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Novel Nanoemulsion Antimicrobials Tested Against Nine Gram-Positive Species

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ABSTRACT

Background: Nanoemulsions (NEs) are oil-in-water emulsions composed of pharmaceutically approved substances in nanodroplets that have an average diameter of 180 or 350 nm. Previous work has shown that NEs penetrate through hair follicles and skin pores to significant levels in both the epidermis and dermis. Three NEs were evaluated against 9 gram-positive species.

Methods: MICs (broth microdilution) and MBCs were determined using CLSI standard methods.

Results: W_{20}5EC, P_{407}5EC and W_{20}5GBA_2ED had MIC_{90} ranges to ≥20 isolates of each species of 1-16, 2-16 and 4-16 µg/ml, respectively, including organisms that were resistant to topical and systemic antimicrobials. MBCs were performed for 35 isolates: W_{20}5GBA_2ED was cidal against all isolates while W_{20}5EC and P_{407}5EC were cidal against 94% of isolates.

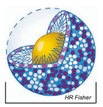
Conclusions: The antimicrobial spectrum and potency of NEs, and their ability to permeate epidermal and dermal tissues, make them ideal candidates for treatment of superficial skin and soft tissue infections caused by leading gram-positive pathogens, including MRSA.

BACKGROUND

The incidence of uncomplicated skin and skin structure infections (uSSTIs) increased by 280% between 2001-2003 (TE Zaoutis, et al, Ped Infect Dis J 2006; 25:343-348). Concomitant with this increase was the emergence of methicillin-resistant *S. aureus* (MRSA) as the major pathogen causing uSSTIs. Mupirocin is the only topical approved for MRSA, but for use in nasal eradication and not for treatment of uSSTIs. A recent study in the US showed that 34 of every 1,000 patients in the survey had active MRSA infections and that 12 were colonized with the superbug, for a total prevalence rate of 46 per 1,000 patients or 35.2 million people hospitalized in 2005 (WR Jarvis, Am J Infect Control. 2007; 35:631-637). Of the patients admitted to a surgical intensive care unit with MRSA, 13.2% of the MRSA were resistant to mupirocin (JC Jones, et al. CID 2007; 45:541-547). Thus, an agent that could eradicate carriage and/or treat uSSTIs caused by MRSA, MSSA and *S. pyogenes* would be a welcome addition to the infectious disease community.

Several topical nanoemulsions were evaluated for microbiological activity against common gram-positive isolates, including defined clones of MRSA community isolates. Nanoemulsions are oil-in-water emulsions composed of nanometer-sized droplets (Figure 1) that were specifically designed to penetrate skin via the follicular route (see M-2135), allowing high concentrations of its active component, cetylpyridinium chloride or benzalkonium chloride, to accumulate in both the epidermis and dermis. Unlike oral or parenteral drugs, there is no systemic exposure to the nanoemulsions, thereby alleviating concern about drug-drug interactions or other adverse side-effects. All three nanoemulsions were active against resistant phenotypes of *S. aureus*, including those with mupirocin resistance. Scanning electron micrographs revealed the cellular damage done when an MRSA isolate is exposed to 4 µg/ml W_{20}5EC for 10 minutes (Figure 2).

Figure 1. Nanoemulsion droplet.



Average diameter: 180 or 350 nm

- Cetylpyridinium chloride or Benzalkonium chloride
- Polysorbate or Poloxamer
- Oil

RESULTS

A. Control (no drug treatment)

B, C. Treatment of MRSA with 4 µg/ml W_{20}5EC for 10 minutes (about 2 log reduction in cfu/ml)

Figure 2. Scanning electron micrographs of MRSA 19001 ± W_{20}5EC.

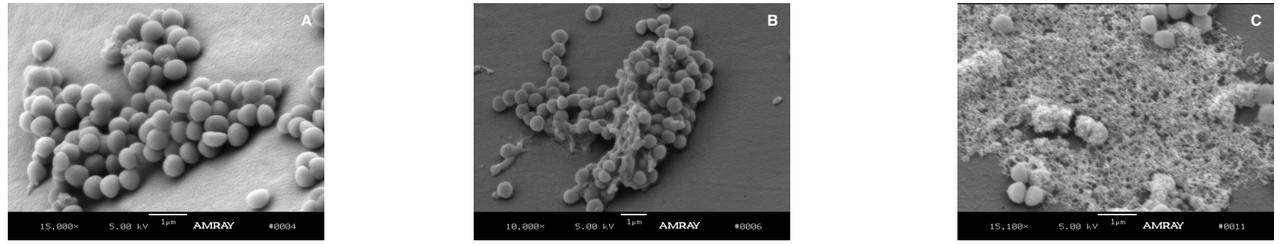


Table 1. Susceptibility of nine gram-positive isolates to three nanoemulsions and comparators.

Antimicrobial agent	*Staphylococcus aureus* (n = 38)[a]				*Streptococcus pyogenes* (n = 21)				*Staphylococcus epidermidis* (n = 20)				*Staphylococcus hemolyticus* (n = 20)				*Enterococcus faecium* (n = 21)				*Enterococcus faecalis* (n = 21)				*Streptococcus agalactiae* (n = 20)				*Streptococcus mitis* (n = 21)				*Streptococcus sanguis* (n = 20)			
	MIC_{50}	MIC_{90}	Range	% susceptible/ resistant[b]	MIC_{50}	MIC_{90}	Range	% susceptible/ resistant	MIC_{50}	MIC_{90}	Range	% susceptible/ resistant	MIC_{50}	MIC_{90}	Range	% susceptible/ resistant	MIC_{50}	MIC_{90}	Range	% susceptible/ resistant	MIC_{50}	MIC_{90}	Range	% susceptible/ resistant	MIC_{50}	MIC_{90}	Range	% susceptible/ resistant	MIC_{50}	MIC_{90}	Range	% susceptible/ resistant	MIC_{50}	MIC_{90}	Range	% susceptible/ resistant
W_{20}5EC	0.5	4	0.5 – 8	- / -	2	2	1 – 2	- / -	1	2	0.25 – 4	- / -	2	4	0.25 – 4	- / -	2	4	2 – 4	- / -	1	1	0.5 – 4	- / -	1	2	0.5 – 2	- / -	4	16	0.5 – 32	- / -	2	16	1 – 16	- / -
P_{407}5EC	2	4	1 – 16	- / -	4	4	2 – 4	- / -	2	4	1 – 4	- / -	4	8	1 – 16	- / -	4	8	4 – 16	- / -	2	4	2 – 8	- / -	2	2	1 – 4	- / -	4	16	1 – 16	- / -	4	16	2 – 16	- / -
W_{20}5G BA_2 ED	2	4	1 – 8	- / -	4	4	4	- / -	2	4	1 – 4	- / -	4	4	0.5 – 4	- / -	4	4	2 – 4	- / -	2	4	2 – 4	- / -	4	4	2 – 8	- / -	8	16	1 – 32	- / -	8	8	2 – 16	- / -
Oxa/Amp/Pen[c]	2	>2	≤0.25 – >2	52.6 / 47.4	≤0.12	≤0.12	≤0.12	100.0 / -	≤0.25	>2	≤0.25 – >2	55.0 / 45.0	1	>2	≤0.25 – >2	25.0 / 75.0	>16	>16	≤8 – >16	4.8 / 95.2	≤8	≤8	≤8	100.0 / 0.0	≤0.12	≤0.12	≤0.12	100.0 / -	≤0.12	>2	≤0.12 – >2	76.2 / 14.3	≤0.12	1	≤0.12 – 2	50.0 / 0.0
Erythromycin	>2	>2	≤0.25 – >2	44.7 / 55.3	≤0.25	≤0.25	≤0.25	100.0 / 0.0	>2	>2	≤0.25 – >2	45.0 / 55.0	>2	>2	0.12 – >2	20.0 / 80.0	>2	>2	>2	0.0 / 100.0	>2	>2	≤0.25 – >2	4.8 / 71.4	2	>2	≤0.25 – >2	45.0 / 55.0	1	>2	≤0.25 – >2	42.9 / 52.4	2	>2	≤0.25 – >2	45.0 / 55.0
Clindamycin	≤0.25	>2	≤0.25 – >2	71.1 / 28.9	≤0.25	≤0.25	≤0.25	100.0 / 0.0	≤0.25	>2	≤0.25 – >2	75.0 / 20.0	≤0.25	>2	≤0.25 – >2	65.0 / 30.0	>2	>2	>2	- / -	>2	>2	>2	- / -	≤0.25	>2	≤0.25 – >2	80.0 / 20.0	≤0.25	≤0.25	≤0.25 – >2	90.5 / 9.5	≤0.25	≤0.25	≤0.25 – >2	90.0 / 10.0
Levofloxacin	2	>4	≤0.5 – >4	47.4 / 50.0	≤0.5	≤0.5	≤0.5 – 1	100.0 / 0.0	≤0.5	>4	≤0.5 – >4	60.0 / 40.0	4	>4	≤0.5 – >4	35.0 / 60.0	>4	>4	≤0.5 – >4	4.8 / 95.2	1	>4	≤0.5 – >4	81.0 / 19.0	≤0.5	1	≤0.5 – 1	100.0 / 0.0	1	2	0.5 – >4	90.5 / 9.5	≤0.5	1	≤0.5 – 1	100.0 / 0.0
Tetracycline	≤2	>8	≤2 – >8	89.5 / 10.5	≤2	≤2	≤2 – >8	95.2 / 4.8	≤2	≤2	≤2 – 4	100.0 / 0.0	≤4	>8	≤4 – >8	68.4 / 26.3	≤2	>8	≤2 – >8	57.1 / 42.9	>8	>8	≤2 – >8	23.8 / 76.2	>8	>8	≤2 – >8	10.0 / 90.0	≤4	>8	≤4 – >8	61.9 / 28.6	≤4	>8	≤4 – >8	55.0 / 20.0
Trimethoprim/ Sulfamethoxazole	≤0.5	≤0.5	≤0.5 – >2	92.1 / 7.9	≤0.5	≤0.5	≤0.5 – >2	- / -	≤0.5	>2	≤0.5 – >2	65.0 / 35.0	≤0.5	>2	≤0.5 – >2	70.0 / 30.0	>2	>2	≤0.5 – >2	- / -	≤0.5	>2	≤0.5 – >2	- / -	≤0.5	≤0.5	≤0.5	- / -	≤0.5	2	≤0.5 – >2	- / -	≤0.5	2	≤0.5 – >2	- / -
Fusidic Acid	0.25	0.5	0.25 – 0.5	- / -	8	8	4 – 16	- / -	0.25	0.5	0.25 – 0.5	- / -	0.25	0.25	0.12 – 0.5	- / -	4	8	0.5 – 8	- / -	8	8	4 – 16	- / -	16	16	8 – 16	- / -	16	32	8 – 32	- / -	16	32	2 – 32	- / -
Mupirocin	≤4	≤4	≤4 – 16	- / -	≤4	≤4	≤4	- / -	≤4	>256	≤4 – >256	- / -	≤8	>8	≤8 – >8	- / -	≤4	≤4	≤4	- / -	256	256	256	- / -	≤4	≤4	≤4	- / -	≤4	≤4	≤4 – 256	- / -	≤4	≤4	≤4	- / -
Linezolid	2	2	1 – 2	100.0 / -	1	1	0.5 – 1	100.0 / -	1	1	0.5 – >8	95.0 / -	1	1	0.5 – 2	100.0 / -	1	2	1 – 2	100.0 / 0.0	1	2	0.5 – 8	95.2 / 4.8	1	1	0.12 – 1	100.0 / -	1	1	0.5 – 2	100.0 / -	0.5	1	0.12 – 1	100.0 / -
Vancomycin	1	2	0.5 – 2	100.0 / 0.0	0.25	0.5	0.25 – 0.5	100.0 / -	2	2	1 – 2	100.0 / 0.0	1	2	0.5 – 4	100.0 / 0.0	>16	>16	0.5 – >16	42.9 / 57.1	2	2	1 – >16	95.2 / 4.8	0.5	0.5	0.25 – 1	100.0 / -	0.5	0.5	0.25 – 1	100.0 / -	0.5	0.5	0.25 – 0.5	100.0 / -

[a]Number of isolates; [b]Criteria as published by CLSI, 2008; [c]Oxa/Amp/Pen = oxacillin for staphylococci, ampicillin for enterococci, and penicillin for streptococci

Table 2. MIC and MBC values against 28 isolates of MRSA community isolates.

Compound	Value (µg/ml)		% susceptible/ resistant
	MIC_{90}	MBC_{90}	
W_{20}5EC	2	8	-/-
P_{407}5EC	2	8	-/-
W_{20}5G BA_2 ED	4	8	-/-
Clindamycin	>64	>64	42.8/57.2
Doxycycline	4	>4	100/0
Erythromycin	>128	>128	14.3/85.7
Levofloxacin	32	16	28.6/71.4
Mupirocin	>64	>64	-/-
Vancomycin	1	2	100/0
Oxacillin	>16	>16	0/100
Sulfamethazole	4	>32	100/-
Trimethoprim	0.5	>4	96.4/3.6

Table 3. Cidality of nanoemulsions against a subset (35) gram-positive isolates.

		Compound (number of strains)			
	MBC/MIC ratio	W_{20}5EC	P_{407}5EC	W_{20}5G BA_2 ED	Fusidic Acid
Cidal ↑	1	13	16	14	1
	2	15	11	17	2
	4	5	7	4	0
Static ↓	>4x	0	0	0	25
	8	2	1	0	7

METHODS

Emulsion manufacturing. Nanoemulsions W_{20}5EC, P_{407}5EC, and W_{20}5GBA_2ED are oil-in-water emulsions manufactured from ingredients that are Generally Recognized As Safe (GRAS) with a cationic detergent (cetylpyridinium chloride, CPC, or benzalkonium chloride, BA) as active ingredients that have proven safe for human use. The emulsion is formed from highly purified oil, ethanol, a nonionic surfactant and water. The average nanoemulsion droplet size is 180nm for W_{20}5EC and 350nm for P_{407}5EC and W_{20}5GBA_2ED as measured by dynamic light scattering using a Malvern Zetasizer Nano 3600 (Malvern Instruments Ltd., Worcestershire, UK).

Source of isolates. The source of the clinical isolates were bloodstream isolates collected by JMI Laboratories or from Barry Kreiswirth (clonally-defined MRSA community clonal skin isolates).

MIC and MBC determinations. MICs were determined as specified by CLSI broth (cation-adjusted Mueller-Hinton, with added 3-5% lysed horse blood [for testing of streptococci]) microdilution method per M7-A7 [2006]. Alamar blue was added to the assay panels two hours post-inoculation for enhanced MIC endpoint detection. MBC values were assessed for nanoemulsions and a comparator compound (fusidic acid) by plating the entire broth content from the MIC well and from those four doubling dilutions above the MIC for each selected organism onto blood agar growth media. Quantitative colony counts were performed on the initial inoculum. The lowest concentration of antimicrobial agent that killed ≥ 99.9% of the starting test inoculum was defined as the MBC endpoint.

CONCLUSIONS

- Nanoemulsions W_{20}5EC, P_{407}5EC, and W_{20}5GBA_2ED have potential for topical use to eliminate carriage and/or treat uSSTIs caused by MRSA, MSSA and *Streptococcus pyogenes*.
- W_{20}5GBA_2ED was bactericidal against all isolates while W_{20}5EC and P_{407}5EC were bactericidal against 94% of isolates.
- No cross-resistance to any known antibiotic was observed for any of the nanoemulsion antimicrobial agents.

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