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ORIGINAL RESEARCH

Spectrum of bactericidal action of amylmetacresol/2,4-dichlorobenzyl alcohol lozenges against oropharyngeal organisms implicated in pharyngitis

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Purpose: Pharyngitis is commonly caused by a self-limiting upper respiratory tract infection (URTI) and symptoms typically include sore throat. Antibiotics are often inappropriately used for the treatment of pharyngitis, which can contribute to antimicrobial resistance, therefore non-antibiotic treatments which have broad antiseptic effects may be more appropriate. Amylmetacresol (AMC) and 2,4-dichlorobenzyl alcohol (DCBA) are present in some antiseptic lozenges and have established benefits in providing symptomatic relief and some in vitro antiviral action.

Methods: Seven bacterial species associated with pharyngitis, namely *Streptococcus pyogenes, Fusobacterium necrophorum, Streptococcus dysgalactiae* subspecies *equisimilis, Moraxella catarrhalis, Haemophilus influenza*, *Arcanobacterium haemolyticum* and *Staphylococcus aureus*, were exposed to an AMC/DCBA lozenge dissolved in artificial saliva. In vitro bactericidal activity was measured as a log reduction in colony-forming units (CFUs).

Results: Bactericidal activity was recorded against all organisms after 1 minute. Greater than 3 $\log_{10}$ reductions in CFUs were observed at 1 minute for *S. pyogenes* ($\log_{10}$ reduction CFU/mL ± SD, 5.7±0.1), *H. influenza* (6.1±0.1), *A. haemolyticum* (6.5±0.0) and *F. necrophorum* (6.5±0.0), at 5 minutes for *S. dysgalactiae* (6.3±0.0) and *M. catarrhalis* (5.0±0.9) and at 10 minutes for *S. aureus* (3.5±0.1).

Conclusion: An AMC/DCBA lozenge demonstrated a greater than 99.9% reduction in CFUs against all tested species within 10 minutes, which is consistent with the time a lozenge remains in the mouth. Patients with uncomplicated bacterial pharyngitis may benefit from the antibacterial action of antiseptic AMC/DCBA lozenges. Furthermore, AMC/DCBA lozenges may be more relevant and appropriate than antibiotics for pharyngitis associated with a self-limiting viral URTI.

Keywords: pharyngitis, bacterial infections, antibacterial agents, Streptococcus, sore throat

Plain language summary

Pharyngitis is a common condition. It can last several days and is usually the result of self-limiting viral infections, such as the common cold, although occasionally, pharyngitis can be caused by a bacterial infection. The most commonly reported symptom is sore throat. Antibiotics do not work against the viruses that in most cases cause pharyngitis but are often prescribed anyway. This contributes to antimicrobial resistance, where bacteria become immune to antibiotics and treatment for infections becomes difficult. Alternative treatments could help reduce inappropriate prescriptions of antibiotics for pharyngitis, and previous studies have demonstrated the antiviral and pain-relieving qualities of some antiseptic lozenges. The authors conducted a laboratory-

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based study to assess the ability of antiseptic lozenges to kill a broad range of bacteria known to cause pharyngitis. They found that, when lozenges containing two antiseptic ingredients were dissolved in a solution similar to human saliva, the mixture killed 99.9% of all pharyngitis-associated bacteria that were tested within 10 minutes. These results suggest that patients with uncomplicated bacterial pharyngitis may benefit from the antibacterial and pain-relieving action of antiseptic lozenges, including those taking antibiotics. Additionally, antiseptic lozenges may be more relevant and appropriate than antibiotics for pharyngitis of a viral origin.

Introduction

Pharyngitis is associated with inflammation of the pharynx[1] and is one of the most common reasons patients seek health care professional advice.[2] Acute pharyngitis is predominantly caused by a viral upper respiratory tract infection such as the common cold[3,4] and is usually self-limiting with symptoms, such as sore throat, lasting ~3–7 days.[5] Despite this, antibiotics are still frequently inappropriately used for the treatment of pharyngitis even though patients consulting their doctor are often primarily seeking reassurance and symptomatic relief.[6,7] Antibiotics are ineffective against the viruses that cause ~90% of cases, do not offer symptomatic relief and inappropriate antibiotic prescription can contribute to antimicrobial resistance, which is a serious threat to global public health.[8] Consequently, there is a need for non-antibiotic treatments,[9,10] which have broad anti-infective effects while meeting patient needs for relief of symptoms.

Antiseptics are a class of antimicrobial agent which kill via a physical action on the bacteria.[11] In addition to bactericidal activity, some antiseptics – such as amylmetacresol (AMC) and 2,4-dichlorobenzyl alcohol (DCBA) – have been shown to have antiviral effects in vitro[12,13] and anesthetic-like effects[14] with established benefits in providing symptomatic relief of pain.[15,16]

Bacterial infections contribute to 5%–15% of pharyngitis cases in adults.[4,17,18] The most common bacterial cause of acute pharyngitis, and the reason for legitimate antibiotic prescribing to prevent complications, is group A β-hemolytic *Streptococcus* (GABHS or *Streptococcus pyogenes*).[3,19] It is responsible for ~30% of cases in children[20] and is less frequent in adults at ~10% of cases,[17] but rarely results in complications.[3] A number of other bacteria have also been implicated in infections of the throat, which may present with a more complicated pathology or represent either opportunistic infection or an underlying medical condition.

Less common species recovered from patients presenting with symptoms of pharyngitis or with a clinical diagnosis of pharyngitis include *Fusobacterium necrophorum*,[21] described in a recent study as a true pathogen rather than a colonizer of the oropharynx,[22] and the *Streptococcus dysgalactiae* subspecies *equisimilis*, which can cause severe or recurrent pharyngitis,[3,17,23] although there is insufficient evidence of a role for *S. dysgalactiae* in other adverse outcomes.[3] *Moraxella catarrhalis* has been frequently isolated from patients with pharyngitis in combination with *S. pyogenes*,[24] which may be significant considering that separate studies have demonstrated that *M. catarrhalis* potentiates the adhesion of *S. pyogenes* to the nasopharyngeal epithelium.[25,26] Other bacteria cultured from patients with pharyngitis include *Haemophilus influenza*,[27] *Arcanobacterium haemolyticum*[28] and the opportunistic pathogen, *Staphylococcus aureus*, although the clinical significance of *S. aureus* association is not known.[19]

In patients diagnosed with tonsillitis, *F. necrophorum*, appears to be a clinically important species, with a prevalence significantly higher in subjects with clinical tonsillitis compared to subjects without tonsillitis.[29] *S. aureus* has also been identified as a common cause of tonsillitis[30,31] and was the most common pathogen isolated from patients undergoing tonsillectomy due to recurrent tonsillitis.[30] *H. influenza* has similarly been recovered from patients with tonsillitis, although the clinical significance is currently unknown.[27]

Non-antibiotic antimicrobial treatments could potentially benefit patients with bacterial pharyngitis by offering not only antimicrobial activity but also symptomatic relief. The in vitro activity of 10 lozenge formulations has previously been investigated against *S. pyogenes* and *S. aureus*.[32] In this study, the in vitro bactericidal activity of AMC/DCBA lozenges against a broader range of potentially pathogenic oropharyngeal bacteria was assessed to evaluate the potential in vivo action of these lozenges against organisms associated with pharyngitis.

Methods and materials

Test samples

For the bactericidal assay, AMC 0.6 mg, DCBA 1.2 mg lozenges (Strepsils Honey and Lemon, Reckitt Benckiser Healthcare Ltd, Slough, UK) were dissolved into 5 mL of artificial saliva medium (0.1% meat extract [VWR International, Lutterworth, UK], 0.2% yeast extract [VWR International], 0.5% protease peptone [Oxoid, Basingstoke, UK], 0.02% potassium chloride [Fisher Scientific, Loughborough, UK], 0.02% sodium chloride [Fisher Scientific], 0.03% calcium carbonate [Fisher Scientific], 0.2% glucose [VWR International], 0.2% mucin from porcine stomach Type II [Sigma Aldrich, Gillingham, Dorset, UK], pH 6.7±0.3) at 44°C±1°C.

Test organisms and incubations

S. aureus (NCTC7445, Public Health England, Salisbury, UK) were cultured on tryptone soya agar (SGL, Corby, UK) at 32°C±2°C; *S. pyogenes* (NCTC12696, Public Health England) were cultured on Columbia blood agar with 5% defibrinated sheep blood (SGL) at 36°C±2°C; *M. catarrhalis* (NCTC3622, Public Health England) were cultured on Columbia blood agar (SGL) at 32°C±2°C; *H. influenza* (NCTC4842, Public Health England) were cultured on chocolate blood agar (SGL) at 32°C±2°C; *F. necrophorum* (NCTC12238, Public Health England) were cultured on anaerobic blood agar (FAA) with 5% horse blood (SGL) at 37°C±2°C anaerobically; *A. haemolyticum* (NCIMB702294, NCIMB, Aberdeen, UK) were cultured on Columbia blood agar with 5% defibrinated sheep blood at 36°C±2°C; *S. dysgalactiae* (ATCC12388, LGC, Teddington, UK) were cultured on Columbia blood agar with 5% defibrinated sheep blood at 36°C±2°C.

Bactericidal assay

The bactericidal assay was performed following a protocol similar to the Clinical and Laboratory Standards Institute approved guideline.[33] Specifically, inoculum cultures were prepared for each challenge organism to give an approximate population of 10^8 colony-forming unit (CFU)/mL in saline (0.9% sodium chloride [Fisher Scientific]). One inoculum suspension was prepared for each replicate tested. Test sample (4.9 mL) was prepared as above and inoculated with 0.1 mL of the inoculum suspension. The solution was vortexed thoroughly to mix and then tested after 1-, 5- and 10-minute contact times, consistent with the time a lozenge takes to dissolve in the mouth,[16] by removing 1 mL of sample/inocula mixture and transferring into 9 mL of neutralizing diluent (0.1% peptone water [VWR International], 0.9% sodium chloride [Fisher Scientific], 0.3% lecithin [MP Biomedicals, Illkirch-Graffenstaden, France], 1% polysorbate 80 [Univar, Bradford, UK], pH 6.6±0.2). Neutralization validation was carried out against all test organisms. Solutions were serially diluted to 10^{-5}, plated onto the appropriate agar medium and incubated for a minimum of 3 days. A positive control sample of 4.9 mL artificial saliva medium and 0.1 mL of the test inoculum for each organism was also prepared without exposure to test samples and assayed at a 30-minute time point. Test control counts were performed to confirm the total population of the culture suspensions used for each test replicate. The test controls were used to calculate the log reduction on exposure to test samples. Mean log reduction in CFUs per milliliter was calculated from three test replicates.

Results

In vitro bactericidal activity of AMC/DCBA lozenges

For all test organisms, evidence of bactericidal activity was recorded at the 1-minute time point (Table 1, Figure 1), and test control counts demonstrated that the test method and media did not affect the survival of the organisms. For *S. pyogenes*, *H. influenza*, *A. haemolyticum* and *F. necrophorum*, the decrease in CFU/mL at 1 minute exceeded 3 $\log_{10}$ (99.9% decrease), whereas greater than 3 $\log_{10}$ reductions were recorded at 5 minutes for *S. dysgalactiae* and *M. catarrhalis* and at 10 minutes for *S. aureus*. Additionally, at all time points, the SD (Table 1) of the replicates was small (≤0.9 $\log_{10}$ CFU/mL), indicating consistent and reproducible observations.

Discussion

This study examined the bactericidal action of an antiseptic lozenge containing AMC and DCBA. The organisms tested included gram-positive cocci (*S. pyogenes, S. aureus, S. dysgalactiae*) and bacilli (*A. haemolyticum*), as well as gram-negative cocci (*M. catarrhalis*) and bacilli (*H. influenza, F. necrophorum*), representing a broad range of bacterial cell structures and sensitivities.

The results demonstrated that the AMC/DCBA lozenge exhibits broad bactericidal activity against a range of organisms implicated in pharyngitis and the rapid activity observed is consistent with the time taken for a lozenge to dissolve in the mouth.[16]

For all test organisms, evidence of bactericidal activity for the AMC/DCBA lozenge was recorded at the 1-minute time point. Of particular interest is the robust bactericidal activity against *S. pyogenes*, the most frequent cause of bacterial pharyngitis.[4] Reductions exceeding 99.9% were achieved by 1 minute for *S. pyogenes*, *H. influenza*, *A. haemolyticum* and *F. necrophorum*, by 5 minutes for *S. dysgalactiae* and *M. catarrhalis* and by 10 minutes for *S. aureus*. The bactericidal activity of an AMC/DCBA lozenge within a 10-minute period is important as it is consistent with the duration that a lozenge remains in the mouth; furthermore, the active ingredients were also tested at the expected concentration achieved when a lozenge is dissolved in the mouth, assuming a volume of 5 mL of saliva.

A previous in vitro evaluation of the bactericidal activity of antiseptic lozenges ([DCBA 1.2 mg, menthol 8 mg, AMC 0.6 mg] and [DCBA 1.2 mg, AMC 0.6 mg]) against *S. pyogenes* and *S. aureus* demonstrated antibacterial effectiveness.[32] Both AMC and DCBA formulations were highly active against the

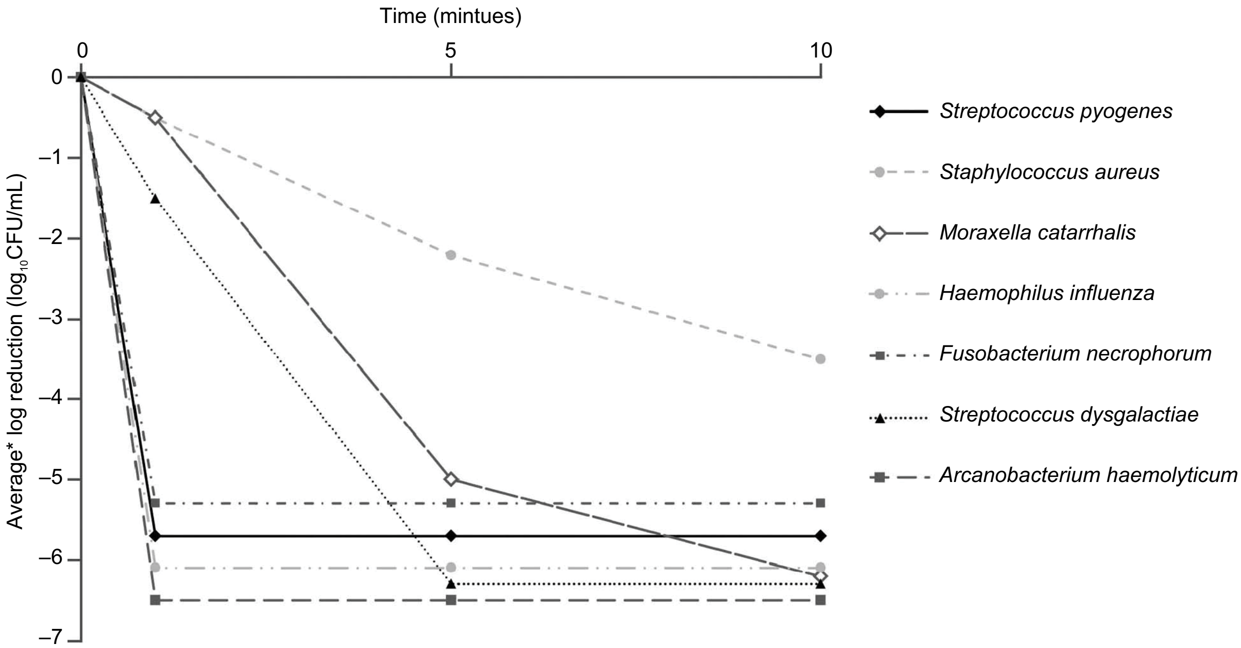

Figure 1 Bactericidal activity of an AMC/DCBA lozenge.
Notes: Bactericidal activity was measured against seven common oropharyngeal organisms over a 10-minute period. Bactericidal activity was defined as a decrease in bacterial count (CFUs per mL), using the average of three test replicates. *Average of the three test replicates.
Abbreviations: AMC, amylmetacresol; CFUs, colony-forming units; DCBA, 2,4-dichlorobenzyl alcohol.

Table 1 Control and test counts of challenge organisms

Challenge organism	Test control count (mean $\log_{10}$ CFUs/mL ± SD)	Log reduction (mean $\log_{10}$ CFUs/mL ± SD)		
		1 minute	5 minutes	10 minutes
Staphylococcus aureus	6.5±0.1	0.5±0.2	2.2±0.1	3.5±0.1
Streptococcus pyogenes	6.7±0.1	5.7±0.1	5.7±0.1	5.7±0.1
Moraxella catarrhalis	7.2±0.1	0.5±0.1	5.0±0.9	6.2±0.1
Haemophilus influenza	7.1±0.1	6.1±0.1	6.0±0.1	6.2±0.1
Fusobacterium necrophorum	6.3±0.0	5.3±0.0	5.3±0.0	5.3±0.0
Arcanobacterium haemolyticum	7.5±0.0	6.5±0.0	6.5±0.0	6.5±0.0
Streptococcus dysgalactiae	7.3±0.0	1.5±0.2	6.3±0.0	6.3±0.0

Abbreviation: CFU, colony-forming unit.

bacteria tested within 5 minutes of exposure, in contrast to the slow and weak action of the local antibiotic tyrothricin.[33] The data generated in this study support and expand upon these previously published observations, providing further evidence of effectiveness against a broader range of bacterial species under in vitro conditions, including those where knowledge of their clinical pathology in pharyngitis is continuing to evolve or those that represent either an opportunistic infection or an underlying medical condition. These data likewise complement recent studies showing the in vitro viricidal effects of lozenges containing AMC/DCBA (and the active ingredients as free substances) against parainfluenza virus type 3, cytomegalovirus, respiratory syncytial virus, influenza A and severe acute respiratory syndrome coronavirus.[12,13] In addition to antimicrobial activity, AMC and DCBA are proven to provide relief from the symptoms of pharyngitis, particularly sore throat, likely through their demonstrated local anesthetic-like action against voltage-gated neuronal sodium channels,[14,34] and therefore may benefit patients presenting with either bacterial or viral pharyngitis. Furthermore, by relieving symptoms and managing patient expectations, the number of instances of inappropriate antibiotic prescribing for viral pharyngitis may be reduced.

A limitation of this study is that these observations were performed in vitro and therefore do not fully reflect the environment of the throat. For example, the throat may contain multiple microorganisms whereas this study tested the bactericidal activity against organisms in isolation. The role of the patient's immune system and swallowing action on the antimicrobial activity of the lozenge or active ingredients can also not be determined using in vitro methodology. However, the incidence of these bacteria is relevantly low in the general population; therefore, studying the bactericidal activity of AMC/DCBA in vivo can be challenging. Consequently, an in vitro approach is advantageous allowing the rapid generation of robust data, for multiple organisms simultaneously, that can be used to evaluate the potential of AMC/DCBA for efficacy in vivo.

Conclusion

These data show that an AMC/DCBA lozenge demonstrates bactericidal activity against all test organisms, representing a broad range of bacterial cell structures, from 1 minute and achieves greater than 99.9% kill for all test organisms within 10 minutes, which is consistent with the duration that a lozenge remains in the mouth.

Therefore, patients with uncomplicated bacterial pharyngitis, including those taking antibiotics, from low-risk populations and without additional risk factors, may benefit from the antiseptic action of AMC/DCBA against a range of bacterial species associated with pharyngitis. Most cases of pharyngitis should not require antibiotics as they are typically self-limiting and often viral in origin. Therefore, over-the-counter antiseptics like AMC/DCBA may be more appropriate, unless the condition deteriorates or a streptococcal infection is diagnosed.

Data sharing statement

All data generated or analyzed during this study are included in this manuscript.

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Author contributions

All authors contributed to data analysis, drafting or revising the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

Disclosure

Derek Matthews, Robert Atkinson and Adrian Shephard are employees of Reckitt Benckiser Healthcare Ltd, UK. The authors report no other conflicts of interest in this work.

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