Case Report

Subconjunctival voriconazole for the treatment of mycotic keratitis in a horse

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Summary

Fungal keratitis in the horse is a common but challenging disease. This report describes the successful treatment of Fusarium keratitis with both topical and subconjunctival voriconazole 1%. Voriconazole is a new triazole antifungal that has demonstrated efficacy against common fungal pathogens. Topical voriconazole is able to penetrate the intact corneal epithelium making it a good choice for superficial or deep mycotic keratitis. Reports of subconjunctival use of antifungal drugs are sparse in the veterinary literature. We found subconjunctival voriconazole was well tolerated and easy to safely administer.

Introduction

Mycotic ulcerative keratitis is a common and often serious ophthalmic disease in horses. Severe cases can lead to corneal perforation with loss of vision or loss of the eye. The secondary anterior uveitis is often difficult to control and can result in blindness from extensive posterior synechia and cataract. The diagnosis of mycotic ulcerative keratitis can usually be made quickly and easily by corneal cytology. Fungal hyphae can be identified by routine cytological stains such as Wrights, Wright-Giemsa and Diff-Quik. However, treatment, can be a challenge for several reasons. Culture and sensitivity results can take weeks by which time the results are clinically useless leading to empirical selection of antifungal therapy. Most antifungal drugs are fungistatic rather than fungicidal (Biswas et al. 2009). If the ulcer epithelialises before resolution of the infection, treatment is further complicated by limited penetration through the intact epithelium. Antifungal drugs for corneal disease in the horse have been used topically and systemically. Topical frequency can be as often as every 2–4 h in attempt to control the infection with fungistatic drugs (Kern et al. 1983; Hendrix et al. 1996, 1997; Martin 2005; Clode 2010). With an intact epithelium even this high frequency has questionable efficacy. Parenteral or oral therapy has increased risk of systemic side effects and is often cost prohibitive and the actual corneal tissue concentration of systemic antifungal drugs is unknown.

This case report describes the topical and subconjunctival use of voriconazole, an antifungal drug, for the treatment of Fusarium keratitis.

Case details

History

A 13-year-old Quarter Horse gelding was presented to the Oklahoma State University Veterinary Teaching Hospital for a suspected fungal ulcer in the left eye. Treatment was initiated 5 days prior to presentation and included atropine, gentamicin and neomycin-polymyxin-bacitracin ophthalmic ointments and flunixin meglumine oral paste. Twenty-four hours prior to presentation, topical medications were changed to voriconazole 1%, ciprofloxacin ophthalmic solution and atropine 1% ointment and oral flunixin meglumine continued. At presentation the horse had received 3 doses of each of the topical medications.

Ophthalmological examination

The horse was sedated with xylazine (AnaSed) 1 (0.3 mg/kg b.w.t. i.v.) for the ophthalmic examination which included biomicroscopy and indirect ophthalmoscopy. Examination findings in the left eye were blepharospasm, epiphora, marked hyperaemia of the bulbar conjunctiva, swelling of the bulbar conjunctiva adjacent to the lateral limbus, lateral corneal oedema, miosis, clear anterior chamber and unremarkable lens and fundus. Fluorescein stain uptake was present in the superficial corneal stroma in a 12 × 5 mm vertical oval pattern, approximately 7 mm from the lateral limbus. The epithelium along the lateral margin of

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the ulcer appeared thickened and the cornea between the ulcer and limbus appeared rough with a very fine, stippled texture. The surface of the ulcer was cultured for aerobic bacteria and cytology of the ulcer was performed using a cytology brush (Microbrush)².

**Diagnosis**

Slides submitted for cytology were stained with aqueous Romanowsky stain. Cytology revealed well differentiated squamous epithelial cells, degenerate neutrophils, 12 × 12 μm extracellular organisms and few nonstaining hyphal structures with rounded borders and parallel sides. The nonhyphal organisms were atypical for mycotic keratitis and a piece of loose epithelium along the lateral ulcer margin was removed using a 64 corneal blade the day following presentation, was placed in saline and submitted for DNA sequencing for definitive diagnosis. The internal transcribed spacer region and D1/D2 region were sequenced resulting in a diagnosis of *Fusarium solani* complex. This was in agreement with later fungal culture results. Bacterial culture at 48 h yielded no growth.

**Treatment and clinical course**

Following ophthalmic examination, sedation was augmented with detomidine (Dormosedan)³ (0.008 mg/kg bwt i.v.) and a subpalpebral lavage tube (Mila)⁴ placed through the superior eyelid with the footplate in the superior fornix. The horse was started on voriconazole (Vfend)⁵ 1% 0.1 ml q. 2 h, ciprofloxacin⁶ 0.3% 0.1 ml q.i.d. and atropine⁶ 1% 0.1 ml q.i.d. through the lavage tube and flunixin meglumine (Banamine)⁷ (1 mg/kg bwt per os b.i.d.) (Day 0). By Day 2 corneal neovascularisation was present at the lateral limbus. In an effort to prevent deep stromal keratomycosis, usually difficult to control and often with a protracted clinical course, we elected to add subconjunctival voriconazole to the treatment regimen. Voriconazole 1% (0.5 ml) was administered subconjunctivally beneath the ventrolateral bulbar conjunctiva. On Day 3 the cornea was restrained and the area of fluorescein stain uptake was approximately one-third of the original area. The neovascularisation was more extensive and progressing further into the cornea toward the ulcer. On Day 4 there was increased corneal oedema and neovascularisation now extended from the 12 to 7 o’clock limbus (Fig 1). Fluorescein stain was negative. The subconjunctival voriconazole 1% (0.5 ml) was repeated, this time beneath the dorsal bulbar conjunctiva. On Day 6 there was no change in the corneal oedema and neovascularisation continued to progress medially. Fluorescein stain remained negative. A small amount of hypopyon was just visible in the ventral anterior chamber; however, the pupil remained dilated. A subconjunctival voriconazole 1% (0.5 ml) injection was repeated beneath the dorsal bulbar conjunctiva. The topical voriconazole was decreased to q. 4 h; all other medications remained the same. On Day 8 the dense lateral corneal oedema remained and light corneal oedema now extended along the ventral limbal cornea. Neovascularisation had progressed, especially from the lateral cornea medially. Fluorescein stain was negative. The hypopyon and pupil size were unchanged. Voriconazole 1% (0.5 ml) was administered subconjunctivally beneath the dorsal bulbar conjunctiva. The horse was discharged with the subpalpebral lavage tube in place and a protective hard cup mask. Medication instructions included voriconazole 1% 5 times per day, atropine 1% q.i.d. and ciprofloxacin 0.3% q.i.d. through the lavage tube, and flunixin meglumine 1 mg/kg bwt per os s.i.d.

On re-examination 6 days following discharge (Day 14), ophthalmic examination revealed decreased corneal oedema and progressive neovascularisation. The cornea adjacent to the lateral limbus where the vessels were no longer active had a dense white appearance similar to fibrosis. Fluorescein stain was negative. The pupil was mid-dilated and the anterior chamber clear. The voriconazole was decreased to q.i.d. and the other medications were continued at the same frequency. On re-examination 17 days later (Day 31), ophthalmic examination revealed resolution of the corneal oedema and neovascularisation. The lateral cornea adjacent to the limbus had a stromal haze with a focal, well defined, pale cream coloured opacity in the deep stroma with few associated thin, inactive blood vessels (Fig 2). The anterior chamber was clear and the pupil maximally dilated. The subpalpebral lavage tube was removed and all medications discontinued.

**Outcome**

A final re-examination one month later (Day 67) revealed a stromal corneal scar adjacent to the lateral limbus from
3 to 4 o’clock and a light superficial stromal haze in the limbal cornea from 1 to 4 o’clock (Fig 3).

Discussion

Voriconazole is a second generation triazole antifungal drug derived from fluconazole. Triazoles inhibit an enzyme required for the synthesis of ergosterol resulting in altered fungal cell membrane permeability, interfere with cell membrane synthesis and repair and can cause accumulation of toxic intracellular products (Regnier 2007a; Pearce et al. 2009). In a recent study evaluating fungal isolates from the south and central United States, voriconazole was the most effective antifungal against all isolates tested. When results were separated by fungal genus, Fusarium species were most susceptible to natamycin first and voriconazole second (Pearce et al. 2009). In the case reported here, at the time the Fusarium was identified, the cornea showed steady clinical improvement and we elected to continue treatment with voriconazole. Sensitivity testing was not performed due to cost. Voriconazole 1% has been shown to penetrate the intact cornea in normal horse eyes achieving an aqueous humour concentration of 2.35 μg/ml, above the estimated, clinically effective, average mean inhibitory concentration (MIC) of 0.5 μg/ml (Clode et al. 2006). To the author’s knowledge, subconjunctival voriconazole has not been evaluated in the horse. Subconjunctival administration of antifungal drugs including fluconazole, miconazole and amphotericin-B is frequently reported in man for fungal keratitis and endophthalmitis; however, no reports of subconjunctival voriconazole have been identified.

Complications of keratomycosis including persistent stromal abscess, penetration of Descemet’s membrane by fungal hyphae, keratomalacia and intractable anterior uveitis were not encountered in the case reported here. Re-epithelialisation occurred quickly (Day 4) which can increase the risk for a corneal stromal abscess as hyphae and neutrophils become sequestered within the stroma. Although no defined abscess was identified on biomicroscopy, the hypopyon noted on Day 6 and the incomplete mydriasis noted on Day 14 suggested a subtle stromal abscess/infection was present inciting the clinical signs of anterior uveitis. Augmenting the topical voriconazole with subconjunctival was an attempt to maximise drug concentration to avoid a prolonged stromal infection and speed resolution of the mycotic keratitis. Also, sensitivity studies for common fungal isolates demonstrated Fusarium has the highest MIC for voriconazole suggesting higher drug concentrations would be more efficacious (Marangon et al. 2004; Clode et al. 2006). Topical and subconjunctival routes are often combined for the treatment of keratomycosis in man to increase drug concentration even when the topical therapy is as frequent as q. 0.5–1 h (Kredics et al. 2007; Mahdy et al. 2010). The subconjunctival route of drug administration provides higher and prolonged drug concentration to the anterior segment of the eye, including the corneal stroma and aqueous humour, compared with topical administration (Matthews 2004; Regnier 2007b; Maggs 2008). Subconjunctival injection of a water-soluble drug can provide therapeutic levels for 8–12 h (Regnier 2007b). Without measuring and comparing the voriconazole tissue concentrations of topical administration alone vs. topical and subconjunctival administration, it is unknown if our combined routes of administration provided a clinically relevant higher concentration of drug. Therefore, in this case, it is impossible to determine how much subconjunctival use contributed to the outcome of the case.

The subconjunctival voriconazole was well tolerated in this patient with no evidence of post injection blepharospasm, epiphora, chemosis or conjunctival
reaction. The subconjunctival injection was also technically easy to perform. We used 2 effective techniques both of which were performed with sedation (detomidine i.v.) and topical anaesthetic (2% lidocaine). A local auriculopalpebral nerve block can also be performed but we found this was usually unnecessary with the sedation. The first technique utilised a Castroviejo eyelid speculum to hold the eyelids open. The speculum was retracted laterally increasing exposure of the lateral bulbar conjunctiva. The second technique involved turning the horse’s head laterally with the affected eye up causing the eye to roll ventrally and exposing the dorsal bulbar conjunctiva. The superior eyelid was then manually retracted for access to the dorsal bulbar conjunctiva. For both techniques once the bulbar conjunctiva was exposed it was gently grasped with small toothed Colibri forceps and the conjunctiva was tented away from the globe. Using a 27 gauge, 12.7 mm needle attached to a 1 ml syringe the needle was inserted beneath the tented conjunctiva and the drug injected. The smaller the needle used the less likelihood of drug leaking from the injection site or inadvertent perforation of the globe. Taking care to keep the needle parallel to the globe will also greatly lessen the risk of globe penetration.

Local tissue concentrations and duration of subconjunctival voriconazole were not determined in this patient. Subconjunctival administration was paired with topical administration of voriconazole, which has been shown to be a relatively effective treatment route for fungal keratitis in previous studies. Further studies are required to determine if subconjunctival voriconazole is a safe and effective form of adjunctive therapy for mycotic keratitis or a means of providing antifungal therapy when frequent topical treatment is not possible.

Author’s declaration of interests

No conflicts of interest have been declared.

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Manufacturers’ addresses

1Akorn, Decatur, Illinois, USA.
2Microbrush International, Grafton, Wisconsin, USA.
3Pfizer Animal Health, Exton, Pennsylvania, USA.
4Mila International, Erlanger, Kentucky, USA.
5Pfizer Ireland Pharmaceuticals, Ringaskiddy, Co. Cork, Ireland.
6Falcon Pharmaceuticals, Fort Worth, Texas, USA.
7Schering-Plough Animal Health, Union, New Jersey, USA.

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