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Antifungals and their use in veterinary ophthalmology

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Mycotic infections can be divided into those that result in superficial disease and those that result in systemic disease. Factors determining the ocular tissue predilection and type of disease caused by a particular fungal organism include fungal species characteristics and host predisposition (large ocular surface area [1], prominent eyes [1], local or systemic immunoprotection [2], and geographic location [3]). Additionally, superficial corneal disease may be exacerbated by exposure to vegetative material (hay, grasses, shavings, and straw) and dust [2], whereas concurrent illness or immunocompromise may predispose to systemic disease. Although horses are most commonly affected with ocular surface fungal infections (keratomycosis), dogs and cats are predisposed to internal ocular infections (anterior uveitis, chorioretinitis, retinal detachment, and secondary glaucoma) and systemic disease. The aim of this article is to provide a general outline of the current knowledge of antifungal agents in veterinary ophthalmology. Few agents are approved by the US Food and Drug Administration (FDA) for the treatment of companion animal fungal infections. Therefore, extralabel use of products approved for human beings and compounding of specific agents may be necessary, particularly in the treatment of keratomycoses.

The biology of fungi

The fungal kingdom comprises yeasts, molds, fungal rusts, and mushrooms [2,4]. Fungi are heterotrophic, nonmotile, multicellular, eukaryotic organisms with a definitive cell wall and no chlorophyll [2,4]. Within the fungal kingdom, pathogens can be divided into three groups: multinucleate

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septate (distinct divisions between cellular elements) filamentous fungi, nonseptate filamentous fungi, and yeasts [5]. Dimorphic species are typified by manifesting a single morphism under specific environmental conditions (eg, the yeast form in the vertebrate host tissue and a hyphal/mycelial form in vitro) [4]. Examples of dimorphic organisms include *Blastomyces dermatitidis*, *Coccioides immitis*, and *Histoplasma capsulatum*. The mold forms give rise to spores that germinate and produce slender, filamentous, branched hyphae that may be septate or nonseptate [2,4]. The mycelial form, present in soil or decaying organic material, is composed of a collection of hyphae. Mycelia produce infective spores that are responsible for inoculating vertebrate tissues [4]. Soil is considered the true reservoir for many fungal organisms because it is the site for essential phases of development [4].

Each hypha has a surrounding cell wall made up of chitins, glucans, and mannans. Chitin is a structural polysaccharide (*N*-acetylglucosamine) that is absent in the vertebrate systems [2]. The cell wall contains complex polysaccharides and glycoproteins, and the nuclear envelope is a porous double membrane containing abundant RNA [4]. Within the cell wall, the plasma membrane contains ergosterol, a cell membrane sterol that is frequently targeted by antifungal agents [5]. Ergosterol regulates permeability of the cell membrane and the activity of membrane-bound enzymes [6,7]. Chitin synthesis is stimulated by low ergosterol content and inhibited by a high concentration. Patchy chitin formation occurs as a result of ergosterol biosynthesis inhibition [6].

Ocular manifestations of fungal infection

Keratomycosis

Keratomycosis is a serious sight-threatening disease in all species; however, species differences exist. It is most commonly reported in the horse and is rare in dogs and cats [8–10]; therefore, information and experience regarding veterinary treatment are based predominantly on literature reports of equine keratomycoses. Fungi are a normal component of the equine conjunctival microflora [1,11,12] but become pathogenic after corneal injury [2,13]. Topical corticosteroids exacerbate keratomycosis [13–18]. After invasion of compromised corneal epithelium, fungi migrate to the deep stroma by hyphal "tip" elongation. Proliferation occurs toward the glycosaminoglycan (GAG)-rich environment of the deep stroma adjacent to Descemet's membrane. This allows the organism to escape natural host ocular surface immune responses.

Fungal pathogens or budding yeasts that infect the equine cornea are usually opportunistic [14]. A study conducted over a 10-year period found isolates from 13 different genera and 20 different species [19]. *Aspergillus* [14] and *Fusarium* [14] organisms are the most frequently isolated filamentous fungi [19,20]. Others, including *Penicillium*, *Cladosporium*, *Mucor*, *Rhizopus*,

Candida, *Cylindrocarpon*, *Pseudallescheria*, and dematiaceous *Alternaria* and *Culveria* organisms, are occasionally isolated [14].

Diagnosis of keratomycosis should be based on history, results of ophthalmic examination, and cytologic findings. Specific antifungal therapy can be initiated after isolation of pathogenic fungi or may be empiric based on known prevalence of unique ocular fungi in specific geographic areas [3]. Frequent topical applications of antibiotics and antifungals in combination with aggressive daily corneal epithelial scrapings are reported to have successful outcomes [21]. Surgical intervention, including lamellar keratectomy with conjunctival grafting or penetrating keratoplasty, is indicated in cases of middle to deep stromal involvement. Surgical management of keratomycoses is discussed elsewhere [15,22].

Ocular manifestations of systemic mycoses

Unlike horses, small companion animals are most commonly affected with systemic infections with ocular manifestations, such as uveitis, chorioretinitis, retinal detachment, and secondary glaucoma [4]. The reported frequency of ocular manifestations in dogs is 20% to 50% [23]. In addition to systemic administration of antifungal medication(s), specific therapy for anterior uveitis or secondary glaucoma is often required [23–26]. The four most common fungal organisms that cause systemic mycoses with ocular manifestations include *B dermatitidis*, *C immitis*, *Cryptococcus neoformans*, and *H capsulatum*. Limited therapeutic options exist for systemic mycoses. Most drugs are not approved by the FDA for use in dogs and cats. Drawbacks of systemic antifungal therapy include expense and toxicity of certain drug regimens.

Classes of antifungal drugs

Antifungals may be categorized into chemical groups based on structure and mode of action. Classes of antifungal agents used in veterinary ophthalmology include polyenes, azoles, allylamines, lipopeptides, and pyrimidines. Other substances used for local control of fungal infections include silver sulfadiazine (SSD) and povidone–iodine. The mode of action may be divided into those that target fungal cell membrane synthesis (azoles, allylamines, lipopeptides, and chitin synthase inhibitors) or function (polyenes and lipopeptides) and those that target nucleic acid synthesis (pyrimidines and SSD). The classes of antifungal agents most commonly used in the treatment of equine keratomycoses are the azoles (miconazole, itraconazole, and fluconazole) and the polyene agent natamycin. The most commonly used classes for treatment of canine and feline systemic mycoses are the polyenes (amphotericin B [AMB] and lipid-complexed AMB) and the azoles (ketoconazole, itraconazole, and fluconazole).

Pathogenic mechanisms, growth requirements, and antifungal drug susceptibility vary considerably among fungal pathogens [27,28]. Initial treatment of patients with suspected fungal keratitis may be empiric, and choice of therapy is defined by previous clinical experience, available drugs, and financial constraints [14]. Because of risk of toxicity associated with systemic administration of antifungal agents, development of safer broad-spectrum antifungal antibiotics with greater potency is ongoing. Progress in development of new agents has been slow [29,30], because fungi are eukaryotic organisms; therefore, agents that inhibit protein, RNA, or DNA biosynthesis in fungi have a greater potential for host toxicity [31,32]. In addition, the incidence of life-threatening fungal infections has been reported as being too low to warrant aggressive product development [33]. The range of antifungal drugs available for systemic use has been limited to a few agents, the most effective of which (AMB) is highly nephrotoxic [34].

Polyenes

Polyene macrolide antibiotics, the first major group of antifungal agents to be discovered [35], are a group of structurally similar products of *Streptomyces* spp. Since the discovery of nystatin (previously known as fungicidin) in 1950 [36], more than 60 members of the class have been described [35]; however, only AMB, natamycin, and nystatin are of practical interest for the treatment of ocular fungal infections [35,37]. Polyenes are unstable insoluble chemicals that are poorly absorbed, with limited penetration into the eye, cerebrospinal fluid (CSF), and joint capsule [35,37–39]. Natamycin, the only topical antifungal agent approved for use on the eye and the only commercially available drug for treatment of ocular fungal keratitis, penetrates the intact cornea poorly [40]. Gastrointestinal absorption of AMB is minimal; therefore, parenteral administration is recommended [41]. After intravenous administration, AMB is highly protein bound and redistributes from the blood to the tissues; however, central nervous system (CNS) penetration is poor. Less toxic formulations of AMB and nystatin have been developed with the use of lipid incorporation, which has been reported to enhance efficacy in treating ocular disease [37,42]. The exact metabolic pathways of AMB are not known; however, a biphasic elimination occurs with an initial half-life of 2 to 4 days, followed by a terminal half-life of 15 days [43], with minimal elimination occurring via the hepatic and renal pathways [41].

Mechanism of action

Polyene antibiotics are fungicidal [5,44] with several proposed mechanisms that have not been fully elucidated. The most widely accepted mechanism of action is membrane barrier disruption. Susceptible cells (fungi, protozoa, and mammalian cells) selectively and irreversibly bind polyenes to ergosterol, the principal sterol in the plasma membrane of fungi [35,45,46]. Binding of

polyenes to ergosterol results in altered membrane permeability [46] and inhibition of cytochrome P-450 and the electron transport chain [39]. As a consequence of increased membrane permeability, leakage of potassium and essential cytoplasmic metabolites [47–49] is followed by impairment of concentrating mechanisms [46] and loss of ammonium, inorganic phosphate, low-molecular-weight carboxylic acids, phosphate esters, nucleotides, and proteins [46,50–52]. Cation leakage secondarily inhibits aerobic and anaerobic glycolysis and respiration [46]. Replacement of lost intracellular cations by hydrogen ions results in a decrease in internal pH that, when less than 5.5, causes lysosome disruption, autolysis, and cell death [46]. The susceptibility or resistance of fungi to these drugs is determined by the relative amounts of sterol in the cell membrane; the most susceptible cells have high sterol/phospholipid ratios, and the least susceptible have low ratios [46].

Spectrum

Polyene antibiotics have the broadest spectrum of antifungal activity of any of the clinically available agents [44]. Specific filamentous fungi include most species of *Aspergillus* [5,18,35,53–58], yeast (especially *Candida* sp [5,44,46,55,59,60]) except for *Candida lusitaniae* [44,46]), Zygomycetes (*Mucor* and *Rhizopus* [5]), *Cryptococcus* [46,55], *Blastomyces*, *Coccidioides*, *Histoplasma* [5], *Paracoccidioides brasiliensis*, *Sporotrichum* sp, *Torulopsis,* (*Candida*) *glabrata* [5,44], and *Sporothrix schenckii* [5,46]. *Trichosporon beigelii* and *Pseudoallescheria boydii* are often resistant [33,61–63]. Activity against *Prototheca* [5], *Curvularia*, *Alternaria*, *Wangiella*, and *Cladosporium* varies [64]. In addition, natamycin is also active against *Trichophyton* sp, *Acremonium* sp, and *P boydii* [37], whereas *Aspergillus* species are frequently resistant [37]. Nystatin is active against *Cryptococcus* [5,46], *Prototheca* [5], some filamentous fungi [5], some dimorphic fungi [5], and *Trichophyton* [46]. The liposomal formulation of nystatin seems to be as active as free nystatin [65]. Systemic doses and ophthalmic preparations of the commonly used polyene antibiotics are listed in Table 1.

Adverse effects

The plasma membranes of mammalian cells contain sterols in the form of cholesterol; therefore, all polyenes are toxic to mammalian cells to some degree [35]. This toxicity may be decreased by the higher affinity of the polyenes for ergosterol in fungal cells than for cholesterol in mammalian cells [33,44,52,66,67].

Amphotericin B. Nephrotoxicity after parenteral administration of AMB remains the greatest adverse effect associated with the polyene antibiotics [44,55,68–70]. The proposed mechanism of action is renal vasoconstriction with a subsequent reduction in glomerular filtration rate or direct renal epithelial cell toxicity [40]. The occurrence of nephrotoxicity may be reduced by pretreatment with 0.9% saline intravenously [71] or concurrent

Table 1
Systemic doses and ophthalmic preparations of antifungal drugs

	Systemic route (IV, SQ, PO)	Ophthalmic route
AMB (Fungizone; Apothecon)	IV: Canine: dose, IV[a], 3 times per week (cumulative dose) Blastomycosis: 0.5 mg/kg (4–6 mg/kg) Histoplasmosis: 0.25–0.5 mg/kg (5–10 mg/kg) Cryptococcus: 0.25–0.5 mg/kg (4–10 mg/kg)[b] Coccidiomycosis: 0.4–0.5 mg/kg (8–11 mg/kg) Feline: dose, IV[a], 3 times per week (cumulative dose) Blastomycosis: 0.25 mg/kg (4 mg/kg) Cryptococcosis: 0.1–0.5 mg/kg (4–10 mg/kg)[b] Histoplasmosis: 0.25–0.5 mg/kg (4–8 mg/kg) SQ: Canine: 0.5–0.8 mg/kg/d Feline: 0.5–0.8 mg/kg/d in 400 mL of 5% dextrose or 0.45% saline[c] (cumulative dose: 4–10 mg/kg)	Topical: 5 mg/mL = 0.5% colloidal suspension 1. Inject 10 mL of sterile water or 5% dextrose solution into the bottle with dry AMB, 50 mg; AMB is incompatible with saline or other electrolyte solutions 2. Shake until transparent, refrigerate, do not filter Subconjunctival: 0.8–2.0 mg Intravitreal: 5 μg Intracameral: 25 μg in 0.05 mL of distilled H_2O 7.4 μg in 0.1 mL of distilled H_2O
AMB liposomal preparation (AmBisome; Fujisawa) (Albecet; The Liposome Co.)	IV: Canine: Blastomycosis/cryptococcosis: Albecet 1 mg/kg, q48 hours (cumulative dose: 8–12 mg/kg) No reported experience with other lipid-based formulations in animals (eg, cholesteryl, Amphotec; colloidal, Amphocil; liposomal, AmBiosome), but recommended human IV dosage might be appropriate in dogs	
Natamycin (Natacyn; Alcon)		Topical: 1 drop of 5% suspension, q1–2 hours initially, then decreasing to 6–8 times daily after a few days for equine fungal keratomycosis

Nystatin (Mycostatin; Apothecon)	Too toxic for parenteral administration Not absorbed after oral administration	Topical: Eyedrop suspension of pure nystain, 100,000 IU, in 5 mL of sterile isotonic, isohydric phosphate buffer solution (20 mL of NaH_2PO_4 (8.0 g in 1000 mL of H_2O) and 80 mL NaH_2PO_4 (9.47 g in 1000 mL of H_2O)) are mixed; NaCl (0.44 g) is added, and the solution is sterilized
Miconazole (Monistat; Janssen)	IV: 20 mg/kg of BW	Topical: Equine: 1% IV miconazole (Monistat, 10 mg/mL) q4–6 hours Miconazole 2% dermatologic creams (Conofite) q6–12 hours for up to 3 weeks without adverse effects Subconjunctival: 1% IV solution, not recommended for use in horses Subtenons: 5–10 mg has been used in horses Intracameral: miconazole, 0.1 mg, with 0.1 mL of sterile 0.9% NaCl
Ketoconazole (Nizoral; Janssen)	PO: Canine: dose[f], q12 hours × months or 30 day after resolution Blastomycosis: 5–15 mg/kg[d] q12 hours, 3 months Histoplasmosis: 10 mg/kg q12–24 hours, 3 months Cryptococcosis: 10 mg/kg[de] q12–24 hours, 3 months Coccidiomycosis: 5–10 mg/kg q12 hours, 8–12 months Feline: Blastomycosis: 10 mg/kg q12 hours[d], 3 months Histoplasmosis: 10 mg/kg q12–24 hours, 3 months Cryptococcosis: 10 mg/kg[de] q12–24 hours, 3 months Coccidiomycosis: 50 mg/cat q12–24 hours, 12 months	Topical: 1%–2% solution from the oral tablets

(*continued on next page*)

Table 1 (*continued*)

	Systemic route (IV, SQ, PO)	Ophthalmic route
Itraconazole (Sporonox; Janssen)	PO: Canine[f]	Topical:
	Blastomycosis: 5 mg/kg/d, 2 months 5 mg/kg q12 hours for 5 days, then q24 hours[h]	Equine: 1% itraconazole with 30% dimethyl sulfoxide ointment q4–6 hours = 7.9 μg/g corneal concentration of itraconazole, mean duration of treatment = 34.6 days
	Histoplasmosis: 5 mg/kg q12 hours, 4–6 months[h]	
	Coccidiomycosis: 5 mg/kg q12 hours, 12 months	
	Feline[f]	
	Blastomycosis: 5–10 mg/kg q12 hours, 2 months	
	Histoplasmosis: 5 mg/kg q12 hours, 4–6 months[h]	
	Cryptococcosis: 5–10 mg/kg q12 hours, 6–10 months[g] 20 mg/kg q24 hours for 6–10 months[g] 25–50 mg/cat q12–24 hours up to 12 months	
Fluconazole (UK 49,858) (Diflucan; Pfizer)	PO: Canine: dose, frequency, duration	Topical: 0.2% saline-based injectable solution
	Blastomycosis: 5 mg/kg q12 hours, 2 months[g]	Subconjunctival: 0.2% saline-based injectable solution
	Histoplasmosis: 2.5–5 mg/kg q12–24 hours, 4–6 months[g]	Intravitreal: 100 μg/0.1 mL
	Cryptococcosis: 5–15 mg/kg q12–24 hours, 6–12 months[g]	
	Coccidiomycosis: 5 mg/kg q12 hours, up to 12 months	
	Feline: dose, frequency, duration	
	Histoplasmosis: 2.5–5 mg/kg q12–24 hours, 4–6 months[g]	
	Cryptococcosis: 5–15 mg/kg q12–24 hours, 6–12 months[g]	
	Coccidiomycosis: 25–50 mg/cat q12–24 hours, up to 12 months	
Econazole (Spectazole; Ortho Dermatological)		Topical: 1% solution 1% dermatologic cream

Clotrimazole (Mycelex; Bayer) (Canesten; Bayer)		Topical: concentrations greater than 10 μg/mL are fungicidal, 1%–2% dermatologic cream or the topical preparation in 1% arachis oil has been shown to be useful
Thiabendazole (Mintezol; Merck)	PO: 25 mg/kg/d	Topical: 1%–4% solution of oral worming mixture and artificial tears or water
Silver sulfadiazine (Silvadene; Marion)		Topical: 1% cream, q4 hours
Povidone–iodine (Betadine 10%; Purdue Frederick)		Topical: 0.2% (1:50 solution)
Flucytosine (Ancobon)	SQ: Canine: Cryptococcosis: 50 mg/kg q6–8 hours, 1–2 months Feline: Cryptococcosis: 25–50 mg/cat q6–12 hours, 1–9 months PO: Canine: Cryptococcosis: 50 mg/kg q6 hours[dg] Feline: Cryptococcosis: 200 mg/kg/d divided q6 hours[dg]	Topical: 1% solution

Abbreviations: AMB, amphotericin B; BW, body weight; IV, intravenous; PO, orally; SQ, subcutaneous.

[a] Measure serum blood urea nitrogen (BUN) and urine sediment for evidence of kidney damage before administration of each dose. On day 1, the total dose is diluted to 5% dextrose 20 mL and 5 mL is given; if no acute anaphylactic response develops in 1 minute, the remainder is given over 45 seconds. Thereafter the total dose is given over 1 minute in 5% dextrose 20 mL for 6–12 weeks, 3 times a week. If BUN exceeds 50 mg/dL, the dose is discontinued or reduced 25%–50% until BUN falls below 40 mg/dL. Treatment of dogs and cats should be continued for a minimum of 60 days or at least 1 month beyond clinical or radiographic resolution of clinical signs.

[b] May be used in combination with flucytosine.

[c] 2–3 times weekly.

[d] With AMB initially.

[e] After AMB/flucytosine for 4–6 months.

[f] Take with food.

[g] For 30 days beyond resolution.

[h] For 60 days beyond resolution.

Data from Refs. [5,24,35,41,44,63,73,76,78,85,101–104,119,132,143–146].

administration of AMB with 5% dextrose over 1 to 5 hours [41,70]. The risk of nephrotoxicity is reduced approximately 8 to 10 times by the use of lipid-complexed AMB drugs [40,44,72,73]; however, these drugs are significantly more expensive [41]. Other adverse effects include anorexia, vomiting, hypokalemia, distal renal tubular acidosis, hypomagnesemia, thrombophlebitis, cardiac arrhythmias, nonregenerative anemia, and fever [40]. In an attempt to delay absorption and reduce toxicity, subcutaneous administration of higher doses of AMB has been used [74,75]. Topical, subconjunctival, or intraocular administration of AMB has been associated with local mild to severe irritation of the tissues [76,77], transitory and reversible iritis, and slight clouding of the lens without permanent sequelae [78]. If injected subconjunctivally, AMB should only be administered after appropriate dilution (see Table 1).

Natamycin. Topical administration of the 5% commercial natamycin suspension is nontoxic; however, low-grade inflammation and local irritation [5] may develop with prolonged use [35]. Toxicity prohibits subconjunctival or intraocular administration.

Nystatin. Occasional gastrointestinal upset has been reported when nystatin is administered systemically at high doses [40]. Nystatin may be compounded for topical ophthalmic use (see Table 1 for formulation).

Resistance

AMB resistance is rare and slow to develop [38]. Known cases of resistance to polyene antibiotics are not commonly reported (see references [79–83]).

Azoles

Azoles are the most widely used of the antifungal agents [37] and are divided into two subclasses: imidazoles and triazoles. Imidazoles include ketoconazole, miconazole, bifonazole, butoconazole, clotrimazole, econazole, enilconazole, fenticonazole, isoconazole, and parconazole. Triazoles include itraconazole, fluconazole, terconazole, and voriconazole. Azoles have activity against dermatophytes, *Cryptococcus*, *Blastomyces*, *Histoplasma*, *Aspergillus*, and *Candida* sp [40,84]. Azoles are water soluble with variable absorption after oral administration. Fluconazole, in contrast to itraconazole, is poorly protein bound with good CSF penetration. Renal elimination occurs after fluconazole administration, whereas itraconazole is eliminated in the bile. Fluconazole has better penetration into the eye and CNS than itraconazole [85] and should be considered with CNS involvement or in individuals refractory to treatment with AMB and itraconazole [41]. The spectrum of activity for fluconazole includes many species of *Candida* and *Cryptococcus* as well as dimorphic fungi, such as *Histoplasma*, *Blastomyces*,

and *Coccioides*; however, it has no efficacy against other fungi, such as *Aspergillus*.

Mechanism of action

Azoles are fungistatic agents used to treat ophthalmic mycoses [46]. Antifungal activity arises from a complex multimechanistic process initiated by the inhibition of ergosterol biosynthesis and the disturbance of lipid organization in cell membranes [86–88]. Specifically, azoles inhibit the fungal cytochrome P450 3A (CYP3A) enzyme lanosterol 14-α-demethylase. This prevents the conversion of lanosterol to ergosterol and disrupts the integrity of membrane-bound enzymes [5] and fungal cell membranes [46,89], which results in increased membrane permeability [35] and leakage of small ions, amino acids, and protein from the fungi [47]. Mammalian cells can be affected as well but can compensate for the temporary effects of the imidazoles by using dietary cholesterol [90]. Variations in affinity for the mammalian CYP3A receptor are the basis for drug–drug interactions with other CYP3A-dependent drugs, such as cyclosporine [91]. Azole inhibition of cytochrome function may also be the basis of interference with steroid biosynthesis [38]. These changes are reversible unless a high dose is administered [92].

Spectrum

Azoles have a broad spectrum of activity against yeasts and filamentous fungi [5,14,35,76,90,93,94], including *Coccidioides*, *Candida* [95], *Cryptococcus* [96], *Histoplasma* sp [48,97], *Paracoccidia*, *Paecilomyces lilacinus* [98,99], *Scopulariopsis brevicaulis* [100], *Aspergillus*, *Mucor*, *Fusarium* sp [12,46,101,102], *Sporothrix*, *Alternaria*, *Blastomyces*, *Sporotrichum* sp, and *Prototheca* [5]. Ketoconazole, considered to be the traditional treatment of choice for coccidiomycosis [41], is less effective than itraconazole, with lower response rates, higher relapse rates, and longer treatment periods in dogs [24,94]. Itraconazole has a similar spectrum of activity as fluconazole but includes *Aspergillus* and is not active against the *Zygomycetes* or *Fusarium* spp. Itraconazole is the treatment of choice for blastomycosis [41], with similar response and recurrence rates between dogs treated with AMB and itraconazole [103]. Itraconazole is also the treatment of choice for histoplasmosis [85,104]. In cats, itraconazole is more effective for histoplasmosis than ketoconazole, with fewer adverse effects [104]. The spectrum of thiabendazole includes *Cladosporium*, *Fusarium*, *Penicillium*, and *Phialophora*. Topical and systemic doses of the azole drugs are listed in Table 1. A limited spectrum of activity and resistance makes use of this drug uncommon.

Adverse effects

Topical formulations of the azoles (miconazole and econazole) are well tolerated [35,63]. Itraconazole (1%)/dimethyl sulfoxide (DMSO; 30%) ointment can be compounded with good results when applied to the cornea [40]. Toxicity is most commonly associated with intravenous administration

of miconazole because of the presence of the solubilizing agent required [5,47,49]. These effects are not seen with subcutaneous injections [105]. Oral administration of ketoconazole, itraconazole, and clotrimazole has been associated with inappetence and vomiting [5,35,90]. Skin changes have also been associated with azole administration and include pruritus, alopecia, reversible leukotrichia (ketoconazole) [90], and drug eruption (itraconazole and fluconazole) [75,106]. Cataracts [107] and hepatitis [90] have been associated with ketoconazole administration in dogs. Cortisol and testosterone suppression and increased progesterone concentrations in dogs have been associated with ketoconazole and itraconazole administration [5,108]; therefore, their use is contraindicated in pregnancy. Cats are more sensitive to ketoconazole and may develop anorexia, depression, weight loss, diarrhea, and fever [5].

Resistance

Known cases of azole resistance are uncommonly reported (see references [79–83]).

Pyrimidines (flucytosine, 5-fluorocytosine, 5-FC)

Flucytosine, a fluorine analogue of a normal cell constituent cytosine, is water soluble and weakly protein bound, with oral absorption that is unaffected by acid [5]. Flucytosine has excellent tissue penetration, including into the CNS.

Mechanism of action

After oral administration, flucytosine is taken up into the cell by a cytosine permease and is rapidly deaminated into 5-fluorouracil (5-FU) by cytosine deaminase, a fungus-specific enzyme [40,109]. 5-FU has fungicidal and fungistatic properties. 5-FU can be converted to 5-fluoro-dUMP, which indirectly inhibits DNA synthesis (-cidal), or 5-fluoroUTP, which disrupts protein synthesis after incorporation into RNA (-static) [109].

Spectrum

Flucytosine is principally active against strains of *Cryptococcus* and *Candida* [40] and is fungistatic against *Aspergillus flavus* and *A fumigatus* in laboratory animals (see Table 1 for doses) [76].

Adverse effects

Reported adverse effects include gastrointestinal disturbances (nausea, vomiting, and diarrhea), bone marrow suppression (anemia, leukopenia, and thrombocytopenia), cutaneous eruption and rash primarily seen on the scrotum and nasal planum (dogs), oral ulceration, and increased levels of hepatic enzymes. Flucytosine should be used with extreme caution in patients with renal impairment, preexisting bone marrow disease, hepatic disease,

hematologic diseases, or treatment with other bone marrow–suppressing drugs. A report of aberrant behavior and seizures in a cat without concurrent CNS infection has also been noted after flucytosine administration [40].

Resistance

Resistance to flucytosine can develop quite rapidly, especially when it is used alone against *Cryptococcus*. Recommendations for the use of AMB, a synergistic drug, in combination with flucytosine have been made for the treatment of cryptococcosis [40].

Lipopeptides (candins)

Candins, cyclic hexamers of amino acids with a lipophilic side chain, are semisynthetic derivatives of pneumocandin and were first isolated from *A nidulans* in 1974 [91]. Both the ring and the side chain are critical for antifungal activity, but variations in the location and composition of small side groups attached to ring members can either enhance or reduce antifungal activity [110–112]. Currently, these antifungal agents are investigational and undergoing clinical trials.

Mechanism of action

Candins inhibit the synthesis of 1,3-D-glucan, a glucose polysaccharide essential for the structural integrity of many fungal cell walls [5,113–115]. This inhibition causes structural damage to the cell wall and, ultimately, cell lysis [5,89].

Spectrum

Although the spectrum of activity of candins is still being defined, in vitro, they are known to include *Candida* spp, *Aspergillus* spp, *H capsulatum*, *B dermatitidis*, *Pneumocystis carinii*, some lesser known filamentous fungi, and possibly *C immitis* and *S schenckii* [89]. Fungi with only small amounts of (1,3)-B-glucan synthase are resistant (eg, *C neoformans*) [89]. Of the candins, echinocandin B and pneumocandin B are likely to be introduced into clinical use [5]. Pneumocandin is named for its activity against *Pneumocystis* and *Candida* [5]. These drugs, which can be used in combination with AMB, are active against fluconazole-resistant *Candida* as well as *Aspergillus* sp and other important filamentous fungi. They are not active against *B dermatitidis*, *C neoformans*, or *Fusarium* sp, because these species lack 1,3-D-glucan synthase [5].

Adverse effects

Candins have a relatively low toxicity compared with the polyenes [116,117]; however, reports in human literature describe thrombophlebitis, vein irritation, hypersensitivity reactions, and anaphylaxis.

Other

Silver sulfadiazine (sulfadiazine silver, Silvadene)

SSD was developed in 1968 by Fox [118], who combined the heavy metal antibacterial action of silver with the antibacterial and antifungal action of sulfadiazine [2,76,118–120]. SSD derives synergistic benefits from sulfonamides and heavy metals and functions as an organic base heavy metal release system by liberating silver [121]. SSD reacts rapidly by binding silver to the DNA of microorganisms and prevents the unzipping of the DNA helix, inhibiting replication [122]. Because no reduced silver is released within the tissues, the risk of argyrosis caused by silver deposition is minimal [123].

Spectrum. SSD has been found to be effective in *Fusarium* keratitis (human) [123], an organism to which miconazole is resistant [35,124]. In addition, SSD can be used alone or in combination with polyenes or imidazoles for topical treatment of fungal keratitis [120]. SSD has been found to be effective against most fungi, namely, *Aspergillus*, yeast-like fungi, and the brown dematiaceous filamentous fungi [123]. In a prospective, randomized, crossover trial conducted in human beings, 1% SSD was compared with 1% miconazole drops in 40 patients with keratomycosis [123]. Both drops were well tolerated, but SSD was superior to miconazole (80% versus 50% success rate) [123].

Adverse effects. SSD is an inexpensive medication with wide availability and without adverse effects [76,123]. It is most commonly used in horses with keratomycosis and seems to be well tolerated and effective, despite the fact that there are warnings on the package against use on the eye [40,76]. Conspicuous epithelial regeneration occurs in the presence of SSD [123], most likely related to the greater amount of DNA in mammalian cells, resulting in a ratio of SSD to microorganismal DNA that is high enough to prevent their division [122]. The corresponding ratio of SSD to epithelial cell DNA is too low to block epithelial cell regeneration (healing), which, in turn, is facilitated by the suppression of microorganismal proliferation [122].

Iodides

Systemic administration. The antimicrobial mechanism of iodides is unknown but may result from enhancement of the immune response of the host by spurring the halide–peroxide killing system of phagocytic cells [5]. AMB and imidazoles also affect the immune system in a similar manner [5]. Sodium iodide has traditionally been the treatment of choice in sporotrichosis before FDA approval of itraconazole [5]. Ketoconazole and sodium iodide administered together seem to have additive effects against sporotrichosis [5].

Topical iodine. Povidone–iodine is an antiseptic agent effective against bacteria, fungi, viruses, and protozoa [125] and can be used therapeutically on corneal ulcers [125]. Iodines are used to treat infectious keratitis in horses [76].

A 1:10 solution to contain a final concentration of 0.1% available povidone-iodine has been shown to be effective against *A niger* in rabbits [126]. Povidone–iodine has recently received attention as a topical antifungal agent, especially against *Fusarium* isolates [127].

Seven percent tincture of iodine is more irritating than organic iodine but penetrates the cornea more effectively and may serve as a stimulus for fibrovascular infiltrates [76]. Because of the presence of alcohol in tincture of iodine, corneal application should be performed with caution [76]. Inadvertent contact with the conjunctiva or palpebral margin may result in transient ocular irritation with conjunctival hyperemia and chemosis [76]. After initial application, iodine tincture may be reapplied in 24 to 48 hours and at 2- to 3-day intervals until neovascularization of the lesion is evident [76].

Chitin synthesis inhibitors

Because chitin is present in fungal cell walls but not mammalian cell walls, chitin synthesis inhibitors are being investigated as new antifungals [128,129]. Chitin synthesis inhibitors are fungicidal because of interference with fungal cell wall formation [39,41]. Chitin and glucan have been targeted directly or indirectly via the enzymes responsible for their synthesis [39,130,131]. Lufenuron (Program) is a chitin synthesis inhibitor approved for control of ectoparasites [41]. An extralabel use has been shown clinically to improve dogs affected with coccidiomycosis and to minimize treatment times [132,133]. Currently, research is being conducted to determine the pharmacokinetic activity of lufenuron in horses [134]. Preliminary studies have demonstrated that lufenuron is absorbed into the equine circulation after oral administration in a dose-dependent manner; however, lufenuron showed no effect on the rate of growth of *Aspergillus* spp in vitro [134]. These studies demonstrate a potential of use in the treatment of equine keratomycosis.

Determination of fungal susceptibility

Minimum inhibitory concentration (MIC) testing has become a useful aid to select the most appropriate therapy [89], because there is a growing incidence of fungal disease, an expanding availability of antifungal drugs, and an increasing development of fungal resistance [89]. Unfortunately, susceptibility of fungi to various drugs is not always predictable [34] because of laboratory variation and the slow development of interpretive criteria of MIC data based on in vivo/in vitro correlation [34]. Fungal MIC determination can vary more than 50,000-fold [34,89,135,136]. Because the underlying immunocompromised state of many patients with fungal infections is an important determinant of the outcome of treatment, correlation of sensitivity to response to therapy may be difficult to determine [34]. In 1983, the National Committee for Clinical Laboratory Standards

(NCCLS) established a subcommittee to standardize fungal MIC determination [34,89,135,136]. This committee arose from the need to standardize inoculum size and preparation, incubation time and temperature, media, and end point determination [34,135,137–141]. Acceptable methods for susceptibility testing of filamentous fungi and criteria for MIC interpretation are under development.

At this time, the relevance or pharmacodynamic correlate of fungal MICs is not firmly established [89]; therefore, it has been suggested to use fungal MIC as a predictor of failure rather than of success [89]. An excellent review of reported MIC values may be found in the report by Vanden Bossche et al [37]. In the absence of veterinary-specific criteria, standards developed for human medicine may be useful [34].

Antifungal drug resistance

Antifungal drug resistance is well recognized and can be intrinsic or acquired after infection (ie, intrinsically resistant fungi), selection amplification of a resistant strain from a population of many strains, or mutation of initially susceptible fungi [34]. The mechanism by which resistance develops depends on the mode of action of the class of antifungal drug and includes reduced drug uptake, drug export through efflux pumps, or reduced affinity of target enzymes [34]. Fortunately, unlike bacterial resistance, transferable drug resistance has not been recognized with fungi, and the spread of resistance has been considerably slower [34]. Prevention of emergence and spread of resistant fungi depends on maximizing the pharmacodynamic properties of the particular drug class, use of local rather than systemic treatment to reduce general exposure of the patient's normal fungal flora to antifungal agents, and practicing good hygiene [34]. This point may be punctuated by the use of topical antifungal agents rather than systemic agents for the treatment of mycotic keratitis. In the case of the flucytosine, combination antifungal therapy is a well-recognized strategy to prevent emergence of flucytosine resistance [34]. For an excellent review of the proposed mechanisms of the development of azole resistance, the reader is referred to the article by Vanden Bossche et al [37].

Summary

Many variables affect the outcome of keratomycosis and systemic fungal infections in animals. These include pathogenicity of the fungal organism (toxins, trophisms, and evasion of host response); previous treatment with topical or systemic corticosteroids, which can have a dramatic negative impact on host defense mechanisms; concurrent systemic illness or immunocompromise; severity/extent of infection; and degree of pain (ie, increased reflex tearing dilutes topical medication) [14]. Experimental work

suggests that antibiotics may occasionally exacerbate fungal infections [142], and some researchers advocate that concurrent antibiotic therapy is contraindicated in horses with yeast infections and septate fungal infections unless bacterial infection is also suspected [14]. Nevertheless, given that normal conjunctival flora often include bacteria and fungi and because care of keratomycoses often includes mixed bacterial and fungal infections, the possible dynamics (natural influences and local competition) between ocular surface microorganisms merit further investigation. There are many unanswered questions regarding the accuracy of in vitro susceptibilities and corneal concentration capabilities for antifungal topical medications [14]. Inherent host resistance or other immune interactions between the patient and fungus are perhaps the most important determinants of the outcome but are currently difficult to measure or assess except by subjective clinical observation [14].

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