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Enhanced topical delivery of non-complexed molecular iodine for Methicillin-resistant *Staphylococcus aureus* decolonization

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ABSTRACT

Staphylococcus aureus, a leading cause of serious human infections in both healthcare and community settings, is increasingly difficult to control due to expanding resistance to multiple antibiotic classes. Methicillin-resistant *S. aureus* (MRSA) strains have disseminated on a global scale and are associated with adverse patient outcomes, increased hospital stays, and significant economic costs to the healthcare system. A proximal step in *S. aureus* infection is colonization of the nasal mucosa, and effective strategies to decolonize high risk patients to reduce the risk of invasive infection and nosocomial spread represent an important clinical priority. With rising resistance to mupirocin, the most common antibiotic utilized for nasal MRSA decontamination, we are examining the use of pure molecular iodine (I_2)-based formulations for this indication. Recently, an iodophor formulation of povidone-iodine (PVP-I) has shown significant promise for nasal MRSA decontamination by swabbing the anterior nares of patients in hospital settings, but the I_2 concentration in this treatment is less than 0.01% of total iodine species present and like all providone-iodine formulations causes skin staining. Here we determine that a novel non-staining formulation of I_2 combined with the safe organic emollient glycerin delivers high local concentrations of the active antimicrobial entity (I_2) with minimal evaporative loss, exhibits activity at ~1 part per million against MRSA and other important Gram-positive and -negative human pathogens. This formulation for I_2 topical delivery produced similar reductions in mean bacterial burden and was associated with fewer treatment failures (< 2-logfold reduction) than PVP-I in a murine model of MRSA nasal decontamination. Formulations of I_2 in glycerin emollient merit further exploration as topical disinfectants for human medical indications.

1. Introduction

For decades we have witnessed increasingly high incidence of serious *Staphylococcus aureus* skin and soft tissue infections and bacteremia, occurring both in healthcare and community settings. Though no patient group is excluded, serious *S. aureus* infections disproportionately affect vulnerable populations including the elderly, juveniles, cancer patients (Big and Malani, 2010), diabetics (Smit et al., 2016) and those in intensive care units, where metastatic diseases such as endocarditis, deep organ abscess, and sepsis can develop (Holland et al., 2014). The case fatality rate of *S. aureus* bacteremia is alarmingly high, estimated between 20 and 30% (Kern, 2010). Disease isolates resistant to front line antibiotics, notably methicillin-resistant (MRSA) strains, have become commonplace in the United States and numerous other countries, with a globally disseminated clone (USA300) of community-acquired MRSA contributing significantly to the increased incidence of severe disease and adverse clinical outcomes (Mediavilla et al., 2012).

The ability of *S. aureus* to establish colonization in its preferred niche, the human nasal mucosa, is a critical first step in the pathogenesis of disease (Krismer et al., 2017). Approximately 20% of people are persistently colonized, and an additional 60% intermittently colonized, with *S. aureus* in their nose (Kluytmans et al., 1997); prior stay in a hospital or chronic care facility and prior antibiotic exposure represent important risk factors for acquisition (Hidron et al., 2005; Xue and Gyi, 2012). Children have higher rates of *S. aureus* colonization than adults (Armstrong-Esther, 1976), and more than two-thirds of normal babies have at least one positive culture for the organism (Peacock et al., 2003). In the setting of hospitalization or immune compromise, nasal carriers of *S. aureus* are at markedly increased risk of

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developing bloodstream infection, and genomic fingerprinting confirms the nasal strain of the individual is usually the agent of the systemic disease (von Eiff et al., 2001). Given this association, many healthcare facilities in the world screen at-risk hospitalized patients for MRSA nasal colonization and, when positive, attempt to decontaminate the patient with a topical antibiotic, typically mupirocin (Bactroban®) (Bode et al., 2010; Septimus and Schweizer, 2016). However, mupirocin is a bacteriostatic drug that requires repeated application over several days to achieve *S. aureus* eradication (Ammerlaan et al., 2009; Mehta et al., 2013), with resistance to this agent on the rise and approaching 30% in some clinical populations (Antonov et al., 2015; Poovelikunnel et al., 2015). Considering alternatives to mupirocin, iodine, an essential element, has a long history as a disinfectant and antibacterial sterilizing agent, especially in surgical skin preparation. In 2010 the 3M Company began marketing a skin and nasal antiseptic preparation (SNP) based on PVP-I as an alternative to topical mupirocin; clinical trials have demonstrated consistent benefits (Perl et al., 2002; Bebko et al., 2015). The use of an iodine-based disinfectant is attractive, as (a) it is well known that iodophors demonstrate rapid and broad-spectrum bactericidal activity (within 10–20 s) and (b) there is no evidence that bacteria can develop resistance to iodine (Houang et al., 1976; Lanker Klossner et al., 1997) since it reacts rapidly with several functional groups (double-bonds, amino groups and sulphydral groups) which results in simultaneous action against multiple molecular targets to cause death. In pilot studies, intranasal application of SNP produced a significant (> 2 log) reduction of *S. aureus* colonization in healthy volunteers (Anderson et al., 2015) and was associated with a reduction in deep surgical site infections among patients undergoing arthroplasty or spine fusion procedures (Phillips et al., 2014).

Iodophors are highly acidic compositions that provide a small concentration of active biocide, i.e. unbound molecular iodine (I_2) (Wada et al., 2016), in equilibrium with large concentrations of iodide/triiodide and polymers that complex I_2 (Favero, 1982; Gottardi, 1999). Complexation of I_2 is necessary since I_2 is unstable in an aqueous environment (Gottardi, 1978, 1981). A paradoxical consequence of this formulation approach is an increase in active biocide upon dilution up to a 100-fold dilution (Ferguson et al., 2003; Gottardi, 1980, 1983). The labeled concentration of "iodine" in iodophors is determined by thiosulfate titration which measures both triiodide and I_2. Consequently, clinicians do not know the concentration of active biocide in the iodophors used in clinical procedures. The concentration of unbound I_2 in the most common iodine-based topical disinfectant formulation, 10% povidone-iodine (PVP-I, Betadine™), is less than 10 ppm or 0.01% of the total iodine atoms present (Gottardi, 1978). Iodophors manufactured with concentrations of I_2 below a critical threshold permit survival of certain bacteria including *S. aureus* (O'Rourke et al., 2003) and have been associated with transmission of nosocomial infections (Weber et al., 2007).

Systemic absorption of iodine across mucous membranes has been demonstrated (Safran and Braverman, 1982) but diffusion of I_2 into and from the epidermis is less well understood. Notably, topical iodine compositions offer the potential to provide a prolonged (> 12 h duration) epidermal antibacterial activity due to a continuous flux or "back diffusion" of absorbed I_2 from treated skin (Gottardi, 1995; McLure and Gordon, 1992). This flux of I_2 is proportional to exposure time and the concentration of I_2 applied to the skin; delivery of pure I_2 without triiodide has been shown to eliminate staining (Kessler, 2001). The low level of unbound I_2 in iodophors mitigates this potential feature of iodine-based skin preps. Also, the presence of several iodine species (e.g. iodide, tri-iodide, hypoiodide, iodate, hypoiodous acid) that do not themselves provide antimicrobial activity increases the risk of systemic toxicity and skin irritation as I_2 per se possesses a benign acute toxicity profile (Duan et al., 1999).

In the present study, we explore basic characteristics of a novel non-staining formulation approach to provide non-staining stabile I_2 which is the actual microbicide in PVP-I; the composition provides emolliency and free I_2 concentrations approximately 100-fold higher than that found in commercially available 10% PVP-I (Gottardi, 1978). Our analysis reveals that formulation with emollient organic carriers (glycerin and propylene glycol) markedly reduces the vapor pressure of I_2 and consequently its loss into the atmosphere by about 100-fold, a property not shared by commercially available iodophors including PVP-I. Minimum inhibitory concentration (MIC) testing against MRSA and additional selected contemporary multidrug-resistant (MDR) bacterial pathogens confirmed potent antimicrobial activity of the new I_2 formulation at or below 1 ppm. Finally, the effectiveness of this formulation in reducing MRSA bacterial burden was examined in a murine model of nasal decolonization.

2. Materials and methods

2.1. Viscosity measurements

Viscosity was measured using a Brookfield Model DV2T (Middleboro, MA) equipped with a Wells-Brookfield cone plate and spindle CPA52Z; thermal control was implemented wih a Lauda Alpha RA-8 (Delran, NJ). Data from vicometric measurements were collected and analyzed using the RheocalcT software package. Viscosity versus shear rate was measured by varying spindle speed up and then down in defined increments at both 25 °C and 33 °C. Two data points were collected at each rpm value; once with spindle speed increasing and once with spindle speed decreasing. The viscosity for 3M SNP was calculated by determining the consistency index; the I2 glycerin formulation exhibited constant viscosity versus shear rate, i.e. Newtonian behavior.

2.2. Iodine vapor pressure study

Saturated solutions of molecular iodine (I_2, Alfa Aesar 14248 Lot 104Z003) were prepared in either: (a) 30 mM acetate buffer, pH 4.5; or (b) emollient organic carriers - glycerin and propylene glycol. The measured concentration of I_2 in each of these samples was 112 ppm (acetate buffer) 632 ppm (propylene glycol) and 1132 ppm (glycerin). One gram of a test article was placed in the bottom of a screw-top glass vial and a lid was tightly fitted to the vial. The inner surface of each lid was fitted with an iodine sensitive paper disc (Fluka #37215, Lot SZBF1310V) held in place against the inner surface of the lid by its threads. The indicator paper was therefore exposed to the atmosphere in the vial which allowed it to react with I_2 in the vapor phase of each vial. At 5 and 20 min and then 1, 6, 24 and 48 h the color of the indicator paper on the inside of the screw-top lids was examined and photographed.

2.3. Bacterial strains and culture conditions

MRSA strain USA300 TCH1516 (ATCC #BAA-1717), isolated from an adolescent patient with severe sepsis syndrome at Texas Children's Hospital in Houston, TX, and vancomycin-resistant *Enterococcus faecalis* (VRE) strain NJ-3 (ATCC #51299), isolated from human peritoneal fluid in St. Louis, MO, were obtained from the American Type Culture Collection (Manassas, VA). Group A *Streptococcus* (GAS) M1T1 serotype strain 5448 was original isolated from a patient with necrotizing fasciitis and toxic shock syndrome (Chatellier et al., 2000). MDR *Pseudomonas aeruginosa* (PA) isolate was isolated from human lung at a tertiary academic hospital in the New York metropolitan area (Fair et al., 2012). MDR *Acinetobacter baumannii* (*AB)* AB5075 was isolated from bone (bone) at Walter Reed Army Medical Center (Zurawski et al., 2012). MRSA and GAS were grown in Todd-Hewitt broth (THB; Difco, BD Diagnostics). VRE was propagated in brain-heart infusion (BHI) broth. PA and AB were grown in Luria-Bertani (LB) broth.

2.4. Bacterial minimum inhibitory concentration (MIC) assay

The I_2 formulation was serial diluted in H_2O in a 96-well flat bottom plate to provide a final concentration range from 0 ppm to 4 ppm. Then 1×10^5 colony forming units (CFU) of each bacterial in a volume of 10 µl H_2O was added to each of the wells. After 5 min at room temperature, a 5 µl aliquot from each well was transferred into a new 96 well flat bottom plate and 200 µl of soft bacterial media agar (0.75% agar in the corresponding bacterial growth media specified above for each strain) was added to each well. Growth of bacterial colonies in the soft agar was analyzed after 12 h incubation in 37 ± 1 °C, and the lowest concentration in which no colonies were observed was defined as the MIC.

2.5. Mice nasal bacterial clearance model

All animal studies were performed under approval by the UC San Diego Institutional Animal Care and Use Committee (IACUC), protocol S00227M ("Mouse Models of Bacterial Infection and Immunity"). Healthy 10 to 12-week-old outbred CD1 mice were randomly assigned to one of three treatment regimens: (a) glycerin (negative control); (b) 3M™ Skin and Nasal disinfectant (positive control); and (c) 400 ppm I_2 in glycerin. All mice in a single cage were assigned to the same treatment regimen. Three experiments were repeated on three separate days as follows: Experiment 1, n = 5 per group, all females; Experiment 2, n = 10 per group, 5 males and 5 females; Experiment 3, n = 10 per group, 5 males and 5 females; Total n = 25/group. Mice were anesthetized with isoflurane and challenged intranasally with 20 µl PBS containing 2×10^8 CFU of MRSA TCH1516. After 24 h, one of the three treatments (10 µl) was applied to the nares of each of the mice. Evaluation of colonized MRSA was performed as previously described (Kiser et al., 1999). After an additional 24 h, the mice were euthanized, and their nasal tissue was excised and dissected with sterile scissors. The nasal cavities were vortexed vigorously (10 s times 3) in phosphate-buffered saline (PBS), and serial dilutions made in PBS and plated in triplicate onto THA plates. Plates were incubated in 37 ± 1 °C for 12 h for CFU determination of the recovered MRSA.

3. Results

3.1. Viscosity of 3M SNP versus I_2-glycerin composition

The viscosity of 3M SNP varied with shear. The consistency index was determined on two separate days, and the apparent viscosity calculated as 5003 centipoise (cP) at 25 °C and 3676 cP at 33 °C (Fig. 1). Viscosity of the I_2-glycerin composition did not change with shear; this composition exhibited classical Newtonian behavior. The viscosities at 25 °C and 33 °C were 885 and 464 cP, respectively.

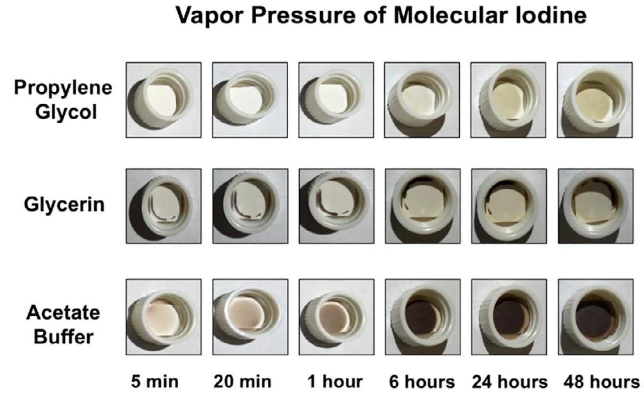


Fig. 2. Organic emollient formulation reduces I_2 vapor pressure and atmospheric loss. Using an iodine-sensitive paper disk assay, the relative vapor pressure of I_2 in glycerin and propylene glycol was compared to its vapor pressure in acetate buffer. Indicator paper stain intensity was visually evaluated using the Munsell Neutral Value Scale. The relative rate of staining per ppm of I_2 in propylene glycol and glycerin as compared to acetate was approximately 270 and 480 times lower.

3.2. Organic emollient formulation reduces I_2 vapor pressure and atmospheric loss

Using an iodine-sensitive paper disk assay, the vapor pressure and atmospheric loss of I_2 was calculated comparing glycerin and propylene glycol emollients to acetate buffer. At 1 h, the control sample of I_2 in acetate buffer was highly colored; by 6 h this paper was 100% black and indistinguishable from the paper at 24 h (Fig. 2). In contrast the indicator paper in the glycerin (1132 ppm) and propylene glycol (632 ppm) samples reacted very little, which indicated that the effective vapor pressure of I_2 was markedly reduced (Fig. 2). Indicator paper stain intensity was visually evaluated using the Munsell Neutral Value Scale (Pantone, Carlstadt, NJ, Cat# M50135). The acetate buffer that contained 112 ppm I2 was scored (N = 8) a 6.68 + 0.108 (standard deviation) at 20 min. After 48 h the propylene glycol (632 ppm I_2) and glycerin (1132 ppm I_2) were scored lighter than the 20 min acetate buffer sample. The observed Munsell lightness unit scores of

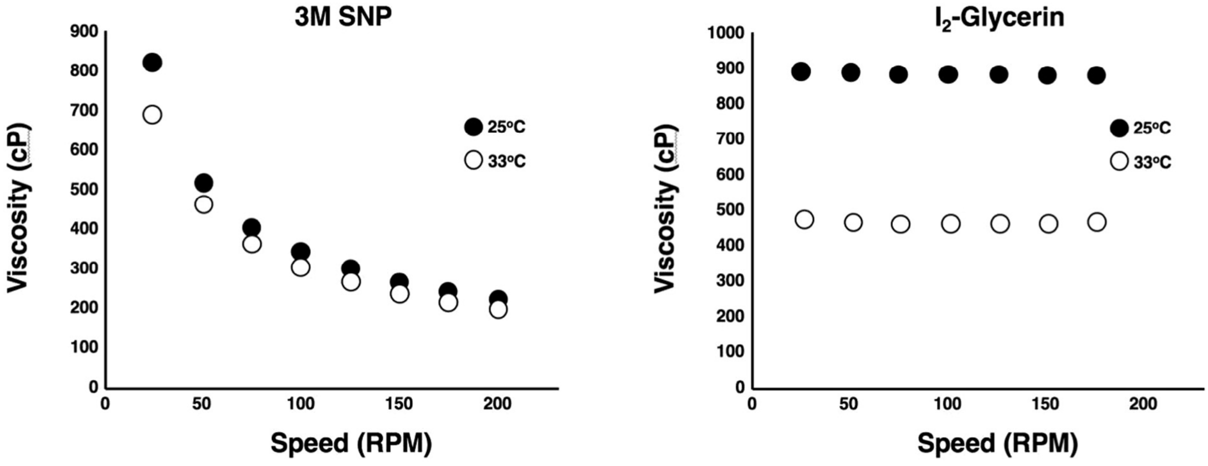
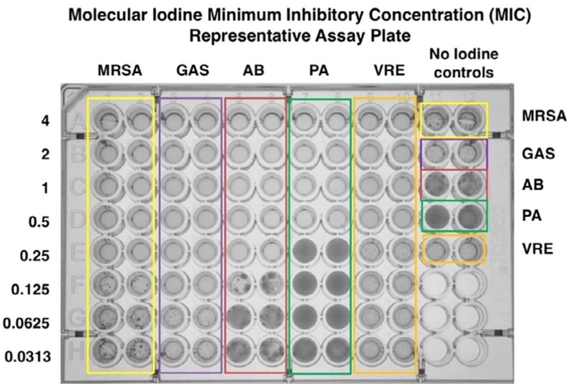


Fig. 1. Stable viscosity of I_2-glycerin in response to shear. In contrast to the viscosity of 3M SNP, the viscosity of the I2-glycerin composition did not change with shear. Rather, this composition exhibited classical Newtonian behavior. The calculated mean viscosities at 25 °C and 33 °C were 885 and 464 centipoise (cP), respectively.



Bacterial Species	MIC [ppm]
Methicillin-resistant *Staphylococcus aureus* (MRSA)	0.25-1
Group A *Streptococcus* (GAS)	0.25-1
Acinetobacter baumannii (AB)	0.125-1
Pseudomonas aeruginosa (PA)	0.5-1
Vancomycin-resistant *Enterococcus faecalis* (VRE)	0.5-2

Fig. 3. The antibacterial potency of the I_2/glycerin formulation by modified microbroth dilution assay with soft agar recovery. The concentration (ppm) at the dilution of product in which no growth of bacteria was detected was defined to represent the minimal inhibitory concentration (MIC); three independent experiments were performed for each bacterial test species.

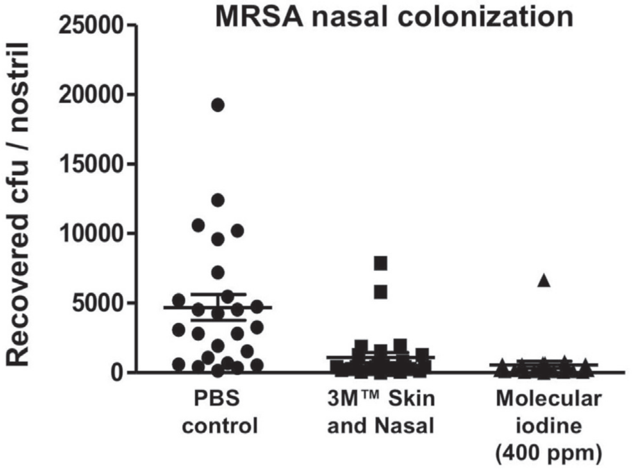
8.18 + 0.108 for propylene glycol and 7.67 + 0.113 for glycerin (N = 8 for each) were assumed to be equivalent to the staining intensity observed at 20 min in the acetate buffer for comparison. The relative rate of staining per ppm of I_2 in propylene glycol and glycerin compared to acetate was reduced by approximately 200- and 400-fold, respectively.

3.3. Inhibitory activity of the I_2/glycerin formulation against MDR pathogens

The antibacterial potency of the I_2/glycerin formulation was assessed in a modified microbroth dilution assay with soft agar recovery (Fig. 3). The concentration (ppm) at the dilution of product in which no growth of bacteria was detected was defined to represent the minimal inhibitory concentration (MIC); three independent experiments were performed for each bacterial test species. The MIC of I_2 against a model USA300 strain of community-associated MRSA was calculated at between 0.25 and 1.0 ppm. Comparable potency (0.25 – 1.0 ppm) was observed against another leading invasive human Gram-positive bacterial pathogen, GAS, capable of producing deep-seated soft-tissue infections, sepsis and toxic shock syndrome (Walker et al., 2014) and VRE (0.5 – 2.0 ppm), a leading MDR Gram-positive opportunistic pathogen seen in catheter-associated bloodstream and urinary tract infections (Miller et al., 2016). The I_2 formulation showed similar potency against two dangerous MDR Gram-negative pathogens currently faced in the hospitals – *P. aeruginosa* (0.5 – 1.0 ppm), a cause of respiratory infections in cystic fibrosis sufferers and ventilated ICU patients, sepsis in neutropenic individuals, and burn infections (Juan et al., 2017), and *A. baumannii* (0.125 – 1.0 ppm), an emerging agent of nosocomial pneumonia, bacteremia and wound infections (Wong et al., 2017).

3.4. In vivo efficacy of the I_2/glycerin formulation in MRSA nasal decolonization

A murine model of nasal colonization with MRSA was used to compare the ability of the I_2/glycerin formulation to reduce bacterial burden in comparison to 3M™ SNP. After 24 h colonization and 24 h of treatment, the dissected nasal cavity was washed in PBS and plated on agar for CFU enumeration. Out of the 25 animals in the control (untreated) arm, six animals exhibited very low colonization levels (CFU per nasal cavity < 1500); however, the average CFU per nasal cavity in the remaining 19 animals was 11,051. Both the 3M™ SNP and the I_2-glycerin treatment significantly reduced MRSA in the nasal cavity; the mean MRSA reduction with 3M™ SNP was 2.15-logfold vs. 2.40-log fold with I_2-glycerin (Fig. 4). However, clinical success in a patient in this application is perhaps more accurately evaluated using binomial statistics, since an average reduction does not incorporate an evaluation of the proportion of individual patients who benefited. To that end, we applied as a criterion for treatment success a minimum two-log reduction in CFU. With this definition, the 3M™ SNP exhibited 9 failures in 25 mice, compared to only 3 failures with I_2-glycerin.



Treatment	Failure[a]	Success
PBS Control	20	5
3M Skin & Nasal Antiseptic	9	16
Glycerin-I_2	3[b]	22

[a] < 2 log reduction MRSA.
[b] p<0.05 Chi-Square

Fig. 4. Murine model of MRSA nasal colonization with MRSA. The ability of the I_2/glycerin formulation to reduce bacterial burden was compared to 3M™ SNP. After 24 h colonization and 24 h of treatment, the dissected nasal cavity was washed in PBS and plated on agar for CFU enumeration. The mean MRSA reduction with 3M™ SNP was 2.15-logfold vs. 2.40-log fold with I_2-glycerin. Using a criterion for treatment success a minimum two-log reduction in CFU, the 3M™ SNP exhibited 9 failures in 25 mice, compared to only 3 failures with I2-glycerin.

4. Discussion

Colonization of the nasal mucosa is a prerequisite for *S. aureus* infection, and new agents to decolonize high risk patients, reduce invasive disease, and check pathogen spread within the hospital are of paramount importance. I_2-based formulations are gaining renewed interest given rising resistance to mupirocin, the most common topical antibiotic for this indication. We report a new non-staining formulation of I_2 combined with glycerin, a common benign pharmaceutical excipient, envisioned for topical delivery, with potent bactericidal activity *in vitro* (~1 ppm vs. MRSA) and therapeutic efficacy in a proof-of-principal murine model of MRSA nasal colonization.

An important concept illustrated in the present study is that the concentration of an iodophor does not correspond to the concentration of active (I_2); in this regard PVP-I is more accurately thought of as an excipient as opposed to an active agent. Indeed, PVP-I is simply one of many excipients that yields a complex equilibrium of chemicals that provides a relatively low concentration of an active biocide, i.e. I_2. Molecular I_2 can inactivate enteric viruses, enteric bacteria, bacterial viruses, protozoan cysts, fungi, mycobacteria and spores (Cheng et al., 2018; Gottardi, 1999; Hoehn, 1976); however, different amounts of I_2 and exposure times are required to inactivate these diverse classes of organisms. Iodophor compositions do not offer the ability to adjust the concentration of I_2 for any particular clinical indication and thus limit its utility in diverse healthcare settings. There are few published studies in which one can confidently conclude that the toxicity of I_2 per se was studied (Duan et al., 1999; Kessler, 2004a,b; Thrall and Bull, 1990; Thrall et al., 1992a; Thrall et al., 1992b). If clinicians knew the true concentration of I_2 in different drug formulations, the potential utility of this active for different therapeutic indications could be evaluated. Another point often misconstrued in the literature regards the inappropriate linkage of iodophor "toxicity" to I_2. Rather, dilution of PVP-I, i.e. increased I_2, is associated with reduced toxicity (York et al., 1988). Evaporation of both I_2 and water can reduce the concentration of unbound I_2 on skin; loss of water shifts the equilibrium binding of I_2 and lowers the concentration of unbound I_2. The glycerin-based I_2 formulation delivered high local concentrations of the active antimicrobial entity with minimal evaporative loss. The loss of I_2 in the glycerin-I_2 mixture is significantly reduced which should provide longer exposure of epidermal tissue to active biocide.

Our results from MIC testing are consistent with a previously published value of 0.2 ppm I_2 that demonstrated a 6-log kill of enteric bacteria (Chang, 1971). However, bacterial inactivation by I_2 has been shown to vary by bacterial species and growth conditions (Cargill et al., 1992; Pyle and McFeters, 1989). I_2 exposure required for inactivation of viruses, cysts, mycobacterium and spores varies widely (Hoehn, 1976). Neutralization of I_2 by organic matter in the nasal cavity or on epidermal surfaces could theoretically lower the effective concentration of active biocide (Gottardi, 1991). I_2 exhibits the unique property of diffusing into human and then back-diffusing out of skin for at least 24 h. This back-diffusion is associated with a topical antibacterial activity (Gottardi, 1999).

The antimicrobial activity of a topical formulation is not solely a function of the active agent as chemical interactions of excipients and the physical properties of the carrier play a role. The dynamic viscosity of the $3M^{TM}$ SNP varies as a function of shear force due to a high concentration of excipients. The thixotropic behavior of $3M^{TM}$ SNP is due to the fact that this composition forms a film as it dries; film forming topical formulations are designed to maintain active on skin. This approach would be logical if the active in $3M^{TM}$ SNP were an antibiotic; however, as indicated in the introduction (Ferguson et al., 2003; Gottardi, 1980, 1983) the concentration of active I_2 is reduced as the concentration of complexing agents is increased, i.e. as a film is formed. At full strength the $3M^{TM}$ SNP contains less than 10 ppm of unbound iodine, 0.5% of thiosulfate titratable iodine, i.e. triiodide and I_2, in a composition that contains 5% total iodine; the comparable numbers for the glycerin-I_2 composition is 800 ppm I2, 0.8% thiosulfate titratable iodine in a composition that contains 0.8% total iodine. The > 80-fold higher concentration of I_2 in the glycerin composition should increase as the $3M^{TM}$ SNP forms a film. The higher concentration of I_2 in the glycerin composition should lead to increased microbicidal capacity in those instances where the nasal cavity contains high levels of mucus which is known to neutralize I_2 via reaction with cysteine. The lower vapor pressure of I_2 in glycerin as compared to the $3M^{TM}$ SNP composition reduces the potential for I_2 diffusion into lung tissue. Likewise, the higher I_2 concentration provides much higher levels of I_2 absorption into skin which confers a durable antimicrobial effect at the cutaneous surface.

A comparison of the formulation characteristics of the 3M product to the glycerin-I_2 composition demonstrates the disparity between the two formulation strategies. The 3M iodophor contains less than 10 ppm of unbound iodine, 0.5% of thiosulfate titratable iodine, i.e. triiodide and I_2, in a composition that contains 5% total iodine; the comparable numbers for the glycerin-iodine composition is 800 ppm I_2, 0.8% thiosulfate titratable iodine in a composition that contains 0.8% total iodine. The > 80-fold higher concentration of active in the glycerin composition should provide increased microbicidal capacity in those instances where the nasal cavity contains high levels of mucus which is known to neutralize I_2 via reaction with cysteine. The lower vapor pressure of I_2 in glycerin as compared to the 3M iodophor composition reduces the potential for I_2 diffusion into lung tissue. Likewise, the higher I_2 concentration provides much higher levels of I_2 absorption into skin which, as shown by (Gottardi, 1999), confers a durable antimicrobial effect.

5. Conclusion

A topical composition that contains greater functional concentrations of I_2 than found in 10% PVP-I should provide a more robust and effective topical agent for MRSA decolonization and other anti-infective indications. Our *in vitro* and *in vivo* proof-of-principle studies with a non-staining formulation of I_2 in a glycerin emollient reveal the potential for this novel topical formulation.

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