Antibacterial activity of silver sulfadiazine against *Streptococcus pyogenes*

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Beta-haemolytic *Streptococcus* is a Gram-positive microorganism that is found in burn wounds and provokes graft failure. *Streptococcus pyogenes* toxins increase the depth and severity of burn wounds. Topical antimicrobial agents can be used to treat burn wounds and improve results after burn infection. However, there are few studies exploring the antimicrobial action of silver sulfadiazine against *S pyogenes*. This study aimed to evaluate 1% silver sulfadiazine antimicrobial activity against *S pyogenes*. We isolated eight *S pyogenes* samples from adult patients at the Hospital Provincial de Rosario, Rosario, Argentina. Six samples were from burn wounds and two from blood culture. The outcomes were compared with the topical antimicrobial agents 2% mupirocin and 1% fusidic acid cream. We tested and compared the susceptibility of *S pyogenes* with these topical antimicrobial agents using agar well diffusion assays and minimum bactericidal concentration tests. The results show that the 1% silver sulfadiazine cream has an inhibitory effect on *S pyogenes* similar to that of 2% mupirocin and 1% fusidic acid cream. Considering its greater antimicrobial spectrum, silver sulfadiazine is a valid alternative to control many infections associated with wounds and burns, including those caused by *S pyogenes*.

Fatality rates from burn wound infection and sepsis have decreased with the advances in infection control measures for burn units and the application of topical antimicrobial agents (Revathi et al, 1998). However, infections remain one of the most common serious complications from burn injury, and approximately 75% of deaths related to burn injuries are associated with infection (Appelgren et al, 2002; Heggers et al, 2002; Norbury et al, 2016).

The incidence of beta-haemolytic *Streptococcus* in burn wounds varies (Bang et al, 1999). *Streptococcus pyogenes* has been associated with the most serious infections in burn patients, leading to severe cellulitis, sepsis and graft failure. Studies have confirmed that *S pyogenes* produces extracellular toxins, increasing the depth and severity of the burn wound. Its pathogenesis can be attributed to the cell membrane M-protein and capsule of this species, which inhibits phagocytosis through polymorphonuclear leucocytes (Moses et al, 1997). Surface structures, such as the M protein family, the capsule and a number of adhesion molecules (including fibronectin, vitronectin collagen-binding proteins and lipoteichoic acid) allow the microorganism to colonise the skin and mucus membranes (Bisno et al, 2003; Gillespie, 2004).

In rare cases, *S pyogenes* can cause necrotising fasciitis and may be complicated unless treated with radical excision and antibiotics (McAdam, 2016).

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Burn wounds infected with *S pyogenes* may exhibit the clinical surgical scarlet fever, inflammatory infiltration boundary and excretion of thin pus. Although *S pyogenes* may not produce an intense systemic response after it infects the donor sites or burns wounds, it typically causes the grafts of those burn sites to slough off, leading to graft failure. Thus, superficial wounds in donor sites may become deep or cause full-thickness skin loss because of this infection. Most *S pyogenes* infections occur within a week after the burn injury, leading to the prescribing of penicillin G to patients in the early post-burn stages (Xiao and Xu, 2015). However, this is not recommended. While it may reduce burn wound infections, colonisation or both, it does not decrease mortality and may increase the risk of selecting resistant microorganisms such as methicillin-resistant *Staphylococcus aureus* (MRSA; Avni et al, 2010; Barajas-Nava et al, 2013).

Using a topical antimicrobial agent is normal practice to treat burn wound infections and improve burn injury outcomes. The therapeutic management of burns involves cleansing, debridement and wound dressing (Cartotto, 2017). However, no consensusis has been reached about optimal antibiotics to control infection or improve wound healing (Wasiak et al, 2013; Norman et al, 2017).

Aim

Although silver sulfadiazine offers a broader antimicrobial spectrum than mupirocin and fusidic acid, few studies have explored its antimicrobial action against *S pyogenes*.

This study aimed to evaluate the 1% silver sulfadiazine antimicrobial activity against *S pyogenes* and compare it with 2% mupirocin and 1% fusidic acid cream.

Methods

Bacterial strains

We studied eight *S pyogenes* strains isolated from eight adult patients admitted to the Hospital Provincial de Rosario, Santa Fe, Argentina. The isolates were recovered from burn wounds of six patients, and from the blood culture of two patients and identified using the Vitek System (bioMérieux, La Balme Les Grottes, France). The bacterial isolates were recovered in different periods (with a difference of 1 to 2 months), which allows the authors to assume that they are not epidemiologically related. The bacterial strains were stored at −70 °C in a preservative medium and subcultured one or two times on blood agar plates.

Topical antimicrobial agents

The topical agents evaluated were 1% silver sulfadiazine (Platsul-A, Soubeiran Chobet, Buenos Aires, Argentina), 2% mupirocin, and 1% fusidic acid.

Silver sulfadiazine is a combination of sodium sulfadiazine and silver nitrate, and it is the most commonly used topical antibiotic agent for both ambulatory and hospitalised burn patients. This agent is an excellent broad-spectrum antibacterial and has been shown to reduce the inflammatory response to burn injury, decrease bacterial colonisation, and provide a firm eschar for easier wound management (Church et al, 2006; Salvador Sanz et al, 2011).

Fusidic acid is a selective antibiotic that reaches a high antimicrobial concentration in the deep skin layers after topical application on both intact and damaged epidermis. It is available in several topical formulations (Bonamonte et al, 2014).

Mupirocin has potent inhibitory activity against *S pyogenes*, staphylococci, and MRSA. Although primarily marketed for nasal decontamination, mupirocin has increasingly been used as a topical agent in burn units (Church et al, 2006; Jagdale et al, 2020).

Susceptibility testing of topical antimicrobial agents

Susceptibility testing of topical antimicrobial agents was conducted using agar well diffusion (AWD) assays (Nathan et al, 1978). Agar plates were inoculated with the test organism and 6 mm wells were cut. The wells were filled with 250 µl of antimicrobial solutions, and all plates were incubated overnight at 35°C. The diameters of the clear zones around the antimicrobial-containing wells were measured after incubation. Results >8 mm indicate the susceptibility of the tested bacterial strain to antimicrobial agents.

Minimum bactericidal concentration

Topical antimicrobial agents with an inhibition diameter >8 mm were analysed to identify their antimicrobial activity against *S pyogenes*. First, each topical agent was diluted with Mueller Hinton broth (MHB), in a range of 0.0001% to 1% (v/v). Then, 1 ml of an *S pyogenes* inoculum (106 UFC/ml) was added to the MHB, and controls without antimicrobial agents were prepared. The samples were incubated and shaking at 37°C for 24 hours. Then, 1 ml of bacterial inoculum was harvested from the tubes and plated onto sheep blood agar (Clinical and Laboratory Standards Institute, 2020). Three

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Table 1: Agar well diffusion assays and broth microdilution test the susceptibility of *S pyogenes* to silver sulfadiazine, fusidic acid and mupirocin.

Strain number	Agar well diffusion (mm)			Minimum bactericidal concentration (%) (w/v)		
	Silver sulfadiazine	Fusidic acid	Mupirocin	Silver sulfadiazine	Fusidic acid	Mupirocin
1	30.3	20.7	40	0.01	0.01	0.001
2	25.7	21.0	36	0.1	0.01	0.001
3	25.3	22.3	37	0.01	0.01	0.0001
4	30.0	21.7	36	0.01	0.01	0.001
5	28.7	18.3	28	0.01	0.01	0.0001
6	30.7	21.0	35	0.01	0.01	0.0001
7	30.0	20.0	32	0.01	0.01	0.001
8	32.3	22.3	38	0.1	0.01	0.001
Range	25.3–32.3	18.3–22.3	28–40	0.01–0.1	0.01	0.0001–0.001

independent assays determined minimum bactericidal concentration (MBC) values (that is, the lowest concentration at which the antimicrobial agent killed the bacterial inoculum (Levison, 2004).

Statistical analysis

The mean inhibition diameter and the mean MBC were determined for each strain and each antimicrobial agent. Student's t-test was conducted to estimate the statistical differences between groups of strains. A *P*-value <0.001 was considered statistically significant.

Results

AWD assays

Eight *S pyogenes* isolates were obtained from clinical samples of eight patients. *Table 1* and *Figure 1* show the inhibition diameters obtained from the AWD assays for the eight strains. The results show that all tested *S pyogenes* isolates were susceptible to silver sulfadiazine (mean inhibition diameters: 25.3 mm and 32.3 mm), mupirocin (mean inhibition diameters: 28 mm and 40 mm), and fusidic acid (mean inhibition diameters: 18.3 mm and 22.3 mm). The inhibition diameters were significantly smaller ($p<0.001$) for fusidic acid.

MBC tests

MBC ranged from 0.01 to 0.1% (w/v) for silver sulfadiazine, from 0.0001 to 0.001% (w/v) for mupirocin, and from 0.01 to 0.1% (w/v) for fusidic acid *[Figure 2]*.

Discussion

Effective treatment with a topical antimicrobial reduces the microbial load on the open burn wound surface and reduces the risk of infection. The selected topical antimicrobial should inhibit microorganisms recovered from wound surveillance cultures of burn patients and infections acquired in hospital burn units. (Church et al, 2006). Nosocomial infections are caused by several microorganisms, so the antimicrobial agent with the broadest spectrum should be used against microorganisms (Glasser et al, 2010).

Fusidic acid has good *in vitro* activity against staphylococci, including both methicillin-sensitive and -resistant strains, beta-hemolytic streptococci, and *Corynebacterium* spp. It is also effective against Gram-positive anaerobes, such as *Clostridium difficile, Clostridium perfringens* and *Peptostreptococcus* spp (Marian et al, 2020).

While the mupirocin spectrum of action includes Gram-positive bacteria and some Gram-negative bacteria (Khoshnood et al, 2019), neither mupirocin nor fusidic acid are effective against *Pseudomonas aeruginosa, Enterobacteriaceae,* and some anaerobes. Therefore, these antibiotics are not an option that should be considered in burn wounds, due to the high prevalence of *P aeruginosa* in burn units and increased bacterial resistance to these compounds (Foster, 2017). Considering its antimicrobial activity, silver sulfadiazine is a suitable alternative to treat *S pyogenes* infection.

This study evaluated the antimicrobial activity of 1% silver sulfadiazine against *S pyogenes* and compared it to 2% mupirocin and 1% fusidic acid creams, using AWD and MBC methods to confirm the inhibitory action of the three antimicrobial agents. While there are no defined cutoff values for inhibition diameters in AWD assays, we obtained precise, easy-to-read diameters for the topical agents. Although the antimicrobial agents' complexity makes it difficult to standardise test conditions, we confirmed the inhibitory effect of three tested agents against *S pyogenes*. The results obtained with the MBC tests were consistent with

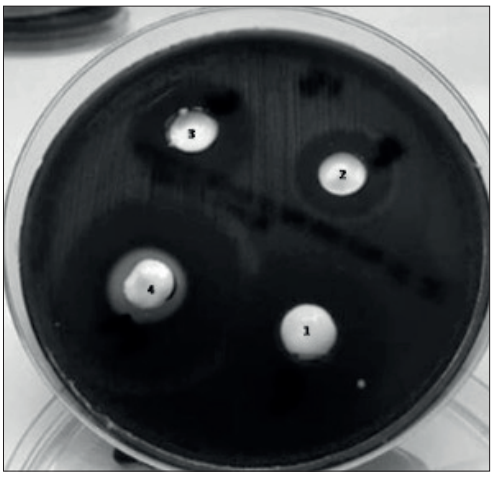

Figure 1. Agar well diffusionassays showing inhibition diameters after testing the different compounds against S pyogenes *strain 2 on trypticase soy agar with 5% sheep blood. 1: silver sulfadiazine; 2–3: fusidic acid; 4: mupirocin.*

Figure 2. Serial confrontations of S pyogenes *strain 2 against the compounds at different concentrations on trypticase soy agar with 5% sheep blood. 1: silver sulfadiazine; 2–3: fusidic acid; 4: mupirocin; 5: without any topical agent. A: 1%; B: 0.1%, C: 0.01%, D: 0.001%, E: 0.0001% (w/v).*

the inhibition diameters observed with the AWD assays. Even though our tests confirm the presence of bactericidal activity in these agents, further pharmacodynamic and pharmacokinetic studies must be conducted to assess their clinical efficacy and antibacterial activity *in vivo* (Pankey and Sabath, 2004).

Many physicians prescribe fusidic acid to patients because it is a topical anti-infective choice with a low risk of contact sensitisation. In addition, its systematic use prevents bacteria from developing cross-resistance to other antibiotics. Fusidic acid is effective in vitro against *S aureus, Staphylococcus epidermidis* and *S pyogenes*.

Alsterholm et al (2010) tested the effect of fusidic acid on *S pyogenes*, with results of a 10 µg/ml MIC for fusidic acid as part of a cream, a 6.25 µg/ml MIC for fusidic acid alone, and a 500 µg/ml MIC for the entire cream. Leclercq et al (2000) reported that fusidic acid was moderately active against *Streptococci* after evaluating its effectiveness against 242 strains of *Streptococci* isolated from soft tissue and skin infections. They obtained MIC levels ranging from 8 µg/ml to 16 µg/ml, with only two strains displaying MICs for fusidic acid ≥64 mg/l.

Nevertheless, the use of topical fusidic acid is firmly discouraged because of the spread of a fusidic acid-resistant clone of *S aureus* associated with impetigo (Simor et al, 2007; Elston, 2009; Shittu et al, 2009). Although mupirocin is effective against Gram-positive skin flora, such as *S aureus* (Dai et al, 2010), its effectiveness as an antimicrobial agent is diminished with the development of resistant strains (Hogue et al, 2010; Vázquez et al, 2019).

Our results show that silver sulfadiazine has similar efficacy against *S pyogenes* as mupirocin and fusidic acid. This topical antimicrobial inhibits the development of the major microorganisms responsible for wound and burns infections, such as *S aureus*, MRSA, *S epidermidis*, beta-hemolytic *Streptococcus, P aeruginosa, Escherichia coli* and other enterobacteria (Snelling et al, 1978; Koo et al, 1989; Nagesha et al, 1996; Palmieri and Greenhalgh, 2002; Olhan et al, 2005; Hussain and Ferguson, 2006; Casabonne et al, 2015). In addition, studies demonstrated the effectiveness of the antimicrobial activity of 1% silver sulfadiazine cream for decreasing colonisation rates in burn wounds (Heyneman et al, 2016; Norman et al, 2017). In many burn centres, silver sulfadiazine is the standard topical antibiotic used to treat burn wounds. Despite its widespread use, its efficacy against *S pyogenes* has not been properly researched. However, the susceptibility of *S pyogenes* to mupirocin and fusidic acid is well documented.

Conclusion

Our study demonstrated that silver sulfadiazine has remarkable antibacterial activity against *S pyogenes*. Due to its broad antimicrobial spectrum, silver sulfadiazine is a suitable alternative to control wounds and burn infections. WINT

Conflicts of interest

The authors declare no conflict of interest.

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