MODULE 2 – COMMON TECHNICAL DOCUMENT SUMMARIES
2.5 Clinical overview (T1680, 1 mg/ml, Eye drops, solution)

MODULE 2.5

CLINICAL OVERVIEW

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ABBREVIATIONS

AK	*Acanthamoeba* keratitis
EC	European Community
HEX	Hexamidine
HEX D	Hexamidine diisetionate
ITT	Intention to treat
MBC	Minimum Bactericidal Concentrations
MIC	Minimum Inhibitory Concentration
PP	Per protocol
SD	Standard deviation
SDU	Single dose unit
SmPC	Summary of Product Characteristics
SSO	Sum of objective signs
SSS	Sum of subjective symptoms

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2.5.1. PRODUCT DEVELOPMENT RATIONALE

2.5.1.1. Nature of the request

This dossier concerns a hybrid application according to the Article 10 (3) of the EC Directive 2001/83/EC to request the marketing authorisation for T1680 (hexamidine diisetionate 0.1%) eye drops solution. T1680 contains the same active substance and excipients in the same concentrations as the currently authorised product, DESOMEDINE® registered by Bausch & Lomb (reference product). The T1680 solution was formulated as sterile eye drops packaged in bottles of 0.6 mL. The therapeutic indications and posology recommended for T1680 are the same as DESOMEDINE®.

T1680 is indicated:

- for the treatment of:
  - purulent bacterial conjunctivitis caused by susceptible microorganisms
  - keratoconjunctivitis
  - blepharitis
  - chronic tear duct infections
- as a preoperative antiseptic for the conjunctival sacs

The recommended dose is one drop into the conjunctival sac of the affected eye(s), 4 to 6 times daily. The total length of treatment should not exceed 8 days.

2.5.1.2. Reference product (DESOMEDINE® eye drops)

The reference product is DESOMEDINE® 1.0 mg/mL eye drops, solution, registered by Bausch & Lomb. Hexamidine diisetionate, the active substance of DESOMEDINE®, is used as an antiseptic for many years. DESOMEDINE® was approved in France and Belgium in 1991. It is also registered in Switzerland and Luxembourg.

2.5.1.3. Scientific background and rationale for use of hexamidine in Ocular Infections

Scientific Background

Bacterial conjunctivitis

Bacterial conjunctivitis is a microbial infection involving the mucous membrane of the surface of the eye. This condition is usually a self-limiting disease. Purulent bacterial conjunctivitis, characterized by mucopurulent discharge and hyperemia, affects subjects of all ages, but is particularly frequent in children. It represents one of the most common ocular diseases in childhood, occurring in approximately 1 in 8 children each year [Bremond-Gignac et al, 2011].

Bacterial infection is a common cause of conjunctivitis and accounts for up to 50% of all cases of conjunctivitis in adults and 70% to 80% of all cases in children. Globally, purulent bacterial conjunctivitis is mainly caused by Gram-positive organisms. The most common causative agents are *Staphylococcus epidermidis* (39% of cases), *Staphylococcus aureus* (22% of cases), and *Streptococcus pneumoniae* (6% of cases). The most common Gram-negative microorganism found in acute conjunctivitis is *Haemophilus influenzae* (9% of cases) [Review in Bremond-Gignac et al, 2011].

Although bacterial conjunctivitis can occur at any age, it frequently occurs in preschool- and school-age children. In these age groups, pathogens are frequently associated with epidemic occurrences of bacterial conjunctivitis. In infants, children and teenagers, the most common

ocular pathogens are *Staphylococcus aureus*, *Haemophilus influenzae*, *Streptococcus pneumoniae*, and also *Moraxella* species [Bremond-Gignac et al, 2011; Leung et al, 2018].

Most cases of acute bacterial conjunctivitis resolve spontaneously within 7-10 days, but a broad-spectrum antibiotic can decrease the disease severity, transmission and also minimize the complications and reinfection rates.

Bacterial keratitis

Corneal infections are among the most common causes of corneal haze, and viral, bacterial, and fungal infections are the leading causes of microbial keratitis [Ung et al, 2019].

Bacterial keratitis is an important ophthalmic emergency and one of the most common causes of corneal blindness [Egrilmez and Yildirim-Theveny, 2020]. A particular feature of bacterial keratitis is its rapid progression. Corneal destruction may be complete in 24-48 hours with some of the more virulent bacteria. Corneal ulceration, stromal abscess formation, surrounding corneal edema, and anterior segment inflammation are characteristic of this disease. Bacterial keratitis is a potentially devastating ocular infection that may occur when the corneal epithelial barrier is compromised due to injury or trauma, leading to ulceration and infiltration of inflammatory cells [Bremond-Gignac et al, 2011].

Despite local and regional variations in bacterial keratitis etiology, the most commonly reported causative organisms appear consistent worldwide, demonstrating a higher proportion of Gram-positive isolates (range 47.6-88.6%; median 72.2%) than Gram-negative isolates (range: 11.4-49.6%; median: 27.0%). The most common pathogens associated with bacterial keratitis are *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pneumoniae*, and *Pseudomonas aeruginosa* [Ung et al, 2019; Egrilmez and Yildirim-Theveny, 2020].

Contact lenses are increasingly involved in keratitis. Contact lens wear now accounts for more than one half of all cases of bacterial keratitis and has become the most important risk factor. Although Gram-negative organisms such as *Pseudomonas aeruginosa* are known to be associated with contact lens-related corneal ulcers, Gram-positive organisms such as *Staphylococcus* and *Streptococcus* species have also been shown to be frequently responsible for a significant proportion of these ulcers, even when Gram-negative organisms are recovered from the lens and lens case. Indeed, a higher incidence of Gram-positive organisms than Gram-negative organisms recovered from infections associated with contact lens wear was reported [Bremond-Gignac et al, 2011].

Pseudomonas is the most frequent etiologic agent of contact lens-associated microbial keratitis, being responsible for up to 2 of 3 cases. Fungi, including *Candida*, account for more than 50% of all culture-proven keratitis cases in tropical and subtropical regions and more than 50% of all cases of endogenous endophthalmitis [Review in Pinna et al, 2020].

Immediate diagnosis and treatment are important to avoid vision-threatening complications, including corneal scarring or perforation. Main treatment agents in bacterial keratitis are topical antibiotics [Egrilmez and Yildirim-Theveny, 2020].

***Acanthamoeba* keratitis**

Free-living amoebas of *Acanthamoeba* genus are the etiologic agent of a painful and sight-threatening infectious disease that affects the human cornea, *i.e.*, *Acanthamoeba* keratitis (AK). It is an aggressive ocular infection that can lead to blindness without treatment [Dart et al, 2009]. It is usually associated with wearing soft contact lenses. Dart et al. documented that in

countries with a high prevalence of contact lens wear, 85%-88% of AK cases occurred in contact lens users [Dart et al, 2009].

According to a recent review [Heaselgrave et al, 2019], there are approximately 4.1 million contact lens wearers in the United Kingdom, and established independent risk factors for developing AK in contact lens wearers include exposure to tap water at home, swimming or bathing when wearing contact lenses, poor lens hygiene, and the use of rigid contact lens in orthokeratology. A recent study from a tertiary hospital in the United Kingdom reported an incidence rate of 2.3% for *Acanthamoeba* over a 12-year period from over 1500 keratitis cases. Due to the small number of patients with AK, many are diagnosed late due to initially being misdiagnosed and treated for bacterial or other forms of keratitis such as fungal and herpes simplex keratitis. A late diagnosis of AK has a massive impact on prognosis, and patients are more likely to develop poorer visual outcome, longer duration of treatment, corneal perforation, and the requirement of penetrating keratoplasty [Heaselgrave et al, 2019]. AK occasionally affects both eyes [Wilhelmus et al, 2008].

The most frequently described symptoms are foreign body sensation, redness, tearing, photophobia, decreased visual acuity, and in some cases, severe pain not predicted by clinical findings. Clinical signs include punctate epithelial erosions, pseudodendritic lesions, subepithelial or stromal infiltrates and perineural infiltrates. The presence of a ring-shaped corneal infiltrate and perineural infiltrates, also known as radial keratoneuritis, are characteristic signs of AK. Although radial keratoneuritis is a strongly suggestive sign of AK in contact lens wearing patients, it has also been reported in bacterial keratitis. In addition, scleritis, anterior uveitis, hypopyon, glaucoma, mydriasis and cataract occur in severe cases or late stages of the disease, as well as corneal abscess, melting and perforation [Review in Carrijo-Carvalho et al, 2017].

Early diagnosis and appropriate therapy are key elements for a good prognosis in AK. A presumptive diagnosis of AK can be made clinically and with *in vivo* confocal microscopy, although a definitive diagnosis requires identification of *Acanthamoeba* on direct scraping, histology, or identification of *Acanthamoeba* DNA by polymerase chain reaction (PCR).

Acanthamoeba castellanii and *A. polyphaga* are the most common of the 8 species reported to cause keratitis [review in Dart et al, 2009; Battaini et al, 2018].

Blepharitis

Blepharitis is a chronic disorder producing inflammation of the eyelid margin. Blepharitis can be classified according to anatomic location: anterior blepharitis affects the base of the eyelashes and the eyelash follicles, and posterior blepharitis affects the Meibomian glands and gland orifices. Blepharitis has traditionally been clinically subcategorized as staphylococcal, seborrheic, Meibomian gland dysfunction, or a combination thereof. Staphylococcal and seborrheic blepharitis mainly involve the anterior eyelid, and both can be described as anterior blepharitis. Meibomian gland dysfunction involves the posterior eyelid margin [Bremond-Gignac et al, 2011].

The organisms most commonly isolated in chronic blepharitis include *Staphylococcus aureus*, coagulase negative *Staphylococcus* spp., *Corynebacterium* spp. and *Propionibacterium acnes*. The cause of inflammation appears to be bacterial by-products (proinflammatory cytokines, lipases) rather than the bacteria themselves, as, although pathogenic bacteria are rare in blepharitis, commensal organisms such as *Staphylococcus epidermidis* and *Staphylococcus aureus* can produce lipolytic exoenzymes and endotoxins. Lipolytic enzymes hydrolyze wax and sterol esters in Meibomian gland secretions with the release of highly irritating fatty acids

and other products resulting in disruption of tear film integrity. These endotoxins can induce the production of proinflammatory cytokines, thus initiating an inflammatory reaction. Reducing the bacterial load on the eyelid margins is therefore part of the treatment of blepharitis [Bremond-Gignac et al, 2011].

Perioperative prophylaxis

Numerous ocular surgical procedures are routinely performed. Although significant technical progress has been made, endophthalmitis is still one of the most serious complications of ocular surgery, particularly cataract surgery. The risk of endophthalmitis reported in 3 prospective studies is as follows: 0.21% and 0.32% in France and 0.38% in Europe [Bremond-Gignac et al, 2011]. The microorganism responsible for endophthalmitis may come from the conjunctival flora, contaminated instruments, irrigating solutions, the implant, or airborne contamination. The best means for preventing endophthalmitis is therefore compliance with strict surgical hygiene. The organisms most commonly isolated from endophthalmitis occurring after cataract surgery are Gram-positive bacteria (86.7% of cases), including *Staphylococcus epidermidis*, *Staphylococcus aureus* and *Streptococcus pneumoniae*, while Gram-negative bacteria represent 13.3% of cases [Bremond-Gignac et al, 2011]. Actually, *Staphylococcus epidermidis* is the organism most commonly isolated from eyes with post-operative endophthalmitis [Pinna et al, 2020].

A variety of practices and procedures have been proposed for a long time in order to minimize the incidence of postoperative infection. One of these approaches consists of reducing the number of microorganisms on the surface of the eye by using topically applied antisepsis and/or antibiotics.

Rationale for Use of Hexamidine in Ocular Infections

Complications from bacterial conjunctivitis are uncommon. However, severe infections can result in keratitis, corneal ulceration and perforation, and blindness [Azari et al, 2013; Hovding, 2008]. Chronic and hyperacute forms of bacterial conjunctivitis are associated with high levels of ocular and systemic morbidity [Azari et al, 2013; Patel et al, 2007].

Topical antimicrobial treatment has been shown to hasten clinical and microbial remission of the disease and may reduce risks for severe ocular complications [Hwang, 1996; Sheikh, 2001].

Eye bacterial infections may be associated with serious ocular complications and develop into a sight-threatening condition, if left untreated. Worsening to corneal abscess may occur. The risk depends on:

- The causative bacteria; some of them have a high-pathogenicity level,
- The severity of the disease; severe bacterial conjunctivitis presents either chemosis, palpebral oedema, severe watering, decreased visual acuity or photophobia [AFSSAPS guidelines July 2004, Hwang, 1996].
- The patient's ocular status, with the possible presence of local diseases such as obstruction of lachrymal ducts, palpebral static disorders, as well as ocular trauma, recent ocular surgery or contact lens wear [AFSSAPS guidelines July 2004]. Disrupted anatomic barriers permits corneal infection with possible infiltration, ulceration and perforation [Foulks et al, 2000; Soukiasian and Baum, 2005].

Uncomplicated bacterial conjunctivitis and other ocular infections can be treated empirically with topical antiseptics.

Mild cases are generally considered to be self-limiting, resolving in 5 to 10 days. However, current consensus supports the use of topical antibiotics [Sheikh et al, 2012; Hwang et al, 1996; Sheikh et al, 2001] as they: 1) provide symptomatic relief, 2) hasten microbial remission, 3) shorten disease duration, 4) reduce risk of developing sight-threatening complications, 5) reduce rate of re-infection, and 6) prevent infection spread.

Acanthamoeba keratitis is an aggressive ocular infection that can lead to blindness without treatment [Dart et al, 2009].

The active substance of the proposed medicinal product T1680 is hexamidine diisetionate (HEX D). It is a hydrosoluble cationic agent and an aromatic diamidine with antimicrobial activity against bacteria, fungi, yeasts and free-living amebae [Grare et al, 2010; Aimard et al, 1998]. This positively charged molecule binds with high affinity to the negatively charged cell walls and membranes of bacteria, thus causing disruption of the target cell by perturbation of the binding sites [Grare et al, 2010]. It has been used in medicine as an antiseptic for over half a century [Grare et al, 2010].

HEX is an “old” topical antimicrobial. However, it has been recently confirmed that the majority of the bacterial species found in the above-mentioned ocular infections are susceptible to HEX. In a recent study [Pinna et al, 2020], HEX D solution showed rapid *in vitro* antimicrobial activity against *Staphylococcus epidermidis*, *Staphylococcus aureus* and *Candida* species, but was poorly active against *Pseudomonas aeruginosa*. These results confirm that HEX D remains an efficient antimicrobial agent for common eye infections.

HEX has also been found to be effective in completely killing 15 different isolates of *Acanthamoeba* and it is currently used as a first-line treatment for *Acanthamoeba* keratitis in combination with chlorhexidine [Siddiqui et al, 2016; Carrijo-Carvalho et al, 2017].

2.5.1.4. Presentation of clinical data in this application

This clinical overview is based on the pertinent bibliographical literature relating to the efficacy and safety of HEX D 0.1% in the treatment of bacterial infections of the eye and its adnexa. The literature search was performed with PubMed and complemented with the Sponsor’s literature database. All publications until October 2020, including (hexamidine or desomedine or hexamidine diisetionate or diamidine) and (eye or ocular) as keywords were analysed and selected whenever relevant. Primarily English or French language peer-reviewed literature was selected initially on the basis of search results including abstracts, and subsequently on the basis of original publications acquired.

2.5.2. OVERVIEW OF BIOPHARMACEUTICS

2.5.2.1. Pharmaceutical form and excipients

The proposed medicinal product T1680 is a sterile eye drops solution for topical ophthalmic use supplied in 0.6-mL bottles and containing hexamidine diisetionate as the active substance.

The proposed concentration of hexamidine diisetionate (0.1%) in T1680 and dosage schedule (one drop in the affected eye(s), 4 to 6 times a day) are the same as the ones recommended for the currently authorised reference product DESOMEDINE®.

The formulation of T1680 is strictly identical to the formulation of DESOMEDINE®. The complete formula is as follows:

Table 2.5-1: Formula of T1680 eye drops

Components	For 100 mL	Function
Active substance		
Hexamidine diisetionate	0.100 g	Active substance
Excipients		
Borax	[redacted]	Buffer agent
Boric acid		Buffer agent
Sodium chloride		Isotonizing agent
Water for injections		Vehicle

All ingredients are listed in the European Pharmacopoeia.

2.5.2.2. Ocular bioavailability

Since there is no difference in formulation between T1680 and DESOMEDINE® eye drops, the active substance hexamidine diisetionate in T1680 is absorbed at the same rate and extent as in the reference product DESOMEDINE®.

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2.5.3. OVERVIEW OF CLINICAL PHARMACOLOGY

2.5.3.1. Pharmacodynamics

2.5.3.1.1. Activities linked to the recommended therapeutic indications

2.5.3.1.1.1. Literature Data

Hexamidine (HEX) is a strong organic base and an aromatic diamidine essentially described as bacteriostatic on Gram-positive cocci. The bactericidal activity is slow [Review in Salvatico et al, 2015]. It has been used in medicine as an antiseptic for over half a century [Grare et al, 2010].

HEX was initially developed as a trypanocidal agent. The anti-protozoal activity of hexamidine was further explored more than 50 years later. In the 1990s, the efficacy of HEX as an amoebicidal agent was demonstrated in a number of studies [Brasseur et al, 1994; Perrine et al, 1995].

The active substance of T1680 is hexamidine diisetionate (HEX D). It is a hydrosoluble cationic agent with antimicrobial activity against bacteria, fungi, yeasts, and free-living amebae [Grare et al, 2010, Aimard et al, 1998]. This positively charged molecule binds with high affinity to the negatively charged cell walls and membranes of bacteria, thus causing disruption of the target cell by perturbation of the binding sites [Grare et al, 2010].

Antimicrobial activity

In terms of antibacterial properties, HEX D has been reported to be effective against *Pseudomonas aeruginosa*, *Proteus*, *Escherichia coli*, *Staphylococcus aureus* and *Tsukamurella paurometabolum* [van Ketel, 1975; Granel et al, 1996].

The efficacy of HEX D against a series of multidrug-resistant Gram-positive bacteria has been demonstrated [Grare et al, 2010]. These authors tested the *in vitro* activity of HEX D against 39 multidrug-resistant Gram-positive bacteria (15 *Staphylococcus aureus*, 12 coagulase-negative staphylococci, and 14 *Enterococcus* spp.) and 30 multidrug-resistant Gram-negative bacteria (20 *Enterobacteriaceae* and 10 non-fermenting bacilli).

The *in vitro* activities of HEX D against *Staphylococcus aureus*, coagulase-negative staphylococci (CoNS), and *Enterococcus* spp., with various resistance phenotypes, are reported in the following table.

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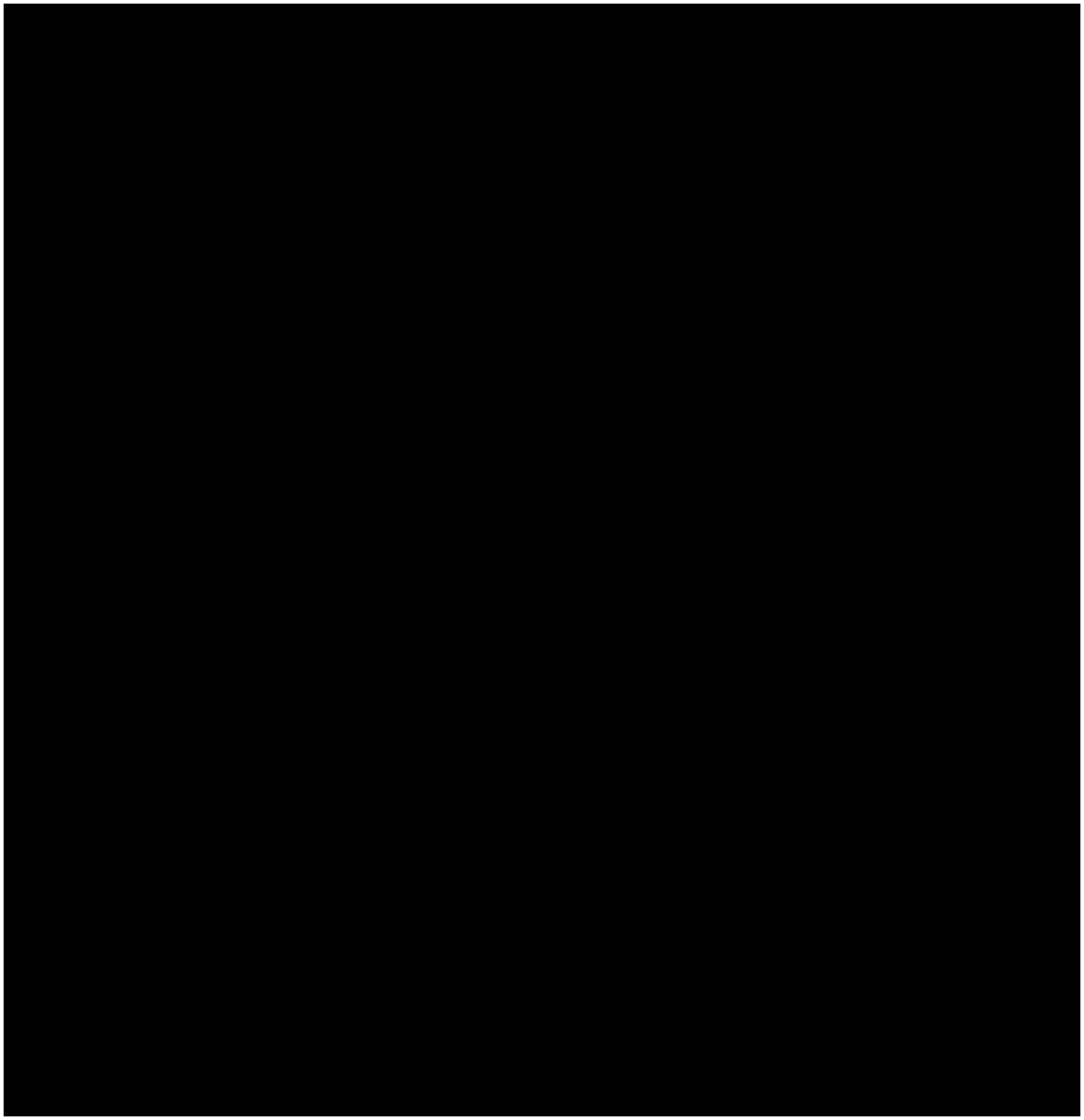
Table 2.5-2: *In vitro* activities of hexamidine against *Staphylococcus aureus*, coagulase-negative *staphylococci* (CoNS) and *Enterococcus spp*. [Grare et al, 2010]



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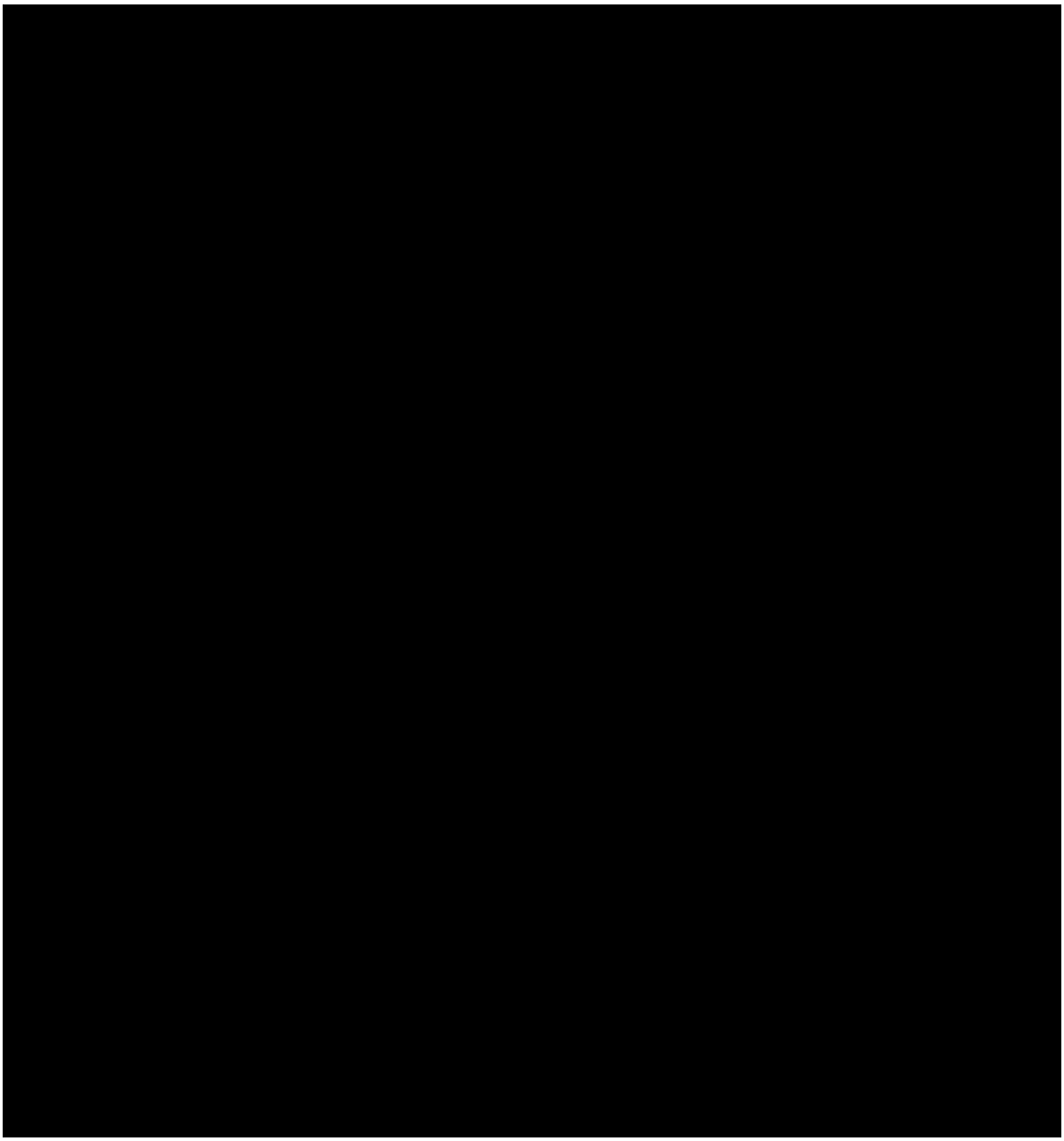
The *in vitro* activities of HEX against *Enterobacteriaceae* with various resistance phenotypes are reported in the following table.

Table 2.5-3: *In vitro* activities of hexamidine against *Enterobacteriaceae* with various resistance phenotypes [Grare et al, 2010**]**



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The *in vitro* activities of HEX against non-fermenting bacilli with various resistance phenotypes are reported in the following table.

Table 2.5-4: *In vitro* activities of hexamidine against non-fermenting bacilli with various resistance phenotypes [Grare et al, 2010]

Staphylococcus aureus, *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* are common causes of eye infection. *Staphylococcus epidermidis* is the organism most commonly isolated from eyes with post-operative endophthalmitis. Furthermore, *Pseudomonas* is the most frequent etiologic agent of contact lens-associated microbial keratitis, being responsible for up to 2 of 3 cases. Fungi, including *Candida*, account for more than 50% of all culture-proven keratitis cases in tropical and subtropical regions and more than 50% of all cases of endogenous endophthalmitis [Review in Pinna et al, 2020].

In a recent study [Pinna et al, 2020], HEX D solution showed rapid *in vitro* antimicrobial activity against *Staphylococcus epidermidis*, *Staphylococcus aureus* and *Candida* species, but was poorly active against *Pseudomonas aeruginosa*. These results confirm that HEX D remains an efficient antimicrobial agent for common eye infections.

Results of this study on the *in vitro* antimicrobial activity of an ophthalmic solution containing HEX D 0.05% [Pinna et al, 2020] are detailed hereafter.

The ability of the ophthalmic solution containing HEX D 0.05% to kill the organisms tested at different exposure times is shown in the Table below.

Table 2.5-5: Microbial growth at different times after exposure to an ophthalmic solution containing hexamidine diisetionate 0.05% [Pinna et al, 2020]

After 1-minute incubation, there was no growth on the plates seeded with *Staphylococcus aureus* ATCC 43300, *Staphylococcus aureus* clinical isolate, *Staphylococcus epidermidis* clinical isolate, and all 5 Candida species tested. Conversely, the ophthalmic solution failed to kill both the clinical isolate and the ATCC reference strain of *Pseudomonas aeruginosa* after 30 minutes exposure and needed 24 hours to eradicate the organisms. Positive controls consistently showed growth at all exposure times. No growth was observed in the plates seeded with the negative control.

In total, the ophthalmic solution containing HEX D showed a good, rapid antimicrobial activity against 5 clinical Candida isolates and multiresistant strains of *S. aureus* and *S. epidermidis*. Conversely, the HEX solution was not so rapidly effective against a clinical isolate and an ATCC reference strain of *P. aeruginosa*, taking more than 30 minutes to eradicate these organisms. These results are consistent with the more former study performed by Grare et al. [Grare et al, 2010] where HEX D was found to show antibacterial activity against Gram-positive organisms, but was poorly active against *Pseudomonas aeruginosa*.

HEX has also been found to be active on *Acanthamoeba* strains. In the 1990s, the efficacy of HEX as an amoebicidal agent was demonstrated in a number of studies [Brasseur et al, 1994; Perrine et al, 1995].

An *in vitro* practical complete kill-assay was developed to determine whether HEX D 0.1% and other agents usually used in the treatment of *Acanthamoeba* keratitis (polyhexamethylene biguanide 0.02%, chlorhexidine digluconate 0.02% and voriconazole 1.0%) were effective in completely killing 15 different isolates of *Acanthamoeba* at time points of 24, 48, and 72 hours in comparison with a saline control [Kowalski et al, 2013]. Results showed that antiacanthamoebal efficacy, determined by the median growth grade and the kill incidence rate, was more prominent for HEX (median growth grade: 0.0; kill incidence rate: 93% [14 of 15 isolates]) and polyhexamethylene biguanide (median growth grade: 0.0; kill incidence rate: 80% [12 of 15 isolates]) than for chlorhexidine digluconate (median growth grade: 1.0; kill incidence rate: 40% [6 of 15 isolates]), voriconazole (median growth grade: 2.0; kill incidence rate: 13% [2 of 15 isolates]), and saline (median growth grade: 3.0; kill incidence rate: 0% [0 of 15 isolates]).

Among the 15 drugs evaluated by Taravaud et al. [Taravaud et al, 2017] for their *in vitro* anti-*Acanthamoeba* activity, the best activity was obtained with HEX, with an IC_{50} at 0.04 µM at 3 and 4 days of treatment, and a value of 0.06 µM at 5 days of treatment. Higher IC_{50} values (respectively, around 10 µM and 1 µM at either 3, 4 or 5 days of treatment) were obtained with

propamidine and pentamidine compared to HEX. According to the authors, these results indicated an increase of the anti-*Acanthamoeba* activity in relation with the length of diamidine alkyl chain. These results are in agreement with a previous study showing that the anti-*Acanthamoeba* activity of diamidines is proportional to the length of their alkyl chain [Perrine et al, 1995].

2.5.3.1.1.2. In vitro study performed with HEXAMIDINE GILBERT 0.1%, single-dose eye drops (equivalent formulation as T1680)

An *in vitro* study has been conducted to compare the antibacterial activity of the generic medicinal product HEXAMIDINE GILBERT® 0.1% single-dose eye drops (equivalent formulation as T1680) and the reference product DESOMEDINE® Eye drops (same formulation as T1680) [Dusart, 2000].

The Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentrations (MBC) were determined for the two products on a panel of 50 strains. These strains included collection strains (n=6) and wild strains isolated at the Montpellier University Hospital - France (n=44).

These were mainly Gram-positive strains (20 *Staphylococcus* including 10 *Staphylococcus aureus*, 10 *Enterococcus* - *Streptococcus* strains, mainly *E. faecalis* strains). Gram-negative bacteria were also tested (10 strains of *Enterobacter*, 5 strains of *Acinetabacter* and 5 strains of *Haemophilus*).

The wild strains tested in this study were chosen because of their involvement in various ocular pathologies.

The MICs were studied by the technique of serial dilutions in liquid medium, in microplates, using successive dilutions of each of the products at HEX D 0.1%, *i.e.* 1000 mg/L (500 - 250 - 125 - 62 , 5 - 31.2 - 15.6 - 7.8 - 3.9 - 1.9 - 0.95 and 0.47 mg/L). Before reading, the microplates were incubated at 35-37°C for 24 to 48 hours under aerobic conditions.

The MBCs were determined by culture on agar after reading the MICs and counting the surviving bacteria.

The MIC and MBC values for the Test product and the Reference product are reported in the following Table.

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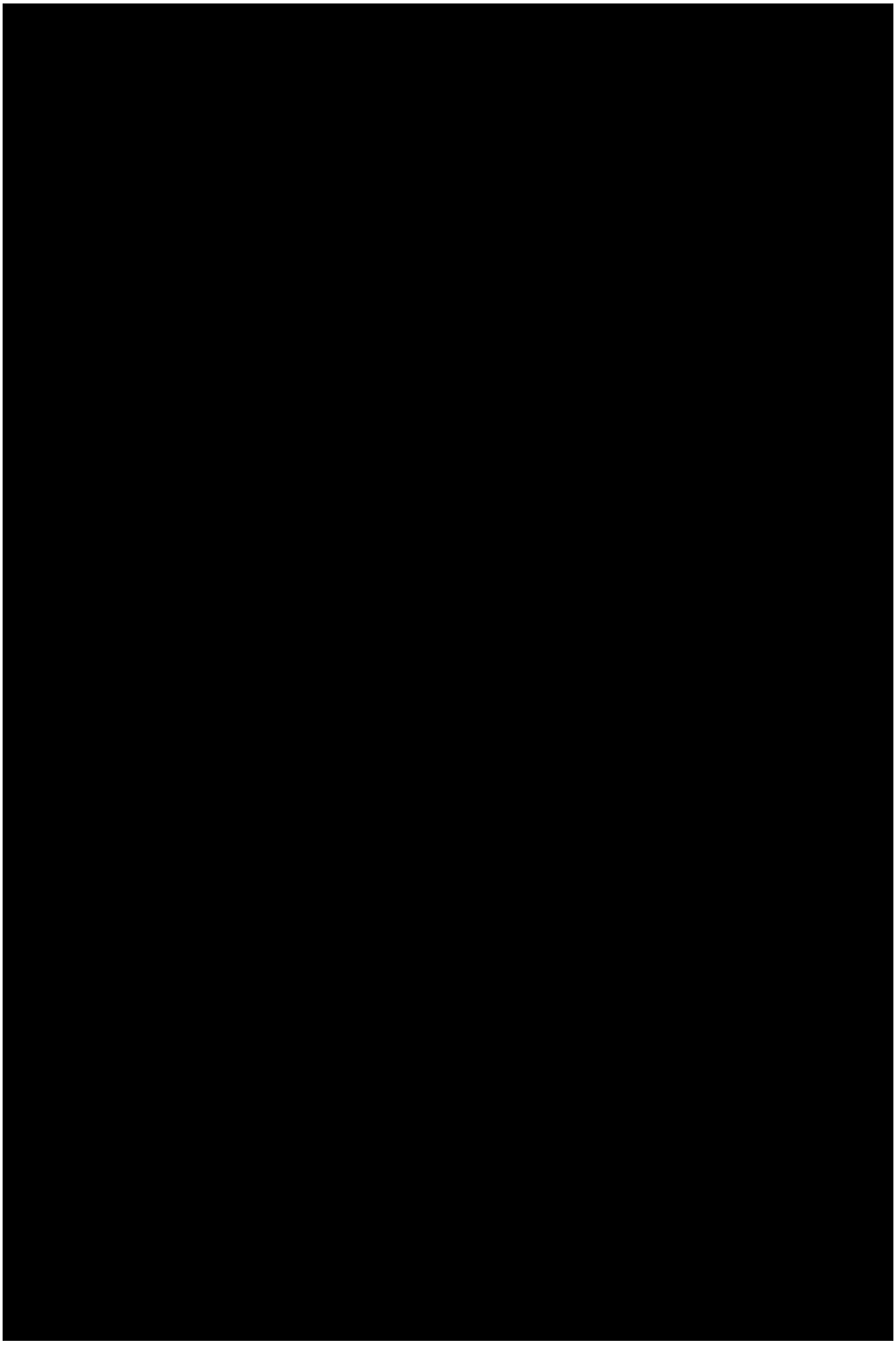
Table 2.5-6: Comparative antibacterial activity of the Test product (HEXAMIDINE GILBERT®) and the Reference Product (DESOMEDINE®)



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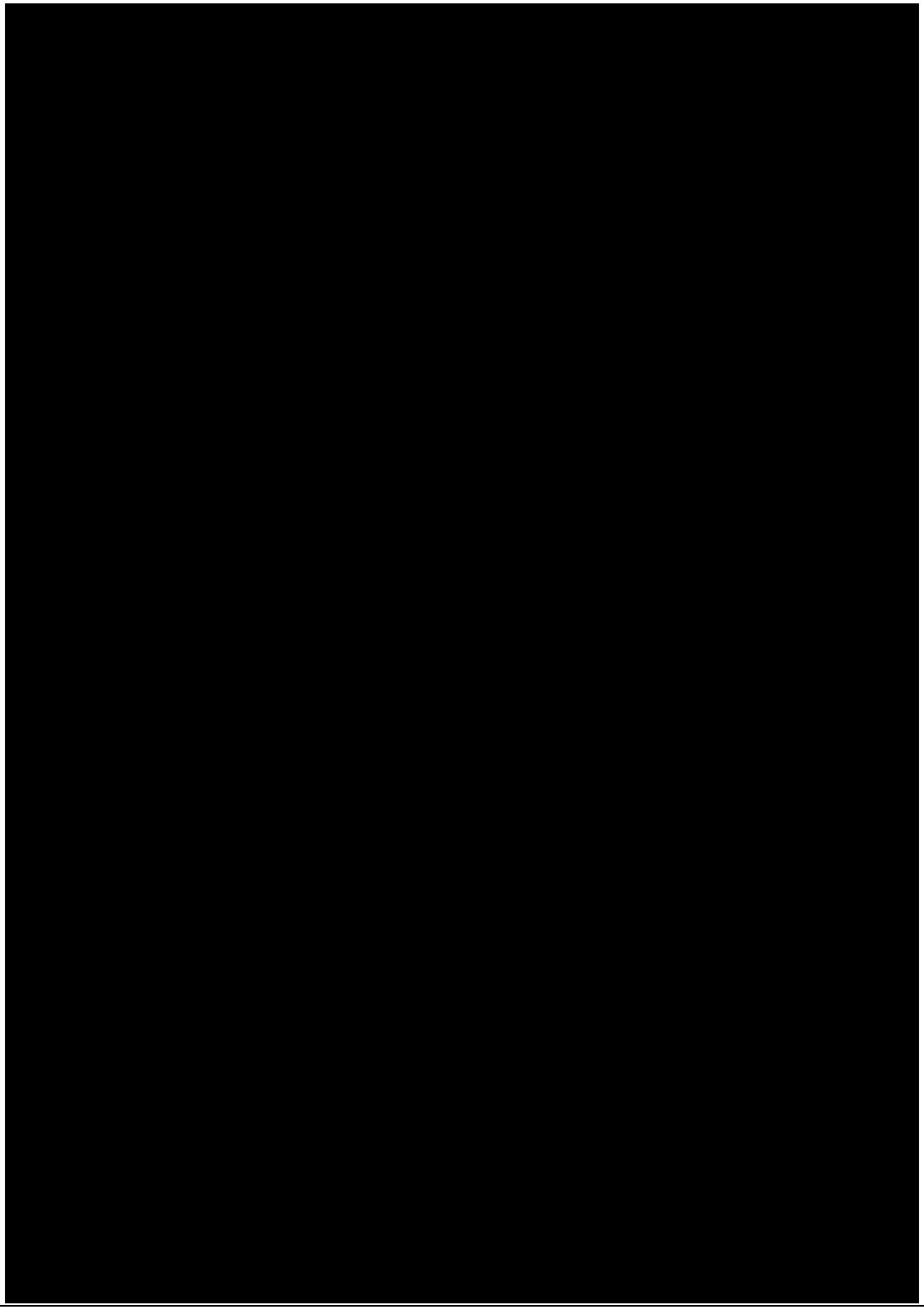


These results showed that the antibacterial activities of both products were comparable. In the very few cases of discrepancy, the values obtained differed only by one dilution, a difference which can be attributed to the sensitivity of the method.

The best bacteriostatic and bactericidal activities were obtained against Gram-positive bacteria, and particularly against strains of *Staphylococci*. The MIC values were 0.47 mg/L and 0.95 mg/L for the most sensitive strains and 7.8 mg/L for the most resistant strains.

The values of MBC obtained on the strains of *Staphylococci* showed a good bactericidal activity, since they were equal to those of the MICs or they differed from them by only three dilutions (*S. aureus* n ° 13 and 23).

Regarding *Enterococci*, the MIC values were homogeneous and equal to 3.9 mg/L, except for the *E. hirae* ATCC 10541 strain (1.9 mg/L). The MBC values differed from those of the MICs by 3 to 4 dilutions, demonstrating a more moderate bactericidal activity than that observed with *Staphylococci*.

Regarding Gram-negative bacteria, the activity of the two products was more variable depending on the species, with MIC values ranging from 0.95 mg/L to 250 mg/L. For MBCs, values ranged from 1.9 to 250 mg/L.

2.5.3.1.2. Mechanism of action

The exact mechanism of HEX D (and other diamidines) antimicrobial efficacy is still unclear. However, due to its native positive charge, it is thought that HEX D binds with high affinity to the negatively charged cell walls and membranes of bacteria and that disruption is brought about by perturbations of the binding sites resulting in inhibition of oxygen uptake and induced leakage of amino acids. In this sense, HEX D might be considered to be acting as a cationic surface-active agent [McDonnell and Russell, 1999].

According to Perrine and colleagues [Perrine et al, 1995], the biocidal activity of the diamidines involves electrostatic interaction with lipids on the plasma membrane, increased membrane permeability and leakage, drug diffusion through the plasma membrane mediated by lipophilic interactions, and denaturation of intracellular proteins. According to this mechanism, the biocide action of the diamidines is associated directly with the cationic groups, while elongation of the alkyl chain to increase lipophilicity may favour diamidine internalization into *Acanthamoeba* in particular. The antimicrobial activity of diamidines would be proportional to the length of their alkyl chain and would explain why HEX has higher amoebicidal activity than propamidine [Review in Carrijo-Carvalho et al, 2017].

2.5.3.2. Conclusion on clinical pharmacology

Hexamidine (HEX) is a strong organic base and has been used in medicine as an antiseptic for over half a century. It belongs to the aromatic diamidine group, essentially described as bacteriostatic on Gram-positive cocci. Diamidines are well known for their antimicrobial effects resulting from the cationic surface-active properties generated from the bipolar structure of the molecules.

The *in vitro* comparative study of the antibacterial activity showed that the antimicrobial profile of the generic product HEXAMIDINE GILBERT® 0.1%, single-dose eye drops (equivalent formulation as T1680) is similar to that of the reference product DESOMEDINE® (same formulation as T1680). The best bacteriostatic and bactericidal activities were obtained against Gram-positive bacteria, and particularly against strains of *Staphylococci*. The MIC values were 0.47 mg/L and 0.95 mg/L for the most sensitive strains and 7.8 mg/L for the most resistant strains.

In a very recent study, HEX D ophthalmic solution showed rapid *in vitro* antimicrobial activity against *Staphylococcus epidermidis*, *Staphylococcus aureus* and *Candida* species, but was

poorly active against *Pseudomonas aeruginosa*. These results confirm that HEX D remains an efficient antimicrobial agent for common eye infections.

HEX has also been found to be effective in completely killing 15 different isolates of *Acanthamoeba*.

Although the mechanisms of action of HEX are not fully understood, some processes involved in the antimicrobial activity of the diamidines have been elucidated. The diamidines act as cationic surface-active agents and cause membrane disruption, leakage of amino acids, and inhibit oxygen uptake.

2.5.3.3. Pharmacokinetics

Since there is no difference in formulation between T1680 and the reference product DESOMEDINE® Eye drops, the active substance HEX D in T1680 has the same pharmacokinetic properties as in DESOMEDINE®.

No pharmacokinetics data after ocular administration of HEX has been found in the literature. However, due to the poor absorption of HEX D after oral administration and dermal application observed in animals [refer to non-clinical data in Module 2.4], no systemic passage of HEX D is expected after ocular administration of the proposed formulation T1680.

In addition, no systemic effects have been reported following ocular instillation of HEX D 0.1% [Desomedine BE SmPC, 2020].

2.5.4. OVERVIEW OF EFFICACY

Hexamidine 0.1% is an antimicrobial agent effective in the treatment of ophthalmic infectious diseases caused by sensitive microorganisms. The *in vitro* evaluation of HEX 0.1% on various pathogens is fully described in Section 2.5.3.1.

Efficacy of HEX-based eye drops is supported by published data and results of one clinical trial performed with the generic product HEXAMIDINE GILBERT® 0.1%, single-dose eye drops (equivalent formulation as the proposed medicinal product T1680) [Morvan and Sechoy, 2001].

HEX D has received increased attention in recent years, because it is particularly effective in the treatment of *Acanthamoeba* keratitis where it is used as first line therapy in combination with biguanide agents.

These supporting efficacy data are analysed in the following sections.

2.5.4.1. Review of Hexamidine-based ophthalmic solution Efficacy in the literature

HEX is known as an antimicrobial agent used in bacterial conjunctivitis and keratoconjunctivitis for a long time. However, no well-controlled study has been published in the literature.

HEX is currently used as a first-line treatment for *Acanthamoeba* keratitis (AK) in combination with chlorhexidine [Siddiqui et al, 2016; Carrijo-Carvalho et al, 2017]. This antiacanthamoebal efficacy has been subjected to publications which are summarized below.

The efficacy of HEX as an amoebicidal agent was firstly demonstrated by Brasseur et al. in two case reports [Brasseur et al, 1994].

As acyclovir ointment, propamidine ointment and neomycine or tobramycin eye drops resulted in no improvement or only limited clinical improvement, it was decided to use HEX 0.1% eye drops (DESOMEDINE®).

The first patient presented superficial ulcerative keratitis and clinical manifestations consistent with an *Acanthamoeba* infection, which was proven by the presence of trophozoites and cysts in corneal scrapings. This patient was treated with HEX 0.1% eye drops four times a day for 4 months. A definitive healing with a final visual acuity of 20/100 due to a central corneal opacity and corneal thinning without new vessels was observed. After 15 months of follow-up, the patient's clinical status remained stable without relapse.

In the second patient, infectious keratitis was milder. HEX 0.1% eye drops were initially prescribed eight times a day then three times a day, and resulted in a rapid improvement without subsequent relapse.

The successful management with HEX 0.1% eye drops of these patients suffering from a corneal infection by *Acanthamoeba* was thus reported in this publication. In both cases, treatment was well tolerated [Brasseur et al, 1994].

An *in vitro* study from Perrine et al. showed that HEX D was effective not only against *Acanthamoeba* trophozoites but also against the dormant cyst forms [Perrine et al, 1995]. Bailly et al. thus hypothesized that the amoebicidal activity of HEX might have been directly related to its capacity to selectively bind DNA [Bailly et al, 1997]. However, although the study showed that HEX D strongly bound DNA, no correlation was found between the amoebicidal potency of the aromatic diamidines series and their DNA binding ability. In contrast, HEX D failed when used against an *Acanthamoeba* and *Hartmannella* corneal coinfection [Aimard et al,

1998] and showed only limited efficacy on six subjects affected by chronic *Acanthamoeba* keratitis [Perez-Santonja et al, 2003].

HEX has been shown to be clinically effective against both the trophozoite and cystic forms of Acanthamibes. The reported mean minimum cysticidal concentration for HEX was 41 µg/mL [Hay et al, 1994]. However, resistant clinical isolates have been described with a minimum cysticidal concentration ranging from 125 to 500 µg/mL [Kilvington, et al, 2002; Perez-Santonja et al, 2003]. For these reasons, diamidines cannot be used as monotherapy for the treatment of amoebic keratitis.

Current medical therapy for AK involves the topical administration of membrane-acting agents such as chlorhexidine or polyhexamethylene biguanide, in combination with a diamidine (HEX or propamidine) for a period of up to one year, with infection recurrence in 10% of cases [Dart et al, 2009; Bang et al, 2010; Bouheraoua et al, 2014; Siddiqui et al, 2016; Carrijo-Carvalho et al, 2017; Review in Taravaud et al, 2017].

HEX has shown a faster amoebicidal effect than propamidine against trophozoite and cystic forms in *in vitro* studies [Perrine et al, 1995]. HEX drops without the preservative benzalkonium chloride showed good activity against trophozoites [Heaselgrave et al, 2019].

In a retrospective, non-comparative, interventional case series study comprising 44 eyes from 42 patients presenting with AK, treated with topical HEX D and topical polyhexamethylene biguanide as first-line therapy, the authors reported that more aggressive medical treatment should be considered when at least one of significant risk factor for surgical treatment is present [Bouheraoua et al, 2013]. This retrospective study showed that late diagnosis, low initial visual acuity, corneal neovascularization, large infiltrates, and preperforated infiltrates were significant risk factors for surgical treatment in patients presenting with AK.

Two non-comparative observational case series have been published to report the use of HEX in the treatment of microsporidial keratoconjunctivitis [Tung-Lien Quek et al, 2011; Kwok et al, 2013].

In human eyes, microsporidia are pathogens initially reported to cause opportunistic infections. It was first described in 1990, when three patients with acquired immunodeficiency syndrome presented with bilateral superficial epithelial keratitis. Patients may develop other complications such as uveitis, corneal neovascularisation, necrosis and perforation. Risk factors for microsporidial keratoconjunctivitis include a history of contact lens wearing, LASIK surgery, previous use of topical corticosteroids, trauma, or exposure to contaminated water or soil [Kwok et al, 2013].

A first retrospective, non-comparative, observational case series reported the use of HEX D 0.1% in the management of microsporidial keratoconjunctivitis in 22 patients (24 eyes) [Tung-Lien Quek et al, 2011].

Of the 22 patients, 90.9% were men, with a mean age of 30.3 years (range: 15-76 years). Two (9.1%) had bilateral involvement, 15 (68.2%) were non-contact lens users, 17 (77.3%) reported contamination with mud within 2 weeks (mean of 6.8 days) of onset of symptoms.

All patients presented with conjunctivitis and coarse, multifocal, punctate epithelial keratitis. Two of 24 eyes (8.3%) had anterior stromal infiltrates, while 8 (33.3%) had anterior uveitis.

Microsporidial spores were identified on modified trichrome staining of corneal epithelial scrapes in all eyes. All eyes were treated with epithelial debridement, topical fluoroquinolone

and HEX D 0.1%. Seven (31.8%) patients received oral albendazole, and all eyes with anterior uveitis received topical steroids. All cases resolved without visually significant sequelae.

In conclusion, microsporidial keratoconjunctivitis occur mainly in males, is usually unilateral, presents as conjunctivitis and coarse, multifocal, punctate epithelial keratitis, and may be associated with anterior uveitis. Soil contamination is an important risk factor. Treatment with debridement, fluoroquinolones, HEX D with or without systemic albendazole is effective, with steroids reserved for any associated anterior uveitis [Tung-Lien Quek et al, 2011].

In the second publication, a cluster of 25 healthy paediatric and teenage individuals with microsporidial keratoconjunctivitis has been investigated [Kwok et al, 2013]. It was a non-comparative, observational case series.

All patients were started on topical moxifloxacin hydrochloride 0.5%, topical HEX D 0.1% or propamidine isethionate 0.1% and ofloxacin 0.3% ointment. The eye drops were instilled every 30 min, alternating with each other. Oral albendazole was continued for 2 weeks in two patients as requested by the parents. This treatment regime was continued for 3 weeks and then gradually tapered according to individual patients' response.

All patients, with a mean age of 13.4 years (range: 5-16), had participated in a rugby match. The onset of symptoms occurred between 10 and 30 days post-exposure. All eyes had multiple superficial coarse punctate keratitis. Four (12%) eyes presented with keratic precipitates.

After treatment, all eyes healed without sequel.

In conclusion, a standardized topical regime including a fluoroquinolone (moxifloxacin) and an antiseptic such as HEX D 0.1% or propamidine isethionate 0.1% was safe and effective in the treatment of microsporidial keratoconjunctivitis in paediatric and teenage individuals [Kwok et al, 2013].

HEX 0.1% eye drops were also found to be successful in treating one case of Thygeson's superficial punctate keratitis [Zonnevylle et al, 2019].

2.5.4.2. Clinical study conducted with HEXAMIDINE GILBERT 0.1%, single-dose eye drops (equivalent formulation as T1680)

A clinical acceptability/tolerance study has been performed to compare the generic product HEXAMIDINE GILBERT® 0.1%, single-dose eye drops (equivalent formulation as T1680) and the reference product DESOMEDINE® (same formulation as T1680) [Morvan and Sechoy, 2001].

Although the main objective of this clinical trial was to compare the acceptability and tolerance of both products [refer to Section 2.5.5.1], the evolution of the objective signs and subjective symptoms of bacterial conjunctivitis were assessed between the initial and the final visits.

Corresponding results are therefore reported in this Efficacy Section.

MODULE 2 – COMMON TECHNICAL DOCUMENT SUMMARIES

2.5 Clinical overview (T1680, 1 mg/ml, Eye drops, solution)

Study Design

The trial was a randomised, multicentre, comparative, open study *versus* reference product.

It was conducted between 30 October 2000 and 04 July 2001, in compliance with GCP and the Declaration of Helsinki.

Patient Selection

Inclusion criteria were the following:

- Patient consulting for bilateral conjunctivitis and presenting at least in one eye the clinical signs of bacterial conjunctivitis [moderate (score 2) or severe (score 3) conjunctival hyperemia and purulent secretions in appearance; absence (score 0) or low intensity (score 1) symptoms of itching and tearing]
- Patient aged 18 and over who signed the written informed consent
- Patient fulfilling the conditions necessary for adherence to the protocol and study treatment

Non-inclusion criteria were as follows:

- Patient not fulfilling one or more inclusion criteria
- History of hypersensitivity to one of the constituents of the study products
- Use of other eye drops prescribed for the same indication
- Concomitant systemic treatment likely to influence the study assessments
- Wearing contact lenses
- Unilateral conjunctivitis

Study Follow-Up

Two mandatory visits were planned:

- Ji (inclusion visit) at Day 0
- Jf (final visit) at the end of treatment, between Day 8 and Day 11

Treatment

Test product: HEXAMIDINE GILBERT 0.1%, eye drops in single dose containers, 2 instillations per eye 4 to 6 times daily for 7 to 10 days

Reference product: DESOMEDINE® (Hexamidine 0.1%) eye drops, 2 instillations per eye 4 to 6 times daily for 7 to 10 days

In accordance with the Summary of Product Characteristics of the reference product, the daily number of instillations (4 to 6 per day), as well as the duration of treatment (7 to 10 days), could vary, depending on the prescription of the investigator.

Evaluation Criteria

Acceptability and tolerance assessments were performed at Ji and Jf.

The intensity of the following ocular symptoms and signs was assessed by the investigator using a 4-point rating scale [0 (symptom/sign absent), 1 (low intensity), 2 (moderate intensity) or 3 (high intensity)]:

Subjective symptomatology:

- stinging
- burning
- itching
- photophobia

Objective signs:

- conjunctival hyperaemia
- conjunctival edema
- secretions
- tearing

Assessments were performed before the first instillation and within minutes (maximum 5 min) of the first instillation.

For all symptoms and signs, a score was assigned for each eye. The sum of the scores for the right eye and the left eye led to the calculation of an **SSS score** (sum of subjective symptoms) from 0 to 24 and an **SSO score** (sum of objective signs) from 0 to 24 for each patient.

At the end of treatment (*i.e.*, during the final visit between Day 8 and Day 11), the same examinations/assessments were carried out.

Statistical methods

Statistical analysis of the results was performed using the SAS software. Data were analysed in the intention to treat (ITT) and per protocol (PP) sets for comparison between Ji and Jf and compliance comparison between groups. The three following successive analyses were performed:

- Comparison of scores before and after instillation at each visit. As the measurements were performed on the same patient, a paired series t test was used.
- Comparison between the initial visit and final visit; this analysis was carried out before and after instillation. A paired series t test was also used.
- Comparison between the two groups of the difference in scores between the initial visit and the final visit. This comparison was carried out before and after instillation by means of a Wilcoxon test (a non-parametric test for comparing means between two groups).

For frequency comparisons, the Chi2 test was used.

Results

Description of the Subjects

A total of 59 patients (n=30 for the Test group and n=29 for the Reference group) were included in 20 study centres in France.

Nineteen (32%) were male and 40 (68%) female. The mean age ± SD was 46.09 ± 20.24 years for the Test group and 57.25 ± 18.04 years for the Reference group.

At baseline (inclusion), the two groups did not show a significant difference for the distribution by sex, the intensity of subjective symptoms (SSS score) and objective signs (SSO score).

Patients in the Reference group had a significantly higher average age than patients in the Test group.

Evolution of clinical symptoms and signs between Ji and Jf

In both groups, a statistically significant and clinically meaningful reduction in SSS and SSO scores was observed between Ji and Jf (see Table below).

Table 2.5-7: Comparison of symptoms and signs scores between both study visits for the Reference and Test groups (PP Set)

Results observed showed a favourable evolution of the ocular symptoms and signs in the two treatment groups between the initial visit and the final visit.

Comparison of the evolution of scores between both groups is provided in the Table below.

Table 2.5-8: Between-group comparison of the evolution of the symptoms and signs scores (PP Set)

The reduction of subjective and objective scores was comparable in the two treatment groups.

In conclusion, although the main objective of this clinical trial was to compare the acceptability and tolerance of the reference and test products, the results have shown a statistically significant and clinically meaningful improvement in ocular signs and symptoms of bacterial conjunctivitis following treatment with HEX D 0.1% eye drops. These results were identical for the two hexamidine-based products [the reference product DESOMEDINE® (same formulation as T1680) and the generic product HEXAMIDINE GILBERT® 0.1%, eye drops in single dose containers (equivalent formulation as T1680)].

2.5.4.3. Overall conclusions on clinical efficacy

Hexamidine 0.1% is an antimicrobial agent effective in the treatment of ophthalmic diseases caused by sensitive microorganisms. The *in vitro* evaluation of HEX D 0.1% on various pathogens is fully described.

Efficacy of HEX D 0.1% in the treatment of bacterial infections of the eye and its adnexa is supported by published data and results of a clinical trial, which compared the reference product DESOMEDINE® (same formulation as T1680) and the generic product HEXAMIDINE GILBERT® 0.1% eye drops (equivalent formulation as T1680).

Although the main objective of this clinical study was to compare the acceptability and tolerance of the reference and test products, results have shown a statistically significant and clinically meaningful improvement in objective signs and subjective symptoms of bacterial conjunctivitis. These results were identical for both HEX-based products: the reference product DESOMEDINE® and the generic product.

HEX D has received increased attention in recent years because it is particularly effective in the treatment of *Acanthamoeba* keratitis where it is used as first line therapy in combination with biguanide agents. This antiacanthamoebal efficacy has been subjected to numerous publications, but no well-controlled study has been reported in the literature.

HEX D has also been found to be effective in the topical treatment of microsporidial keratoconjunctivitis in combination with a fluoroquinolone.

2.5.5. OVERVIEW OF SAFETY

2.5.5.1. Clinical study conducted with HEXAMIDINE GILBERT 0.1%, single-dose eye drops (equivalent formulation as T1680)

A clinical acceptability/tolerance study has been performed to evaluate the tolerance and acceptability of the generic product HEXAMIDINE GILBERT® 0.1% eye drops (equivalent formulation as T1680) in comparison to the reference product DESOMEDINE® (same formulation as T1680) [Morvan and Sechoy, 2001].

This study was conducted between 30 October 2000 and 04 July 2001, in compliance with GCP and the Declaration of Helsinki.

The **objectives of this study** were:

- to assess the tolerance and acceptability of the generic product HEXAMIDINE GILBERT® 0.1% eye drops in single dose containers, in comparison to the reference product DESOMEDINE® (HEX 0.1%) eye drops, 2 instillations per eye 4 to 6 times daily for 7 to 10 days in the treatment of bacterial conjunctivitis,
- to establish that the generic product has an acceptable tolerance, at least equivalent to that of the reference product DESOMEDINE® Eye drops.

Study Design

The trial was a randomised, multicentre, comparative, open study *versus* reference product.

Patient Selection

Inclusion criteria and **non-inclusion criteria** are detailed in Section 2.5.4.2.

Study Follow-Up

Two mandatory visits were planned:

- Ji (inclusion visit) at Day 0
- Jf (final visit) at the end of treatment, between Day 8 and Day 11

Treatment

Tested product: HEXAMIDINE GILBERT® 0.1% eye drops in single dose containers, 2 instillations per eye 4 to 6 times daily for 7 to 10 days

Reference product: DESOMEDINE® (Hexamidine 0.1%) eye drops, 2 instillations per eye 4 to 6 times daily for 7 to 10 days

Justification of choice of dosage regimen

The duration of treatment was, according to the investigator's prescription, from 7 to 10 days. This duration was considered sufficient to assess the local tolerance of the products tested.

In accordance with the Summary of Product Characteristics (SmPC) of the reference product, the daily number of instillations (4 to 6 per day), as well as the duration of treatment (7 to 10 days), could vary, depending on the prescription of the investigator.

Evaluation Criteria

Acceptability and tolerance assessments were performed at Ji and Jf.

The intensity of the following ocular symptoms and signs was assessed by the investigator using a 4-point rating scale [0 (symptom/sign absent), 1 (low intensity), 2 (moderate intensity) or 3 (high intensity)]:

Subjective symptomatology:

- stinging
- burning
- itching
- photophobia

Objective signs:

- conjunctival hyperaemia
- conjunctival edema
- secretions
- tearing

Assessments were performed before the first instillation and within minutes (maximum 5 min) of the first instillation.

For all symptoms and signs, a score was assigned for each eye. The sum of the scores for the right eye and the left eye led to the calculation of a **SSS score** (sum of subjective symptoms) from 0 to 24 and an **SSO score** (sum of objective signs) from 0 to 24 for each patient.

At the end of the treatment during the final visit (*i.e.*, between Day 8 and Day 11), the same examinations/assessments were carried out.

Adverse effects (serious and non-serious) were collected by the investigator during the initial visit after the first application, and during the final visit after completion of the entire treatment.

Justification of choice of measurement parameters

All the parameters evaluated, whether they are subjective symptoms (stinging, burning, pruritus, photophobia), objective signs (conjunctival hyperaemia and edema, secretions, tearing) or adverse events are commonly used parameters for tolerance studies on eye drops.

In addition, **compliance to treatment** has been studied as follows:

An individual monitoring log was given to the patient at the inclusion visit for the report of the times and the number of instillations. This notebook allowed the investigator to determine a degree of compliance for each patient, according to the number of instillations not administered between the 1st instillation (of Day 2) and the last instillation (of the day preceding the final visit), using the following scale:

- 0 instillation not performed: Very good compliance
- 1 to 4 instillations not performed: Good compliance
- 5 to 8 instillations not performed: Moderate compliance
- more than 8 instillations not performed: Poor compliance

All treatment units (vials used in whole or in part) had to be returned by the patient to the investigator at the final visit.

Statistical methods

Performed statistical analyses are detailed in Section 2.5.4.2.

Results

Description of the Subjects

A total of 59 patients (n=30 for the Test group and n=29 for the Reference group) were included in 20 study centres in France.

One patient in the Reference group did not come to the planed visits and twelve patients did not completed the study:

- 5 patients in the Reference group (DESOMEDINE® Eye drops): 2 patients stopped their treatment prematurely following non-serious adverse events (redness of the conjunctivae and eyelids), 2 patients stopped as they were cured, and 1 patient stopped for lack of product.
- 7 in the Test group (HEXAMIDINE GILBERT 0.1%, single-dose eye drops): 3 patients stopped their treatment prematurely following a non-serious adverse event (dry eye sensation, irritation and secretion, acute conjunctivitis and edema), 2 patients stopped their treatment for insufficient efficiency, 2 patients stopped as they were cured.

Nineteen (32%) patients were male and 40 (68%) female. The mean age ± SD was 46.09 ± 20.24 years for the Test group and 57.25 ± 18.04 years for the Reference group.

At baseline, the two groups did not show a significant difference at inclusion for the distribution by sex, the intensity of subjective symptoms (SSS score) or objective signs (SSO score). Patients in the Reference group had a significantly higher average age than patients in the Test group.

Extent of Exposure

All subjects received 1 or 2 instillations of HEX 0.1% eye drops per eye 4 times daily for 7 to 10 days whatever the study group.

The mean treatment duration was 6.98 ± 1.87 days in the Test group and 7.43 ± 1.87 days in the Reference group.

Safety Data

Adverse Events (AEs)

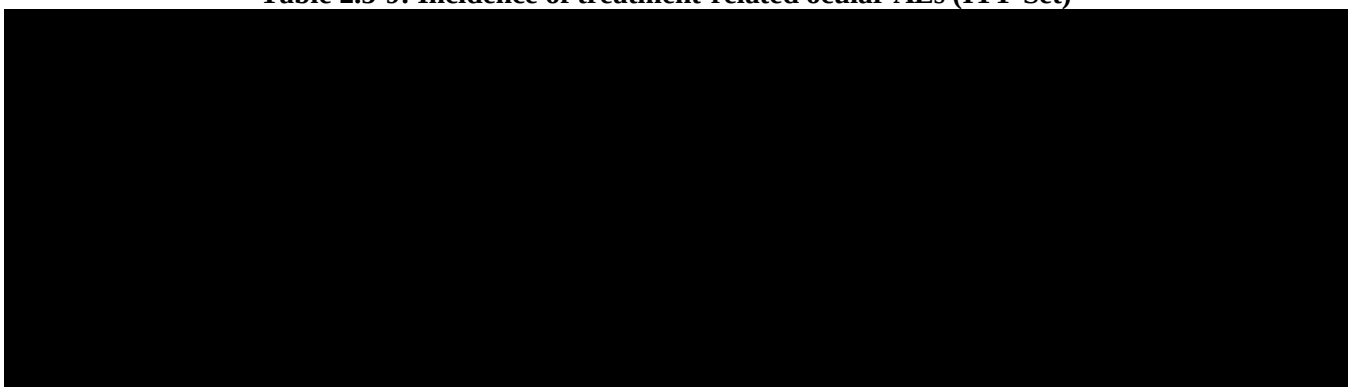
No serious AEs (SAEs) were reported, and none of the non-serious AEs reported were systemic AEs. Experienced AEs, which were slight to moderate in severity, led to premature study discontinuation.

Ocular AEs were reported for 5 subjects (8.5%), but the nature of the AE was not described for 2 subjects (1 in each group).

Ocular AEs were described for 3 patients (1 in the Reference group and 2 in the Test group). These ocular AEs were considered as treatment-related by the investigators.

The incidence of **treatment-related ocular AEs** is summarised in the table below.

Table 2.5-9: Incidence of treatment-related ocular AEs (ITT-Set)



In this situation of treatment of bacterial conjunctivitis, it is necessary to underline the difficulty in distinguishing between the pre-existing symptoms of irritation due to the disease and those which may be linked to the instillation of the studied eye drops.

Effect of treatments on ocular symptoms and signs before and 5 minutes after instillation

For both products, the subjective symptoms score and objective signs score before and after instillation were not statistically different, neither at Ji (initial visit) nor at Jf (final visit).

The immediate tolerance of the 2 eye drops was therefore comparable.

Evolution of ocular symptoms and signs between Ji and Jf

In both groups, a statistically significant and clinically meaningful reduction in SSS and SSO scores was observed between Ji and Jf (see Table 2.5-7 in Section 2.5.4.2).

Results observed showed a favourable evolution of the ocular symptoms and signs in the two groups between the initial visit and the final visit.

The between-group comparison of the evolution of the SSS and SSO scores is provided in Table 2.5-8 (see Section 2.5.4.2).

The reduction of subjective and objective scores was comparable in the two treatment groups.

Compliance to treatment

More than 80% of the patients were compliant to treatment in each group.

In conclusion, this clinical study showed the good ocular tolerance of the generic product, which was comparable with that of the reference product DESOMEDINE® (same formulation as T1680).

2.5.5.2. Overview of clinical safety from literature data

2.5.5.2.1. Topical adverse effects

The safety of HEX and HEX D was assessed by the Cosmetic Ingredient Review Expert Panel [CIR Expert Panel, 2007], which concluded that both actives are safe when used in cosmetics at concentrations less than or equal to 0.1%. This opinion was subsequently confirmed by the European Parliament and the Council of European Union [EP, 2009] which fixed the maximum allowed concentration of HEX and its salts in cosmetic products at 0.1%.

Recently, the safety of HEX with reference to skin application has been reviewed by Parisi et al. [Parisi et al, 2017]. This full review concluded that HEX and its salts are generally safe to use.

Several cases of allergic contact dermatitis have been reported with local application of HEX to the skin and the first was reported by Gougerot et al. in 1950 [review in Parisi et al, 2017]. A total of 147 cases of sensitization to HEX D has been observed in 8 years [Sidi et al, 1969]. Further cases were described by van Ketel [van Ketel, 1975], Robin [Robin, 1978], Dooms-Goosens et al. [Dooms-Goosens et al, 1989], and Brand and Ballmer-Weber [Brand and Ballmer-Weber, 1995]. One case of particular interest was reported by Mullins [Mullins, 2006] who observed an allergic systemic reaction due to topical application of HEX D. Furthermore, in a study aimed at comparing 75 cases of contact dermatitis caused by antiseptics, HEX was the strongest sensitizer with 20 positive patch tests [Barbaud et al, 2005]. However, studies involving larger numbers of subjects have shown that sensitization to HEX is not a common phenomenon. For example, Roul et al. tested 269 children aged 3 to 15 years with 34 allergens and attributed only one allergic reaction to HEX [Roul et al, 1999]. In a larger study, 641 children less than 16 years of age with atopic dermatitis were patch tested with 7 actives, which are commonly used for the topical treatment of this disease [Mailhol et al, 2009]. The results showed that HEX D caused allergic contact dermatitis to only three children (0.5% of the tested population). A photosensitivity reaction to a HEX solution was reported in a 19 year-old male eczema patient [Boulitrop-Morvan et al, 1993]. Finally, in a sensitization study in 100 male and 100 female subjects (aged between 15 and 60 years), topical application of HEX D 0.1% did not cause primary irritation, inflammation or sensitization in human subjects.

2.5.5.2.2. Ocular adverse effects

As reported by Bouheraoua et al. [Bouheraoua et al, 2014], clinically used diamidines are well tolerated by eye tissues, although prolonged treatment with propamidine can lead to toxic keratitis [Bacon et al, 1993].

Evaluation of propamidine and pentamidine toxicity *in vitro* showed that the drugs have minor toxic effects on corneal epithelial and endothelial cells with short-term exposure. It has been reported that cellular contact with the diamidines for prolonged times at concentrations effective against *Acanthamoeba* species produced cytotoxic effects. However, there are no comparative data on the effect of HEX on corneal cells [Carrijo-Carvalho et al, 2017].

2.5.5.2.3. Systemic adverse effects

No systemic adverse effects after ocular administration of HEX has been found in the literature. However, due to the poor absorption of HEX D after oral administration and dermal application observed in animals [refer to non-clinical data in Module 2.4], no systemic passage of HEX D is expected after ocular administration of the proposed formulation T1680.

In addition, according to the current SmPC of the reference product DESOMEDINE®, no systemic adverse effects have been reported following ocular instillation of HEX [Desomedine BE SmPC, 2020].

2.5.5.2.4. Safety in special groups and situations

Paediatric population

A topical regime including HEX D 0.1% or propamidine isethionate 0.1% and moxifloxacin hydrochloride 0.5% was found to be safe and effective in the treatment of microsporidial keratoconjunctivitis in a cluster of 25 paediatric and teenage individuals [Kwok et al, 2013]. In this study, all patients, with a mean age of 13.36 years (range: 5-16), had participated in a rugby match. The onset of symptoms occurred between 10 and 30 days post-exposure. All eyes had

multiple superficial coarse punctate keratitis. Four (12%) eyes presented with keratic precipitates. All patients were started on topical moxifloxacin hydrochloride 0.5% (daytime), topical propamidine isethionate 0.1% or HEX D 0.1% (daytime) and ofloxacin 0.3% ointment (nocte). The eye drops were instilled every 30 min, alternating with each other. This treatment regime was continued for 3 weeks and then gradually tapered according to individual patients' response. After treatment, all eyes healed without sequel. No adverse effect was reported.

A [redacted] patient diagnosed with AK related to soft contact lens wearing responded extremely well to the combination of HEX 0.1% and chlorhexidine digluconate 0.02% eye drops [Elabjer et al, 2009]. In this case report, after confirmation of *Acanthamoeba* spp. in the corneal sample, HEX 0.1% combined with chlorhexidine digluconate 0.02% eye drops were introduced with the following scheme: hourly for two days and nights, then hourly only days, then two-hourly days for three weeks and continued 4-6 times for ten months. Corneal healing was recorded after two months of continuous combined therapy and progressed until 10 months of treatment. The best corrected visual acuity was improved. No adverse effect was reported.

The administration of a therapy including topical HEX D 0.1% in a [redacted] child diagnosed with AK without any history of trauma or contact lens use was not associated with any adverse event [Demirci et al, 2006]. In this case report, after confirmation of *Acanthamoeba* spp. in the corneal sample, intensive therapy with topical HEX D 0.1%, chlorhexidine diacetate 0.02% and oral ketoconazole was initiated. At the end of the first month, topical prednisolone acetate 1% was added to reduce inflammation, chlorhexidine diacetate 0.02% and oral ketoconazole were discontinued, and HEX 0.1% was lowered to 4 x 1 and administered for an additional 4 months. At the end of the 5-month treatment, all medications were withdrawn.

An [redacted] patient diagnosed with Thygeson's superficial punctate keratitis was successfully treated with HEX 0.1% eye drops [Zonnevylle et al, 2019]. After failure of topical ciprofloxacin treatment and since the patient did not tolerate topical steroids, a new treatment with HEX 0.1% eye drops 3 times a day and artificial tears eye gel once a day was initiated in both eyes. This showed objective and subjective improvements in both eyes after ten days. The same treatment regimen was continued. Six weeks after initial presentation, only very discrete subepithelial lesions were still visible. HEX eye drops were tapered off with one drop a week after four months and artificial tear gel drops were continued. Clinical remission was achieved six months after initiating this treatment. No recurrent lesions were seen during a follow-up period of three years. No adverse effect was reported in this paediatric patient.

The available literature data showed that HEX 0.1% is safe in paediatric population. The posology for the paediatric population is the same as for adults.

Drug interaction

Given the possible drug interaction (antagonism, inactivation), in particular with anionic compounds, the simultaneous or successive use of other antiseptics is not recommended.

Pregnancy and lactation

Currently, there are no known contraindications to the use of hexamidine during pregnancy and breastfeeding.

T1680 can be used during pregnancy and breastfeeding.

2.5.5.3. Post-marketing surveillance data

Not applicable.

2.5.5.4. Overall conclusions on safety

No ocular or systemic adverse effects were reported in the literature following the ophthalmic use of HEX D solution. A full safety review of HEX with reference to skin application concluded that HEX and its salts are generally safe to use, apart from possible local reactions such as irritation or sensitization. The comparative clinical study showed a good tolerance and acceptability of the generic product HEXAMIDINE GILBERT® 0.1% eye drops (equivalent formulation as T1680), which was comparable to that of the reference product DESOMEDINE® (same formulation as T1680).

2.5.6. BENEFITS AND RISKS CONCLUSIONS

2.5.6.1. Clinical pharmacology

Hexamidine (HEX) is a strong organic base and has been used in medicine as an antiseptic for over half a century. It belongs to the aromatic diamidine group, essentially described as bacteriostatic on Gram-positive cocci. Diamidines are well known for their antimicrobial effects resulting from the cationic surface-active properties generated from the bipolar structure of the molecules.

The *in vitro* comparative study showed that the antimicrobial activity of the generic product HEXAMIDINE GILBERT® 0.1%, single-dose eye drops (equivalent formulation as T1680) is similar to that of the reference product DESOMEDINE® (same formulation as T1680). The best bacteriostatic and bactericidal activities were obtained against Gram-positive bacteria, and particularly against strains of *Staphylococci*. The MIC values were 0.47 mg/L and 0.95 mg/L for the most sensitive strains and 7.8 mg/L for the most resistant strains.

In a very recent study, HEX D ophthalmic solution showed rapid *in vitro* antimicrobial activity against *Staphylococcus epidermidis*, *Staphylococcus aureus* and *Candida* species, but was poorly active against *Pseudomonas aeruginosa*. These results confirm that HEX D remains an efficient antimicrobial agent for common eye infections.

HEX has also been found to be effective in completely killing 15 different isolates of *Acanthamoeba*.

Although the mechanisms of action of HEX are not fully understood, some processes involved in the antimicrobial activity of the diamidines have been elucidated. The diamidines act as cationic surface-active agents and cause membrane disruption, leakage of amino acids, and inhibited oxygen uptake.

No pharmacokinetics data after ocular administration of HEX in human has been found in the literature. However, in view of the poor absorption of HEX D after oral administration and dermal application observed in animals, no systemic passage of HEX D in the proposed formulation T1680 is expected after ocular administration. In addition, no systemic adverse effects have been reported following ocular instillation of HEX.

2.5.6.2. Efficacy

Hexamidine 0.1% is an antimicrobial agent effective in the treatment of ophthalmic infectious diseases caused by sensitive microorganisms. The *in vitro* evaluation of HEX D 0.1% on various pathogens is fully described.

Efficacy of HEX D 0.1% in the treatment of bacterial infections of the eye and its adnexa is supported by published data and results of a comparative clinical trial conducted on the generic product HEXAMIDINE GILBERT® 0.1%, single-dose eye drops (equivalent formulation as T1680) and the reference product DESOMEDINE® (same formulation as T1680).

Although the main objective of this clinical study was to compare the acceptability and tolerance of the reference and test products, results showed a statistically significant and clinically meaningful improvement in objective signs and subjective symptoms of bacterial conjunctivitis. These results were identical for both HEX-based products, *i.e.*, the reference product DESOMEDINE® and the generic product.

HEX D has received increased attention in recent years, because it is particularly effective in the treatment of *Acanthamoeba* keratitis where it is used as first line therapy in combination with biguanide agents. This antiacanthamoebal efficacy has been subjected to publications, but no well-controlled study has been reported in the literature.

HEX D has been found to be effective in the topical treatment of microsporidial keratoconjunctivitis in combination with a fluoroquinolone.

2.5.6.3. Safety

No ocular or systemic adverse effects were reported in the literature following the ophthalmic use of HEX D solution. A full safety review of HEX with reference to skin application concluded that HEX and its salts are generally safe to use. Allergies and hypersensitivity reactions can be observed as with all topically applied medicinal products.

The comparative clinical study showed a good tolerance and acceptability of HEXAMIDINE GILBERT® 0.1%, single-dose eye drops (equivalent formulation as T1680), which was comparable to that of the reference product DESOMEDINE® (same formulation as T1680).

2.5.6.4. Benefit/risk evaluation

HEX is a strong organic base and is an aromatic diamidine. It has been used for its biocidal actions in topical preparations since the 1950s. It is primarily used as the diisetionate salt. HEX D is a hydrosoluble cationic agent with antimicrobial activity against bacteria, fungi, yeasts and free-living amebae.

Laboratoires THEA developed a medicinal product T1680, a sterile preservative-free solution for ophthalmic use containing 1 mg/mL hexamidine diisetionate (0.1%). T1680 contains the same active substance and excipients in the same concentrations as the currently authorised product, DESOMEDINE® registered by Bausch & Lomb (reference product). The therapeutic indications and posology recommended for T1680 are the same as DESOMEDINE®.

T1680 is indicated:

- for the treatment of:
    - purulent bacterial conjunctivitis caused by susceptible microorganisms
    - keratoconjunctivitis
    - blepharitis
    - chronic tear duct infections
- as a preoperative antiseptic for the conjunctival sacs

The recommended dose is one drop into the conjunctival cul-de-sac of the affected eye(s) 4 to 6 times a day. The total duration of treatment should not exceed 8 days to avoid the emergence of resistant strains.

Staphylococcus aureus, *Staphylococcus epidermidis*, and *Pseudomonas aeruginosa* are common causes of eye infection. Actually, *Staphylococcus epidermidis* is the organism most commonly isolated from eyes with post-operative endophthalmitis. Furthermore, *Pseudomonas* is the most frequent etiologic agent of contact lens-associated microbial keratitis, being responsible for up to 2 of 3 cases. Fungi, including *Candida*, account for more than 50% of all culture-proven keratitis cases in tropical and subtropical regions and more than 50% of all cases of endogenous endophthalmitis.

Results of the comparative *in vitro* study showed that the antimicrobial activity of HEXAMIDINE GILBERT® 0.1%, single-dose eye drops (equivalent formulation as T1680) is

similar to that of the reference product DESOMEDINE® (same formulation as T1680). In a very recent study, HEX D solution showed rapid *in vitro* antimicrobial activity against *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Candida* species, but was poorly active against *Pseudomonas aeruginosa*. These results confirm that HEX D currently remains an efficient antimicrobial agent for common eye infections.

HEX has also been found to be effective in completely killing 15 different isolates of *Acanthamoeba* and it is currently used as a first-line treatment for *Acanthamoeba* keratitis in combination with chlorhexidine.

The exact mechanism of action of diamidines (including HEX) is unknown, but they have been shown to inhibit oxygen uptake and induce leakage of amino acids, as would be expected with cationic surface-active agents.

No pharmacokinetics data after ocular administration of HEX in human has been found in the literature. However, in view of the poor absorption of HEX D after oral administration and dermal application observed in animals, no systemic passage of HEX D in the proposed formulation T1680 is expected after ocular administration. In addition, no systemic effects have been reported following ocular instillation of HEX.

The efficacy of HEX D 0.1% in the treatment of bacterial infections of the eye and its adnexa is supported by published data and results of a comparative clinical trial with the generic product HEXAMIDINE GILBERT® 0.1%, single-dose eye drops (equivalent formulation as T1680) and the reference product DESOMEDINE® (same formulation as T1680).

Despite the lack of well-designed clinical trials, it can be stated that HEX D eye drops are efficient in the treatment of ocular diseases caused by susceptible microorganisms. The anti-microbial effect of HEX D ophthalmic solution (0.1% or 0.05%) has been proven in a number of *in vitro* studies.

The satisfactory safety profile of T1680 ophthalmic solution is supported by relevant data available in the literature on the safety of HEX D. No ocular or systemic adverse events were reported following the ophthalmic use of HEX D solution. A good tolerance and acceptability, comparable to that of the reference product DESOMEDINE® (same formulation as T1680), have been demonstrated for HEXAMIDINE GILBERT® 0.1%, single-dose eye drops in a comparative clinical study.

Review of these data supports the view that HEX has a positive benefit/risk ratio, with a high therapeutic index, for patients presenting with ocular conditions requiring a topical anti-microbial treatment sensitive to HEX and it is considered acceptable that Laboratoires THEA has not conducted any new clinical trial in the claimed therapeutic indications.

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MODULE 2 – COMMON TECHNICAL DOCUMENT SUMMARIES

2.5 Clinical overview (T1680, 1 mg/ml, Eye drops, solution)

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