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PHARMACOLOGY AND PHARMACODYNAMICS OF ANTIFUNGAL AGENTS (N BEYDA, SECTION EDITOR)

Updates in Ocular Antifungal Pharmacotherapy: Formulation and Clinical Perspectives

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Abstract

Purpose of Review In this review, a compilation on the current antifungal pharmacotherapy is discussed, with emphases on the updates in the formulation and clinical approaches of the routinely used antifungal drugs in ocular therapy.

Recent Findings Natamycin (Natacyn® eye drops) remains the only approved medication in the management of ocular fungal infections. This monotherapy shows therapeutic outcomes in superficial ocular fungal infections, but in case of deep-seated mycoses or endophthalmitis, successful therapeutic outcomes are infrequent, as a result of which alternative therapies are sought. In such cases, amphotericin B, azoles, and echinocandins are used off-label, either in combination with natamycin or with each other (frequently) or as standalone monotherapies, and have provided effective therapeutic outcomes.

Summary In recent times, amphotericin B, azoles, and echinocandins have come to occupy an important niche in ocular antifungal pharmacotherapy, along with natamycin (still the preferred choice in most clinical cases), in the management of ocular fungal infections.

Keywords Ocular fungal infections · Antifungal drugs · Off-label use · Antifungal pharmacotherapy · Formulation approaches · Clinical evaluations

Introduction

Ocular keratitis infections affect about 1 million Americans annually in the USA with about 30,000 new cases reported each year [1, 2••, 3••]. Out of these, nearly half require emergency ambulatory care, and more than two thirds require the prescription of an antifungal and/or antibacterial agent [1, 4]. Ocular keratitis coupled with other corneal infections account for an expenditure approximating $175 million in direct healthcare expenditures in the USA [1].

Amongst all the ocular fungal infections, fungal keratitis, endophthalmitis, conjunctivitis, and blepharitis are some of the major clinical concerns, and if left clinically untreated could lead to blindness and vision loss [3••, 4, 5••]. These ocular infections can occur due to numerous factors such as eye surgeries, ocular trauma, excessive use and/or contamination of ocular products, immunocompromised morbidities, and systemic fungal infections and are usually found localized in ocular tissues such as cornea, aqueous and/or vitreous humor, sclera, and the other ocular coats [5, 6•, 7].

These fungal infections have been predominantly found to be caused by filamentous fungal species such as *Aspergillus* and *Fusarium* and non-filamentous fungal species such as *Candida* [8]. In the pharmacotherapy of the ocular fungal infections caused by these organisms, antifungal drugs belonging to polyene, azole, and echinocandin classes are being routinely used clinically [3••, 5••]. Amongst these, natamycin (a polyene antifungal drug) is the only US FDA-approved drug for ocular fungal keratitis. Despite natamycin being the only approved drug for ocular infections, the other polyene (amphotericin B), azole, and echinocandin antifungals have been routinely used clinically in view of their potent and broad-spectrum antifungal activity, fewer cases of resistance and cross-resistance (amphotericin B and echinocandins), use

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of lipid-based polyene formulations (amphotericin B) that exhibit greater safety and lower toxicity profiles, and a favorable safety/toxicity profiles associated with the echinocandin antifungals [9–13].

To understand the locus of these antifungal drugs in the current ocular antifungal pharmacotherapy, this review focuses on two important aspects; first being the current antifungal regimen that is clinically practiced in ocular fungal infections and second being the updates in the domain of ocular antifungal pharmacotherapy with respect to formulation development and clinical evaluations for polyene, azole, and echinocandin antifungals in ocular antifungal pharmacotherapy.

Recent Updates in Current Antifungal Pharmacotherapy

The transition of preclinical antifungal candidates to antifungal drugs used in clinical ocular antifungal pharmacotherapy has been slower owing to numerous factors such as anatomical and physiological limitations presented at the ocular milieu, unfavorable physicochemical properties exhibited by the antifungal candidates, manifestation of unwarranted toxicity owing to the non-selectivity of antifungal candidates–host (human) versus causative organism (fungal species), elicitation of systemic and ocular toxicities, emergence of resistance and cross-resistance, lack of robust in vitro–in vivo correlation of the activities associated with the antifungal drugs [3••, 14]. Despite these challenges, polyene, azole, and echinocandin antifungals still remain the mainstay clinical drugs in the management of ocular fungal infections (Fig. 1).

Natamycin

Natamycin is a tetraene polyene antifungal that has remained the only approved antifungal drug for ocular fungal infections. Natamycin elicits its fungicidal activity by binding to ergosterol which is an essential structural component of the fungal cell membrane, thereby causing a loss in the fungal cell integrity [15]. Natamycin has found favor as the first-line therapeutic agent in ocular fungal infections owing to its selective and potent activity against the filamentous fungal species—*Aspergillus* and *Fusarium*, which are the major ocular infection causing fungal organisms in the USA [16•]. The marketed formulation of natamycin is an ophthalmic suspension that is administered topically as eye drops [8]. Due to this formulation and route of administration, natamycin eye drops have a low retention and bioavailability at the ocular surface, necessitating frequent administration for an effective therapy [4]. Also, the topical administration mode provides an effective therapeutic outcome in case of superficial fungal infections such as fungal keratitis but not in cases of deep-seated ocular keratomycosis [5••]. Due to these reasons, concomitant administration of other antifungal drugs, either systemic or subconjunctival, is needed which increases the cost of therapy and risks of side effects and does not lead to effective therapeutic concentrations in the deeper ocular tissues [17]. Despite these challenges, natamycin remains one of the mainstay antifungal drugs clinically, due to its favorable safety/toxicity profile and fewer side effects. Therefore, in recent times, to further improve upon the current natamycin therapy and utilize its aforementioned clinical advantages, various formulation strategies and clinical evaluations have been investigated to improve and advance the current therapy associated with natamycin which are discussed below.

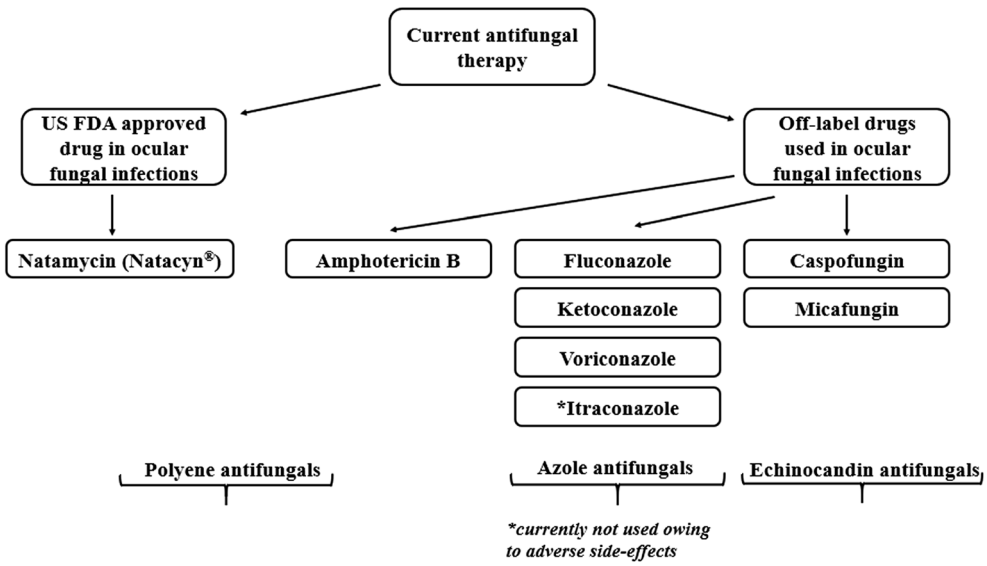

Fig. 1 US FDA-approved drugs and off-label drugs used in ocular antifungal infections

i. *Updates in formulation approaches*: Formulation approaches have been one of the most common routes undertaken to improve the delivery of antifungals to the ocular tissues with the potential aim of improving the therapy associated with the drug. A few studies in recent times have sought to improve the ocular delivery of natamycin by evaluating various formulation strategies. In a comparative study reported by Patil et al., natamycin-loaded PEGylated nanolipid carriers (NT-PEG-NLC) (0.3%) were formulated and compared with the marketed formulation Natacyn® (5%). NT-PEG-NLCs exhibited significantly higher transcorneal natamycin (NT) permeability and flux in vitro and provided concentrations statistically similar to the marketed suspension in the inner ocular tissues such as iris–ciliary bodies and aqueous and vitreous humors, in vivo, indicating its utility as a potential alternative to the ophthalmic suspension during the course of fungal keratitis therapy [4]. In another study reported by Janga et al., ion-sensitive in situ gels of natamycin bilosomes were fabricated with an intent to enhance natamycin ocular delivery. The bilosomes entrapped in in situ gels showed efficient ocular penetration with a 6- to 9-fold increase in the transcorneal flux of natamycin with superior permeability characteristics over the marketed suspension Natacyn®. The study also reported that dose-normalized natamycin concentrations obtained from the formulations were significantly higher in the ocular tissues, indicating the potential feasibility of these careers in ocular fungal infections [18]. Paradkar and Parmar also reported the formulation of natamycin-loaded niosomal in situ gel which improved corneal retention time and transcorneal permeation and extended drug release up to 24 h in comparison to Natacyn® [19]. Zhang et al. reported the development of NT-hydroxypropyl-beta-cyclodextrin (NT-CD) inclusion complex in situ gels (NT-CD-IG) (0.2%) to prolong ocular retention time and enhance corneal permeability. The ocular concentrations of NT-CD-IG in tears, cornea, and aqueous humor were significantly higher than those for the controls–marketed formulation and NT-CD solution [20].

ii. *Updates in clinical evaluations*: Majority of the recent clinical evaluations for natamycin have focused on evaluating the potency of natamycin in cases of superficial ocular fungal infections such as keratitis. In a clinical evaluation reported by Lalitha et al., it was found that natamycin was effective against 100 clinical isolates of *Fusarium* and *Aspergillus* species recovered from keratitis-infected eyes, and natamycin was more effective than other antifungals such as amphotericin B, caspofungin (acetate), itraconazole, voriconazole, posaconazole, fluconazole, and terbinafine on *Fusarium* and *Aspergillus* fungal species recovered from corneal keratitis eyes [21–23]. Also, the activity of natamycin was observed against *Aspergillus*, *Fusarium*, and *Alternaria* species isolated from keratomycosis infected eyes [24]. Additionally, the activity of natamycin was found to be more potent against *Fusarium* species (lower minimum inhibitory concentration (MIC)) in comparison to *Aspergillus* species (higher MIC) which were isolated from eyes infected with keratitis [25]. In a comparative clinical evaluation by Prajna et al., it was observed that the therapeutic outcomes associated with voriconazole and natamycin monotherapies were similar; however, it was found that natamycin therapy was safer than the voriconazole therapy [26]. In yet another comparative study that compared the effectiveness of natamycin in superficial and deep-seated mycosis, it was found that therapeutic outcomes with the former were higher than with the latter, indicating the favored utility of natamycin in superficial ocular fungal infections such as keratitis [27].

Amphotericin B

Amphotericin B is a broad-spectrum antifungal agent that was initially utilized in the treatment of systemic fungal infections and was later used to treat localized fungal infections owing to its ability to control disseminated fungal infections [16•]. Amphotericin B has shown to be effective against *Candida*, *Aspergillus*, *Fusarium*, *Penicillium*, and *Cryptococcus* spp. [16•, 28–30]. Therefore, amphotericin B has been routinely used off-label in the treatment of ocular fungal infections. In case of fungal keratitis, topical application of amphotericin B is effective only when the cornea has been abraded since amphotericin B is not found to penetrate the intact cornea owing to its high molecular weight. This implies the ineffectiveness of the drug during the healing stages of corneal fungal infections when the cornea beings to restore its integrity [31]. Therefore, intravenous (IV) administrations of the drug were sought, that required the administration of a higher dose of amphotericin B (to reach the ocular sites post systemic administration) which led to the manifestation of systemic toxicity such as nephrotoxicity [32, 33]. Despite these challenges associated with ocular amphotericin B delivery, various formulation and clinical strategies were sought for the delivery of amphotericin in cases of ocular fungal infections.

i. *Updates in formulation approaches*: Various formulation approaches also have been undertaken for improving amphotericin B delivery to the eye. In a study reported by Fu et al., amphotericin B-loaded chitosan-modified nanostructured lipid carriers (AmB-CH-NLC) were developed for fungal keratitis [34]. The in vivo ocular

pharmacokinetic studies indicated improved corneal bioavailability of amphotericin B from AmB-CH-NLC in comparison to amphotericin B eye drops (higher area under curve (AUC) and maximum concentration (C_{max})) without any irritation to the rabbit eyes. Another comparative study reported by Chhonker et al. compared their developed amphotericin B-loaded lecithin/chitosan nanoparticles (0.15%) with the marketed formulation Fungizone® (0.15%) [35]. The nanoparticles exhibited ~ 2.04-fold improved bioavailability and ~ 3.36-fold improved precorneal residence time in New Zealand albino rabbit eyes compared to the marketed formulation, indicating their enhanced preclinical effectiveness owing to the mucoadhesive properties of lecithin/chitosan combination. Positively charged nanosuspension and nanoparticles of amphotericin B, formulated using Eudragit RS 100 and RL 100, exhibited activity against *Fusarium solani* and showed no signs of ocular irritation upon in vivo application [36, 37]. A study by da Silveira et al. reported the formulation of amphotericin B microemulsion systems for improved ocular permeation [38]. The in vitro susceptibility testing of amphotericin B microemulsion showed high activity against *Candida* strains and lesser toxicity against red blood cells when compared to Fungizone® indicating its feasibility as a potential eye drop system in *Candida* ocular infections. In another study reported by Zhou et al., amphotericin B-loaded self-aggregating nanoparticles (AmB/PLA-g-CS) (0.15%) using amphiphilic poly-(lactic acid)-grafted-chitosan (PLA-g-CS) copolymer were fabricated for ocular delivery [39]. The nanoparticles showed antifungal activity not significantly different to that of free amphotericin B against *Candida albicans*. The nanoparticles did not cause any ocular irritation after instillation into rabbit eyes and could permeate into the cornea where they prolonged the amphotericin B residence time at the corneal surface.

ii. *Updates in clinical evaluations*: Amphotericin B has been clinically evaluated in determining its effectiveness in the management of ocular fungal infections, particularly keratomycosis and endophthalmitis. Intracameral administration (0.15%) of amphotericin B was found to be more effective in the treatment of ocular keratomycosis than its topical application (0.15%) by bringing about faster healing and quicker disappearance of the fungal hypopyon [40]. The intracameral effectiveness of amphotericin B was also corroborated by a clinical evaluation by Yilmaz et al., in which intracameral amphotericin B proved effective in treating refractory keratomycosis and endophthalmitis that was unresponsive to the conventional fluconazole therapy [41]. Intracameral injections of amphotericin B in the treatment of ocular fungal infections owing to *Aspergillus niger* (along with oral itraconazole), *Colletotrichum graminicola*, and *Aspergillus flavus* were found to be safe and effective in all the clinical evaluations [42–44]. A combination of intracameral and intrastromal injection of amphotericin B was found to be effective in the treatment of severe fungal keratitis that was unresponsive to the conventional therapy involving fluconazole, natamycin, and itraconazole [45]. Similarly, in another clinical evaluation, it was observed that intrastromal injection along with intravitreal injection of amphotericin B was effective in the management of recurrent fungal keratitis and endophthalmitis post penetrating keratoplasty procedure [46]. These clinical evaluations indicate that the use of amphotericin B, particularly via the intracameral injections, could be a potential route in the effective management of severe keratitis, endophthalmitis, and recurrent fungal infections.

Fluconazole

Fluconazole is a drug belonging to the azole group of antifungals that has found off-label clinical application in ocular fungal infections owing its ability to permeate the ocular tissues [47]. Fluconazole has shown ocular permeation to reach therapeutic levels upon topical, oral, and systemic administration thereby finding utility in various ocular fungal infections affecting the front and back of the eye, that is, in cases of superficial and deep-seated mycosis, respectively [48–51]. Additionally, this drug also exhibits a favorable safety/toxicity profile, high bioavailability, and potent antifungal activity against *Candida* and *Aspergillus* spp. [5••, 52]. Hence, fluconazole, like amphotericin B, has also found favor as an off-label agent in ocular antifungal pharmacotherapy. However, one of the major challenges associated with the fluconazole therapy is the development of resistance and cross-resistance upon prolonged exposure or an incomplete therapeutic regimen, as a result of which it is frequently utilized along with other antifungals for a quicker therapeutic outcome [53, 54].

i. *Updates in formulation approaches*: The formulation approaches investigated for the ocular delivery of fluconazole have been discussed below. Moustafa et al. reported the development of hyalugel-integrated liposomes of fluconazole (0.9%) that sustained the fluconazole release up to 24 h, in vitro. Ex vivo corneal permeation studies indicated that the liposomes increased the corneal uptake and permeation of fluconazole by 4.2-fold and 2.6-fold in comparison to fluconazole suspension (0.9%) [55]. In a study reported by Silva et al., fluconazole-loaded poly-lactide-co-glycolide implants (25% *w*/*w*) were developed for fungal endophthalmitis and compared to fluconazole

suspension (0.18% *w/v*). It was found that implants released fluconazole for a prolonged period (detected until 6 weeks) compared to the fluconazole suspension (detected only until 120 min post intravitreal administration). The implants showed in vitro biocompatibility and ocular tolerance, thereby providing a considerable alternative system to locally treat the fungal endophthalmitis induced by *Candida* species [56]. In another study reported by Fetih et al., fluconazole-encapsulated niosomal vesicles were incorporated into poloxamer gelling and chitosan gelling systems with an intent for ocular drug delivery. The gelling systems containing niosomes exhibited higher flux and permeation than the control (only drug in gel). Both the gel formulations showed antifungal activity against *C. albicans*, *Chrysosporium tropicum*, and *Penicillium chrysogenum* [57]. Despite these promising formulation strategies, there is a need for intensive preclinical investigations of fluconazole formulations before transitioning them into clinical evaluations.

ii. *Updates in clinical evaluations*: Fluconazole alone or in combination has been routinely used in the clinical setup as an off-label antifungal agent in ocular infections. Subconjunctival injection of fluconazole (2%) was effective in the treatment of severe fungal keratitis and fungal ulcers characterized by dense fungal hypopyons than its topical or IV administration [49]. Another clinical study reported by El-Sayed et al. revealed that subconjunctival fluconazole (0.2%) was more effective in treating fungal keratitis and ulcers than topically administered amphotericin B (0.05%) [58]. However, in another clinical evaluation, subconjunctival administration of fluconazole (0.2%) along with amphotericin B topical application was found to be both effective and safe in the management of fungal keratitis caused by *Candida* and filamentous fungal species indicating the superiority of the combination therapy [59]. Fluconazole (0.2%), administered intrastromally, was also reported to be effective in severe fungal keratitis in conjunction with lamellar keratectomy [60]. From the above updates it is evident that fluconazole has been primarily utilized in the clinical management of severe keratitis and ulcers via the subconjunctival route.

Ketoconazole

Ketoconazole is an imidazole antifungal that has been used as an adjuvant in most ocular fungal infections [5••]. It has been used primarily as an adjuvant because of its activity against only a few filamentous and yeast-like fungal species and because of its major side-effect of photochemical toxicity [61, 62]. However, ketoconazole finds clinical application in deep-seated mycosis, since it shows high concentrations in the anterior ocular chamber upon oral administration [63]. Despite this, the oral ketoconazole therapy for a prolonged duration is manifested by the occurrence of side effects such as decreased libido, oligospermia, menstrual changes, and hepatoxicity [16•]. Owing to these reasons, there has been limited utility of ketoconazole in ocular fungal infections and the utility has been mostly as an adjuvant in concomitant antifungal therapy.

i. *Updates in formulation approaches*: A few formulation approaches have been evaluated for the potential ocular delivery of ketoconazole in ocular fungal infections. Ketoconazole-loaded proniosomal gels for ocular keratitis were reported by Abdelbary et al. Following topical application, it was observed that the C_{max} and concentration of the drug in aqueous humor was significantly higher from the gel compared to the control (drug suspension) indicating an improvement in the bioavailability [64]. Ketoconazole poly(lactide-co-glycolide) nanoparticles loaded into an alginate-chitosan in situ gelling system were formulated for ophthalmic drug delivery. It was found that the gelling system provided a significantly higher permeation across epithelial cell lines and a significantly higher antifungal activity in comparison to the controls (pure drug solutions) indicating the feasibility of in situ gel in the ocular delivery of ketoconazole [65]. In a study reported by Kakkar et al., ketoconazole-loaded polyethylene glycol-based solid lipid nanoparticles were developed for ocular drug delivery. Pharmacokinetic studies indicated superior C_{max} and AUC for the drug from the nanoparticles than from the suspension (control) in aqueous and vitreous humors indicating their potential feasibility in delivering the drug at the ocular sites during antifungal regimen [66].

ii. *Updates in clinical evaluations*: Ketoconazole has been evaluated orally and topically in the management of ocular mycoses despite there being no specifically marketed dosage forms of ketoconazole indicated for ophthalmic fungal infections [6•. Topically administered ketoconazole eye drops exhibited effectiveness in treating *Aspergillus* and *Fusarium* corneal infections, whereas in another study, the utility of oral ketoconazole along with natamycin did not significantly affect the therapeutic outcomes [67, 68]. In certain cases of ocular infections due to *Phaeohyphomycetes* species, oral and/or topical application of ketoconazole have shown favorable therapeutic outcomes [6•]. However, in recent times, other azole antifungals such as fluconazole and/or voriconazole and the echinocandin antifungals have been the common standalone and/or adjuvants (along with natamycin and amphotericin B) in the management of ocular fungal infections.

Voriconazole

Voriconazole is a triazole antifungal that has been widely used off-label in the management of clinical ocular fungal infections owing to its potent activity against *Candida*, *Aspergillus*, *Fusarium*, and other filamentous fungal species [69–71]. Voriconazole has been commonly administered orally for treating ocular fungal infections owing to its high bioavailability and permeability across the ocular tissues, thereby improving patient compliance [72]. Furthermore, reports on emergence of resistance and cross-resistance have been scanty, and oral/systemic routes have exhibited infrequent and inconsistent occurrences of side effects such as skin rashes, visual flashes, and hepatotoxicity [73]. Owing to these advantages and relatively fewer challenges, intensive investigations into ocular delivery of voriconazole have resulted and are elaborated below.

i. *Updates in formulation approaches*: Multiple formulation approaches have been undertaken for the delivery of voriconazole in ocular fungal infections. In a study reported by Kumar et al., voriconazole-loaded microemulsion for ocular delivery was fabricated. The microemulsion exhibited higher permeation, flux, and amount of voriconazole deposited at the corneal surface in comparison to the drug suspension, ex vivo, indicating its feasibility in the improved delivery of the drug at the ocular surface [74]. Andrade et al. developed voriconazole-loaded nanostructured lipid carriers and compared them to the marketed voriconazole formulation (Vfend®). Ex vivo ocular experiments indicated that the nanolipid carriers were able to deliver therapeutically relevant drug amounts to the cornea after only 30 min even though the nanolipid carriers contained approximately 40% of Vfend's voriconazole dose. The formulation was reported to be well tolerated and non-irritant, thereby making it a good candidate for prospective preclinical evaluations [75]. Additionally, solid lipid nanoparticles of voriconazole have been fabricated as delivery vehicles to the ocular surface that exhibited drug release and corneal permeation of voriconazole without affecting the corneal hydration and demonstrated a 2-year shelf life; however, in better evaluating their feasibility as ocular delivery platform, it is essential to study in vivo profiles of the drug-loaded delivery forms [76]. In yet another study, voriconazole-loaded solid lipid nanoparticles were fabricated into in situ gelling system, which demonstrated antifungal activity against *Candida albicans*, and did not exhibit damage to cornea, iris, and conjunctiva and a shelf life of at least 3 months, thereby making it a promising candidate for further preclinical evaluations [77].
ii. *Updates in clinical evaluations*: Voriconazole has been one of the most frequently clinically used off-label antifungal drugs. Topical and oral administration of voriconazole has shown effectiveness in healing the cases of fungal keratitis caused by *Candida albicans*, *Alternaria* spp., *Fusarium* spp., and *Scopulariopsis* spp. along with concomitant administration of either natamycin or amphotericin B eye drops or a combination of both with voriconazole [78]. Similarly, topical and oral voriconazole empirical therapy has also shown efficacy, with slight discomfort in treating endophthalmitis and corneal ulcer caused due to *Candida parapsilosis* and fungal keratitis refractory to conventional antifungal therapy involving amphotericin B [79]. In yet another clinical evaluation, voriconazole monotherapy (systemic and/or topical) was effective in the complete management of at least 50% of the fungal keratitis cases caused by *Fusarium* and *Candida* species [80]. Topical and systemic administration of voriconazole was effective in treating *Aspergillus fumigatus* keratitis that was unresponsive to the standard natamycin eye drop therapy and subsequently to topical amphotericin B and systemic ketoconazole concomitant therapy [81]. In a comparative clinical study between topically applied natamycin (5%) and voriconazole (1%), it was found that voriconazole was as effective as natamycin in treating ocular keratitis caused by *Aspergillus* and *Curvularia* spp. However, in case of *Fusarium* keratitis, it was found that natamycin was more effective in treatment outcomes than voriconazole natamycin in a prospective, double-blinded, and randomized clinical trial comprising 118 patients [82, 83•]. In a clinical study reported by Prajna et al., it was found that administration of oral voriconazole along with topical voriconazole (1%) or topical natamycin (5%) did not benefit the therapeutic outcomes in severe filamentous mycotic ulcers [84]. Thus, it is evident that voriconazole can provide positive therapeutic outcomes associated with fungal keratitis, endophthalmitis, and mycotic ulcers, thereby being an alternative to the conventional natamycin antifungal therapy.

Itraconazole

Itraconazole is also a triazole antifungal that has been used in some countries in the form of a topical eye drop (Itral®) in the treatment of ocular fungal infections. However, the ocular usage of this drug has been reported to be associated with various side effects such as dry mouth, convulsions, dizziness, nausea, vomiting, irregular heartbeat, and abdominal pain. In the USA, itraconazole has not been approved for ocular use owing to scanty and clinically inconsistent data over its ocular effectiveness [5••]. Also, itraconazole has been reported to be negligibly active against *Fusarium* spp.; its use has been associated with the development of resistance and cross-

resistance and occurrence of the aforementioned side effects, thereby making its utility only as an adjuvant in some rare cases in ocular antifungal pharmacotherapy [85–89].

i. *Updates in formulation approaches*: The reports on the formulations of itraconazole for ocular drug delivery in fungal infections remain scanty and only a couple of studies have reported the development of itraconazole formulations specifically for ocular delivery. A study by Ahuja et al. reported the formulation of chitosan-based nanosuspension of itraconazole. The chitosan-nanosuspension (1%) exhibited significantly higher (1.73-fold) corneal permeability of itraconazole than the commercial nanosuspension (1%) across goat cornea, ex vivo. The nanosuspension also showed a 12-fold increase in aqueous saturation solubility of itraconazole thereby providing a promising approach for ophthalmic delivery of itraconazole with enhanced solubility [90]. In another study by Mohanty et al., development of itraconazole-loaded solid lipid nanoparticles for ocular fungal infections was reported. The nanoparticles exhibited permeation across excised goat cornea and antifungal activity against *Aspergillus flavus* [91].
ii. *Updates in clinical evaluations*: Even though itraconazole exhibits a broad spectrum of activity, the clinical application of itraconazole has been limited due to the adverse side effects associated with it. It has been reported that topical and systemic administration of itraconazole failed to provide a therapeutic outcome in case of severe fungal keratitis caused by *Trichophyton* species [92]. In another clinical evaluation, it was reported that itraconazole alone, and in combination with fluconazole, was effective in treating and preventing the spread of deeper fungal infections before, during, and after performing keratoplasty procedure in patients suffering from severe fungal keratitis [93]. Itraconazole co-administered systemically and topically helped in the successful management (over 77% success rate) of corneal ulcers caused by *Aspergillus*, *Penicillium*, and *Fusarium* species [88]. Apart from these, reports on the use of itraconazole in ocular mycoses have been rare and coupled with side effects, and better and consistent therapeutic outcomes have been evidenced with the other antifungals such as natamycin and off-label use of amphotericin B, fluconazole, voriconazole, and the echinocandins, thereby not making itraconazole a favored off-label drug in ocular antifungal therapy [6•].

Caspofungin

Caspofungin, a high molecular weight lipopeptide antifungal drug, was first to be approved from the echinocandin class in the management of systemic invasive fungal infections and was later evaluated for its utility in ocular fungal infections. Caspofungin acts by inhibiting β-(1,3)-D-glucan synthesis which plays an essential role in fungal cell integrity [94]. Caspofungin has shown to elicit a potent antifungal activity against *Candida* and *Aspergillus* spp. but only a weak activity against *Fusarium* species, due to which it has found clinical off-label application in ocular fungal infections caused by *Candida* and *Aspergillus* spp. In preclinical evaluations, caspofungin administered topically was found to be as efficacious as amphotericin B in *Candida* keratitis, and intravitreal administration of caspofungin was found to be effective, safe, and non-toxic in the treatment of deep-seated ocular fungal infections [95–99]. Although caspofungin has shown favorable preclinical outcomes with respect to the efficacy and safety, it has been only majorly used as an adjuvant or sometimes individually, owing to the higher cost of the therapy associated with it.

i. *Updates in formulation approaches*: Formulation approaches investigated for the ocular delivery of caspofungin have been scarce and the IV formulation of caspofungin (Cancidas®) has been used in ocular mycoses, given either systemically or topically (such as eye drops) or in some cases intravitreally (deep-seated mycoses) [100]. In case of ocular fungal infections, caspofungin is known to penetrate the ocular tissues through the topical and systemic routes when the anatomical ocular barriers are disrupted; however, it exhibits poor permeation in uninfected/uninflamed eyes [101]. Since, extemporaneously prepared solution of caspofungin (of desired dosage strength) has shown favorable therapeutic outcomes (monotherapy or concomitant therapy), there has been a lack of development of ocular delivery forms for caspofungin [5••].
ii. *Updates in clinical evaluations*: In a clinical study reported by Spriet et al., caspofungin (IV), along with voriconazole (IV) and posaconazole (IV) as adjuvants, was successful in treating fungal endophthalmitis caused by *Oxysporum* species. In the study, it was also observed that therapeutic concentrations of caspofungin were obtained in the aqueous humor owing to the disruption of the blood-retinal barrier due to the infection; this indicates that caspofungin could be a drug of choice in treating ocular mycoses [102]. Also, caspofungin has shown to be effective in treating postoperative endophthalmitis caused by *Aspergillus* and *Scopulariopsis* species, when used concomitantly with amphotericin B and voriconazole, respectively [100, 103]. Additionally, topical application of caspofungin solution (1%) was effective and safe in treating *Candida* keratitis that was refractory to voriconazole and in treating recurrent *Candida* keratitis infections [104, 105]. Topical caspofungin, along with

intrastromal voriconazole and an independent caspofungin intrastromal therapy, has shown effectiveness in treating *Alternaria* keratitis that had shown poor clinical outcomes with a prior natamycin therapy [106, 107]. These clinical studies indicate that caspofungin has been commonly used off-label as an independent alternative and/or concomitantly with other azole antifungals (primarily voriconazole) in the management of refractory and/or recurrent ocular fungal infections.

Micafungin

Micafungin, like caspofungin, belongs to the class of echinocandin antifungals and exhibits activity against *Candida* and *Aspergillus* spp. but only a weak activity against *Fusarium* species [108]. Micafungin was also evaluated preclinically (in rabbit models) as an alternative in ocular antifungal pharmacotherapy wherein topical micafungin administration exhibited similar safety and efficacy to topically administered natamycin in *Aspergillus* keratitis [109, 110]. Intravitreal injection of micafungin was also effective in treating *Aspergillus* keratitis with its concentration localized in the vitreous for a prolonged period [111], intravitreal injection of micafungin was also as effective as voriconazole and amphotericin B in treating *A. fumigatus* infections [112], and the IV administration of micafungin led to MIC concentrations in the posterior ocular chamber [113]. Owing to these preclinical successes, micafungin has been evaluated clinically to determine its locus in ocular antifungal pharmacotherapy.

i. *Updates in formulation approaches*: Like caspofungin, micafungin (Mycamine®) has also been administered either systemically or topically (extemporaneously prepared from the IV formulation). This approach has shown favorable response in the management of keratitis and endophthalmitis, thereby decreasing the pressing need for further investigation and exploration into formulation strategies for micafungin ocular delivery in antifungal therapeutic regimen.
ii. *Updates in clinical evaluations*: Clinically, micafungin concomitant therapy with voriconazole has shown safety and efficacy in treating fungal keratitis caused by *Beauveria bassiana* when monotherapies with natamycin and voriconazole failed, postoperative endophthalmitis caused by *Aspergillus* species, keratitis caused by *Wickerhamomyces anomalus*, and endophthalmitis caused by *Trichosporon* species [114–117]. Also, concomitant micafungin therapy with fluconazole was found to be more effective than fluconazole monotherapy in treating *Candida* endophthalmitis [118]. Topical micafungin, at half the dosage strength than fluconazole, exhibited similarity in potency, efficacy, and safety to fluconazole in the management of *Candida* keratitis [119]. Topical and IV administration of micafungin was found to be effective and safe in the management of refractory *Pestalotiopsis clavispora* keratitis upon the failure of voriconazole and natamycin (pimaricin)-associated therapy [120]. From the above clinical investigations, it is evident that micafungin administered concomitantly with azoles (fluconazole and voriconazole; latter preferred) shows a better therapeutic outcome owing to a synergistic activity provided by the two drugs, thereby making their off-label combination an alternative in the ocular keratitis and endophthalmitis.

Table 1 summarizes the aforementioned discussion on the formulation and clinical updates and investigations of the abovementioned antifungal drugs.

Future Directions

The development of antifungals agents has been nearly static, with the introduction of only three classes of antifungal drugs in the past seven decades for some of the most severe fungal diseases. Due to the continued use of most of these antifungal drugs, there have been reports of failure of clinical therapies owing to the emergence of resistant fungal strains, thereby making the therapy a daunting challenge. Therefore, there is an undeniable clinical need to not only identify new antifungal drug classes but also to optimize and improve the current therapies against the ocular fungal infections. Despite this clear need, there has been only one FDA-approved medication (Natacyn®) for the management of ocular fungal infections. This formulation also suffers from its own challenges of poor ocular retention and permeation, since it is being administered topically. Therefore, one of the strategies that needs to be intensely investigated in current antifungal therapy is the development of newer carriers, such as nanoparticles, liposomes, surface-modified nanoparticles, and nano- and microemulsions, for improved ocular delivery of the currently available antifungal drugs. Moreover, although several combination therapies are already being clinically adopted, there needs to be further investigation into these approaches to identify more favorable combinations with respect to higher success rates, cost-effectiveness, and decreased side effects/improved safety profile.

Summary

Despite natamycin being the only antifungal approved in the management of ocular fungal infections, antifungals belonging to polyene, azole, and echinocandin classes have also come to occupy an important niche in the current antifungal

Table 1 Summary on the recent updates in formulation approaches and clinical evaluations of the antifungal drugs

Antifungal drugs	Updates		References
	Formulation approaches	Clinical evaluations	
Natamycin	a. PEGylated nanostructured lipid carriers: PEGylated formulation (0.3%) exhibited significantly higher transcorneal permeability and flux of NT, in vitro; statistically similar concentrations to the marketed suspension (5%) in inner ocular tissues at 1/16th dose. b. Niosomal in situ gelling system: in situ gel (0.3%) improved corneal retention time, transcorneal permeation, and extended drug release up to 24 h in comparison to Natacyn® (5%). c. Bilosome-loaded in situ gelling system: formulation (0.1%) showed significantly higher ocular penetration, transcorneal flux, and dose-normalized concentrations in ocular tissues over the marketed suspension Natacyn® (5%). d. Cyclodextrin inclusion complex in situ gels: gels (0.2%) prolong ocular retention time and enhance corneal permeability; ocular concentrations of gels in tears, cornea and aqueous humor were significantly higher than the control suspension (5%).	a. Monotherapy: i. Natamycin (5%) was effective against clinical isolates of *Fusarium*, *Aspergillus*, and *Alternaria* species recovered from infected eyes. ii. Therapeutic outcomes with voriconazole (1% and 200 mg bid) and natamycin (5%) monotherapies were similar; however, natamycin therapy was safer than the voriconazole therapy.	[4, 18–27]
Amphotericin B	a. Chitosan-modified nanostructured lipid carriers: improved bioavailability of comparison to amphotericin B eye drops (higher AUC and C_{max}); no irritation. b. Lecithin/chitosan nanoparticles (0.15%): exhibited improved bioavailability and precorneal residence time compared to the marketed formulation (0.15%). c. Nanosuspension: exhibited activity against *Fusarium solani*; no ocular irritation upon in vivo application. d. Microemulsion: activity against *Candida* strains and lesser toxicity against red blood cells when compared to Fungizone®. e. Self-aggregating nanoparticles (0.15%): activity against *Candida albicans*; no irritation; permeate into the cornea where they prolonged drug residence time.	a. Monotherapy: i. Intracameral administration (5 μg) was found to be effective in treating ocular keratomycosis, refractory keratomycosis, and endophthalmitis. ii. Intracameral amphotericin B (7.5 μg in 0.1 mL) was effective and safe in the treatment of ocular fungal infections due to *Colletotrichum graminicola* and *Aspergillus flavus*. b. Combination: i. Intracameral amphotericin B (0.005 mg) along with oral itraconazole (10 mg) was effective in the treatment of *Aspergillus niger* ocular infections.	[34–46]
Fluconazole	a. Liposomes (0.7%): increased corneal uptake and permeation of fluconazole from formulations in comparison to suspension (0.9%). b. Implants (25% *w/w*): release of fluconazole from implants for a prolonged period compared to the fluconazole suspension (0.18% *w/v*); in vitro biocompatibility; ocular tolerance. c. Gelling systems: gels (0.5%) showed higher flux and permeation than the control; gel formulations showed antifungal activity against *C. albicans*, *C. tropicum*, and *P. chrysogenum*.	a. Monotherapy: i. Subconjunctival and intrastromal injection of fluconazole (2 mg/mL) effective in the treatment of severe fungal keratitis and fungal ulcers. b. Combination: i. Subconjunctival administration of fluconazole (2 mg/mL) along with topical amphotericin B (0.5 mg/mL) was effective and safe in the management of fungal keratitis caused by *Candida*.	[55–60]
Ketoconazole	a. Gels: C_{max} and concentration of the drug from gel was significantly higher than the control in aqueous humor. b. Alginate-chitosan gelling system (0.3%): higher permeation across epithelial cell lines; significantly higher antifungal activity in comparison to pure drug solutions (0.3%). c. Solid lipid nanoparticles (0.25%): superior C_{max} and AUC for the drug from nanoparticles than suspension (0.25%) in aqueous and vitreous humors.	a. Monotherapy: i. Topical ketoconazole (2%) exhibited effectiveness in treating *Aspergillus* and *Fusarium* corneal infections. ii. Oral (200–400 mg/day) and/or topical (1–2%) ketoconazole has shown effectiveness in treating ocular infections due to *Phaeohyphomycetes* species.	[6•, 64–68]
Voriconazole	a. Microemulsion: higher flux, permeation, and amount of drug deposited at corneal in comparison to the control. b. Nanostructured lipid carriers (3.5%): therapeutically relevant drug amounts to the cornea after only 30 min (drug content was approximately 40% of Vfend's	a. Combination therapy: i. Topical (1%) and oral voriconazole with either natamycin (5%) or amphotericin B (0.2%) eye drops or a combination of both with voriconazole: effective against fungal keratitis caused by *Candida albicans*, *Alternaria* spp., *Fusarium* spp., *Scopulariopsis* spp.	[74–82, 83•, 84]

Table 1 (continued)

Antifungal drugs	Updates		References
	Formulation approaches	Clinical evaluations	
	voriconazole dose); well tolerated and non-irritant, ex vivo evaluations. c. Solid lipid nanoparticles (0.02%): 2-year stability; drug release and corneal permeation without affecting the corneal hydration. d. In situ gelling system (0.05%): antifungal activity against *Candida albicans*; no damage to the cornea, iris, and conjunctiva; shelf life of at least 3 months.	b. Monotherapy: i. Topical (1%) and oral voriconazole (200 mg): effective in treating endophthalmitis and corneal ulcer caused due to *Candida parapsilosis* and fungal keratitis. ii. Topical (1%) and/or systemic: effective in fungal keratitis cases caused by *Fusarium*, *Candida*, and *Aspergillus* species.	
Itraconazole	a. Nanosuspension: formulation (1%) exhibited significantly higher corneal permeability than marketed nanosuspension (1%). It also showed 12-fold increase in aqueous saturation solubility of itraconazole. b. Solid lipid nanoparticles (0.05%): improved permeation across cornea and activity against *Aspergillus flavus*.	a. Monotherapy: i. Systemic and topical (1%) itraconazole successful in managing corneal ulcers caused by *Aspergillus*, *Penicillium*, and *Fusarium* species. b. Combination: i. Itraconazole (300 mg) with fluconazole (0.2%) was effective in treating and preventing the spread of deeper fungal infections after keratoplasty procedure.	[88, 90–93]
Caspofungin	No formulation approaches investigated. IV formulation of caspofungin (Cancidas®) used topically (extemporaneously reconstituted as eye drops) and systemically.	a. Monotherapy: i. Topical caspofungin (1%) successful in treating *Candida* keratitis. ii. Intrastromal caspofungin (0.5%) has shown effectiveness in treating *Alternaria* keratitis. b. Combination: i. Caspofungin (IV) (loading dose 70 mg, maintenance dose 50 mg) along with voriconazole (IV) (loading dose 400 mg, maintenance dose 300 mg) and posaconazole (IV) (200 mg) was successful in treating fungal endophthalmitis caused by *Oxysporum* species. ii. Caspofungin (loading dose 70 mg, maintenance dose 50 mg/day) with amphotericin B (3 mg/kg/day) and voriconazole (4 mg/kg, bid) was effective in treating postoperative endophthalmitis caused by *Aspergillus* and *Scopulariopsis* species. iii. Topical caspofungin (0.5%) with intrastromal voriconazole (50 μg/0.1 mL) was effective in treating *Alternaria* keratitis.	[100–107]
Micafungin	No formulation approaches investigated. IV formulation of micafungin (Mycamine®) used topically (extemporaneously reconstituted as eye drops) and systemically.	a. Monotherapy: i. Topical micafungin (0.1%) was safe and effective in the management of *Candida* keratitis. ii. Topical (0.1%) and IV administration of micafungin (50 mg) was effective and safe in refractory *Pestalotiopsis clavispora* keratitis. b. Combination: i. Micafungin (0.1%) with voriconazole (0.1%) has shown safety and efficacy in treating fungal keratitis caused by *Beauveria bassiana*, in postoperative endophthalmitis caused by *Aspergillus*, keratitis caused by *Wickerhamomyces anomalus*, and endophthalmitis caused by *Trichosporon* species. ii. Micafungin (75 mg) with fluconazole (50 mg) was effective in treating *Candida* endophthalmitis.	[114–120]

therapy. Natamycin has primarily provided safe and effective therapy in the superficial fungal infections of the eye such as fungal keratitis but has not shown comparable success rates in treating deep-seated ocular fungal infections. In such cases, the off-label use of other antifungals—amphotericin B, fluconazole, ketoconazole, voriconazole, caspofungin, and micafungin—has provided effective therapeutic outcomes in the management of both superficial and deep-seated ocular fungal infections. These off-label antifungals have been frequently used in combination with each other or natamycin and as monotherapies in cases of failure of natamycin therapy and/or refractory/recurrent fungal infections. However, to better

optimize the current antifungal therapy and improve the therapeutic outcomes associated with it, it is essential to further investigate these antifungal agents both preclinically and clinically, with respect to their alternative formulation strategies and potential combination therapies.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflicts of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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