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Antimicrobial Activity of Xibornol and a Xibornol-Based Formulation Against Gram-Positive Pathogens of the Respiratory Tract

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Abstract

Xibornol is known since the 70s and a xibornol-based formulation is commercialized as spray suspension for the antisepsis of the oral cavity and as adjuvant in pharyngeal infections caused by Gram-positive microorganisms. Herein, we evaluated the antimicrobial activity of xibornol and the xibornol-based formulation against common pathogens of the upper and lower respiratory tract.

Our results indicate that xibornol alone and the xibornol-based formulation have strong antibacterial action against *Streptococcus pneumoniae*, *Streptococcus pyogenes*, and *Staphyloccus aureus,* as well as against the two emerging pathogens *Actinomyces israelii* and *Corynebacterium ulcerans*. These findings highlight the antimicrobial potential of these drugs in the topical control of pathogenic Gram-positive bacteria of the respiratory tract.

Keywords

Antimicrobial activity · Bornilene · Topical drug · Upper respiratory tract · Xibornol

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1 Introduction

The normal microbiota of the human throat is a complex mix of hundreds of bacterial species. It includes streptococci, lactobacilli, staphylococci, corynebacteria, and actinomyces. In some conditions, most of the microorganisms that normally reside in the upper respiratory tract may be responsible for mild to severe infections, not only limited to mouth cavity or teeth (Bosch et al. 2013). Bacterial infections of the throat are very

common and are mainly caused by Group A β-haemolytic streptococci such as *Streptococcus pyogenes* (Oliver et al. 2018). Infections are generally limited to sore pharyngitis, but complications are frequent and include both suppurative (i.e otitis media, cervical lymphadenitis, mastoiditis, sinusitis) and non-suppurative (i.e. acute rheumatic fever, acute glomerulonephritis) diseases. Despite with less frequency, upper respiratory tract infections are also caused by the emerging pathogen *Corynebacterium ulcerans* (Hacker et al. 2016), which is occasionally responsible for tonsillitis, pharyngitis, sinusitis, and pneumonia (Otsuji et al. 2017).

The oral cavity is an important source of bacteria able to cause infections of the lungs and other body districts. In fact, microorganisms that cause community-acquired pneumonia (i.e *Streptococcus pneumoniae* and *Actinomyces spp.*) normally reside in the oropharynx. *S. pneumoniae* can colonize the respiratory tract of asymptomatic healthy carriers and spread from this area becoming responsible for a plethora of severe infections, which include pneumonia, meningitis, bronchitis, rhinitis, sinusitis, and otitis media (Paju and Scannapieco 2007). *Actinomyces israelli* is a normal constituent of the human microbiota. From the mouth, it can occasionally spread and produce actinomycosis, a severe and chronic invasive disease (Valour et al. 2014). Actinomycosis includes infections of odontogenic origin that mainly concern the perimandibular region, as well as infections of the respiratory tract (pneumonia, bronchitis and laryngitis) and other body sites. *S. aureus* is another important human pathogen long described as a constituent of the human microbiota of the upper respiratory tract, despite its role in the oral health and disease is still debated (McCormack et al. 2015). Due to the impact of the emergence of methicillin-resistant *S. aureus* (MRSA) strains, the control of this microorganism in the oral cavity is some concern, particularly in subjects undergoing oral surgery or hospitalization.

Xibornol (6-isobornyl-3-4 xylenol; Fig. 1) is an antiseptic drug for topical use. The drug is a phenolic derivative of bornan and shows strong lipophilic nature, making its water solubility very poor. Although xibornol was discovered in the late '60s (Capponi 1969), only few data reported in the '80s are available describing its effectiveness and very few recent data are available on this drug. The antimicrobial activity of xibornol was demonstrated against *S. aureus* and other Gram-positive bacteria, viruses and fungi (Morandini et al. 1985; Combe et al. 1988; Scaglione 2009; Verani et al. 2017; Fabbri et al. 1988). Detailed mechanism of action this drug remains unknown, but bacteria exposed to xibornol present a marked reduction in cellular division and in the synthesis of nucleic acids, proteins and peptidoglycans (Combe et al. 1988). Several studies demonstrated the therapeutic efficacy of xibornol in treating upper respiratory tract infections (Morandini et al. 1985; Olivieri et al. 1984) and the good tolerability of this drug (Morandini et al. 1985; Scaglione et al. 1988).

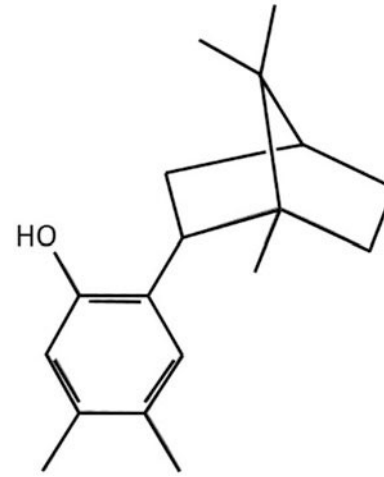

Fig. 1 6-isobornyl-3-4 xylenol (Xibornol)

A xibornol-based formulation is currently available on the Italian market sold as Bornilene (Agenzia Italiana del Farmaco – AIFA 2016). Bornilene is a complex spray drug that contains 3% xibornol and several excipients that facilitate spray dispersion and implement mucus adhesivity of the drug in the oral cavity (Scaglione 2009). Bornilene is sold for the topical treatment of infections and inflammatory states of the throat (pharyngitis, laryngitis, acute and chronic tonsillitis), for sustaining the hygiene of the mouth in

pre- and post-surgery, and as antiseptic in dental practice. With the exception of information on Bornilene reported on an AIFA document (AIFA 2016), there are no published data reporting the antimicrobial activity of this formulation. The aim of this work was evaluate the antimicrobial activity of xibornol and Bornilene against a panel of Gram-positive microorganisms that can cause infections of the respiratory tract.

2 Methods

2.1 Drug Formulations

Xibornol powder, Bornilene, and a placebo formulation were kindly provided by Abiogen Pharma (Abiogen Pharma S.p.a. Pisa, Italy). Bornilene and the placebo solution were contained in 30 ml bottles of plastic glass equipped with a pre-dosed metric valve and dispenser spout. Bornilene spray is a mixture of 3% xibornol and contains various excipient (glicerol, 96% (v/v) ethanol, micro-crystalline cellulose and 30% carmellose sodium, simeticon emulsion, clathrate menthol, ammonium glycyrrhizinate, hemihidrate chlorine buthanol, sodium saccharin, citral clathrate, anhydrous colloidal silica, castor oil, polyethoxylate, potassium). The placebo solution contained the solution of excipients of Bornilene but did not contain xibornol. Bornilene and the placebo solution were nebulized in a sterile tube, using the provided nebulizing bottle. 12% (w/v) solutions of xibornol were prepared in DMSO. Nebulized compounds and xibornol solutions were used immediately.

2.2 Bacterial Strains and Culture Media

Streptococcus pneumoniae NCTC 7465 (corresponding to ATCC 33400), *Streptococcus pyogenes* ATCC 12344, *Corynebacterium ulcerans* ATCC 51799, *Staphylococcus aureus* DSM 799 (corresponding to ATCC 6538), and *Actinomyces israelii* ATCC 12102 were used. *S. aureus* was propagated in TSA (Triptone Soy Agar; Oxoid, Basingstoke, UK), *S. pyogenes* and *S. pneumoniae* were propagated in TSA supplemented with 5% defibrinated horse blood, *C. ulcerans* and *A. israelii* were propagated on BHIA (Brain Heart Infusion Agar; Biolife, Milan, Italy). Cultures were incubated at 37 °C in aerobic condition (*S. aureus*, *C. ulcerans*), or 5% CO2 (*S. pyogenes* and *S. pneumoniae*), or in anaerobiosis (*A. israelii*).

2.3 MIC and MBC Determination

Minimal inhibitory concentrations (MIC) and minimal bactericidal concentrations (MBC) were determined in order to assess antimicrobial activity of the three different formulations (xibornol solution, Bornilene, placebo). Microdilution assays were performed according to the European Committee on Antimicrobial Susceptibility Testing standards (EUCAST 2017) in a 200 µl volume/well using, in the first well, 100 µl of the formulations prepared as described above. Mueller Hinton (MH; Oxoid) and Mueller Hinton-Fastidious (MH-F, Mueller Hinton supplemented with 5% defibrinated horse blood and 20 mg/L β-NAD; Oxoid) were used for conventional and fastidious microorganisms respectively. Incubation was performed at 37 °C in aerobiosis, 5% CO2, or anaerobiosis depending on microorganisms. Bacterial colonies were suspended in sterile 0.9% NaCl solution to 0.5 McFarland, a 1:10 dilution was performed and 10 µl (about 5 x 10^4 CFUs) used to inoculate microplate wells. For each assay, two consecutive plates (containing 24 dilutions each) were prepared. Due to the low water solubility of xibornol, the drug has been used starting from a 3% (w/v) concentration from a 12% stock solution in DMSO. The Bornilene and placebo solutions were used as supplied starting from a 1:2 dilution in the first well. Non-inoculated medium (C-), medium alone inoculated with bacteria (C+), DMSO dilutions (the same as xibornol) were used as controls for each assay. Assays were performed in triplicate. MIC was determined following the EUCAST reading guide (EUCAST 2017) and by seeding

appropriate dilutions of each well and counting CFUs after incubation at 37 °C for 24 h. MBC were calculated by seeding appropriate dilutions of aliquots of suspensions deriving from the wells corresponding to the MIC and higher drug concentrations and by CFU counting after incubation in the appropriate culture medium.

3 Results and Discussion

The oropharyngeal cavity is normally colonized by a wide variety of microorganisms that constitute a complex microbiome. From this district, pathogens may spread and cause local or systemic diseases (Avila et al. 2009). Although antibiotics are largely used worldwide to treat and prevent infections that originate from the upper respiratory tract, development of bacterial resistance is of great concerns. For this reason, the use of alternative or additional medications, such as oral antiseptics, should be strongly encouraged in view to control pathogen spreading, as well as dental (Slots 2002) and oral health (Shi et al. 2013).

Several antiseptics are available to control potential pathogenic microorganisms residing in the upper respiratory tract and include the widely used chlorhexidine (Karpiński and Szkaradkiewicz 2015) and povidone-iodine (Kanagalingam et al. 2015). Other antiseptics, such as octenidine (Assadian 2016), polyhexanide (Fjeld and Lingaas 2016), hexetidine (Kapić et al. 2002), and xibornol (Scaglione 2009) are also available.

Xibornol has long history of safe use as adjuvant and antiseptic and some studies regarding xibornol activity against bacteria do exist (Morandini et al. 1985; Combe et al. 1988; Scaglione 2009). However, the strong lipophilic nature that affects handling of xibornol in antimicrobial dilution assays probably contributed to limit the studies performed on this compound. Xibornol is commercially available as Bornilene, a well-tolerated drug containing 30 mg/ml xibornol (6-isobornyl-3-4-xylenol) that is used for topical administration in the oral cavity and the upper respiratory tract (AIFA 2016). To make xibornol more usable for topic action in the upper respiratory tract, a complex mixture of excipients was used in the formulation Bornilene.

In this work, we choose a panel of Gram-positive microorganisms that can be harmful for the human respiratory tract. In addition to the most famous *S. pneumoniae*, *S. pyogenes*, and *S. aureus*, we included the emerging pathogens *C. ulcerans* and *A. israelii*, which have an established role in causing respiratory and extra-respiratory infections. The activity of xibornol, Bornilene, and the placebo solution contained in Bornilene was determined by microdilution assays. As shown in Table 1, results indicate that the MIC values of xibornol against the tested bacteria ranged from 0.003% for *S. aureus* and the two streptococci, to a concentration of 0.0005% for *A. israelii* that resulted the most sensitive among the considered microorganisms. Xibornol showed bactericidal action against *S. aureus*, *A. israelii*, and *C. ulcerans* (MBC: 0.003%, 0.0005%, 0.001%, respectively), being the MIC and MBC values identical for these organisms. For *S. pyogenes* and *S. pneumoniae*, MBC values (0.012%) were two dilutions higher than MICs, indicating that at the MIC values the drug had bacteriostatic effect against these two pathogens. As expected, similar results were obtained with Bornilene. The MIC values of this complex formulation ranged from 1:1024 (0.0029% xibornol) for *S. pyogenes* to 1:4096 (0.0007% xibornol) for *S. aureus* and *C. ulcerans*. Similar to xibornol, Bornilene showed bactericidal effect against *S. aureus* (MBC 1:4096), *A. israelii* (MBC 1:2560) and *C. ulcerans* (MBC 1:4096). MIC values against *S. pneumoniae* and *S. pyogenes* (1:20148 and 1.1024, respectively) were lower than MBC values, thus indicating that, similar to xibornol, at the MIC values Bornilene has bacteriostatic effect on these organisms. The placebo solution had no activity against all the tested microorganisms.

In the case of *S. pneumoniae* and *S. aureus*, MIC and MBC values of Bornilene were slightly lower than those of xibornol alone (Table 1). This effect could result from the presence of the excipients contained in Bornilene. Although excipients alone do not show antimicrobial

Table 1 MIC and MBC values of xibornol, Bornilene, and placebo against Gram-positive microorganisms

	xibornol		Bornilene		placebo	
Bacterial species	MIC	MBC	MIC (% xibornol)	MBC (% xibornol)	MIC	MBC
S. pneumoniae	0.003%	0.012%	1:2048 (0.0015)	1:1024 (0.0029)	n	n
S. pyogenes	0.003%	0.012%	1:1024 (0.0029)	1:512 (0.0058)	n	n
S. aureus	0.003%	0.003%	1:4096 (0.0007)	1:4096 (0.0007)	n	n
A. israelii	0.0005%	0.0005%	1:2560 (0.0012)	1:2560 (0.0012)	n	n
C. ulcerans	0.001%	0.001%	1:4096 (0.0007)	1:4096 (0.0007)	n	n

n: no activity

activity, their presence in Bornilene could increase xibornol bioavailability and activity towards these organisms.

Bornilene is used without dilution by direct spraying it on tissues of the oral cavity and pharynges. Our finding that Bornilene is active at high dilutions suggests that this drug is very efficacious when administered *in vivo*. In addition, the presence of xibornol in a glycerol-based formulation, which displays strong mucoadhesion properties (Jones et al. 2007), most likely confers longer persistence of the drug on mucosal tissues, thus reducing its wash-out and prolonging its activity.

4 Conclusion

In the present paper, we evaluated MIC and MBC of xibornol and the highly water-insoluble xibornol-based mixture Bornilene against Gram-positive pathogens of the upper respiratory tract. We found that both the drug alone and the drug suspended in excipients have strong antibacterial action against *S. aureus*, *S. pneumoniae*, *S. pyogenes*, and the two emerging pathogens *C. ulcerans* and *A. israelii*.

Our findings indicate that xibornol-based formulations can be very effective in the topical control of pathogenic Gram-positive bacteria relevant in upper and lower respiratory tract, odontostomatologic, as well as systemic infections. Due to the dramatic increase of bacterial resistance to a number of commonly used antibacterial agents, xibornol-based formulations, such as Bornilene, appear as valid antiseptic for controlling and reducing bacterial colonization and infections also caused by antibiotic-resistant bacteria.

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