface, with some penetration of the superficial layers resulting in precipitation in the stratum corneum as silver sulfide [2]. Systemic silver is mostly excreted through the liver and kidneys but hair and nail growth also provides a minor avenue of excretion [2]. The uptake and metabolism of silver have not been well studied with exception of some work with burn antimicrobials such as silver sulfadiazine and silver nitrate. Silver nitrate has been utilized as a topical burn treatment since 1965 and silver sulfadiazine has been a mainstay of burn care since 1968 [13–15]. As much as 10% of silver sulfadiazine may be absorbed through partial thickness burns that have good vascularity [2,16] with blood silver levels of >300 μg/L measured [2,16–18]. The absorption of silver is greatest during the inflammation and cell proliferation phases of wound healing [6,19,20]. Urinary silver excretion may increase a thousand-fold when silver compounds are used to treat large open wounds (burns) for prolonged periods of times [11,18]. This appears to have no clinical significance [11].

Constable et al. [21] investigated systemic absorption of Ag^{111} tagged silver nitrate solutions applied to open wounds in a rat model. They found significant silver isotope uptake in the liver with lower levels present in the kidneys. After cessation of silver nitrate treatment, the isotope rapidly cleared from the liver with 40% remaining after one week and 25% after two weeks.

In burn patients being treated with silver sulfadiazine, plasma silver levels can reach a level of 50–310 μg/L and urine excretion may reach a maximum of 400 μg/day [3]. Silver sulfadiazine tagged with radioactive Ag^{110} demonstrates that silver tends to accumulate in superficial wound layers and is completely cleared in 28 days [3,22]. Elevated blood and urine silver levels in conjunction with increased liver enzymes have been documented in burn patients treated with 'nanometer silver' or nanocrystalline burn dressings [3,23,24]. These resolved after treatment was discontinued.

Pure metallic silver is inert and does not react with human tissue or kill microorganisms until it is ionized [6]. There is a direct correlation between bacterial lethality and free silver ion concentration in the medium. Silver ion that is bound, chelated or precipitated into insoluble complexes with tissue exudate or secretions is not available for antimicrobial action. Ionic silver is highly reactive and will combine with halides (particularly chloride), inorganic compounds, organic acids, negatively charged proteins, DNA and RNA [25,26]. Because many of these compounds can be found in wounds, topical silver released into a wound "can be rapidly consumed" [25]. Chloride ion seems to be a particular problem as wound exudate has a high percentage of Cl^- ions, which bind with Ag^+ to form the biologically inactive precipitate silver chloride (AgCl) [11]. The amount of silver required for efficacy in complex wound broth models is 80–2000 times higher than requirements in simple aqueous solutions [26–29]. Some experts argue that when excess Cl^- is present, it is possible to overcome this precipitation (and to restore antimicrobial action) with the delivery of relatively massive amount of silver [11,28,30]. Clinical experience bears this out, and most commercially available silver dressings purport to deliver high silver ion levels for this reason. One study testing the antimicrobial effects of a silver dressing in simulated wound fluid concluded that the silver-containing dressing is still likely to provide a barrier against infection presumably because of large levels of delivered silver ion [3,31].

2.1. *Antimicrobial effects*

In addition to binding anions and proteins in biologic systems, silver ion (Ag^+) avidly binds to cell surface receptors of bacteria, yeasts and fungi [2]. Silver cation also strongly binds to electron donor groups of biologic molecules containing sulfur, oxygen and nitrogen [2]. The binding of silver ion to sulfhydryl groups and proteins on cell membranes appears to be critical to antimicrobial action [2]. The ionizing capacity of various silver compounds is critical in comparing the antimicrobial activities [2]. Ionization of silver is also proportional to the surface area of dressing that is exposed to the wound. Applied electrical current increases ionization [2]. The combination of silver-nylon dressings and weak direct current has been extensively studied in a number of animal models [32–43] and in limited human studies [44,45].

While it is generally recognized that ionic silver is responsible for the antimicrobial activity of silver due to the dissociation of ions from the oxidized metal surface, the actual mechanism by which ionic silver kills bacterial cells has not been established unequivocally. There are four plausible mechanisms that have been postulated for the antimicrobial effects of silver [6,11,46–50]. Regardless of the intra-cellular mechanism, binding of silver to the cell membrane with intracellular absorption is an obligatory first step and sensitive bacteria accumulate silver against a concentration gradient until lethality is reached [2,18].

The first proposed mechanism involves the inhibition of life-sustaining enzymes by chemical interaction with silver ion. Silver ion is capable of blocking the electron transport system in bacteria [51,52]. Concentrations of 15 μg/mL of ionic silver have been found to inhibit the oxidation of glucose, glycerol, fumarate, succinate, D-lactate, L-Lactate, and other endogenous substances in *E. coli* [53]. Ionic silver has been shown to inhibit the enzymes of the respiratory chain at two specific sites: between the b cytochromes and cytochrome d, and between the site of substrate entry into the respiratory chain and flavoprotein in the NADH and

succinate dehydrogenase regions. At concentrations as low as 2 μg/mL of ionic silver, the uptake of inorganic phosphate was inhibited and the efflux of accumulated phosphate occurred [53]. Silver ion interacts with thiol groups on enzymes. Thiol groups are present in enzymes that contain the amino acid cysteine, and when ionic silver binds to this group, the enzyme is deactivated, which results in bacterial cell death. However, a cell may circumvent this mechanism by producing large amounts of glutathione or reduced cysteine in the protoplasm, which has the potential to prevent thiol–silver binding [54].

The second mechanism by which ionic silver kills bacterial cells is through interaction and rupture of the cell membrane or cell wall. A bacterial cell membrane contains both cationic and anionic charges on its surface. In solution, the ionic silver will electrostatically bind to the anionic portions of the membrane. This can inhibit the movement of the organism or cause the membrane to rupture or leak. Ionic silver has been demonstrated to induce the leakage of mannitol, succinate, glutamine, and proline from bacterial cell membranes [53]. In addition, the binding of silver to a membrane can inhibit the passage of nutrients through the membrane, and/or interfere with normal concentration gradients between the cell and surrounding environment, leading to cell death.

A third mechanism involves the interaction of ionic silver with bacterial cell DNA. While eukaryotic cells are not affected by this mechanism (since DNA is contained within the nucleus), prokaryotic cells, such as bacteria do not have a nucleus and have DNA present in the cytoplasm. Ionic silver has been shown to interact with the guanine–cytosine and adenine–thymine base pairs. The interaction of ionic silver with guanine–cytosine involves the N [7] atom of guanine binding to silver, while interaction with the adenine–thymine pair causes thymine dimerization in the presence of UV light [54]. Both of these interactions will result in mutation of the DNA and ultimately in the death of a bacterial cell.

A fourth interaction involves the destruction of a bacterial cell by silver free-radicals. These free-radicals possess high antimicrobial potency due to the presence of unpaired electrons on the silver atom. Ionic silver can bind to many amino acids in a bacterial cell, including arginine and glutamic acid. When silver binds to amino acids, an organometallic complex is formed. If the silver-containing bond of the complex later breaks, a silver free-radical can be generated inside the cell. Silver free-radicals accumulating in the cell can impair the electron transport chain, inactivate bacterial DNA and RNA, damage and rupture the cell membrane, and bind and precipitate proteins with cysteine and thiol groups causing cell death [55,56].

The fact that silver resistance is extremely uncommon suggests that several of the proposed mechanisms of action may occur simultaneously. In order to produce a completely silver-resistant microorganism in the lab, it is necessary to overcome all 4 possible mechanisms of action of the silver ion.

2.2. *Oligodynamic effect*

Silver ions are antimicrobial at very low concentrations. Antimicrobial efficacy of silver has been determined through the use of minimum inhibitory concentration (MIC) testing, a method that evaluates the lowest concentration of an antimicrobial agent that will visibly inhibit the growth of a microorganism. In different studies, the MIC for silver has shown wide variation. Ten-fold variations in MIC (8–80 mg silver/mL for *Staphylococcus aureus* and 8–70 mg silver/mL for *Pseudomonas aeruginosa*) have been reported to inhibit bacterial growth at bacterial concentrations of 10^5–10^7 colony-forming units (CFU)/mL [57]. The effectiveness of very low levels of metal ions against these high bacterial concentrations is explained by the oligodynamic effect.

The ''oligodynamic effect'' (Gr. *Oligos* meaning few, *dynamis* meaning power) was described in the 1895 by von Nageli, who performed the first systematic studies of the antibacterial effects of metals [58]. The term 'oligodynamic' comes from the observation that the lethal effect on bacteria is observed at very low concentrations of silver and other metals [2]. Thiele and Wolf first demonstrated the oligodynamic phenomenon in 1809 by placing silver on an agar plate that had been inoculated with bacteria. After incubation, they noted regions around the silver metal where no growth of bacteria occurred. A fundamental observation was noted during the course of these experiments was that if the metal surface was rigorously cleaned, either chemically or mechanically, the silver lost its antimicrobial effectiveness. This led to the general conclusion that high-purity silver (>99%) was devoid of activity, while oxidized silver surfaces released ionic silver, which was responsible for the observed oligodynamic effect. For most microorganisms, an antimicrobial effect is seen at silver ion levels of one part-per-million or lower [2,11,48,59].

Using the worst case scenario with the reported MIC data above, 8 mg/mL of ionic silver (the low side of the reported MIC range) combined with 10^7 CFU/mL (the high side of the bacterial levels used in MIC testing) would still yield an incredibly high silver ion: bacterial cell ratio as follows:

Number of silver ions in each milliliter of solution:

$$\frac{8\times10^{-6}\ \text{g Ag}^+}{\text{mL}}\times\frac{1\ \text{mole Ag}^+}{107.86\ \text{g Ag}^+}\times\frac{6.02\times10^{23}\ \text{Ag}^+}{\text{mole Ag}^+}=\frac{4.46\times10^{16}\ \text{Ag}^+}{\text{mL}}$$

Since each milliliter of solution contains 1×10^7 CFU/mL, the silver ion:bacterial cell ratio would be:

$$\frac{4.46\times10^{16}\ \text{Ag}^+\ \text{ions per mL}}{1\times10^7\ \text{bacteria per mL}}=4.46\times10^9\ \text{ions per cell}$$

Thus, the oligodynamic advantage provides over one *billion* silver ions for e*ach bacterial cell* present in solution. This fits well with clinical experience. Clarke in 1937 noted that bacteria, trypansomes and yeasts are killed by silver concentrations of from 10^5 to 10^7 ions per cell which he termed the 'estimated number of enzyme-protein molecules per cell'' [2]. When viewed from another perspective, most pathogenic organisms are killed at silver ion concentrations of 5–40 parts per *million* (ppm), with some resistant organisms, including methicillin-resistant *Staphylococcus epidermis* killed at 60 ppm [26,60–63].

2.3. *Antimicrobial resistance*

True microbial resistance to silver is extremely uncommon, although the exact incidence remains undefined [64]. The few

articles that can be found consist of in vitro studies, letters to the editor or collective reviews [64]. Chopra in a 2007 literature review noted that there have been fewer than 20 documented cases of bacterial resistance to silver since 1975 [57,64]. Nevertheless, silver-resistant strains of *Staphylococcus aureus*, *Acinetobacter baumannii*, *Escherichia coli*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Pseudomonas stutzeri* and *Salmonella typhimurium* have been reported [65–73]. Some were intentionally created in the laboratory [66,73], while others were isolated from veterinary [65] or human sources, or from the environment (e.g. silver mines) [71]. Many of these reports appeared after the widespread use of silver nitrate or silver sulfadiazine became common for the management of burn patients [74]. Two strains of *Enterobacter cloacae* resistant to silver sulfadiazine were isolated from a burn center in 1973. Interestingly, both strains retained sensitivity to silver nitrate [74]. The resistance to silver sulfadiazine was overcome by exposure of the strains to low levels of ampicillin and oxacillin intended to induce cell wall damage and increase cellular ingress of silver sulfadiazine [74]. On this basis, the authors postulated that the structural basis of silver sulfadiazine resistance involved the cell wall [74].

Silver-resistance may be encoded in plasmids [47,66,67,70,71,75] but the presence of plasmids does not appear to be necessary for the production of silver resistance. Li et al. were able to experimentally create silver-resistance in some strains of *E. coli* lacking plasmids [66]. Rosenkranz et al. reported two strains of *Enterobacter cloacae* resistant to silver sulfadiazine [74]. The resistant bacteria harbored episomes for resistance to carbenicillin and kanamycin, however resistance to silver sulfadiazine could not be transferred by these episomes [74].

There is a theoretic basis for plasmid-mediated silver resistance in bacteria. Loh et al. [65] examined the prevalence of three genes coding for silver resistance (*sil*E, *sil*P and *sil*S) in 33 strains of methicillin-resistant *Staphylococcus aureus* (MRSA) and 8 strains of methicillin-resistant coagulase negative Staphylococci (MR-CNS) obtained from animal and human wounds and nasal cavities. Two of the resistance genes (*sil*P and *sil*S) were absent in all specimens tested. Two MRSA and one MR-CNS strain were positive for the *sil*E gene. Further testing of these strains showed that a silver-containing dressing was effective in killing all strains with or without the resistance gene: in other words, *the presence of a silver-resistance gene did not afford protection to the organism against silver dressings* [64,65]. The investigators felt that this was related to a high level of silver release. Percival et al. [76] used polymerase chain reaction (PCR) to screen for 3 silver resistance transcriptional units (*sil*E, *sil*S and *Sil*P) in 112 bacterial isolates taken from human diabetic foot ulcers. Two silver-resistant strains of *E. cloacae* were identified, with the author noting that this organism is rarely implicated as a primary pathogen in chronic wounds. No resistance was demonstrated in 24 isolates of *Staphylococcus aureus* or 9 isolates of *Pseudomonas aeruginosa* [76].

Because silver ion acts at multiple cellular sites, for an organism to become silver-resistant, all of the mechanisms and sites of action must be overcome [25]. Li et al. [66] were able to experimentally create silver resistance in *E. coli* bacteria by a multi-step exposure to silver nitrate or silver sulfadiazine starting at half the expected MIC value (2–4 mg/L), and increasing exposure levels with each generation. The result was an isolate resistant to silver levels greater than 1024 mg/L showing complete cross-resistance to both silver compounds [25,66]. The proposed mechanism of resistance was a combination of energy-dependent active efflux of silver from the cells combined with decreases in outer cell membrane permeability to silver [66]. All resistant variants produced were deficient in major porins, either OmpF or OmpF plus OmpC [66]. The resistant cells had up to a fourfold decrease in uptake of tagged silver nitrate. Li was unable to duplicate this result using a one-step procedure, suggesting that multiple cellular mechanisms were involved [25]. Warriner and Burrell were able to create a similar resistance in *Pseudomonas aeruginosa* using a 3-step procedure [25]. Kaur and Vadehra created silver resistance in a strain of *Klebsiella pneumonia* [73]. This required 10 or 11 transfers of exposure to increasing concentrations of silver nitrate [73]. The uptake of silver was three to four times lower in the silver-resistant strain indicating altered membrane permeability or cell surface-associated changes, although the authors believed that more than one resistance mechanism may be involved [73].

'Silver does not induce resistance if used at adequate concentrations' [25]. When ionic silver levels are kept appropriately high, multiple simultaneous mutations would have to occur, one for each mechanism of action [25]. Warriner and Burrell state that 'very few cases of resistance were reported in the past when silver nitrate (0.5% or 3176 mg/L) and silver sulfadiazine (1% or 3025 mg/L) were the primary source of silver' [25]. They further caution that introduction of newer dressings that release silver at concentrations below the minimum inhibitory concentrations for many organisms may result in more resistance being discovered [25].

To summarize, silver resistance is exceptionally rare and generally of no clinical significance. It is difficult to experimentally induce silver resistance, as multiple mechanisms of action must be overcome. Silver resistance may be plasmid-mediated but plasmids are not required for resistance to occur, and the presence of silver-resistance genes does not protect bacteria against high levels of silver. Clinicians should preferentially choose dressings that release high levels of ionic silver and demonstrate rapid bactericidal activity to minimize the risk of silver resistance [57,64].

2.4. *Adverse effects*

Adverse physiological effects attributed to silver include allergy, skin staining, and interference of silver-containing dressings with medical imaging procedures. The incidence of true allergy is undefined but thought to be very low. Unlike nickel and other metallic elements, silver is not a skin sensitizer. Reports of allergic reactions to silver sulfadiazine compounds are more likely due to the sulfa component [77]. Skin staining can be further divided into temporary and permanent skin discoloration (argyria). Temporary skin staining of devitalized tissue can occur with topical silver compounds, but this usually involves tissue that is nonviable or sloughing, and resolves once topical therapy ceases. Permanent skin staining is almost always limited to oral

ingestion of silver-containing compounds, although at least one topical product (nanocrystalline silver) has also been implicated.

The term argyria was first used by Fuchs in 1840 [8]. Argyria is defined as a blue-gray discoloration caused by the accumulation of metallic silver or silver sulfide in the subepithelial portions of the skin, conjunctiva, nails or gums [78–80]. Silver particles can also be found in nerves, capillary walls, macrophages, fibroblasts, perifollicular sheaths and elastic fibers [8]. The degree of skin discoloration directly correlates with the amount of silver present [8]. Argyria may occur over large areas of the body and is more common in areas exposed to the sun [80]. The diagnosis is confirmed by biopsy demonstrating gray, brown or black granules scattered in the extracellular dermis. Silver granules are absent in the epidermis but increased melanization of the epidermis and dermal melanophages can be seen [8]. Darkfield microscopy will often demonstrate bright refractory granules and is a useful technique when argyria is suspected but not confirmed by light microscopy [8]. Argyria is a permanent effect, but appears to be a cosmetic problem that may not be otherwise harmful to health [5,80]. The bluish color imparted to the skin often is confused with cyanosis, prompting extensive (and negative) diagnostic workups for causes of hypoxia.

Argyria is almost always associated with *oral ingestion* of silver products, particularly colloidal silver. Proponents of colloidal silver have claimed or advertised that the compound can treat or cure 650 different diseases or disease organisms; eliminate all pathogens in the human body in 6 min or less; and kill every destructive bacterial, viral, and fungal organism in the body including anthrax, Hanta, Ebola, and flesh-eating bacteria [81,82]. Others have claimed that colloidal silver can be used to treat HIV, cancer, tuberculosis, malaria, lupus, syphilis, scarlet fever, shingles, herpes, typhus, tetanus, bubonic plague, cholera, warts, Menieres disease, hemorrhoids, ringworm, prostatism, and appendicitis [8,83]. Ingestion of colloidal silver purportedly clears acne and other infections of the skin; improves nasal discharge and sinus troubles; and improves sexual performance and enjoyment [82].

Published reports of argyria are usually single-case studies. Baker [79] reported a case of a patient who self-treated a skin tumor by the daily ingestion of a homemade colloidal silver solution over a 2 year period. Other than esthetic concerns, the patients' only complaint was a normocytic anemia. Gulbranson et al. reported a case of argyria from ingestion of a colloidal silver dietary supplement for cold and allergy prophylaxis [84]. Baker et al. reported an 11-year old boy with skin discoloration following ingestion of colloidal silver as an alternative-medicine therapy to facilitate mucus clearance for cystic fibrosis [85]. Kim et al. reported a case of diffuse blue-gray skin discoloration in a women who ingested 1 L of colloidal silver solution daily for 16 months as a traditional remedy [86]. Toth et al. reported facial argyria in a vegetarian patient who had ingested colloidal silver for two years to stimulate his immune system [87]. Kwon et al. reported a case of argyria in a 73-year old male who had consumed colloidal silver for over 5 years as an alternative medical remedy. In addition to brown-black extracellular granules seen in the dermis on skin biopsy, the patient also had silver particles in the mucosa of the colon [88]. Schrauben et al. reported a diabetic schizoaffective patient who developed argyria of the face and neck after self-treating his symptoms by ingesting colloidal silver-proteins for approximately 10 years [89]. Wadhera and Fung reported a case of systemic argyria in a 38 year old male who consumed 16 oz of a 450 ppm colloidal silver solution three times a day for 10 months as an internet-recommended treatment for arthritis. The colloidal silver solution was homemade using a battery-operated chamber to leach silver from pure silver wire [8]. Presumably, instructions for building the chamber were also found on the internet.

The US National Institutes of Health/National Center for Complementary and Alternative Medicine states that colloidal silver is not safe or effective for treating any disease or condition [80]. The United States Pharmacopeia and the National Formulary have not listed colloidal silver products since 1975 [8].

In addition to argyria, consumption of large amounts of colloidal silver can result in coma, pleural edema, agranulocytosis or hemolysis [8]. Oral administration of colloidal silver may interfere with the absorption of certain antibiotics, thyroxine and penicillamine [80].

In 1996, the US Food and Drug Administration (FDA) proposed regulations regulating over-the counter products containing colloidal silver ingredients [83]. In 1999 the FDA finalized regulations prohibiting the sale of over-the counter drugs containing colloidal silver or silver salts because they had not been shown to be safe and effective [80,82]. Despite federal restriction, troublesome side effects and demonstrated lack of efficacy, colloidal silver products continue to be both popular and widely available from internet sources.

Although true argyria is probably only seen after oral silver intake, under certain circumstances, skin staining can occur after application of topical silver-containing dressings. Despite the universal use of silver-containing compounds for burn care, Wang et al. note that 'surprisingly, few studies have reported the discolouration and argyria-like appearance in scars, skin and mucus membranes' after the topical application of silver dressings [90]. Most of these reports involve the use of silver nitrate or silver sulfadiazine [17,90–92], however some studies have documented the potential for nanocrystalline silver dressings (Acticoat™) to cause skin staining. Using a porcine deep-partial thickness burn model, Wang et al. reported that a number of wounds treated with nanocrystalline silver had 'a slate-gray appearance', and that brown-black pigment was documented within the burn scars [90]. The mean tissue silver level following use of nanocrystalline silver dressings was 136 μg/g, compared to $<$0.7 μg/g in controls [90]. The level of silver and the severity of tissue discoloration correlated with the length of use of the nanocrystalline silver dressings [90]. A second (case) report of reversible grayish discoloration of the face was reported in a 17 year old burn patient where nanocrystalline silver dressings were employed for 6 days to treat a 30% burn [24]. Walker et al. compared the skin-staining potential of hydrofiber-silver and nanocrystalline silver dressings in a fresh cadaver skin model. When water was used to hydrate the dressings, the nanocrystalline dressing released significantly more silver resulting in approximately 30 times more silver deposition [93]. Using saline rather than water resulted in lower silver release.

Finally, concern has been raised about possible dangers of silver (and other metal-based dressings) that are left in place during magnetic resonance imaging procedures. Cosmetic or decorative skin tattoos may contain ferromagnetic particles, which, in theory, may either heat up or distort the imaging quality during magnetic resonance procedures [94–97]. On this basis, most manufacturers of silver-based wound dressings recommend removal prior to imaging procedures, and in particular prior to magnetic resonance imaging [94]. These recommendations are usually not based upon any specific studies [94].

Chaudhry et al. [94] used a pig model to study the effects of MRI on three standard silver dressings commonly used for burn care. The study was performed independent of any industry funding. Skin temperature and image quality were assessed. None of the silver-containing dressings exhibited significant temperature increases or caused significant distortion of the image quality. On this basis, the authors concluded that it is unnecessary to remove silver-containing dressings prior to MRI studies, and that the three silver-based wound dressings examined were safe for patients and suitable for use in an MRI [94].

In summary, many of the reported complications of silver-based therapies are a result of the inappropriate ingestion of colloidal silver or silver salts for unsubstantiated indications. Short-term use of silver-based wound dressings appears to have few complications. It may not be necessary to remove such dressings prior to magnetic resonance imaging, although most practitioners continue to do so.

2.5. *Clinical aspects: test methods*

There is disagreement over the correct methodology of assessing the antimicrobial effects of silver. Many of the studies on the efficacy of new silver products are sponsored by the manufacturer and tend to promote the benefits of the product under investigation [26,55]. Most are in vitro studies of zones of inhibition on culture plates [26]. Zones of inhibition are 'broadly seen as least reliable' and least-applicable to the actual clinical wound situation [26]. Zone of inhibition data also seems to correlate poorly with log-reduction assays [25]. The zone of inhibition test is not recommended for silver because zone sizes are not proportional to silver release [25,98–100].

Some manufacturers emphasize 30-min kill rates, as this methodology favors products that deliver large silver boluses [26,28]. Other alternative methods to zone of inhibition testing include minimum inhibitory concentrations (MIC), minimum bacteriocidal concentrations (MBC) and log reductions [25,26]. The MIC test indicates susceptibility of organisms to silver but does not yield information about the most effective treatment dose [25]. The treatment dose should be higher than the mutant prevention concentration (MPC) (which is greater than the MIC) to avoid inducing resistance [25]. The MBC and log-reduction tests both assess killing activity [25]. A 1-log reduction is a kill of 90% of the population in the time allotted, with the term *bacteriocidal* usually indicative of a 3-log or greater reduction [25]. To achieve a broad-spectrum bacteriocidal effect or 3-log reduction, studies suggest delivery of a silver ion (Ag^+) concentration of at least 30–40 mg/L in complex fluids containing organic material and chloride [25,61,101,102].

It is difficult to compare log reduction data between different studies, as investigators use different incubation times, different media and different organisms [25]. Warriner and Burrell compared log-reduction data from several silver product studies and found that when silver concentrations were greater than 36 mg/L, 67.9% of the test points showed greater than 3-log reductions [25]. There is considerable difference in amount of silver released from different commercial dressings varying from <10 mg/100 cm^2 to over 100 mg/cm^2 but all release more silver than the 10–40 ppm deemed necessary for antimicrobial action [2,103].

To summarize, several standard microbiological assays are utilized by manufacturers to demonstrate antimicrobial properties of silver-containing dressings. None of these assays closely simulate clinical conditions. There is a paucity of published log-reduction data from actual wounds, either in animal studies or from human clinical trials. Fluids found in acute and chronic wounds contain ions (Cl^-) and organic compounds known to bind silver ion, and future development of a clinically accurate antimicrobial assay must take this into account.

2.6. *Clinical aspects: studies of silver-containing dressings*

Meaningful clinical trials of silver-containing wound products are difficult to perform or interpret. There is wide variation in carrier dressing design, claimed levels of silver delivery, source of silver (metallic silver versus ionic compounds) and study end-points. Proper prospective, double-blinded randomized clinical trials are frequently impossible to perform because of small sample size (burn patients), disagreement on end-point, long durations required for healing, multiple co-morbidities common to the chronic-wound population, and inability to blind clinical investigators to the dressings in use.

The type of dressing used influences the efficacy of the biologically active agent [11,104]. Data generated for one type of silver-containing dressing cannot be easily extrapolated to other types [11]. The source of silver differs among dressings and may include ionic compounds, such as silver calcium phosphate and silver chloride, or metallic compounds such as nanocrystalline silver [25,28]. The nanocrystalline silver dressings commercially available in the US and Europe differ in composition, rate of silver release, mechanism of action, and supposedly oxidation state of the bioactive silver moiety [6].

In comparing different dressings 'since the efficacy of silver itself is not at stake' the basic issues are characteristics of the carrier dressing and delivery of silver to the wound [26]. Conformity of the dressing to the wound is probably more important than the silver levels present in a wound dressing [3]. A highly conformable dressing prevents voids and dead spaces where bacteria may flourish [3].

The end-point or determinant of success in a clinical trial is important. The ability to reduce a wound size by 30% might reach statistical significance but is clinically meaningless if the result is a 20 cm decubitus ulcer reduced to a 13 cm still-open deep wound. The only currently accepted endpoint of effectiveness is complete wound healing [105]. Many

published wound care trials last only 4–8 weeks and show little difference between complete wound healing of the treatment and control arms [105]. Robson et al. demonstrated that wound healing tends to either occur quickly or take prolonged periods of time, with the time estimated for wound closure in the prolonged group to be at least 9 months [105,106]. It is difficult to conduct a randomized clinical trial lasting this long, and many evidence-based medicine scoring schemes down-rate the quality of studies that have dropout rates exceeding 20% [105].

Patients with chronic wounds are usually older, are frequently diabetic, have multiple medical co-morbidities and may have nutritional or other issues impacting wound healing. They have high rates of wound infection and recurrence [105]. Often, underlying conditions must be treated before effective wound healing can begin [105]. In clinical practice, wound durations of years are common [105]. These patients are typically treated with a succession of different wound therapies depending on the stage and condition of the wound. Silver dressings (and all other modalities) are almost never used for the entire duration of the wound course [105], making it difficult to compare the efficacy of different dressings in the promotion of wound closure.

Clinical studies in print tend to be case reports or clinical observation series [26]. There are very few adequately designed or adequately powered randomized prospective clinical trails (RCT), and this methodology may be inappropriate and impossible to perform in some wound patient populations. In wound care, it is usually not possible to conduct double-blind clinical trials and therefore impossible to perform the grade A studies demanded by evidence-based medicine advocates [11].

Gravante et al. [51] literature-searched clinical trials involving nanocrystalline silver and found only 5 prospective randomized studies comparing nanocrystalline silver to either silver nitrate (1 study) or silver sulfadiazine (4 studies). Combined, the five studies had only 105 patients in the nanocrystalline group and 180 patients in the SSD or silver nitrate groups [51]. A Cochrane review of the use of topical silver for preventing wound infection found only 26 randomized controlled clinical trials comprising a total of 2066 patients, including 20 studies involving burn patients [107]. The authors stated that 'most studies were small and of poor quality' [107]. Heterogeneity of treatments and outcomes precluded meta-analysis. A similar Cochrane review of the effect of silver-containing wound dressings and topical applications for the treatment (rather than the prevention) of infected wounds found similar results with only three randomized clinical trials comprising a total of 847 patients [108].

The burn population is too small to conduct properly sized randomized clinical trials. Burns of over 50% total body surface area (TBSA) comprise only 4% of burn center admissions in the United States or less than 1000 patients per year [109]. In 2001, the American Burn Association published practice guidelines for burn care based upon literature review by expert panels [110]. There were insufficient data to support evidence-based treatment standards for all 12 topics examined [110]. Since that time, consensus statements have been written on several of the topics reviewed, but in the absence of a sufficient research population, expert opinion, rather than large-scale randomized trials will have to remain the basis of care.

3. Summary

Although the use of silver ion for burn and wound dressings is a recent occurrence, the interaction of humans and silver is not. Silver has been used since antiquity for currency, body adornment, food handling, and water storage/purification without adverse effect on health. Silver is not an essential nutrient and has no physiologic role in human biology, but is found at low levels in the body secondary to inhalation and ingestion of silver from natural sources. Silver is not a known sensitizer, carcinogen or mutagen. True allergy to silver is highly uncommon.

Ionic silver has a broad antimicrobial spectrum. Silver-containing compounds have been the mainstay of burn wound care for nearly 50 years. The reported incidence of antimicrobial resistance is exceptionally low and the multiple mechanisms and sites of antimicrobial action make development of resistance very unlikely. High levels of silver ion will overcome resistance even in bacteria possessing genes that confer silver resistance.

The management of chronic wounds represents an important niche for silver-based dressings. Chronic wounds, by definition, take months or years to heal. Over time, the microbial flora of the wound becomes resistant to multiple antibiotics. Because of inadequate blood supply, systemically administered antibiotics may not reach effective levels within in a chronic wound. Sub-therapeutic antibiotic levels lead to more resistance. Topical antimicrobial therapy does not rely on inadequate blood circulation, delivers agent directly to the site of colonization or infection, and avoids systemic complications. Silver-ion based topical wound dressings can be designed to deliver predictably high and consistent levels of broad-spectrum antimicrobial therapy that is unlikely to induce resistance.

At present, the science supporting a broader use of silver-based dressings has limitations. There is no consensus on the proper method to assay the effectiveness of silver-containing dressings. It is difficult to extrapolate the results of standard in vitro antimicrobial assays to clinical efficacy in acute or chronic wounds. In designing bench or clinical studies, the silver-binding capacity of chloride ion and other species present in wound fluid need to be kept in mind. To avoid problems of binding and resistance, silver products intended for burn or wound care should be designed to release high and sustained levels of silver ion over long periods of time.

The prospective, blinded, randomized trial may represent the 'gold standard' of clinical research, but, for many reasons, is an inappropriate 'silver standard' when applied to burns or chronic wounds. It is probably not possible to produce large-scale prospective studies of burn patients because the affected population is too small. The chronic wound population is large enough for such trials but also infinitely diverse: most patients have confounding systemic diseases such as vascular insufficiency or diabetes mellitus, and most wounds will receive a variety of topical treatments during a prolonged healing

course. The only appropriate endpoint of such studies would be complete wound healing, which may take years to occur. In the absence of the ability to perform truly blinded prospective studies, appropriate standards of care must rely on expert opinion.

Conflict of interest statement

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