Current efforts and the potential of nanomedicine in treating fungal keratitis

Taís Gratieri[1], Guilherme M Gelfuso[1], Renata FV Lopez[1] and Eliana B Souto[†2,3]

[1]Universidade de São Paulo, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Av. do Café, s/n. Ribeirão Preto, 14040-903, São Paulo, Brazil

[2]Faculty of Health Sciences, Fernando Pessoa University, Rua Carlos da Maia, Nr. 296, Office S.1, P-4200-150 Porto, Portugal

[3]Centre of Genetics and Biotechnology, University of Trás-os-Montes and Alto Douro (IBB/CGB-UTAD), PO Box 1013, 5000-801 Vila Real, Portugal

[†]Author for correspondence:
Tel.: +351 225 074 630
Fax: +351 225 074 637
eliana@ufp.edu.pt

Fungal infection of the cornea (mycotic or fungal keratitis, keratomycosis) is a serious disease that can lead to loss of vision if not diagnosed and treated promptly and effectively. The pharmacological approach of management of fungal keratitis involves administration of antifungal agents. However, owing to the physiologic constraints of the eye, only a few drugs define sufficient bioavailability. The need for more potent antifungals with increased activity, shorter treatment durations and fewer adverse effects simultaneously stimulates the drive for the development of new antifungal agents with a broader spectrum and improved pharmacokinetic profile, and the development of advanced novel formulations for drug delivery that could increase drug bioavailability while reducing the adverse effects. In this article, the efforts and scientific potential of these two avenues are discussed. First, the classical and novel antifungal drugs are presented. Second, the classical formulations are compared with the advanced novel nanomedicines, and their potential clinical applications are discussed.

Fungal infection of the cornea (mycotic or fungal keratitis, keratomycosis) was described for the first time in 1879 in Germany, in a patient who had a corneal ulcer caused by *Aspergillus* spp. Until 1951, only 63 cases were reported in the literature [1], but nowadays fungal keratitis has spread worldwide, with a continuous increase in the number of cases. The distribution pattern varies widely with geographic location and season, factors that determine the prevalence of etiological agents. The overall incidence tends to be higher in tropical and subtropical regions, with *Fusarium* (20–83.6%), *Aspergillus* (16.5–75%) and *Candida* (1–63%) being the most frequent fungi causing keratitis worldwide [2]. While *Fusarium* and *Aspergillus* are the most common fungi isolated from patients in the tropics, *Candida albicans* is the most common pathogen of mycotic keratitis in temperate regions [1,3]. Other pathogens are isolated to a minor extent, and include *Penicillium* (incidence: 0.1–10%), *Curvularia* (incidence: 2.64–15.7%), *Alternaria* (incidence: 0.3–5%) and *Rhizopus* (incidence: 0.06–1%) [4–11]. A study conducted in north China reported that fungal keratitis represented approximately 62% of all cases of severe infective keratitis among the inpatients from January 1999 to December 2004 [12]. Such high incidence is also reported in other places such as India (44%) [13,14], Brazil [15], Australia [16], Thailand (38%) [17], south Florida (35%) [18], Nepal (17%) [19], Saudi Arabia [20] and Ghana (37.6%) [21]. However, in temperate climates, such as Britain and the northern USA, the incidence of fungal keratitis is comparatively low [9–11].

Mycotic keratitis is a serious disease that can lead to loss of vision if not diagnosed and treated promptly and effectively [1]. Regardless of the cause of keratitis, migration of inflammatory cells into the cornea can result in a disruption of the critical condition that maintains transparency, leading to corneal opacification or complete blindness [22]. However, the high morbidity of this condition is not only related to the migration of inflammatory cells, but also to the physical damage caused by the presence of fungal organisms, secondary damage from fungal toxins and enzymes [23], frequently delayed diagnosis, and poor response to available therapeutic options [24].

Fungi cannot penetrate the intact healthy corneal epithelium and do not enter the cornea from episcleral limbal vessels. Hence, trauma is related

10.1586/EOP.10.19

to most fungal keratitis cases reported in developing countries [5,15,25], especially among agricultural or outdoor workers [26,27] exposed to corneal trauma with plant or soil matter [4,28], which could either introduce the fungus directly into a corneal epithelial defect or, alternatively, cause a defect to become infected following trauma. Morbidity in these cases can be aggravated by malnutrition [29] and lack of access to healthcare [30].

Another risk factor for fungal keratitis in industrialized countries is contact lens wear [31]. One hypothesis suggests that microscopic defects are introduced by lens wear that enhance microorganism adherence to the otherwise nonadherent corneal epithelium [32,33]. *Candida* is the principal cause of keratitis associated with therapeutic contact lenses, although cases by filamentous fungi have been reported [34,35]. Recently, there have been epidemic increases in *Fusarium* keratitis associated with particular contact lens solutions in several parts of the world [36–39].

Less frequently reported risk factors include prolonged use of topical corticosteroids [9,10] and antibacterials, systemic diseases such as diabetes mellitus [40], immunosuppressive diseases [11], prolonged chemo- or immunosuppressive therapy [41], previous eye surgery [42] and chronic eye surface diseases [11].

The diagnosis is difficult since the symptoms are usually nonspecific; they include tearing, pain, photophobia, a decrease in vision and redness [43]. Another problem is that features of keratitis caused by yeasts may resemble bacterial keratitis, misleading the diagnosis. In addition, since many of the filamentous fungi grow slowly, the disease often remains unrecognized and untreated for days or weeks until growth is visually detected [32]. In advanced suppurative cases, ulcerative lesions or granular infiltrations in the corneal epithelium may be seen [29]. Pathologic specimens of filamentous fungal keratitis demonstrate hyphae following the tissue planes of the cornea, lying parallel to the corneal collagen lamellae [32]. Neovascularisation may occur as a result of inflammation, which may lead to severe scarring of the cornea. Associated signs indicating the severity of inflammation include the presence of hypopyon and ciliary injection. It is important to determine the etiologic agent of the corneal ulcer. Diagnosis is usually achieved by scraping material from the base of the ulcer and culturing the material on solid and liquid media [24].

Pharmacological treatments

The pharmacological approach to the management of fungal keratitis involves the administration of antifungal agents. However, owing to the physiologic constraints of the eye, only a few drugs present adequate bioavailability [44]. Although surgical options (e.g., therapeutic keratoplasty) have a high incidence of infection recurrence [45], in most cases surgery may be recommended [46]. In extremely severe cases, enucleation or evisceration is needed [47,48].

Until the 1940s, relatively few agents were available for the treatment of systemic fungal infections. Nystatin was the first polyene antifungal to be identified in the late 1940s; however, its use has been discontinued owing to corneal toxicity and poor ocular penetration. By the late 1950s, the broader spectrum, more effective amphotericin B represented a major advance in

the treatment of fungal infections [49,50]. However, its clinical use is associated with numerous adverse effects [51]. The search for newer systemic antifungals led to the discovery of the azoles in the 1960s, with the release of ketoconazole in the early 1980s followed by fluconazole and itraconazole in the early 1990s [52]. These agents were available in oral formulations and demonstrated a relatively improved safety profile compared with that of amphotericin B. Nevertheless, they still present a less than optimal pharmacokinetic profile and, in some cases, a narrow spectrum of activity.

The need for more potent antifungals with increased activity against resistant pathogens, shorter treatment durations and fewer adverse effects stimulates both the drive for the development of new antifungal agents with broader spectrum and better pharmacokinetic profile, and the development of advanced novel formulations for drug delivery that could increase drug bioavailability while reducing the adverse effects.

In this article, the efforts and potential of these two avenues are discussed. First, the classical and novel antifungal drugs are presented. The classical formulations are then compared with the advanced novel formulations proposed, and the potential of these are discussed.

Classical antifungal drugs

Polyenes

Polyenes exert their antifungal effect by binding to the ergosterol in the fungal cell membrane, blocking fungal growth or altering membrane permeability [53]. Amphotericin B can also induce oxidative damage, which may contribute to its fungicidal action [54]. The extent of damage to fungal membranes is dose related; however, beyond a certain concentration, human cells may be affected, which accounts for the polyenes' toxic effects. Natamycin and amphotericin B are the two antifungal agents of this class in current use for the treatment of ophthalmic mycoses.

Natamycin

Natamycin, a tetraene polyene, has long been considered the mainstay of treatment for filamentous fungal keratitis [55,56]. As natamycin is poorly soluble in water it is presented as a 5% topical ophthalmic suspension. The initial dosage is normally one drop every hour. Therapy is generally continued for 14–21 days or until there is resolution of active fungal keratitis [301]. It is reported to have a broad spectrum of activity against various fungi, including species of *Fusarium*, *Aspergillus*, *Candida* and *Penicillium* [2,57,58], although its main limitation is its poor penetration after topical application. This has been attributed to the tissue binding, since 97% of the drug that enters the cornea quickly becomes biologically inactivated [59]. Therapeutic concentrations can still be achieved in the aqueous humor with intense topical administration after removal of the corneal epithelium [59].

Amphotericin B

Amphotericin B is a macrolid polyene with two special physicochemical properties: amphiphilic behavior owing to the apolar and polar sides of the lactone ring and amphoteric behavior owing

to the presence of ionizable carboxyl and amine groups (TABLE 1). As a consequence of its amphiphilic and zwitterionic nature and the asymmetrical distribution of hydrophobic and hydrophilic groups, amphotericin B is poorly soluble in all aqueous solvents and in many organic solvents [60].

The primary advantages of amphotericin B include its fungicidal activity against most clinically relevant pathogens [58] and the low occurrence of resistance [61]. It has been widely administered by intravenous, topical, intracameral and intravitreal routes for therapy of ocular infections [56,62–64]. The intravenous administration is the treatment of choice for invasive fungal infections, but this route may cause poor corneal bioavailability and severe nephrotoxicity [65]. Similarly to natamycin, the corneal penetration of amphotericin B is reduced in the presence of an intact corneal epithelium [59]. The topical regime often includes administration every 30 min for the first 24 h and every hour for the second 24 h, before being slowly tapered according to the clinical response [301]. Subconjunctival injection has been reported to lead to severe toxic effects, and is no longer recommended. Intracameral injections of amphotericin B may be an effective adjunctive treatment for fungal keratitis unresponsive to conventional antifungal therapy [66], although cataract may occur [67]. A case reported the use of intrastromal corneal injections combined with intravitreal injection of amphotericin B that led to the eradication of the corneal fungal plaques and the intraocular infection [68]. Intravitreal administration, although commonly used [69], has been reported to cause retinal necrosis and detachment if the injection is not made slowly and exactly in the center of the vitreous, as far as possible from the retina [2].

Azoles
The azoles, discovered in the late 1960s, are totally synthetic. They are inhibitors of a cytochrome P450 fungal enzyme involved in the conversion of lanosterol into ergosterol, an essential sterol in fungal cell membranes. The decrease in ergosterol synthesis leads to increased permeability of the fungal cell membrane, alteration of membrane enzymes, inhibition of growth and death of the fungal cell [70].

The azoles are classified as imidazoles or triazoles, on the basis of whether they have two or three nitrogens in the five-membered azole ring. The imidazoles include clotrimazole, isoconazole, econazole, miconazole and ketoconazole, the last two mostly being used in the treatment of ocular fungal infections. The triazoles include fluconazole and itraconazole.

Imidazoles
Miconazole is usually reserved as a second-line drug in the management of fungal keratitis. Very low miconazole levels are obtained in the cornea after intravenous injection, but following the subconjunctival injection higher levels can be noted in corneas with debridement of corneal epithelium. Similarly, after topical administration the penetration is almost ten times higher in debrided corneas [71]. Topical, subconjunctival and intravenous administrations of miconazole have been reported in the 1980s to

result in successful outcomes [72–75]. Nonetheless, corneal toxicity has been reported, manifesting itself as a row of pinpoint vesicular elevations in the corneal epithelium associated with surrounding superficial punctate keratitis [74,76]. With the later discovery of other antifungal agents with better pharmacokinetic properties, its use has declined. Notwithstanding, miconazole 1% is one of the most common topical antifungal drugs employed in veterinary cases of fungal keratitis [77–79].

Ketoconazole has pharmacological properties similar to that of miconazole; however, it is absorbable from the gastrointestinal tract and less toxic [80]. It is currently available as oral preparation worldwide. The adult dose of ketoconazole is normally 200–400 mg/day, which can be increased to 800 mg/day. However, gynecomastia, oligospermia and decreased libido have been reported in 5–15% of patients who have been taking 400 mg/day for a long period [301]. Recent *in vitro* susceptibility studies have shown that the majority of the ocular fungal isolates, including *Aspergillus*, *Candida* and some *Fusarium* species, were sensitive to ketoconazole [81,82]. The oral preparation is often used concomitantly to other topical antifungal agents [83–85], although it can also be administered topically without significant corneal toxicity signs [86]. However, the drawback is its poor water solubility [87]. Case reports can be found in the literature of patients with laboratory-proven fungal corneal infections that were successfully treated with topical ketoconazole. The clinical signs of corneal infection normally disappear after 3–7 weeks of therapy [86].
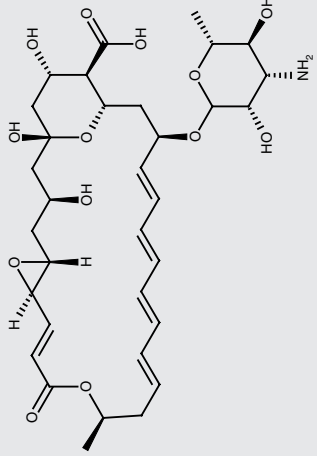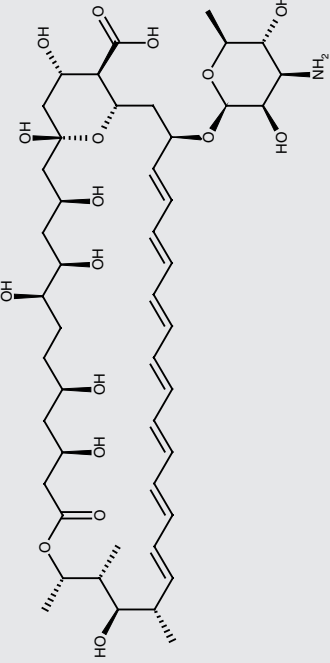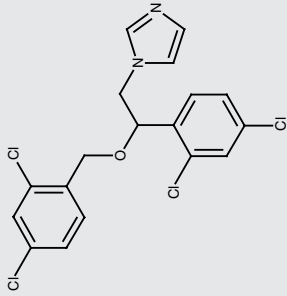
Triazoles
Fluconazole is a bistriazole antifungal compound with improved physical and pharmacokinetic properties. Fluconazole is a stable, nontoxic, water-soluble, low-molecular-weight (306.2 Da) compound that can be administered by several routes, such as topical [88–92], subconjunctival [93], intravitreal [94] and systemic [95,96]. The subconjunctival regime consists of fluconazole 2% up to 1.0 ml twice daily for at least 5 days [93].

Abbasoglu *et al.* achieved a fluconazole aqueous humor peak concentration in humans upon single- and multiple-drop applications of a 0.2% solution of 3.35 ± 0.64 and 7.13 ± 0.79 µg/ml, respectively, after 15 min [97]. Antifungal susceptibility tests have reported that among the most common etiological agents in fungal keratitis, *Fusarium* is the most resistant genera to fluconazole, exhibiting an *in vitro* MIC of 32–64 µg/ml [98]. Some studies reported lower MIC values for *Alternaria alternata* (12 µg/ml), *Aspergillus* (8 µg/ml), *Candida* (0.2–0.8 µg/ml), *Penicillium* (4 µg/ml), *Curvularia* (6–64 µg/ml) and *Rhizopus* (4–32 µg/ml) [99–102]. Based on this data, topically applied fluconazole may only be effective for the treatment of less resistant fungi. It is possible that higher aqueous humor concentrations, which would cover the MIC for most pathological agents, could be obtained with multiple-dose administration.
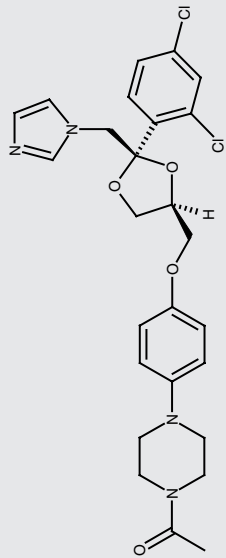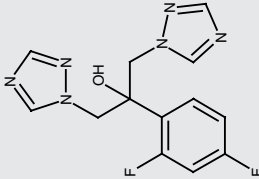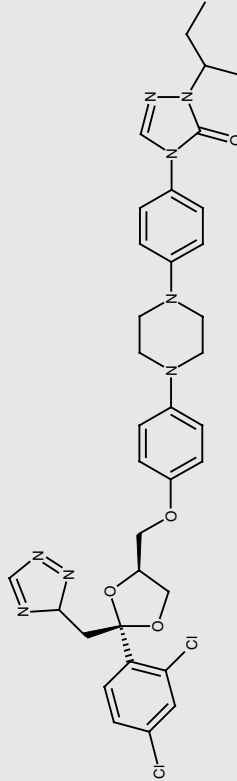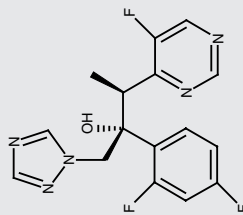
Itraconazole, a dioxolane triazole, is very hydrophobic with a relatively higher molecular weight (705.6 Da). It is well absorbed orally, although more than 90% binds to protein in serum [103]. The major drawback of using itraconazole by the oral route for therapy of ocular

Table 1. Physicochemical properties, advantages and disadvantages of the classical antifungal compounds used for the treatment of ocular fungal infections.

Drug	Structure	pKa	MW	Log P	Advantage/disadvantage
Polyenes					
Natamycin			665.7	-3.7	Broad spectrum of activity Poor intact corneal penetration after topical application Low bioavailability
Amphotericin-B		5.7 10.0	924.1	-2.8	Fungicidal activity against most clinically relevant pathogens Nephrotoxicity Retinal necrosis and detachment following intravitreal administration
Azoles					
Miconazole		6.5	16.1	6.2	Subconjunctival administration generally well tolerated Corneal toxicity Poor intact corneal penetration

Compiled from [302,303].

Table 1. Physicochemical properties, advantages and disadvantages of the classical antifungal compounds used for the treatment of ocular fungal infections.

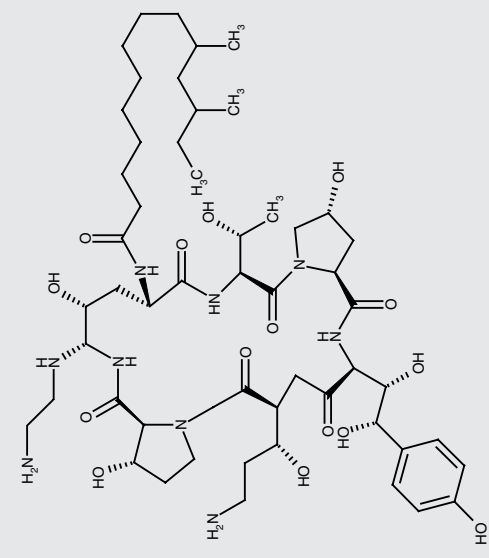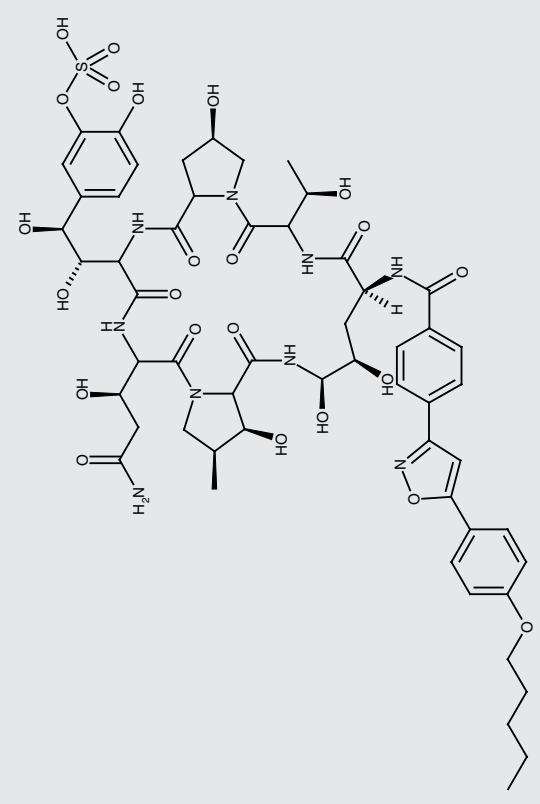| Drug	Structure	pKa	MW	Log P	Advantage/disadvantage
Azoles (cont.)					
Ketoconazole		2.9 6.5	531.4	4.3	The oral preparation is often used concomitantly to other topical antifungal agents with good outcome Poor water solubility Requires acid pH for absorption
Fluconazole		2.0	306.2	0.5	Excellent safety profile Stable, water-soluble Good intraocular penetration Poor *in vitro* activity *vs* most strains of *Aspergillus* and *Fusarium* spp.
Itraconazole		3.7	705.6	5.6	Excellent safety profile Hydrophobic, high molecular weight: poor bioavailabily Narrow spectrum coverage
New azoles					
Voriconazole		4.9 12.0	349.3	1.8 2.5	Broader spectrum of activity Greater efficacy Reversible disturbance of vision and skin rashes as common side effects Rapid vitreal clearance

Compiled from [302,303].

Table 1. Physicochemical properties, advantages and disadvantages of the classical antifungal compounds used for the treatment of ocular fungal infections.

Drug	Structure	pKa	MW	Log P	Advantage/disadvantage
Echinocandins					
Caspofungin		--	1093.3	-2.8	Fungicidal activity against fluconazole-resistant fungi strains High-molecular-weight rends poor intact corneal topical penetration
Micafungin		9.1	1270.3	-0.4	Water soluble Excellent *in vitro* activity against both *Candida* and *Aspergillus* High-molecular-weight rends poor intact corneal topical penetration

Compiled from [302,303].

fungal infections is its poor penetration into the cornea, aqueous humor and vitreous compared with fluconazole and ketoconazole. However, oral itraconazole was found to be effective in a case of fungal keratitis of the eye caused by *Pichia anomala* when used in combination with topical amphotericin B and natamycin [64], and in a case of fungal keratitis caused by *Scedosporium apiospermum* [104].

Topical itraconazole also proved to be useful for treating infections caused by *Aspergillus* or *Curvularia* spp. [105,106]. Topical itraconazole has also been reported as effective in treating animal models of *Fusarium* keratitis [107], even though its spectrum coverage is narrow against these species [57,82].

Allylamines
Allylamines prevent fungal ergosterol biosynthesis via specific and selective inhibition of fungal squalene epoxidase, thereby interfering with the integrity of fungal cell membrane [108]. Allylamines are less frequently used in the treatment of ocular fungal infections compared with polyenes and azoles. Antifungal agents belonging to this class include amorolfine, butenafine, naftifine and terbinafine, with terbinafine being the most commonly used compound [109].

Novel antifungal drugs
Novel antifungal drugs were developed with the aim of solving classical antifungal therapy problems such as severe toxicity (polyenes), narrow antifungal spectrum (especially against filamentous fungi), rapid development of resistance (most azoles), and fungistatic rather than fungicidal effects at the achieved ocular concentrations. Some of these problems are not yet completely solved, but the advances made are presented.

Newer azoles
The newest triazole agents, including ravuconazole, isavuconazole, posaconazole and voriconazole, are synthetic derivatives of fluconazole but have a significantly broader spectrum of activity [57]. Voriconazole is the better studied compound, and up until now there are no records of the clinical efficacy of the other new azole agents against fungal keratitis, with the exception of a few reports describing the use of posaconazole [110–112].

Voriconazole
Voriconazole is a new antifungal drug derived from fluconazole by the addition of a methyl group to the propyl backbone and by the substitution of a triazole moiety with a fluoropyrimidine group [113]. The molecular alterations conferred to voriconazole a broader spectrum of activity and greater efficacy than its parent compound, fluconazole. However, voriconazole presents more side effects and drug interactions. The most common side effect is a reversible disturbance of vision (photopsia), which may include blurred vision, altered color discrimination and photophobia. These symptoms are related to changes in electroretinogram tracings, which revert to normal when treatment with the drug is stopped; no permanent damage to the retina has been noted. Skin rashes are the second most common adverse effect and elevations in hepatic enzyme levels may also occur [114].

The mechanism of action is the same as the other azole agents, but voriconazole also inhibits the 24-methylene dihydrolanasterol demethylation in certain yeast and filamentous fungi [113,114]. The greater efficacy can be confirmed by *in vitro* susceptibility tests. In general, the MIC of voriconazole for *C. albicans* is 1–2 log lower than the MIC of fluconazole [115]. It also appears to be very effective in the management of ocular infections caused by many filamentous fungi [40], especially in the management of *Aspergillus* ocular infections, as compared with other antifungals [82]. Numerous case reports indicate that voriconazole treatment has been successful where natamycin, amphotericin B or fluconazole have failed, even in cases of drug-resistant fungal keratitis and endophthalmitis [116–122].

Voriconazole is well absorbed following oral administration, with a bioavailability of 90%. A study by Hariprasad *et al.* demonstrated that orally administered voriconazole achieves therapeutic aqueous and vitreous levels in the noninflamed human eye [123]. After two doses, the mean plasma concentration of voriconazole was 2.13 µg/ml, which resulted in voriconazole concentrations of 0.81 µg/ml in the vitreous and 1.13 µg/ml in the aqueous. The activity spectrum appeared to appropriately encompass the most frequently encountered mycotic species involved in the various causes of fungal endophthalmitis. A similar result was described in a case report of an eye with *Scedosporium apiospermum* keratitis that went on to corneal transplant. It was found that aqueous voriconazole levels following 12 days of oral treatment in the aqueous humor was 1.8 µg/ml, almost seven times higher than the MIC for that specific strain [124].

Topical therapy may also be used in conjunction with oral therapy to increase the amount of drug in the anterior chamber [125]. The topical administration of voriconazole 1% solution every 2 h for 1 day in noninflamed human eyes prior to planned vitrectomy surgery resulted in a mean concentration of 6 µg/ml of the drug in the aqueous and 0.15 µg/ml in the vitreous, demonstrating that the drug penetrates well beyond the cornea when applied topically [126]. These results are in accordance with other studies that applied voriconazole topical solution. Recently, a prospective open-label trial involving ten participants that received topically administered 1% voriconazole solution hourly for four doses or four times a day for 3 days, obtained voriconazole concentrations ranging from 0.1 to 1.1 µg/ml in the vitreous humor [127]. In a similar study, 13 human subjects scheduled for elective anterior segment eye surgery received hourly 2% voriconazole eye drops at 4 h presurgery. Significantly, the voriconazole concentration in the aqueous humor of the eye was similar to that reported for the 1% voriconazole solution, suggestive of concentration-independent absorption through an intact infection-free cornea [128]. This is consistent with observations in a recent animal study, where the voriconazole level in the corneas of horses with fungal keratitis did not change when the administered voriconazole eye drop concentration was changed from 1 to 3% [129]. In addition, in the study conducted by Lau *et al.* it was also observed that no accumulation of voriconazole in the vitreous humour could be detected with a four-times-a-day dosing regimen, suggesting that voriconazole is cleared very rapidly from the posterior

chamber [127]. This hypothesis is also consistent with results by Shen *et al.*, where the concentration of intravitreal voriconazole at various time points was reported to exhibit exponential decay with a half-life of 2.5 h after single intravitreal injections in a rabbit model [130]. This suggests that in severe cases of fungal keratitis, where pathogens have already spread into the eye or there is a risk of fungal endophthalmitis, considerably higher concentrations of voriconazole or a slow-release formulation would be necessary to sustain therapeutic drug levels in the posterior chamber.

Echinocandins

Echinocandins are lipopeptides that have been synthetically modified from the fermentation broths of various fungi, and have recently emerged as valuable antifungal agents.

They possess a unique mechanism of action, inhibiting β-(1,3)-D-glucan synthase, an enzyme that is necessary for the synthesis of essential components of the cell wall of several fungi. The depletion of these components results in an abnormally weak cell wall unable to withstand osmotic stress [131]. The echinocandins display fungistatic activity against *Aspergillus* spp. and fungicidal activity against most *Candida* spp., including strains that are fluconazole resistant. Overall, resistance to echinocandins is still rare and all agents are well tolerated, with similar adverse effect profiles and few drug–drug interactions [132].

Three echinocandins have been approved by the US FDA, namely caspofungin, micafungin and anidulafungin, but up until now there is no record of anidulafungin applied for the treatment of keratitis.

Caspofungin

Caspofungin was the first approved member of the class; it has the most available data and the most indications of the echinocandins [122,133].

Several studies have recently compared the efficacy of topical caspofungin with that of topical amphotericin B. When using an animal model of *C. albicans* keratitis the authors observed comparable results for 0.5% caspofungin and 0.15% amphotericin B [134]. Similar results were also found for 1% caspofungin and 0.15% amphotericin B topical solutions in an animal model of *Fusarium solani* keratitis [135].

Since caspofungin has a high molecular weight of 1093.5 Da, the topical administration without corneal epithelium abrasion resulted in no detectable amounts of the drug in the aqueous humor. However, after corneal epithelial abrasion, therapeutic drug levels that cover the MIC of most fungi could be reached [136].

Micafungin

Micafungin is a water-soluble echinocandin with excellent *in vitro* activity against *Candida*, *Aspergillus* and some fungi resistant to other antifungal agents [137,138].

Hiraoka *et al.* evaluated the efficacy of subconjunctival injection of 0.1% micafungin in the treatment of experimental *C. albicans* keratitis and observed complete healing of the corneal lesions in six out of eight eyes treated [139]. The remaining two eyes where the drug was not effective presented deeper corneal lesions. Although corneal penetration of micafungin has not been studied yet, the penetration into the deep corneal stroma through an intact epithelial layer seems limited because of its high molecular weight (1292.26 Da). There has been one case report of the clinical application of topical micafungin eyedrops in the treatment of refractory yeast-related corneal ulcers with a satisfactory outcome [140]. Moreover, topical instillation of micafungin solution had no apparent toxicity to the cornea [141].

Classical formulations

The eye is characterized by physiological barriers that limit drug entrance from the blood circulation to its inner structures. These are the blood–aqueous and the blood–retinal barriers [142]. As a consequence, systemic or oral drug therapy requires large drug dosages to reach the site of action in proper amounts, which may cause significant systemic side effects [143]. Intravitreal, periocular and subconjunctival injections could minimize systemic exposure of the drug, but the use of these systems is followed by a series of disadvantages. The intravitreally injected drug is rapidly eliminated by the eye's natural circulatory process and therefore frequent injections may be required. Likewise, large doses are often needed, giving rise to toxicological problems. Besides, there are also relevant side effects, such as pain, discomfort, increased intraocular pressure, intraocular bleeding, increased chances for infection and the risk of retinal detachment. The major complication for intravitreal injection is endophtalmitis, which can result in severe vision loss [144–146]. In addition, ocular injections are not well accepted by patients. The topical administration is the most convenient route for the management of ocular fungal infections, especially for infection affecting the cornea and anterior chamber structures. Therefore, although sometimes not the most efficient, the topical route is the first choice for starting the administration of drugs on the treatment of ocular fungal infections. The classical formulations applied include topical solutions or suspensions in the form of eye drops or ointments in the form of night creams. More recently, lipid complexes of amphotericin B have also been applied.

Topical eye drops

In several cases, intensive topical antifungal therapy involves the use of multiple antifungal eye drops in very short administration intervals (e.g., half an hour) [147]. Protection mechanisms of the human eye such as lachrymal secretion and blinking reflex cause rapid drainage of the topically applied eye drops [148]. The short precorneal residence time allied with cornea impermeability results in low bioavailability, and frequent dosing is usually needed to compensate for the rapid precorneal drug loss.

Water-soluble drugs can be administered in the form of solutions and relatively insoluble drug substances in an aqueous vehicle as a form of suspensions. In this case, the vehicle must contain suitable suspending and dispersing agents to allow good drug redispersibility, maintaining the uniformity of drug dosage. Controlled flocculation of suspensions can be accomplished by adding electrolytes, ionic or nonionic surfactants, or even water-soluble polymers [149]. Owing to the particles' tendency to be retained in the cul-de-sac, the contact time and duration of action of a suspension exceed

those of a solution [150]. The retention may increase with particle size; however, it is recommended that particles should not exceed 10 μm so that they do not cause discomfort.

Several antifungal drugs have been tested in the form of topical eye drops. These drugs may include natamycin [83], amphotericin B [62,107,151], miconazole [74,152], ketoconazole [86], fluconazole [88–92], itraconazole [107], voriconazole [107,125], caspofungin [134,135] and micafungin [140].

The contact time with the target ocular tissue may depend on the physicochemical properties of the drug and the body's clearance mechanisms, but may also be highly influenced by the vehicle chosen for drug delivery. Even for the newer antifungal compounds, it has been observed that corneal penetration is insufficient. A recent study concluded that to achieve a sustained high level of caspofungin as an effective antifungal therapy for corneal keratitis, the drug should be administered topically every 30 min after removal of the corneal epithelium [136]. However, developing a sustained-release ocular preparation would overcome the requirement for a frequent dosing.

Formulations with enhanced solubility
The most important drawback to the formulation of most common antifungal agents is their scarce solubility in water. Such are the cases of amphotericin B (solubility: 0.001 mg/ml; pKa: 5.7) [60], miconazole (solubility: ≤0.00103 mg/ml; pKa: 6.5), ketoconazole (solubility: 0.017 mg/ml; pKa: 6.5) and itraconazole (solubility: 1.8 mg/ml; pKa: 3.7) [153]. Several attempts have been made to obtain drug formulations suitable for intravenous and topical ophthalmic administration with adequate drug concentrations.

Cyclodextrins have been used to increase ketoconazole aqueous solubility [154]. When hydroxypropyl β-cyclodextrin was used, it produced more than a twelvefold bioavailability increase after topical instillation in rabbit corneas when compared with the classical ketoconazole suspension [87]. The solubilities of voriconazole, ketoconazole and clotrimazole were also significantly improved with this cyclodextrin in aqueous media [155].

The solubilizer effect of acetate, phosphate and gluconate solutions, along with ethanol, glycerol, macrogol 400, propylene glycol, and surfactants such as polysorbate 20, 60, 80 and sodium taurocholate, were studied in binary or ternary combinations. Ternary combinations were capable of solubilizing more than 30 mg/ml miconazole and more than 135 mg/ml of ketoconazole [153]. Nevertheless, for the ocular administration of these solutions further tolerability studies must be performed.

Another example is the colloidal dispersion of amphotericin B with sodium deoxycholate (Fungizone®; Bristol-Myers Squibb Co., NJ, USA), which became available in 1958 for the treatment of fungal infections [60]. However, the topical application of such a formulation is known to induce corneal lesions [26,156]. More recent studies have focused on the development of more biocompatible micelles. Micelles composed of a block copolymer poly(2-ethyl-2-oxazoline)-block-poly(aspartic acid) containing amphotericin B (Fungizone) were able to increase drug solubility and efficiency with lower cytotoxicity [157].

Ointments
Enhanced ocular retention of oily vehicles has been reported for more than 30 years [158], being attributed to their interaction with the superficial oily layer of the tear film. As a consequence, initial attempts to overcome the poor bioavailability of topically instilled drugs typically involved the use of ointments.

Ointments ensured superior drug bioavailability by increasing contact time with the eye, minimizing dilution by tears and resisting nasolachrymal drainage. However, these vehicles have the major drawback of being uncomfortable and causing blurred vision. Consequently, they are mainly used for either administration overnight or for treatment on the outside and edges of eyelids [159]. A series of antifungal drugs have already been formulated in ointments, such as natamycin [152], amphotericin B [160], miconazole [161] and itraconazole [83,84], although in most cases a combined therapy is used.

Lipid complexes
To increase the therapeutic index of amphotericin B, lipid complexes were developed. In the commercial drugs Abelcet® (The Liposome Company, NJ, USA) and Amphocil® (Sequus Pharmaceuticals, Inc., CA, USA), amphotericin B has been formulated with two phospholipids in a 1:1 drug to lipid molar ratio. Amphotec® (Sequus Pharmaceuticals, Inc., CA, USA) is an amphotericin B formulation with cholesterol sulfate in equimolar concentrations. Amphotec particles resemble discs and have a similar antifungal efficacy to Fungizone but with lower cytotoxic and hemolytic effects. The reduction of renal toxicity has been attributed to the strong affinity of amphotericin B to the cholesterol moieties, which reduces the amount of free amphotericin B in the circulation [60]. A case of *Fusarium solani* keratitis that progressed to fungal endophthalmitis was successfully treated systemically with the amphotericin B lipid complex Abelcet [162].

Advanced novel formulations
The clinical efficacy of an antifungal agent in ophthalmic mycoses depends, to a great extent, on the concentration achieved in the target ocular tissue [163]. Unfortunately, in several cases, topical treatment with classical formulations is not effective enough.

The ability of a drug to penetrate the eye is primarily dependent on its physicochemical properties, such as molecular weight, pKa (which determines the nonionized/ionized proportion of the molecule at a certain pH) and log P, which provides information about its lipophilicity.

With respect to drug delivery, the cornea can be divided into three layers, namely the outer epithelium (lipophilic in nature), the stroma (hydrophilic in nature) and the inner endothelium (also lipophilic) [164,165]. In the human eye, the epithelium contains five to seven layers of cells, each connected by tight junctions, which provide a large barrier that is permeable only to small lipophilic molecules. Because the cornea has hydrophilic as well as lipophilic tissues, it provides an effective bifunctional barrier for the absorption of both lipophilic and hydrophilic compounds. In this way, the overall absorption of moderately lipophilic compounds across the cornea is favored (log P 2–3) [166]. Regardless of the

administration route, most of the antifungal drugs available do not possess the required physicochemical properties to be absorbed and reach or enter target tissues (Tables 1 & 2).

A promising strategy to overcome these problems involves the development of suitable drug-carrier systems. The *in vivo* fate of the drug is no longer dependent on the properties of the drug but on the carrier, which should maximize precorneal drug absorption, minimize precorneal drug loss and allow a controlled and localized release of the active drug, while maintaining the simplicity and convenience of the dosage form.

Since only a limited percentage of the administered drug reaches the target tissue, patient compliance is an important aspect to consider when developing an ophthalmic delivery system. As such, attention should be paid to the facility of administration and to the sensorial feeling after the administration, since discomfort (e.g., burning sensation) could induce tear production, followed by drug dilution and drainage through nasolachrymal duct.

Other important aspects to be considered are the retention time, drug-loading capacity and drug protection from metabolic degradation. In fact, if the drug-carrier system is able to prolong the retention while loading a sufficient amount of drug in a protected manner, the interval between administrations can be lengthened. For instance, in the case of intravitreal injections, the reduction in the number of injections would also reduce the potential side effects. Apart from these, all the factors that would influence the overall costs should also be considered, such as the possibility of scaling up production, sterilizing, and the physical and chemical storage stability of the product.

Novel colloidal delivery systems such as polymeric nano- and microparticles, liposomes, solid lipid nanoparticles, and nanostructured lipid carriers, are currently being studied in attempt to fulfill all these requirements.

Polymeric micro- & nanoparticles

A controlled-release strategy is to encapsulate the drug in polymeric microparticles (1–1000 µm) or nanoparticles (1–999 nm). These systems consist of various biocompatible polymeric matrices in which the drug can be adsorbed, entrapped or covalently attached [167]. Biodegradable and biocompatible synthetic polymers such as poly(D,L-lactide-co-glycolide) (PLGA) and polyalkylcyanoacrylates are preferred for nanoparticle production. Nonetheless, use of polysaccharides (e.g., curdlan) and macromolecules (e.g., chitosan, albumin and gelatin) has been very well described in the literature [167–169].

Nanoparticulate technologies in general offer interesting benefits such as solubilization of hydrophobic drugs, bioavailability improvement, modification of pharmacokinetic parameters, and protection of drug molecules from physical, chemical and/or

biological degradation [168]. Increased residence time of drugs and maintenance of their therapeutic concentrations for longer time intervals could reduce the number of subconjunctival and intravitreal injections required in some treatments, while allowing higher doses without toxicity from initial concentration. A drawback is that intravitreal injections of particulate systems may cause vitreal clouding [170]. However, microparticles tend to sink to the lower part of the vitreal cavity, whereas nanoparticles are more likely to cause clouding in the vitreous [145]. It is also suggested that nanoparticles increase the residence time owing to their bioadhesive nature, a property that would be especially useful for topical delivery. Different polymers can be used to coat nanoparticles and improve adhesion. Studies have shown, for example, that the bioavailability of encapsulated indomethacin doubled when poly(ε-caprolacton) nanoparticles were coated with chitosan [171]. In addition, microparticles formed of PLGA and poly(ethylene glycol) (PEG) as a core material and mucoadhesion promoter, respectively, showed prolonged residence time in rabbit eyes [172]. In this way, the ideal size and composition of a polymeric colloidal system would depend on the target. For instance, microparticles can be more effective than nanoparticles for intravitreal administration, but if they are larger than 10 µm they could cause an uncomfortable 'sand-like' feeling after topical administration [172,173]. In addition, depending on the drug, higher encapsulation efficiency can be obtained in microparticles than nanoparticles.

The encapsulation of antifungal agents in nanoparticulate carriers has been used with the objective of modifying the pharmacokinetics of drugs, resulting in more efficient treatments with fewer side effects. Although there are no records to date of applying these systems for the treatment of ophthalmic fungal infections, they have been studied for the treatment of similar infections in other organs with promising results.

Several recently published works describe the production of nanoparticles containing amphotericin B aiming to control drug delivery and reduce toxicity [174–177]. For example, amphotericin B

Table 2. General *in vitro* MIC$_{50}$ values for the different antifungals against the most common pathogens in fungal keratitis.

Antifungals	MIC$_{50}$ (µg/ml)			Ref.
	Fusarium	*Aspergillus*	*Candida*	
Natamycin	4–8	2–32	4–33[†]	[57,221–224]
Amphotericin B	0.5–32	0.25–2	0.25–1	[57,58,81,82,98,99,137,221,222,225]
Miconazole	8	2	1–10[†]	[58,226]
Ketoconazole	2–16[†]	0.06–4[†]	0.008–0.4[†]	[58,81,82]
Fluconazole	32–64	8–64	0.2–51	[58,81,82,98,99,115,225]
Itraconazole	8–32	0.125–4	0.016–0.256	[57,58,82,137,222]
Voriconazole	0.5–12	0.12–0.5	0.008–0.25[†]	[57,115,222,225,227]
Caspofungin	16–128[†]	0.12–1.0[†]	0.06–8[†]	[57,225,227]
Micafungin	>256[†]	0.004–0.03[†]	0.002–4[†]	[225,227]

It is important to note that the results of *in vitro* antifungal susceptibility tests may not necessarily predict clinical outcome in keratitis. Host factors, stage of infection, patient compliance to the therapeutic regime and drug levels are all important factors that may influence the clinical response.
[†]Fungi used were not isolated from cases of keratitis.

Advanced novel formulations	Advantages	Disadvantages	Incorporated antifungal agents	Ref.
Polymeric nanoparticles	May be biocompatible and biodegradable Able to entrap both hydrophilic and hydrophobic drugs Controlled release Protect drug from metabolic degradation Prolonged residence time – bioadhesive properties	Burst effect Limited drug loading May cause vitreous clouding High cost	Amphotericin B Voriconazole Fluconazole Itraconazole	[174–180] [181] [182] [184]
Polymeric microparticles	Can be prepared by spray drying – large-scale production May be biocompatible and biodegradable Able to entrap both hydrophilic and hydrophobic drugs Controlled release Protect drug from metabolic degradation Prolonged residence time – bioadhesive properties	Burst effect May cause uncomfortable sensation if ≥10 μm	Fluconazole	[183]
Liposomes	Biocompatible and biodegradable Able to entrap both hydrophilic and hydrophobic drugs Controlled release Protect drug from metabolic degradation Prolonged residence time – precorneal and in vitreous	Poor stability Difficult to prepare and sterilize High cost	Amphotericin B Fluconazole	[194–196] [191–193]
SLNs	Easy preparation – large-scale production Easy sterilization Improved ocular bioavailability Prolonged precorneal residence time Controlled release	Limited drug loading	Clotrimazole Ketoconazole Itraconazole Miconazole Econazole	[209–211] [212] [214] [215] [216]
NLCs	Easy preparation – large-scale production Easy sterilization Drug loading of lipophilic and possibly hydrophilic drugs Improved ocular bioavailability Prolonged precorneal residence time Controlled release	Hydrophilic drugs can show burst effects	Clotrimazole Ketoconazole	[209,210] [212]

NLC: Nanostructured lipid carrier; SLN: Solid lipid nanoparticle.

entrapped into PLGA nanoparticles was shown to improve the oral bioavailability and minimize the adverse effects observed in classical systemic amphotericin B therapy [178]. Nonetheless, nanoparticles have also been used for targeting drug delivery. Amphotericin B-loaded PLA-β-PEG nanoparticles coated with polysorbate 80 have been efficiently produced for brain targeting [179]. Since these systems have been shown to efficiently cross the blood–brain barrier, they represent a promising tool for crossing the retinal–blood barrier and increasing intraocular bioavailability after systemic administration. Further studies should be carried out in this area. In addition, intraperitoneal administration of amphotericin B nanoparticles based on PLGA and dimercaptosuccinic acid in mice showed antifungal efficacy, fewer undesirable effects and a favorable extended dosing interval [180].

Poly(D,L-lactide-co-glycolide) nanoparticles loaded with voriconazole were prepared by the emulsion–solvent evaporation technique. The mean particle size was 132.8 nm when using sodium hexametaphosphate to avoid particle agglomeration. Both *in vitro* and *in vivo* studies in mice showed greater antifungal efficacy of drug-loaded nanoparticles by contrast with the drug alone [181]. Nano- or microparticle production has been described for other antifungal agents, such as fluconazole [182,183] and itraconazole [184].

Liposomes

Liposomes are biocompatible and biodegradable phospholipid vesicles formed by one or several lipid bilayers. In each bilayer, the nonpolar fatty acid tails are placed in the interior whereas the polar heads are turned outside, containing an aqueous phase both inside and between the bilayers. Owing to their amphiphilic character, liposomes are able to entrap both hydrophilic and hydrophobic compounds in the aqueous compartments or within the lipid bilayers, respectively [185,186]. Liposomes can provide controlled release of incorporated drugs as the spherical lipid shield formed by bilayer membranes provides a permeability barrier to drug release. In this way, the drug is protected from degradation and clearance, and toxicity resultant from high peak concentration is avoided. This property can be especially useful for posterior segment applications [187]. Similarly to polymeric nano- and microparticles, liposomes can minimize some of the adverse side effects encountered by the intraocular administration routes, increasing therapeutic effectiveness [188–190].

Gupta *et al.*, studying the pharmacokinetics of plain and liposome-encapsulated fluconazole after intravitreal injection in rabbit eyes, observed a rapid vitreal clearance and a short half-life (3.08 h) for plain fluconazole, whereas liposome-entrapped fluconazole showed an extended half-life (23.40 h) [191]. The constant

terminal elimination of the liposome-loaded drug from the vitreous was seven times less than the plain drug [191]. However, the same authors later discouraged the use of fluconazole as a sole therapy for endophthalmitis. They reported inferior outcomes for liposome-entrapped fluconazole in a candidal endophthalmitis rabbit model, probably owing to heterogenous distribuition throughout the vitreous cavity and initial low drug concentration [192].

Liposomal formulations containing fluconazole for ophthalmic controlled release were also prepared using the reverse-phase evaporation technique [193]. Soya bean phosphatidylcholine and cholesterol in specific weight ratios were used, and selected formulations tested for their *in vivo* ocular antifungal effect. Conversely, the authors of this work reported that, after *in vivo* administration in a model of *Candida* keratitis, fluconazole liposomal formulations achieved complete healing in a shorter time than plain fluconazole solution. In addition, the frequency of instillation could be reduced [193].

A reduction in ocular toxicity of subconjunctival injection of liposomal amphotericin B has also been reported. Comparisons were made with conventional amphotericin B deoxycholate formulation in a rabbit model. The study reported that subconjunctival injection of amphotericin B deoxycholate formulation or deoxycholate alone induced severe corneal and conjunctival edema with necrosis and infiltration of inflammatory cells, whereas the liposomal formulation induced only mild inflammation near the injection site. The authors also observed satisfactory concentrations in corneal stroma after the liposomal formulation injections [194]. In fact, a liposomal formulation named AmBisome® (Vestar, Inc., CA, USA) containing amphotericin B is commercially available. The formulation is supplied lyophilized as a powder and must be reconstituted in water directly before use, producing liposomes with a mean diameter of 60–70 nm [60]. Because of its hydrophobicity, amphotericin B binds predominantly to the lipid bilayer rather than being placed in the small hydrophilic core of the liposome. The liposomal material consists of hydrogenated soy phosphatidylcholine and distearoylphosphatidylglycerol. Moreover, the negative charge of the distearoylphosphatidylglycerol can interact with the positive amino group of the amphotericin B, forming an ionic complex in the bilayers [60]. In addition, a broad antifungal activity spectrum has been defined by the liposomal formulation [195]. In a recent study, the corneal availability following systemic administration of parenteral amphotericin B lipid complex or liposomal amphotericin B was compared with that of amphotericin B deoxycholate in a rabbit model [196]. The authors

Table 4. Aspects to be considered on choosing an ophthalmic delivery system and the performance of advanced novel delivery systems.

Aspects to consider	Advanced novel formulations				
	Nanoparticles	*Microparticles*	*Liposomes*	*SLNs*	*NLCs*
Facility of administration	+ +	+ +	+ +	+ +	+ +
Sensorial feeling after administration† (blurred vision, burning sensation, lacrimation)	+ + +	+ +	+ + +	+ + +	+ + +
Drug loading capacity	+ +	+ + +	+ +	-	+ +
Possibility of drug targeting	+ + +	+ + +	+ + +	+ +	+ +
Precorneal retention time	+ + +	+ + +	+ +	+ +	+ +
In vitreous residence time	+ + +	+ + +	+ +	‡	‡
Controlled drug release	+ + +	+ + +	+ +	+ +	+ +
Avoidance of burst effect	-	-	+ +	-	+ +
Avoidance of toxicity	+ +	+ +	+ + +	+ +	+ +
Scaling up of production	+	+ +	-	+ + +	+ + +
Easy to sterilize	+ + +	+ + +	-	+ + +	+ + +
Storage stability	+	+ +	-	+ +	+ +

It is important to consider the form in which formulations are dispensed. The overall storage stability tends to be significantly higher if the formulations are dispensed in lyophilized form.
†The scale indicates the absence of such events, + + + being indicative of the lowest probability of the formulation to cause undesirable sensorial feeling after administration.
‡Not reported.
-: Poor; +: Good; + +: Very good; + + +: Excellent; NLC: Nanostructured lipid carrier; SLN: Solid lipid nanoparticle.

reported that no drug could be detected in the corneas of the non-inflamed eyes, but in a uveitis-induced model the penetration into the cornea was significantly higher after systemic administration of liposomes, followed by lipid complexes and conventional amphotericin B deoxycholate [196].

It needs to be considered, however, that the type of vesicles formed and the formulations constituents may interfere with the final toxicity and antifungal activity of the drug. It has been observed that small unilamellar vesicles [197–199], multilamellar vesicles [200–202] or large multilamellar vesicles [201] containing amphotericin B perform differently [198]. Similarly, fluconazole showed different MIC values in different vesicle types [198,203]. Inhibition of the antifungal activity of miconazole and ketoconazole by phospholipids has also been reported. Such an effect seems to be dependent on the phospholipid concentration [198]. Moreover, sterols present in the formulation may interfere with the fungicidal activity of liposomal amphotericin B. It has been observed that ergosterol- and cholesterol-containing liposomes were less effective against *C. albicans* compared with the sterol-free liposomes [204].

Significant progress has been made in demonstrating the advantages of liposome-mediated drug delivery in ophthalmology. In some cases, liposomes have shown to improve efficacy, reduce toxicity, prolong activity and provide site-specific delivery. Despite

these reasons, which make liposomes a potentially useful system for ocular delivery, until now there have been very few attempts to apply them for the treatment of ophthalmic fungal infections. Problems usually encountered were the short shelf life, limited drug-loading capacity, use of aggressive conditions for preparation and sterilization issues [165]. Temperatures required for autoclaving can cause irreversible damage to vesicles while filtration reduces the vesicle to an average of 200 nm, limiting its use to small vesicles.

Solid lipid nanoparticles & nanostructured lipid carriers

Solid lipid nanoparticles (SLNs) are the first generation of nanoparticles composed of lipids that are solid at room and body temperatures, stabilized with an emulsifying layer in an aqueous dispersion. They offer the possibility of a controlled drug delivery, since drug mobility in a solid lipid is lower compared with an oily phase. Other advantages of such carriers include the use of physiological compounds in the composition, the fast and effective production process, including the possibility of large-scale production, the avoidance of organic solvents in the production procedures, and the possibility of producing high concentrated lipid suspensions [205]. The main disadvantage, however, is the low drug-loading capacity [206], which is mainly related to the possibility of drug expulsion during storage [207].

Nanostructured lipid carriers (NLCs) are another type of lipid nanoparticle being developed to overcome some limitations of SLNs. NLCs are prepared not only from solid lipids but from a blend of a solid lipid with a certain amount of oil, to maintain a melting point above 40°C. Mixing very different molecules, such as long-chain glycerides of the solid lipid with short-chain glycerides of the liquid lipid, creates crystals with many imperfections [208]. Apart from localizing the drug inbetween fatty acid chains or lipid lamellae, these imperfections provide a location for the additional loading of drug molecules. These drug molecules can then be incorporated in the particle matrix in a molecularly dispersed form, or be arranged in amorphous clusters. There is also more flexibility for modulation of drug release, increasing the drug loading and preventing its leakage.

Lipid nanoparticles (SLNs and NLCs) are interesting systems for the ocular delivery of drugs. Similar to emulsions, they are composed of accepted excipients, and can be produced on a large industrial scale using an established and low-cost homogenization process. In addition, SLNs and NLCs show the advantages of a solid matrix similar to polymeric nanoparticles, having the ability to protect chemically labile drugs and to modulate release (from very fast to extremely prolonged release). Surface modifications can be used to prolong precorneal residence time. Similarly to liposomes, several SLNs and NLCs have been successfully prepared for the incorporation of antifungal drugs but aimed for different administration routes, such as transdermal drug delivery.

Clotrimazole-loaded SLNs and NLCs have been prepared by hot high-pressure homogenization with entrapment efficiency higher than 50%. After 3 months of storage at different temperatures the mean diameters of SLNs and NLCs remained below 1 µm [209]. The entrapment efficiency and the drug-release profile were dependent on the concentration and the lipid mixture employed. NLCs showed higher entrapment efficiency owing to their liquid parts. In agreement with these results, NLCs also depicted a faster release rate in comparison to SLNs with the same lipid concentration. Incorporated clotrimazole in tripalmitine-based SLNs and NLCs stabilized with tyloxapol were also obtained. The particles displayed a spherical shape and a narrow size distribution with a mean diameter smaller than 200 nm [210]. The SLN containing clotrimazole displayed a prolonged release character [211].

Lipid particles containing ketoconazole were also obtained using the hot high-pressure homogenization technique, using Compritol® (Compritol 888 ATO, Gattefossé, Weil am Rhein, Germany) as the solid lipid and the natural antioxidant α-tocopherol as the liquid lipid compound for the preparation of NLCs. The authors verified that the SLN matrix was not able to protect the chemically labile ketoconazole against degradation under light exposure. By contrast, the NLCs were able to stabilize the drug, but the aqueous NLC dispersion showed size increase during storage. Possible solutions would be light-protected packaging for the SLNs or NLCs physically stabilized in a gel formulation [212]. In accordance, another study revealed that after a shelf life of 2 years, more than 95% of clotrimazole and less than 30% of ketoconazole incorporated in SLNs and NLCs were detected in the developed formulations. Still, these values were shown to be higher than those obtained with reference emulsions of similar composition and droplet sizes [213].

Other antifungal agents that were successfully incorporated in SLNs include itraconazole [214], miconazole [215] and econazole [216].

Therefore, it is expected that in the near future lipid nanoparticles will become available for the treatment of ophthalmic fungal infections. Despite the drug-loading difficulties, several compounds commonly used in the treatment of ocular diseases have been incorporated into lipid nanoparticles, such as tobramycin [217], gatifloxacin [218], cyclosporine [219] and timolol maleate [220]. Lipid nanoparticles have shown sustained release and enhancement of drug bioavailability in all such cases [217].

Expert commentary

For the treatment of ocular fungal infections, one should keep in mind that there are no ideal antifungal agents or administration regimens. As such, the pharmacological treatment should be chosen considering disease-specific conditions, possible side effects, and the drug's ability to reach the site of infection and achieve therapeutic concentrations.

Few significant advances have been reached in treating ophthalmic fungal infections. The major problem encountered is the poor water solubility of most of the drugs. Larger molecule sizes (>500 Da) also restrict their intrinsic permeability. Although some formulations with enhanced drug solubility can be easily prepared using cyclodextrins, polymers or suitable surfactants, these solutions may suffer from the drawback of having low residence time at the ocular surface and being

rapidly drained. Owing to short residence time and corneal impermeability to most compounds, the topical treatment is often not effective.

Polymeric nano- and microparticles could therefore be a suitable alternative. Despite not yet being applied for the treatment of fungal keratitis, promising results have been shown for other targets. It is believed that polymeric particles containing antifungal agents could be used to increase drug availability, reduce toxicity and prolong interval of administration. Similarly, liposomes and SLNs offer sustained drug delivery with low toxicity. However, the former represents a challenge when considering large-scale production, whereas the latter has a lower drug-loading capacity. NLCs have emerged as a novel delivery system that could incorporate the advantages of those lipid-based delivery systems and overcome their limitations. In the last few years, NLC formulations have been successfully prepared for the incorporation of antifungal drugs but have not yet been fully employed in the treatment of ocular diseases. It is expected that in the next few years more studies will be performed using polymeric particles and lipid-based systems for the ocular route, resulting in more efficient therapeutic options.

Five-year view
It is expected that in the near future more knowledge will be available on the corneal permeation profile of novel antifungal agents. From that point it is also expected that novel nanomedicines would be applied for the ocular delivery of antifungal agents, leading to higher bioavailability and fewer adverse effects.

Key issues

- Fungal keratitis occurs throughout the world, but the overall incidence tends to be higher in tropical and subtropical regions. The most frequent fungi causing keratitis worldwide are *Fusarium* (incidence 20–83.6%), *Aspergillus* (incidence 16.5–75%) and *Candida* (incidence 1–63%).
- Fungal keratitis risk factors include trauma, contact lens wear, prolonged use of topical corticosteroids, immunosuppressive diseases, previous eye surgery and chronic eye surface diseases.
- The pharmacological approach of management of fungal keratitis involves the administration of antifungal agents. However, owing to the physiologic constraints of the eye, only a few drugs present adequate bioavailability.
- Classical antifungal drugs act mainly in the fungal cell membrane. The two most commonly used classes are the polyenes and the azoles. The first includes nathamycin and amphotericin B, while the second includes miconazole, ketoconazole, fluconazole and itraconazole.
- Novel drugs have been developed with the aim of solving classical antifungal therapy problems. The novel azole voriconazole is more potent but leads to some adverse effects. The new class echinocandins possesses a broad spectrum but the compounds belonging to this class will probably have low corneal penetration owing to their high molecular weights.
- Nanoparticulated systems containing antifungal drugs could be used to prolong drug delivery and reduce toxicity.
- Liposomes containing antifungal drugs may be useful for intraocular administration. They can minimize some of the adverse side effects encountered by these administration routes and prolong drug residence time, increasing therapeutic effectiveness when no other options are available.
- Antifungal agents have successfully been incorporated into solid lipid nanoparticles and nanostructured lipid carriers, but have not yet been fully employed in the treatment of ocular diseases.

References

Papers of special note have been highlighted as:
- of interest
- of considerable interest

1   Shukla PK, Kumar M, Keshava GB. Mycotic keratitis: an overview of diagnosis and therapy. *Mycoses* 51(3), 183–199 (2008).
- **A recent overview of fungal keratitis aspects. Diagnosis methods and the most common therapies are presented.**

2   Thomas PA. Current perspectives on ophthalmic mycoses. *Clin. Microbiol. Rev.* 16(4), 730–797 (2003).

3   Galarreta DJ, Tuft SJ, Ramsay A, Dart JK. Fungal keratitis in London: microbiological and clinical evaluation. *Cornea* 26(9), 1082–1086 (2007).

4   Bharathi MJ, Ramakrishnan R, Vasu S, Meenakshi R, Palaniappan R. Epidemiological characteristics and laboratory diagnosis of fungal keratitis. A three-year study. *Indian J. Ophthalmol.* 51(4), 315–321 (2003).

5   Carvalho ACA, Ruthes HI, Maia M *et al.* Ceratite fúngica no estado do Paraná- Brasil: aspectos epidemiológicos, etiológicos e diagnósticos. *Rev. Iberoam. Micol.* 18(2), 76–78 (2001).

6   Gopinathan U, Sharma S, Garg P *et al.* Review of epidemiological features, microbiological diagnosis and treatment outcome of microbial keratitis: experience of over a decade. *Indian J. Ophthalmol.* 57(4), 273–279 (2009).

7   Panda A, Satpathy G, Nayak N, Kumar S, Kumar A. Demographic pattern, predisposing factors and management of ulcerative keratitis: evaluation of one thousand unilateral cases at a tertiary care centre. *Clin. Experiment. Ophthalmol.* 35(1), 44–50 (2007).

8   Perez-Balbuena A, Vanzzini-Rosano V, Valadez-Virgen JD, Campos-Moller X. *Fusarium* keratitis in Mexico. *Cornea* 28(6), 626–630 (2009).

9   Tanure MA, Cohen EJ, Sudesh S, Rapuano CJ, Laibson PR. Spectrum of fungal keratitis at Wills Eye Hospital, Philadelphia, Pennsylvania. *Cornea* 19(3), 307–312 (2000).

10   Tuft SJ, Tullo AB. Fungal keratitis in the United Kingdom 2003–2005. *Eye* 23(6), 1308–1313 (2009).

11   Ritterband DC, Seedor JA, Shah MK, Koplin RS, McCormick SA. Fungal keratitis at the New York Eye and Ear Infirmary. *Cornea* 25(3), 264–267 (2006).

12   Xie LX, Zhong WX, Shi WY, Sun SY. Spectrum of fungal keratitis in north China. *Ophthalmology* 113(11), 1943–1948 (2006).

13   Leck AK, Thomas PA, Hagan M *et al.* Aetiology of suppurative corneal ulcers in Ghana and south India, and epidemiology of fungal keratitis. *Br. J. Ophthalmol.* 86(11), 1211–1215 (2002).

14   Gopinathan U, Garg P, Fernandes M, Sharma S, Athmanathan S, Rao GN. The epidemiological features and laboratory results of fungal keratitis: a 10-year review at a referral eye care center in south India. *Cornea* 21(6), 555–559 (2002).

15   Ibrahim MM, Vanini R, Ibrahim FM *et al.* Epidemiologic aspects and clinical outcome of fungal keratitis in southeastern Brazil. *Eur. J. Ophthalmol* 19(3), 355–361 (2009).

16   Thew MRJ, Todd B. Fungal keratitis in far north Queensland, Australia. *Clin. Experiment. Ophthalmol.* 36(8), 721–724 (2008).

17   Sirikul T, Prabriputaloong T, Smathivat A, Chuck RS, Vongthongsri A. Predisposing factors and etiologic diagnosis of ulcerative keratitis. *Cornea* 27(3), 283–287 (2008).

18   Liesegang TJ, Forster RK. Spectrum of microbial keratitis in South Florida. *Am. J. Ophthalmol.* 90(1), 38–47 (1980).

19   Upadhyay MP, Karmacharya PC, Koirala S *et al.* Epidemiologic characteristics, predisposing factors, and etiologic diagnosis of corneal ulceration in Nepal. *Am. J. Ophthalmol.* 111(1), 92–99 (1991).

20   Khairallah SH, Byrne KA, Tabbara KF. Fungal keratitis in Saudi Arabia. *Doc. Ophthalmol.* 79(3), 269–276 (1992).

21   Hagan M, Wright E, Newman M, Dolin P, Johnson G. Causes of suppurative keratitis in Ghana. *Br J. Ophthalmol.* 79(11), 1024–1028 (1995).

22   Hall LR, Lass JH, Diaconu E, Strine ER, Pearlman E. An essential role for antibody in neutrophil and eosinophil recruitment to the cornea: B cell-deficient (microMT)

mice fail to develop Th2-dependent, helminth-mediated keratitis. *J. Immunol.* 163(9), 4970–4975 (1999).

23   Kaur IP, Rana C, Singh H. Development of effective ocular preparations of antifungal agents. *J. Ocul. Pharmacol. Ther.* 24(5), 481–493 (2008).

24   FlorCruz NV, Peczon I. Medical interventions for fungal keratitis. *Cochrane Database Syst. Rev.* 1, CD004241 (2008).

25   Srinivasan M. Fungal keratitis. *Curr. Opin. Ophthalmol.* 15(4), 321–327 (2004).

26   Thomas PA. Mycotic keratitis – an underestimated mycosis. *J. Med. Vet. Mycol.* 32(4), 235–256 (1994).

27   Wang LY, Sun ST, Jing Y, Han L, Zhang HM, Yue J. Spectrum of fungal keratitis in central China. *Clin. Experiment. Ophthalmol.* 37(8), 763–771 (2009).

28   Godoy P, Cano J, Gene J, Guarro J, Hofling-Lima AL, Lopes CA. Genotyping of 44 isolates of Fusarium solani, the main agent of fungal keratitis in Brazil. *J. Clin. Microbiol.* 42(10), 4494–4497 (2004).

29   Thomas PA. Fungal infections of the cornea. *Eye (Lond.)* 17(8), 852–862 (2003).

30   Salera CM, Tanure MAG, Lima WTM, Trindade FC, Moreira JA. Perfil das ceratites fúngicas no Hospital São Geraldo Belo Horizonte – MG. *Arq. Bras. Oftalmol.* 65, 9–13 (2002).

31   Alfonso EC, Miller D, Cantu-Dibildox J, O'Brien TP, Schein OD. Fungal keratitis associated with non-therapeutic soft contact lenses. *Am. J. Ophthalmol.* 142(1), 154–155 (2006).

32   Klotz SA, Penn CC, Negvesky GJ, Butrus SI. Fungal and parasitic infections of the eye. *Clin. Microbiol. Rev.* 13(4), 662–685 (2000).

33   Klotz SA, Au YK, Misra RP. A partial-thickness epithelial defect increases the adherence of *Pseudomonas aeruginosa* to the cornea. *Invest. Ophthalmol. Vis. Sci.* 30(6), 1069–1074 (1989).

34   Ahearn DG, Zhang S, Stulting RD *et al.* *Fusarium* keratitis and contact lens wear: facts and speculations. *Med. Mycol.* 46(5), 397–410 (2008).

35   Rao SK, Lam PTH, Li EYM, Yuen HKL, Lam DSC. A case series of contact lens-associated *Fusarium* keratitis in Hong Kong. *Cornea* 26(10), 1205–1209 (2007).

36   Chang DC, Grant GB, O'Donnell K *et al.* Multistate outbreak of *Fusarium* keratitis associated with use of a contact lens solution. *JAMA* 296(8), 953–963 (2006).

37   Gaujoux T, Chatel MA, Chaumeil C, Laroche L, Borderie VM. Outbreak of contact lens-related *Fusarium* keratitis in France. *Cornea* 27(9), 1018–1021 (2008).

38   Khor WB, Aung T, Saw SM *et al.* An outbreak of *Fusarium* keratitis associated with contact lens wear in Singapore. *JAMA* 295(24), 2867–2873 (2006).

39   Gorscak JJ, Ayres BD, Bhagat N *et al.* An outbreak of *Fusarium* keratitis associated with contact lens use in the northeastern United States. *Cornea* 26(10), 1187–1194 (2007).

40   Granados JM, Puerto N, Carrilero MJ. Efficiency of voriconazole in fungal keratitis caused by *Candida albicans*. *Arch. Soc. Esp. Oftalmol.* 79(11), 565–568 (2004).

41   Krishnan S, Manavathu EK, Chandrasekar PH. *Aspergillus flavus*: an emerging non-*fumigatus Aspergillus* species of significance. *Mycoses* 52(3), 206–222 (2009).

42   Mendicute J, Orbegozo J, Ruiz M, Saiz A, Eder F, Aramberri J. Keratomycosis after cataract surgery. *J. Cataract Refract. Surg.* 26(11), 1660–1666 (2000).

43   Nayak N. Fungal infections of the eye: laboratory diagnosis and treatment. *Nepal. Med. Coll. J.* 10(1), 48–63 (2008).

44   Behrens-Baumann W. Topical antimycotics in ophthalmology. *Ophthalmologica* 211(Suppl. 1), 33–38 (1997).

45   Ti SE, Scott A, Janardhanan P, Tan DTH. Therapeutic keratoplasty for advanced suppurative keratitis. *Am. J. Ophthalmol.* 143(5), 755–762 (2007).

46   Yuan XY, Wilhelmus KR, Matoba AY, Alexandrakis G, Miller D, Huang AJW. Pathogenesis and outcome of *Paecilomyces* keratitis. *Am. J. Ophthalmol.* 147(4), 691–696 (2009).

47   Banitt M, Berenbom A, Shah M, Buxton D, Milman T. A case of polymicrobial keratitis violating an intact lens capsule. *Cornea* 27(9), 1057–1061 (2008).

48   Bashir G, Hussain W, Rizvi A. *Bipolaris hawaiiensis* keratomycosis and endophthalmitis. *Mycopathologia* 167(1), 51–53 (2009).

49   Gupta AK, Sauder DN, Shear NH. Antifungal agents: an overview. Part I. *J. Am. Acad. Dermatol.* 30(5 Pt 1), 677–698 (1994).

50   Smith EB. History of antifungals. *J. Am. Acad. Dermatol.* 23(4 Pt 2), 776–778 (1990).

51   Luke RG, Boyle JA. Renal effects of amphotericin B lipid complex. *Am. J. Kidney Dis.* 31(5), 780–785 (1998).

52   Kauffman CA, Carver PL. Antifungal agents in the 1990s. Current status and future developments. *Drugs* 53(4), 539–549 (1997).

53   te Welscher YM, ten Napel HH, Balagué MM *et al*. Natamycin blocks fungal growth by binding specifically to ergosterol without permeabilizing the membrane. *J. Biol. Chem.* 283(10), 6393–6401 (2008).

54   Georgopapadakou NH, Walsh TJ. Human mycoses: drugs and targets for emerging pathogens. *Science* 264(5157), 371–373 (1994).

55   O'Day DM, Ray WA, Head WS, Robinson RD. Influence of the corneal epithelium on the efficacy of topical antifungal agents. *Invest. Ophthalmol. Vis. Sci.* 25(7), 855–859 (1984).

56   Loh AR, Hong K, Lee S, Mannis M, Acharya NR. Practice patterns in the management of fungal corneal ulcers. *Cornea* 28(8), 856–859 (2009).

57   Lalitha P, Shapiro BL, Srinivasan M *et al*. Antimicrobial susceptibility of *Fusarium*, *Aspergillus*, and other filamentous fungi isolated from keratitis. *Arch. Ophthalmol.* 125(6), 789–793 (2007).

58   Xie LX, Zhai HL, Zhao J, Sun SY, Shi WY, Dong XG. Antifungal susceptibility for common pathogens of fungal keratitis in Shandong Province, China. *Am. J. Ophthalmol.* 146(2), 260–265 (2008).

59   O'Day DM, Head WS, Robinson RD, Clanton JA. Corneal penetration of topical amphotericin B and natamycin. *Curr. Eye Res.* 5(11), 877–882 (1986).

60   Torrado JJ, Espada R, Ballesteros MP, Torrado-Santiago S. Amphotericin B formulations and drug targeting. *J. Pharm. Sci.* 97(7), 2405–2425 (2008).

61   DiDomenico B. Novel antifungal drugs. *Curr. Opin. Microbiol.* 2(5), 509–515 (1999).

62   Al Assiri A, Al Jastaneiah S, Al Khalaf A, Al Fraikh H, Wagoner MD. Late-onset donor-to-host transmission of *Candida glabrata* following corneal transplantation. *Cornea* 25(1), 123–125 (2006).

63   Levy J, Benharroch D, Peled N, Lifshitz T. *Blastoschizomyces capitatus* keratitis and melting in a corneal graft. *Can. J. Ophthalmol.* 41(6), 772–774 (2006).

64   Park KA, Ahn K, Chung ES, Chung TY. *Pichia anomala* fungal keratitis. *Cornea* 27(5), 619–620 (2008).

65   Khoo SH, Bond J, Denning DW. Administering amphotericin B: a practical approach. *J. Antimicrob. Chemother.* 33(2), 203–213 (1994).

66   Kaushik S, Ram J, Brar GS, Jain AK, Chakraborti A, Gupta A. Intracameral amphotericin B: initial experience in severe keratomycosis. *Cornea* 20(7), 715–719 (2001).

67   Yilmaz S, Ture M, Maden A. Efficacy of intracameral amphotericin B injection in the management of refractory keratomycosis and endophthalmitis. *Cornea* 26(4), 398–402 (2007).

68   Garcia-Valenzuela E, Song CD. Intracorneal injection of amphothericin B for recurrent fungal keratitis and endophthalmitis. *Arch. Ophthalmol.* 123(12), 1721–1723 (2005).

69   Gupta A, Srinivasan R, Kaliaperumal S, Saha I. Post-traumatic fungal endophthalmitis – a prospective study. *Eye* 22(1), 13–17 (2008).

70   Koltin Y, Hitchcock CA. The search for new triazole antifungal agents. *Curr. Opin. Chem. Biol.* 1(2), 176–182 (1997).

71   Foster CS, Stefanyszyn M. Intraocular penetration of miconazole in rabbits. *Arch. Ophthalmol.* 97(9), 1703–1706 (1979).

72   Anderson RL, Carroll TF, Harvey JT, Myers MG. *Petriellidium* (*Allescheria*) *boydii* orbital and brain abscess treated with intravenous miconazole. *Am. J. Ophthalmol.* 97(6), 771–775 (1984).

73   Fitzsimons R, Peters AL. Miconazole and ketoconazole as a satisfactory first-line treatment for keratomycosis. *Am. J. Ophthalmol.* 101(5), 605–608 (1986).

74   Foster CS. Miconazole therapy for keratomycosis. *Am. J. Ophthalmol.* 91(5), 622–629 (1981).

75   Ishibashi Y, Matsumoto Y, Takei K. The effects of intravenous miconazole on fungal keratitis. *Am. J. Ophthalmol.* 98(4), 433–437 (1984).

76   Zaidman GW. Miconazole corneal toxicity. *Cornea* 10(1), 90–91 (1991).

77   Galan A, Martin-Suarez EM, Gallardo JM, Molleda JM. Clinical findings and progression of 10 cases of equine ulcerative keratomycosis (2004–2007). *Equine Vet. Educ.* 21(5), 236–242 (2009).

78   Myers DA, Lsaza R, Ben Shlomo G, Abbott J, Plummer CE. Fungal keratitis in a gopher tortoise (*Gopherus polyphemus*). *J. Zoo Wildl. Med.* 40(3), 579–582 (2009).

79   Andrew SE, Brooks DE, Smith PJ, Gelatt KN, Chmielewski NT, Whittaker CJG. Equine ulcerative keratomycosis: visual outcome and ocular survival in 39 cases (1987–1996). *Equine Vet. J.* 30(2), 109–116 (1998).

80   Hemady RK, Chu W, Foster CS. Intraocular penetration of ketoconazole in rabbits. *Cornea* 11(4), 329–333 (1992).

81   Therese KL, Bagyalakshmi R, Madhavan HN, Deepa P. *In vitro* susceptibility testing by agar dilution method to determine the minimum inhibitory concentrations of amphotericin B, fluconazole and ketoconazole against ocular fungal isolates. *Indian J. Med. Microbiol.* 24(4), 273–279 (2006).

82   Marangon FB, Miller D, Giaconi JA, Alfonso EC. *In vitro* investigation of voriconazole susceptibility for keratitis and endophthalmitis fungal pathogens. *Am. J. Ophthalmol.* 137(5), 820–825 (2004).

83   Manikandan P, Varga J, Kocsube S *et al*. Mycotic keratitis due to *Aspergillus nomius*. *J. Clin. Microbiol.* 47(10), 3382–3385 (2009).

84   Kredics L, Varga J, Kocsube S *et al*. Infectious keratitis caused by *Aspergillus tubingensis*. *Cornea* 28(8), 951–954 (2009).

85   Dursun D, Fernandez V, Miller D, Alfonso EC. Advanced *Fusarium* keratitis progressing to endophthalmitis. *Cornea* 22(4), 300–303 (2003).

86   Torres MA, Mohamed J, Cavazos-Adame H, Martinez LA. Topical ketoconazole for fungal keratitis. *Am. J. Ophthalmol.* 100(2), 293–298 (1985).

87   Zhang JJ, Wang LY, Gao CF, Zhang L, Xia HY. Ocular pharmacokinetics of topically-applied ketoconazole solution containing hydroxypropyl β-cyclodextrin to rabbits. *J. Ocul. Pharmacol. Ther.* 24(5), 501–506 (2008).

88   Behrens-Baumann W, Klinge B, Ruchel R. Topical fluconazole for experimental *Candida* keratitis in rabbits. *Br. J. Ophthalmol.* 74(1), 40–42 (1990).

89   Chung PC, Lin HC, Hwang YS *et al*. Paecilomyces lilacinus scleritis with secondary keratitis. *Cornea* 26(2), 232–234 (2007).

90   Sonego-Krone S, Sanchez-Di Martino D, Ayala-Lugo R *et al*. Clinical results of topical fluconazole for the treatment of filamentous fungal keratitis. *Graefes Arch. Clin. Exp. Ophthalmol.* 244(7), 782–787 (2006).

91   Holgado S, Luna JD, Juarez CP. Postoperative *Candida* keratitis treated successfully with fluconazole. *Ophthalmic Surg.* 24(2), 132 (1993).

92   Sodhi PK, Mehta DK. Fluconazole in managment of ocular infections due to *Curvularia*. *Ann. Ophthalmol.* 35(1), 68–72 (2003).

93 Yilmaz S, Maden A. Severe fungal keratitis treated with subconjunctival fluconazole. *Am. J. Ophthalmol.* 140(3), 454–458 (2005).

94 Su CY, Lin CP, Wang HZ *et al.* Intraocular use of fluconazole in the management of ocular fungal infection. *Kaohsiung J. Med. Sci.* 15(4), 218–225 (1999).

95 Avunduk AM, Beuerman RW, Warnel ED, Kaufman HE, Greer D. Comparison of efficacy of topical and oral fluconazole treatment in experimental *Aspergillus* keratitis. *Curr. Eye Res.* 26(2), 113–117 (2003).

96 Akler ME, Vellend H, McNeely DM, Walmsley SL, Gold WL. Use of fluconazole in the treatment of candidal endophthalmitis. *Clin. Infect. Dis.* 20(3), 657–664 (1995).

97 Abbasoglu OE, Hosal BM, Sener B, Erdemoglu N, Gursel E. Penetration of topical fluconazole into human aqueous humor. *Exp. Eye Res.* 72(2), 147–151 (2001).

98 Li L, Wang Z, Li R, Luo S, Sun X. *In vitro* evaluation of combination antifungal activity against *Fusarium* species isolated from ocular tissues of keratomycosis patients. *Am. J. Ophthalmol.* 146(5), 724–728 (2008).

99 Fungtomc JC, Minassian B, Huczko E, Kolek B, Bonner DP, Kessler RE. *In vitro* antifungal and fungicidal spectra of a new pradimicin derivative, BMS-181184. *Antimicrob. Agents Chemother.* 39(2), 295–300 (1995).

100 Gonzalez G. *In vitro* activities of isavuconazole against opportunistic filamentous and dimorphic fungi. *Med. Mycol.* 47(1), 71–76 (2009).

101 Li LH, McCarthy P, Hui SW. High-efficiency electrotransfection of human primary hematopoietic stem cells. *FASEB J.* 15(3), 586–588 (2001).

102 Qiu WY, Yao YF, Zhu YF *et al.* Fungal spectrum identified by a new slide culture and *in vitro* drug susceptibility using Etest in fungal keratitis. *Curr. Eye Res.* 30(12), 1113–1120 (2005).

103 Savani DV, Perfect JR, Cobo LM, Durack DT. Penetration of new azole compounds into the eye and efficacy in experimental *Candida* endophthalmitis. *Antimicrob. Agents Chemother.* 31(1), 6–10 (1987).

104 Saracli MA, Erdem U, Gonlum A, Yildiran ST. *Scedosporium apiospermum* keratitis treated with itraconazole. *Med. Mycol.* 41(2), 111–114 (2003).

105 Kalavathy CM, Parmar P, Kaliamurthy J *et al.* Comparison of topical itraconazole 1% with topical natamycin 5% for the treatment of filamentous fungal keratitis. *Cornea* 24(4), 449–452 (2005).

106 Martinez-Ramos M, Claros B, Vale-Oviedo MA *et al.* Effect of the vehicle on the topical itraconazole efficacy for treating corneal ulcers caused by *Aspergillus fumigatus*. *Clin. Experiment. Ophthalmol.* 36(4), 335–338 (2008).

107 Yavas GF, Oztuerk F, Kusbeci T *et al.* Antifungal efficacy of voriconazole, itraconazole and amphotericin B in experimental *Fusarium solani* keratitis. *Graefes Arch. Clin. Experiment. Ophthalmol.* 246(2), 275–279 (2008).

108 Abdel-Rahman SM, Nahata MC. Oral terbinafine: a new antifungal agent. *Ann. Pharmacother.* 31(4), 445–456 (1997).

109 Schelenz S, Goldsmith DJA. *Aspergillus* endophthalmitis: an unusual complication of disseminated infection in renal transplant patients. *J. Infect.* 47(4), 336–343 (2003).

110 Tu EY, Park AJ. Recalcitrant *Beauveria bassiana* keratitis: confocal microscopy findings and treatment with posaconazole (noxafil). *Cornea* 26(8), 1008–1010 (2007).

111 Amiel H, Chohan AAB, Snibson GR, Vajpayee R. Atypical fungal sclerokeratitis. *Cornea* 27(3), 382–383 (2008).

112 Vyzantiadis TA, Kioumi A, Papadakis E *et al.* Rhino-cerebral zygomycosis resistant to antimycotic treatment: a case report. *Mycoses* 52(1), 87–90 (2009).

113 Sabo JA, Abdel-Rahman SM. Voriconazole: a new triazole antifungal. *Ann. Pharmacother.* 34(9), 1032–1043 (2000).

• **Currently available information on the pharmacology, pharmacokinetics, efficacy, adverse effects, drug interactions and dosage guidelines of voriconazole is presented.**

114 Johnson LB, Kauffman CA. Voriconazole: a new triazole antifungal agent. *Clin. Infect. Dis.* 36(5), 630–637 (2003).

115 Ruhnke M, Schmidt-Westhausen A, Trautmann M. *In vitro* activities of voriconazole (UK-109,496) against fluconazole-susceptible and -resistant *Candida albicans* isolates from oral cavities of patients with human immunodeficiency virus infection. *Antimicrob. Agents Chemother.* 41(3), 575–577 (1997).

116 Bunya VY, Hammersmith KM, Rapuano CJ, Ayres BD, Cohen EJ. Topical and oral voriconazole in the treatment of fungal keratitis. *Am. J. Ophthalmol.* 143(1), 151–153 (2007).

117 Creti A, Esposito V, Bocchetti M *et al.* Voriconazole curative treatment for *Acremonium* species keratitis developed in a patient with concomitant *Staphylococcus aureus* corneal infection: a case report. *In Vivo* 20(1), 169–171 (2006).

118 Durand ML, Kim IK, D'Amico DJ *et al.* Successful treatment of *Fusarium* endophthalmitis with voriconazole and *Aspergillus* endophthalmitis with voriconazole plus caspofungin. *Am. J. Ophthalmol.* 140(3), 552–554 (2005).

119 Freda R. Use of oral voriconazole as adjunctive treatment of severe cornea fungal infection: case report. *Arq. Bras. Oftalmol.* 69(3), 431–434 (2006).

120 Kramer M, Kramer MR, Blau H *et al.* Intravitreal voriconazole for the treatment of endogenous *Aspergillus* endophthalmitis. *Ophthalmology* 113(7), 1184–1186 (2006).

121 Al Badriyeh D, Leung L, Davies GE, Stewart K, Kong D. Successful salvage treatment of *Scedosporium apiospermum* keratitis with topical voriconazole after failure of natamycin. *Ann. Pharmacother.* 43(6), 1139–1142 (2009).

122 Tu EY. *Alternaria* keratitis: clinical presentation and resolution with topical fluconazole or intrastromal voriconazole and topical caspofungin. *Cornea* 28(1), 116–119 (2009).

123 Hariprasad SM, Mieler WF, Holz ER *et al.* Determination of vitreous, aqueous, and plasma concentration of orally administered voriconazole in humans. *Arch. Ophthalmol.* 122(1), 42–47 (2004).

124 Nulens E, Eggink C, Rijs AJ, Wesseling P, Verweij PE. Keratitis caused by *Scedosporium apiospermum* successfully treated with a cornea transplant and voriconazole. *J. Clin. Microbiol.* 41(5), 2261–2264 (2003).

125 Klont RR, Eggink CA, Rijs AJMM, Wesseling P, Verweij PE. Successful treatment of *Fusarium* keratitis with cornea transplantation and topical and systemic voriconazole. *Clin. Infect. Dis.* 40(12), E110–E112 (2005).

126 Vemulakonda GA, Hariprasad SM, Mieler WF, Prince RA, Shah GK, Van Gelder RN. Aqueous and vitreous concentrations following topical administration of 1% voriconazole in humans. *Arch. Ophthalmol.* 126(1), 18–22 (2008).

127 Lau D, Leung L, Ferdinands M *et al.* Penetration of 1% voriconazole eye drops into human vitreous humour: a prospective, open-label study. *Clin. Experiment. Ophthalmol.* 37(2), 197–200 (2009).

128 Al Badriyeh D, Leung L, Roydhouse T *et al*. Prospective open-label study of the administration of two-percent voriconazole eye drops. *Antimicrob. Agents Chemother.* 53(7), 3153–3155 (2009).

129 Clode AB, Davis JL, Salmon J, Michau TM, Gilger BC. Evaluation of concentration of voriconazole in aqueous humor after topical and oral administration in horses. *Am. J. Vet. Res.* 67(2), 296–301 (2006).

130 Shen YC, Wang MY, Wang CY *et al*. Clearance of intravitreal voriconazole. *Invest. Ophthalmol.Vis. Sci.* 48(5), 2238–2241 (2007).

131 Hector RF. Compounds active against cell walls of medically important fungi. *Clin. Microbiol. Rev.* 6(1), 1–21 (1993).

132 Sucher AJ, Chahine EB, Balcer HE. Echinocandins: the newest class of antifungals. *Ann. Pharmacother.* 43(10), 1647–1657 (2009).

133 Cleary JD. Echinocandins: pharmacokinetic and therapeutic issues. *Curr. Med. Res. Opin.* 25(7), 1741–1750 (2009).

134 Goldblum D, Frueh BE, Sarra GM, Katsoulis K, Zimmerli S. Topical caspofungin for treatment of keratitis caused by *Candida albicans* in a rabbit model. *Antimicrob. Agents Chemother.* 49(4), 1359–1363 (2005).

135 Ozturk F, Yavas GF, Kusbeci T *et al*. Efficacy of topical caspofungin in experimental *Fusarium* keratitis. *Cornea* 26(6), 726–728 (2007).

136 Vorwerk CK, Tuchen S, Streit F, Binder L, Hofmuller W, Behrens-Baumann W. Aqueous humor concentrations of topically administered caspofungin in rabbits. *Ophthalmic Res.* 41(2), 102–105 (2009).

137 Tawara S, Ikeda F, Maki K *et al*. *In vitro* activities of a new lipopeptide antifungal agent, FK463, against a variety of clinically important fungi. *Antimicrob. Agents Chemother.* 44(1), 57–62 (2000).

138 Temesgen Z, Barreto J, Vento S. Micafungin: the newest echinocandin. *Drugs Today* 45(6), 469–478 (2009).

139 Hiraoka T, Kaji Y, Wakabayashi T, Nanbu PN, Okamoto F, Oshika T. Comparison of micafungin and fluconazole for experimental *Candida* keratitis in rabbits. *Cornea* 26(3), 336–342 (2007).

140 Matsumoto Y, Dogru M, Goto E, Fujishima H, Tsubota K. Successful topical application of a new antifungal agent, micafungin, in the treatment of refractory fungal corneal ulcers: report of three cases and literature review. *Cornea* 24(6), 748–753 (2005).

141 Hiraoka T, Wakabayashi T, Kaji Y *et al*. Toxicological evaluation of micafungin ophthalmic solution in rabbit eyes. *J. Ocul. Pharmacol. Ther.* 21(2), 149–156 (2005).

142 Barar J, Javadzadeh AR, Omidi Y. Ocular novel drug delivery: impacts of membranes and barriers. *Expert Opin. Drug Deliv.* 5(5), 567–581 (2008).

143 Schalenbourg A, Leys A, de Courten C, Coutteel C, Herbort CP. Corticosteroid-induced central serous chorioretinopathy in patients with ocular inflammatory disorders. *Klin. Monbl. Augenheilkd.* 219(4), 264–267 (2002).

144 Cunningham MA, Edelman JL, Kaushal S. Intravitreal steroids for macular edema: the past, the present, and the future. *Surv. Ophthalmol.* 53(2), 139–149 (2008).

145 Del Amo EM, Urtti A. Current and future ophthalmic drug delivery systems. A shift to the posterior segment. *Drug Discov. Today* 13(3–4), 135–143 (2008).

146 Moshfeghi DM, Kaiser PK, Scott IU *et al*. Acute endophthalmitis following intravitreal triamcinolone acetonide injection. *Am. J. Ophthalmol.* 136(5), 791–796 (2003).

147 Kredics L, Varga J, Kocsube S *et al*. Case of keratitis caused by *Aspergillus tamarii*. *J. Clin. Microbiol.* 45(10), 3464–3467 (2007).

148 Wolf EJ, Braunstein A, Shih C, Braunstein RE. Incidence of visually significant pseudophakic macular edema after uneventful phacoemulsification in patients treated with nepafenac. *J. Cataract Refract. Surg.* 33(9), 1546–1549 (2007).

149 Yasueda S, Inada K, Matsuhisa K, Terayama H, Ohtori A. Evaluation of ophthalmic suspensions using surface tension. *Eur. J. Pharm. Biopharm.* 57(2), 377–382 (2004).

150 Bourlais CL, Acar L, Zia H, Sado PA, Needham T, Leverge R. Ophthalmic drug delivery systems: recent advances. *Prog. Retin. Eye Res.* 17(1), 33–58 (1998).

151 Giobbia M, Rossi MC, Conti E *et al*. Post-traumatic *Aspergillus fumigatus* keratitis. *J. Mycol. Med.* 12(1), 32–33 (2002).

152 Suzuki T, Hori N, Miyake T, Hori Y, Mochizuki K. Keratitis caused by a rare fungus, *Malassezia restricta*. *Jpn. J. Ophthalmol.* 51(4), 292–294 (2007).

153 Kovacs K, Stampf G, Klebovich I, Antal I, Ludanyi K. Aqueous solvent system for the solubilization of azole compounds. *Eur. J. Pharm. Sci.* 36(2–3), 352–358 (2009).

154 Esclusa-Diaz MT, Guimaraens-Mendez M, Perez-Marcos MB, Vila-Jato JL, Torres-Labandeira JJ. Characterization and *in vitro* dissolution behaviour of ketoconazole/β- and 2-hydroxypropyl-β-cyclodextrin inclusion compounds. *Int. J. Pharm.* 143(2), 203–210 (1996).

155 Owens PK, Fell AF, Coleman MW, Berridge JC. Complexation of voriconazole stereoisomers with neutral and anionic derivatised cyclodextrins. *J. Incl. Phenom. Macrocycl. Chem.* 38(1–4), 133–151 (2000).

156 Cohen T, SauvageonMartre H, Brossard D *et al*. Amphotericin B eye drops as a lipidic emulsion. *Int. J. Pharm.* 137(2), 249–254 (1996).

157 Wang CH, Wang WT, Hsiue GH. Development of polyion complex micelles for encapsulating and delivering amphotericin B. *Biomaterials* 30(19), 3352–3358 (2009).

158 Brauninger GE, Shah DO, Kaufman HE. Direct physical demonstration of oily layer on tear film surface. *Am. J. Ophthalmol.* 73(1), 132–134 (1972).

159 Nanjawade BK, Manvi FV, Manjappa AS. *In situ*-forming hydrogels for sustained ophthalmic drug delivery. *J. Control. Release* 122(2), 119–134 (2007).

160 Hirose H, Terasaki H, Awaya S, Yasuma T. Treatment of fungal corneal ulcers with amphotericin B ointment. *Am. J. Ophthalmol.* 124(6), 836–838 (1997).

161 Delpalacio A, Perezblazquez E, Cuetara MS *et al*. Keratomycosis due to *Scedosporium-apiospermum*. *Mycoses* 34(11–12), 483–487 (1991).

162 Goldblum D, Frueh BE, Zimmerli S, Bohnke M. Treatment of postkeratitis fusarium endophthalmitis with amphotericin B lipid complex. *Cornea* 19(6), 853–856 (2000).

163 Manzouri B, Vafidis GC, Wyse RK. Pharmacotherapy of fungal eye infections. *Expert Opin. Pharmacother.* 2(11), 1849–1857 (2001).

164 Prausnitz MR, Noonan JS. Permeability of cornea, sclera, and conjunctiva: a literature analysis for drug delivery to the eye. *J. Pharm. Sci.* 87(12), 1479–1488 (1998).

165 Sahoo SK, Dilnawaz F, Krishnakumar S. Nanotechnology in ocular drug delivery. *Drug Discov. Today* 13(3–4), 144–151 (2008).

166 Wang W, Sasaki H, Chien DS, Lee VHL. Lipophilicity influence on conjunctival drug penetration in the pigmented rabbit: a comparison with corneal penetration. *Curr. Eye Res.* 10(6), 571–579 (1991).

167 Lockman PR, Mumper RJ, Khan MA, Allen DD. Nanoparticle technology for drug delivery across the blood–brain barrier. *Drug Dev. Ind. Pharm.* 28(1), 1–13 (2002).

168 Date AA, Joshi MD, Patravale VB. Parasitic diseases: liposomes and polymeric nanoparticles versus lipid nanoparticles. *Adv. Drug Deliv. Rev.* 59(6), 505–521 (2007).

169 Couvreur P, Vauthier C. Nanotechnology: intelligent design to treat complex disease. *Pharm. Res.* 23(7), 1417–1450 (2006).

170 Hsu J. Drug delivery methods for posterior segment disease. *Curr. Opin. Ophthalmol.* 18(3), 235–239 (2007).

171 Calvo P, VilaJato JL, Alonso MJ. Evaluation of cationic polymer-coated nanocapsules as ocular drug carriers. *Int. J. Pharm.* 153(1), 41–50 (1997).

172 Bin Choy Y, Park JH, Prausnitz MR. Mucoadhesive microparticles engineered for ophthalmic drug delivery. *J. Phys. Chem. Solids* 69(5–6), 1533–1536 (2008).

173 Chiang CH, Tung SM, Lu DW, Yeh MK. *In vitro* and *in vivo* evaluation of an ocular delivery system of 5-fluorouracil microspheres. *J. Ocul. Pharmacol. Ther.* 17(6), 545–553 (2001).

174 Espuelas MS, Legrand P, Loiseau PM, Bories C, Barratt G, Irache JM. *In vitro* antileishmanial activity of amphotericin B loaded in poly(ε-caprolactone) nanospheres. *J. Drug Target.* 10(8), 593–599 (2002).

175 Espuelas MS, Legrand P, Irache JM *et al.* Poly(ε-caprolacton) nanospheres as an alternative way to reduce amphotericin B toxicity. *Int. J. Pharm.* 158(1), 19–27 (1997).

176 Sangeetha S, Venkatesh DN, Adhiyaman R, Santhi K, Suresh B. Formulation of sodium alginate nanospheres containing amphotericin B for the treatment of systemic candidiasis. *Trop. J. Pharm. Res.* 6(1), 653–659 (2007).

177 Tiyaboonchai W, Limpeanchob N. Formulation and characterization of amphotericin B–chitosan–dextran sulfate nanoparticles. *Int. J. Pharm.* 329(1–2), 142–149 (2007).

178 Italia JL, Yahya MM, Singh D, Kumar MNVR. Biodegradable nanoparticles improve oral bioavailability of amphotericin B and show reduced nephrotoxicity compared to intravenous Fungizone. *Pharm. Res.* 26(6), 1324–1331 (2009).

• **Amphotericin B entrapped into poly(lactide-co-glycolide) nanoparticles was shown to improve the oral bioavailability and minimize the adverse effects observed in classical systemic amphotericin B therapy.**

179 Ren TB, Xu N, Cao CH *et al.* Preparation and therapeutic efficacy of polysorbate-80-coated amphotericin B/PLA-b-PEG nanoparticles. *J. Biomater. Sci. Polym. Ed.* 20(10), 1369–1380 (2009).

•• **Amphotericin B/poly(lactic acid)-β-poly(ethylene glycol) nanoparticles coated with polysorbate 80 have been efficiently produced for brain targeting.**

180 Amaral AC, Bocca AL, Ribeiro AM *et al.* Amphotericin B in poly(lactic-co-glycolic acid) (PLGA) and dimercaptosuccinic acid (DMSA) nanoparticles against paracoccidioidomycosis. *J. Antimicrob. Chemother.* 63(3), 526–533 (2009).

181 Peng HS, Liu XJ, Lv GX *et al.* Voriconazole into PLGA nanoparticles: improving agglomeration and antifungal efficacy. *Int. J. Pharm.* 352(1–2), 29–35 (2008).

182 de Assis DN, Mosqueira VCF, Vilela JMC, Andrade MS, Cardoso VN. Release profiles and morphological characterization by atomic force microscopy and photon correlation spectroscopy of (99m)technetium-fluconazole nanocapsules. *Int. J. Pharm.* 349(1–2), 152–160 (2008).

183 Rivera PA, Martinez-Oharriz MC, Rubio M, Irache JM, Espuelas S. Fluconazole encapsulation in PLGA microspheres by spray-drying. *J. Microencapsul.* 21(2), 203–211 (2004).

184 Choi C, Jung H, Nam JP, Park Y, Jang MK, Nah JW. Preparation and characterization of deoxycholic acid-conjugated low molecular weight water-soluble chitosan nanoparticles for hydrophobic antifungal agent carrier. *Polymer (Korea)* 33(4), 389–395 (2009).

185 Ebrahim S, Peyman GA, Lee PJ. Applications of liposomes in ophthalmology. *Surv. Ophthalmol.* 50(2), 167–182 (2005).

186 Mehanna MM, Elmaradny HA, Samaha MW. Ciprofloxacin liposomes as vesicular reservoirs for ocular delivery: formulation, optimization, and *in vitro* characterization. *Drug Dev. Ind. Pharm.* 35(5), 583–593 (2009).

187 Bejjani RA, Jeanny JC, Bochot A, Behar-Cohen F. The use of liposomes as intravitreal drug delivery system. *J. Fr. Ophtalmol.* 26(9), 981–985 (2003).

188 Alghadyan AA, Peyman GA, Khoobehi B, Milner S, Liu KR. Liposome-bound cyclosporine: clearance after intravitreal injection. *Int. Ophthalmol.* 12(2), 109–112 (1988).

189 Barza M, Stuart M, Szoka F Jr. Effect of size and lipid composition on the pharmacokinetics of intravitreal liposomes. *Invest. Ophthalmol. Vis. Sci.* 28(5), 893–900 (1987).

190 Liu KR, Peyman GA, Khoobehi B, Alkan H, Fiscella R. Intravitreal liposome-encapsulated trifluorothymidine in a rabbit model. *Ophthalmology* 94(9), 1155–1159 (1987).

191 Gupta SK, Velpandian T, Dhingra N, Jaiswal J. Intravitreal pharmacokinetics of plain and liposome-entrapped fluconazole in rabbit eyes. *J. Ocul. Pharmacol. Ther.* 16(6), 511–518 (2000).

192 Gupta SK, Dhingra N, Velpandian T, Jaiswal J. Efficacy of fluconazole and liposome entrapped fluconazole for *C. albicans* induced experimental mycotic endophthalmitis in rabbit eyes. *Acta Ophthalmol. Scand.* 78(4), 448–450 (2000).

193 Habib FS, Fouad EA, Abdel-Rahman MS, Fathalla D. Liposomes as an ocular delivery system for fluconazole: *in vivo* study. *Bull. Pharmaceut. Sci.* 31, 249–263 (2008).

•• **Fluconazole liposomal formulations administered in an animal model of *Candida keratitis* demonstrated more complete healing in a shorter time than plain fluconazole solution. The frequency of instillation could be reduced for the liposomal formulation.**

194 Kaji Y, Yamamoto E, Hiraoka T, Oshika T. Toxicities and pharmacokinetics of subconjunctival injection of liposomal amphotericin B. *Graefes Arch. Clin. Experiment. Ophthalmol.* 247(4), 549–553 (2009).

195 Carrillo-Munoz AJ, Quindos G, Tur C *et al.* Comparative *in vitro* antifungal activity of amphotericin B lipid complex, amphotericin B and fluconazole. *Chemotherapy* 46(4), 235–244 (2000).

196 Goldblum D, Rohrer K, Frueh BE, Theurillat R, Thormann W, Zimmerli S. Corneal concentrations following systemic administration of amphotericin B and its lipid preparations in a rabbit model. *Ophthalmic Res.* 36(3), 172–176 (2004).

197 Anaissie E, Paetznick V, Proffitt R, Adler-Moore J, Bodey GP. Comparison of the *in vitro* antifungal activity of free and liposome-encapsulated amphotericin B. *Eur. J. Clin. Microbiol. Infect. Dis.* 10(8), 665–668 (1991).

198 De Logu A, Fadda AM, Pellerano ML, Diana G, Schivo ML. Prevention by L-α-phosphatidylcholine of antifungal activity *in vitro* of liposome-encapsulated imidazoles determined by using time–killing curves. *Int. J. Antimicrob. Agents* 15(1), 43–48 (2000).

199 Lee JW, Amantea MA, Francis PA *et al.* Pharmacokinetics and safety of a unilamellar liposomal formulation of amphotericin B (AmBisome) in rabbits. *Antimicrob. Agents Chemother.* 38(4), 713–718 (1994).

200 Szoka FC Jr, Milholland D, Barza M. Effect of lipid composition and liposome size on toxicity and *in vitro* fungicidal activity of liposome-intercalated amphotericin B. *Antimicrob. Agents Chemother.* 31(3), 421–429 (1987).

201 Wasan KM, Brazeau GA, Keyhani A, Hayman AC, Lopez-Berestein G. Roles of liposome composition and temperature in distribution of amphotericin B in serum lipoproteins. *Antimicrob. Agents Chemother.* 37(2), 246–250 (1993).

202 Wasan KM, Rosenblum MG, Cheung L, Lopez-Berestein G. Influence of lipoproteins on renal cytotoxicity and antifungal activity of amphotericin B. *Antimicrob. Agents Chemother.* 38(2), 223–227 (1994).

203 Singh M, Singh MP, Maiti SN, Gandhi A, Micetich RG, Atwal H. Preparations of liposomal fluconazole and their *in vitro* antifungal activity. *J. Microencapsul.* 10(2), 229–236 (1993).

204 Hopfer RL, Mills K, Mehta R *et al. In vitro* antifungal activities of amphotericin B and liposome-encapsulated amphotericin B. *Antimicrob. Agents Chemother.* 25(3), 387–389 (1984).

205 Martins S, Sarmento B, Ferreira DC, Souto EB. Lipid-based colloidal carriers for peptide and protein delivery: liposomes versus lipid nanoparticles. *Int. J. Nanomedicine* 2(4), 595–607 (2007).

206 Wissing SA, Kayser O, Muller RH. Solid lipid nanoparticles for parenteral drug delivery. *Adv. Drug Deliv. Rev.* 56(9), 1257–1272 (2004).

207 Muller RH, Radtke M, Wissing SA. Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) in cosmetic and dermatological preparations. *Adv. Drug Deliv. Rev.* 54(Suppl. 1), S131–S155 (2002).

208 Muller RH, Radtke M, Wissing SA. Nanostructured lipid matrices for improved microencapsulation of drugs. *Int. J. Pharm.* 242(1–2), 121–128 (2002).

209 Souto EB, Wissing SA, Barbosa CM, Muller RH. Development of a controlled release formulation based on SLN and NLC for topical clotrimazole delivery. *Int. J. Pharm.* 278(1), 71–77 (2004).

210 Souto EB, Muller RH. Investigation of the factors influencing the incorporation of clotrimazole in SLN and NLC prepared by hot high-pressure homogenization. *J. Microencapsul.* 23(4), 377–388 (2006).

211 Souto EB, Muller RH. Rheological and *in vitro* release behaviour of clotrimazole-containing aqueous SLN dispersions and commercial creams. *Pharmazie* 62(7), 505–509 (2007).

212 Souto EB, Muller RH. SLN and NLC for topical delivery of ketoconazole. *J. Microencapsul.* 22(5), 501–510 (2005).

213 Souto EB, Muller RH. The use of SLN (R) and NLC (R) as topical particulate carriers for imidazole antifungal agents. *Pharmazie* 61(5), 431–437 (2006).

214 Mukherjee S, Ray S, Thakur RS. Design and evaluation of itraconazole loaded solid lipid nanoparticulate system for improving the antifungal therapy. *Pak. J. Pharm. Sci.* 22(2), 131–138 (2009).

215 Bhalekar MR, Pokharkar V, Madgulkar A, Patil N, Patil N. Preparation and evaluation of miconazole nitrate-loaded solid lipid nanoparticles for topical delivery. *AAPS PharmSciTech* 10(1), 289–296 (2009).

216 Sanna V, Gavini E, Cossu M, Rassu G, Giunchedi P. Solid lipid nanoparticles (SLN) as carriers for the topical delivery of econazole nitrate: *in vitro* characterization, *ex vivo* and *in vivo* studies. *J. Pharm. Pharmacol.* 59(8), 1057–1064 (2007).

217 Cavalli R, Gasco MR, Chetoni P, Burgalassi S, Saettone MF. Solid lipid nanoparticles (SLN) as ocular delivery system for tobramycin. *Int. J. Pharm.* 238(1–2), 241–245 (2002).

218 Kalam MA, Sultana Y, Ali A, Aqil M. Gatifloxacin-loaded solid lipid nanoparticles for topical ocular delivery. *J. Pharm. Pharmacol.* 61, A75 (2009).

219 Niu M, Shi K, Sun Y, Wang J, Cui F. Preparation of CyA-loaded solid lipid nanoparticles and application on ocular preparations. *J. Drug Deliv. Sci. Technol.* 18(4), 293–297 (2008).

220 Attama AA, Reichl S, Muller-Goymann CC. Sustained release and permeation of timolol from surface-modified solid lipid nanoparticles through bioengineered human cornea. *Curr. Eye Res.* 34(8), 698–705 (2009).

221 Xu Y, Pang GR, Zhao DQ *et al. In vitro* activity of thimerosal against ocular pathogenic fungi. *Antimicrob. Agents Chemother.* 54(1), 536–539 (2010).

222 Iqbal NJ, Boey A, Park BJ, Brandt ME. Determination of *in vitro* susceptibility of ocular *Fusarium* spp. isolates from keratitis cases and comparison of Clinical and Laboratory Standards Institute M38-A2 and E test methods. *Diagn. Microbiol. Infect. Dis.* 62(3), 348–350 (2008).

223 Lalitha P, Vijaykumar R, Prajna NV, Fothergill AW. *In vitro* natamycin susceptibility of ocular isolates of *Fusarium* and *Aspergillus* species: comparison of commercially formulated natamycin eye drops to pharmaceutical-grade powder. *J. Clin. Microbiol.* 46(10), 3477–3478 (2008).

224 Oday DM, Ray WA, Robinson RD, Head WS. Correlation of *in vitro* and *in vivo* susceptibility of *Candida albicans* to amphotericin-B and natamycin. *Invest. Ophthalmol. Vis. Sci.* 28(3), 596–603 (1987).

225 Ikeda F, Saika T, Sato Y *et al.* Antifungal activity of micafungin against *Candida* and *Aspergillus* spp. isolated from pediatric patients in Japan. *Med. Mycol.* 47(2), 145–148 (2009).

226 Barasch A, Griffin AV. Miconazole revisited: new evidence of antifungal efficacy from laboratory and clinical trials. *Future Microbiol.* 3(3), 265–269 (2008).

227 Isham N, Ghannoum MA. Determination of MICs of aminocandin for *Candida* spp. and filamentous fungi. *J. Clin. Microbiol.* 44(12), 4342–4344 (2006).

Websites

301 Singh D, Verma A. Keratitis, Fungal: Treatment & Medication
http://emedicine.medscape.com/article/1194167-treatment

302 Drug Bank
www.drugbank.ca

303 SRC PhysProp Database
www.syrres.com