Current Therapeutic Research 83C (2016) 8–12

Contents lists available at ScienceDirect

Current Therapeutic Research

journal homepage: www.elsevier.com/locate/cuthre

A Novel Topical Combination Ointment with Antimicrobial Activity against Methicillin-Resistant *Staphylococcus aureus*, Gram-Negative Superbugs, Yeasts, and Dermatophytic Fungi

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ARTICLE INFO

Article history:
Accepted 7 July 2016

Key words:
methicillin-resistant *Staphylococcus aureus*
multiple-drug resistant organisms
topical antibiotics

ABSTRACT

Background: The use of topical antimicrobial agents for management of minor skin infections is a clinical strategy that is commonly practiced in the community. Coupled with the use of topical antimicrobial agents is the emergence of antibiotic-resistant strains of pathogens leading to the need for alternative treatments.
Objective: A novel topical combination ointment consisting of salicylic acid, oak bark extract, benzoic acid, and polyethylene glycol (Bensal HP, Sonar products Inc., Carlstadt, NJ) with antimicrobial properties was assessed to determine its spectrum of activity.
Methods: One hundred eighty-four bacterial and fungal isolates from culture collections that included multidrug-resistant bacteria such as methicillin-resistant *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Acinetobacter* spp, and gram-negative so-called superbugs, as well as yeasts and filamentous fungi, were investigated by cylinder diffusion and agar dilution assays.
Results: All 184 bacterial and fungal isolates were susceptible to the combination ointment at the clinically applied concentration and there was no evidence of cross-resistance between Bensal HP and other classes of antimicrobials. In time-kill tests, Bensal HP was rapidly bactericidal against *P aeruginosa* ATCC 27853 and methicillin-resistant *S aureus* SA179 at 4 × the MIC, a concentration that is applied clinically.
Conclusions: The results of this study suggest that this combination ointment has a broad in vitro spectrum of antimicrobial activity against both more common bacterial and fungal pathogens and may be particularly useful for treatment of infections by multidrug-resistant organisms. Additional studies are warranted to investigate the full clinical utility as a therapeutic agent and also for possible infection control interventions.

Introduction

Antibiotic resistance is a serious health threat and has the potential for dire consequences. The Centers for Disease Control and Prevention estimate that more than 2 million individuals in the United States develop illness resulting from antibiotic-resistant infections on an annual basis and published *Antibiotic Resistance Threats in the United States, 2013*,[1] which provides a snapshot of the complex problem of antibiotic resistance. The threats were prioritized as urgent, serious, and concerning. Of particular concern is increasing multidrug resistance coupled with cessation of antibiotic discovery programs by most major pharmaceutical companies. This situation has created a major global health crisis in which there are few or no effective agents to treat common bacterial infections or infections caused by less common pathogens, including *Mycobacterium* spp,[2,3] filamentous fungi, and yeasts.[4–6] Furthermore, alternative second- and third-line agents that are effective are also associated with safety issues. Most current concerns about antibiotic resistance focus on infections in hospital

This study was presented as a poster at the South Central Association of Clinical Microbiologists, April 19, 2014, Cincinnati, Ohio as well as the 114th General Meeting of American Society for Microbiology, May 20, 2014, Boston, Massachussets.

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http://dx.doi.org/10.1016/j.curtheres.2016.07.001

Table I
Summary of cylinder test zone sizes for test isolates.

Organism	No. tested	Range of zone (mm)	Drug-sensitive zone*
Escherichia coli (M)	17	11–16	12
Klebsiella pneumoniae (M)	13	12–18	13
Serratia marcescens (M)	10	13–19	13
Pseudomonas aeruginosa (M)	11	13–18	13
Acinetobacter baumannii (M)	13	14–18	16
Methicillin-sensitive *Staphylococcus aureus*	12	16–23	16
Methicillin-resistant *Staphylococcus aureus* (M)	11	20–22	
Enterococcus faecalis (M)	11	16–21	17
Streptococcus pyogenes	12	10–15	0
Nocardia brasiliensis	10	18–42	0
Mycobacterium fortuitum	10	22–36	0
Candida albicans	10	14–19	0
Candida glabrata	10	12–17	0
Trichophyton rubrum	12	21–31	0
Trichophyton tonsurans	10	18–37	0
Trichophyton mentagrophytes	10	22–27	0
Propionibacterium acnes	1	27	0
Cryptococcus neoformans	1	18	0
Total	184		

M = multidrug-resistant organisms included.

* Zone of 0 mm indicates resistant. Note that zones for drug-sensitive isolates of *Escherichia coli*, *K pneumoniae*, *Serratia marcescens*, *Pseudomonas aeruginosa,* and *Enterococcus faecalis* tended to be smaller (suggesting they were more resistant) than those of their multidrug-resistant counterparts.

settings requiring parenteral agents.[3] Little is known about the activity of topical agents against multidrug-resistant organisms (MDROs), some of which are likely to be compromised because they contain agents to which resistance has already been reported. These include neomycin, polymyxin B, bacitracin, and mupirocin.[7,8] Bensal HP (Sonar Products Inc., Carlstadt, NJ) is a combination topical ointment with antimicrobial properties with activity against methicillin-resistant *Staphylococcus aureus* (MRSA) and common bacterial and fungal skin pathogens.[9–11] The current study was designed to assess the in vitro activity of Bensal HP against a broad range of contemporary pathogens, including MDROs such as MRSA, vancomycin-resistant *Enterococcus*, gram-negative so-called superbugs, *Mycobacterium fortuitum*, *Nocardia brasiliensis*, yeasts, and filamentous fungi.

Materials and Methods

Test agent

Bensal HP contains salicylic acid (30 mg/g), benzoic acid (60 mg/g), QRB-7 (oak bark extract) (30 mg/g), and vehicle polyethylene glycol 400 and polyethylene glycol 3350. The test agent was provided by SMG Pharmaceuticals, Cary, North Carolina.

Organisms

In vitro activity was investigated against 184 bacterial and fungal isolates from the culture collections of Creighton University, Omaha, Nebraska; the Alegent Creighton Hospital Microbiology Laboratory, Omaha, Nebraska; and the University of Louisville Hospital Microbiology Laboratory, Louisville, Kentucky. The bacterial isolates were from US and international sources and included well characterized non-MDRO and MDRO isolates of Enterobacteriaceae (n = 40), *Pseudomonas aeruginosa* (n = 11); *Acinetobacter baumannii* (n = 13); *S aureus* (n = 23), including MRSA and methicillin-susceptible *S aureus*; and *Enterococcus faecalis* (n = 11), including vancomycin-resistant *Enterococcus*, Group A *Streptococcus* (*Streptococcus pyogenes* [n = 12]), *Propionibacterium acnes* (n = 1), *M fortuitum* (n = 10), and *N brasiliensis* (n = 10). The fungal isolates were *Candida albicans* (n = 10), *Candida glabrata* (n = 10), *Cryptococcus neoformans* (n = 1), *Trichophyton rubrum* (n = 12), *T tonsurans* (n = 10), and *T mentagrophytes* (n = 10). The gram-negative bacteria were previously characterized for resistance mechanisms by phenotypic, biochemical, and molecular methods.[2] These included isolates of Enterobacteriaceae, *Pseudomonas* spp, and *Acinetobacter* spp producing the extended spectrum β-lactamases TEM-52, SHV-4, SHV-12, OXA-45, CTX-M-1, CTX-M-9, CTX-M-12, CTX-M-14, CTX-M-15, CTX-M-17, CTX-M-18, and CTX-M-19; chromosomal and plasmid-mediated AmpC β-lactamases, including FOX-like and CMY-2 enzymes; and carbapenemases of the IMP, VIM, KPC, OXA, and NDM families. The *Pseudomonas aeruginosa* isolates included some with upregulated MexAB, MexEF, and MexXY efflux pumps, and downregulation of the OprD porin. The isolates included organisms described in the media as superbugs because of their resistance to most available antibacterial agents. ATCC reference isolates included in the study were *Escherichia coli* ATCC 25922, *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, and *T mentagrophytes* ATCC 9533.

Cylinder diffusion susceptibility testing

All isolates were tested by a cylinder diffusion procedure[12,13] that was a modification of the Clinical and Laboratory Standards Institute (CLSI) disk diffusion method.[14,15] In this procedure a cylinder containing Bensal HP was substituted for the impregnated filter paper disks of the CLSI method. Bensal HP liquefied by heating to 56°C for 10 minutes and 40 μL was pipetted into a sterile metal cylinder placed on a lawn culture of the test organism. The lawn culture of the test isolate was prepared according to CLSI methodology and inoculated onto appropriate media (see Media). The tests with gram-negative pathogens, staphylococci, streptococci, and enterococci were incubated as recommended by CLSI; that is, overnight, typically 18–20 hours. All other isolates were incubated for as long as necessary to be able to visualize sufficient growth to allow measurement of an inhibition zone; that is, 48–72 hours. After incubation, inhibition diameters around the cylinders were measured and recorded according to the CLSI method. In the absence of CLSI interpretive criteria, any zone of inhibition was interpreted to indicate susceptibility and the absence of an inhibition zone indicated resistance. This interpretation was adopted to correlate with the occurrence or absence of activity at the undiluted concentration of Bensal HP that is used therapeutically.

MIC testing

Bensal HP MICs were determined by CLSI agar dilution methodology.[14,15] The test isolates were 73 representative bacterial isolates that were capable of overnight growth at 35°C on Mueller-Hinton agar.

Time-kill testing

Using concentrations based on the agar dilution MICs, the bactericidal activity of Bensal HP against *Pseudomonas aeruginosa* ATCC 27853 and MRSA SA179 was determined by time-kill methodology. The Bensal HP concentrations tested were 4 × the MIC and 1 × the MIC. Drug-free and antibiotic-supplemented Mueller-Hinton broths were inoculated to provide an initial inoculum of $\geq 5 \times 10^5$ CFU/mL of each isolate. Growth rates