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Ocular toxicity of experimental intravitreal itraconazole

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Abstract

We investigated itraconazole, a new triazole antifungal agent that poorly penetrates ocular tissues after oral administration. We injected itraconazole in doses from 10 to 100 micrograms dissolved in 100% dimethyl sulfoxide into the eyes of New Zealand rabbits. Ocular toxicity studies performed five weeks after administration showed no substantial retinal or histopathologic changes in eyes injected with either 100% dimethyl sulfoxide or 10 micrograms of itraconazole. Higher doses caused focal areas of retinal necrosis. Our results indicated that intravitreal doses of 10 micrograms or less of itraconazole may be beneficial in the treatment of fungal endophthalmitis.

Introduction

Treatment of fungal endophthalmitis is complicated by many factors, including drug toxicity [14], delay in diagnosis and treatment [14, 15], size and location of the inoculum [5], development of resistance by offending organisms to antimycotic agents [2], the ability of organisms to produce exotoxins and proteolytic enzymes [14], and the limited number of antifungal agents available [19]. Inadequate antifungal penetration inside the vitreous cavity by agents following parenteral [19, 11] or subconjunctival [11] administration has resulted in the unsuccessful treatment of intraocular mycotic infections, even after the causative agent has been identified.

Intravitreal injection overcomes anatomic barriers to achieve therapeutic drug concentrations in the vitreous, and is an accepted method of treating fungal endophthalmitis [14]. Amphotericin B has been the standard drug for most mycotic intraocular infections [2], even though it produces systemic and intraocular toxicity [6, 17].

The triazole compounds represent an important development in the continuing search for antifungal agents with low toxicity and a broad spectrum of activity. Itraconazole is a new triazole derivative with broad spectrum antifungal activity *in vitro* and in animal models. This antifungal drug is lipophilic and practically insoluble in water [9, 1, 13]. We evaluated the toxicity of itraconazole after intravitreal injection, since it penetrates ocular structures poorly following parenteral administration [4].

Materials and methods

Eight New Zealand white rabbits, weighing 2 to 3 kg each, were anesthetized with 1 ml of an intramuscular injection containing a 50:50 mixture of

ketamine (100 mg/ml) and xylazine hydrochloride (20 mg/ml). Before treatment, all pupils were maximally dilated with 1% cyclopentolate and 2.5% phenylephrine eyedrops, and a dark-adapted electroretinogram (ERG) was performed.

For each ERG, both eyes were stimulated simultaneously, following 30 minutes of dark adaptation, using a photostimulator (Grass PS22) within a Ganzfeld sphere. Eyes were positioned 20 cm from the light source at intensity 116 (maximal flash intensity for this unit is approximately 1,500,000 candle power).

Twelve eyes received an injection of itraconazole in concentrations ranging from 10 to 100 μg. Being poorly water-soluable [9], itraconazole was dissolved in a 100% dimethylsulfoxide (DMSO) solution.

An anterior chamber paracentesis was performed before each injection (0.1 ml of aqueous was removed through clear cornea, using a 30-gauge needle attached to a tuberculin syringe) to reduce the risk of damage to intraocular structures caused by increased intraocular pressure. The appropriate dose of itraconazole diluted in 0.1 ml of 100% DMSO was injected through the pars plana, 2 mm from the limbus, using a 27-gauge needle directed toward the center of the vitreous cavity. Three eyes received injections of 100% DMSO alone, and one eye was not injected and was excluded from the study.

Eyes were examined by biomicroscopy and indirect ophthalmoscopy preoperatively and at one hour, 2, 6, and 8 days, and 5 weeks after intravitreal injection; thereafter, the animals were killed and the eyes enucleated.

Eyes were enucleated and fixed in a 10% formaldehyde solution, dehydrated in a series of graded alcohols, and embedded in paraffin. Sections were cut on a microtome (American Optical), stained with hematoxylin and eosin, and evaluated by light microscopy.

Results

Scotopic ERGs performed five weeks after intravitreal injection of either 100% DMSO or itraconazole in concentrations ranging from 10 to 100 μg failed to demonstrate any changes from preoperative values. Clinical examination carried out at intervals ranging from one hour to five weeks after injection appeared normal.

Histopathologic examination by light microscopy showed no damage to the retina, retinal pigment epithelium, or choroid in eyes injected with either 100% DMSO or 10 μg of itraconazole. Focal areas of retinal necrosis were present in eyes injected with more than 10 μg of itraconazole. The focal areas of retinal necrosis were discrete and located near the site where the eye was injected, and may have been a sequela of the injection technique [14, 12]. Diffuse retinal changes were not present in any eye (Table 1).

Discussion

Penetration into the inflamed and uninflamed rabbit eye was evaluated by Perfect and associates [13] four hours following oral administration of itraco-

Table 1. Toxicity of intravitreal injections of itraconazole in a solution of 100% dimethyl sulfoxide (DMSO).

No of eyes	Dose (volume injected/ml)	Electroretinographic findings (5 weeks after injection)	Histopathologic findings
3	0 μg(0.1) in 100% DMSO	normal	normal
3	10 μg(0.1) in 100% DMSO	normal	normal
3	20 μg(0.1) in 100% DMSO	normal	abnormal*
3	50 μg(0.1) in 100% DMSO	normal	abnormal*
3	100 μg(0.1) in 100% DMSO	normal	abnormal*

* Focal areas of retinal disorganization.

nazole given in a 200 mg dose. Levels of drug measured in the cornea, sclera, aqueous, and vitreous were either undetectable or extremely small [4].

Intravitreal injection of antifungal agents alone, or in conjunction with vitrectomy has been effective, in selected instances, in treating mycotic intraocular infections [8]. Based on this data, we decided to evaluate ocular toxicity following intravitreal injection of intraconazole.

Itraconazole is a new third-generation triazole with significant antifungal activity *in vitro,* ranging from molds such as *Aspergillus fumigatus* to yeasts such as *Candida albicans.* Clinically, itraconazole has been efficacious in the treatment of vaginal candidiasis, systemic candidiasis in neutropenic patients, and systemic histoplasmosis. The reported incidence of adverse reaction was very low [7, 3]. The antifungal activities of intraconazole are thought to be derived from a selective interaction with fungal microsomal cytochrome P-450, which leads to a disturbance in ergosterol synthesis in fungal cell membranes that results in cell death [7].

Preliminary studies suggest that itraconazole is considerably more active against *Candida albicans* than ketaconazole. Both agents were tested *in vitro* against 58 clinical isolates from vaginal isolates of *C. albicans* derived from women with vaginal candidiasis. The 90% mean inhibitory concentrations (MICS) for itraconazole and ketaconazole were 2.0 and 4.8 μg/ml, respectively [16].

In vitro data and animal studies suggest that intravitreal administration of itraconazole in doses which are nontoxic to the eye may provide effective antimicrobial levels in the vitreous for treatment of fungal endophthalmitis caused by different ocular fungal pathogens. However, *in vitro* susceptibility test results do not always correlate with an *in vivo* response to therapy or predict clinical effectiveness [15, 18]. Extrapolation of data from animal to human eyes is not always reliable [10].

Significant size and structural differences exist between rabbit and human eyes [18]. Subsequently, evaluation of itraconazole toxicity in primate eyes and the determination of its efficacy in experimental models of fungal endophthalmitis must be completed before clinical use is considered in humans.

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