Treatment of *Aspergillus fumigatus* Keratitis in Rabbits With Oral and Topical Ketoconazole


*Aspergillus fumigatus* keratitis was established in rabbits by intrastromal injection of a suspension containing 5,000 spores. After a 48-hour incubation period, the animals were treated with oral and topical ketoconazole alone and in combination with topical natamycin for five days. Colony counts per cornea were obtained at the conclusion of the treatment period.

Based on these colony counts, neither oral nor topical ketoconazole was effective despite moderate in vitro sensitivity of the fungus to ketoconazole. Oral and topical ketoconazole used in conjunction with natamycin, however, appeared to augment sterilization of *A. fumigatus* in this model of fungal keratitis.

Fungal infections of the cornea are difficult to treat even when diagnosed promptly and therapy is guided by antifungal sensitivity testing. There are few antifungal agents available that are both effective and nontoxic to the eye.

The introduction of natamycin, a broad-spectrum, topical, polyene antifungal agent, markedly improved treatment of keratomycosis. Natamycin is useful for superficial infections but penetrates the cornea poorly and is much less effective for deep fungal corneal infections or when intraocular penetration of the mycosis has occurred.

Ketoconazole, a new oral imidazole antifungal agent, has recently been used to treat several patients with fungal keratitis. After successfully treating two patients with fungal keratitis, Ishibashi suggested that oral ketoconazole would be useful as therapy for human keratomycosis. This drug is active in vitro against dermatophytes, yeasts (including *Candida*), and dimorphic fungi like *Coccidioides immitis*, *Blastomyces dermatitidis*, *Histoplasma capsulatum*, and *Paracoccidioides brasiliensis*. It penetrates the cornea and aqueous humor well when administered orally, topically, or subconjunctivally but penetrates the vitreous poorly by any route of administration.

Ketoconazole does not significantly retard closure of experimentally produced corneal epithelial defects in rabbits and is well tolerated topically.

Despite reports of favorable responses in animal models of *Aspergillus flavus* keratitis, sensitivity of other species of *Aspergillus* to ketoconazole is not routine. Therefore, we conducted a randomized, prospective study comparing natamycin and ketoconazole, alone and in varying combinations, in treatment of experimental *Aspergillus fumigatus* keratitis in rabbits.

### Material and Methods

**Animals**—We used 24 New Zealand white rabbits, averaging 1.4 kg in weight, in two experiments. All the animals were obtained from the same rabbitry and were free of corneal disease. After an initial 14-day isolation and observation period, each eye was injected subconjunctivally with 0.2 ml of triamcinolone acetonide suspension (40 mg/ml) on the day before and the day of corneal inoculation with the fungus.

**Organism**—We used a human ocular isolate of *A. fumigatus* to infect each rabbit cornea. The fungus was grown on brain-heart infusion media for three days at 35 C. Spores were harvested from the media with a wire loop and suspended in normal saline and counted by hemacytometer. The volume of saline was adjusted to obtain a count of 5 x 10⁶ spores/ml. The hemacytometer spore count was confirmed by standard pour plate culture techniques. The spore suspension was free of hyphal fragments.
tube dilution minimum inhibitory concentrations of ketoconazole and natamycin for this fungus were 4 μg/ml for both agents.

**Corneal inoculation**—Intramuscular xylazine and ketamine were used to anesthetize the animals. We rinsed each cornea with sterile balanced salt solution before inoculation. Under microscopic control, a Rizzuti Spirrizi needle was used to penetrate through Bowman’s membrane in the mid-cornea. We injected a volume of 10 μl of the spore suspension (5,000 spores) through the entry hole into the corneal stroma with a calibrated microliter syringe. Both eyes of each rabbit were infected by this technique.

**Treatment groups**—After 48 hours of incubation, 12 rabbits were randomly assigned to receive oral ketoconazole. These rabbits received a single oral daily dose of 60 mg kg of body weight of ketoconazole in a gelatin capsule without regard to food intake. The remaining rabbits did not receive any systemic treatment with oral ketoconazole.

Once the two systemic treatment groups were established (oral ketoconazole and no oral ketoconazole), topical therapy was randomly assigned to each rabbit eye. One of the following topical agents was used in each rabbit eye: 5% ketoconazole in arachis oil, 5% natamycin, 0.02% benzalkonium chloride solution as a control for natamycin, and arachis oil as a control for 5% ketoconazole. Four rabbit eyes received topical therapy with both 5% ketoconazole and 5% natamycin.

Topical therapy was administered hourly for ten consecutive hours each day for five days. In eyes receiving both topical 5% ketoconazole and 5% natamycin, one drop of each suspension was administered concurrently each hour. The medication administered first each hour was alternated. Clinical estimates of corneal infectivity were made daily on the treatment days by two of us in a masked fashion using a previously described scoring system.

A second experiment was conducted to confirm the findings of the first experiment. Sixteen eyes from eight rabbits were divided into four equal groups for treatment with topical natamycin, oral ketoconazole and topical natamycin, oral ketoconazole, and topical ketoconazole, or topical arachis oil (control). The three treatment groups were chosen for retesting in this second experiment based on their statistical effectiveness in the first experiment. The same experimental protocol was used.

**Colony count determination**—The animals were killed at the conclusion of the five-day treatment period with N-[2-(m-methoxy-phenyl)-2-ethyl-butyryl(1)] gamma-hydroxy butyramide (161 euthenasia solution). Each cornea was first rinsed with sterile balanced salt solution, excised at the limbal margin regardless of size, and then pulverized with a tissue homogenizer in 5 ml of normal saline. From this suspension, 0.1 ml was plated onto trypticase soy agar with a spin plate technique. One of us, who did not know the identity of the various treatment groups, counted the number of fungal colonies per culture plate after 48 hours of incubation. Each count was multiplied by 50 to obtain the total number of fungal colonies per cornea.

**Statistical analysis**—We compared the colony counts by means of a two-tailed Student’s t-test.

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**Results**

All eyes were clinically infected after 48 hours of incubation. A portion of the spore injectate was lost in one eye in the first experiment and we excluded this eye from the data analysis. One animal died during the second experiment; no evidence of systemic infection was found.

**Experiment 1**—Topical natamycin significantly reduced the number of organisms per cornea compared to control (P < .01). Neither oral nor topical ketoconazole alone or in combination was better than control in significantly reducing the number of fungal organisms per cornea (Figure). Combinations of ketoconazole and topical natamycin gave the lowest colony counts in this experiment. The combinations of topical natamycin with oral ketoconazole (P < .01) and topical natamycin with both oral and topical ketoconazole (P < .01) were significantly better than the control in reducing fungal colony counts per cornea. Ketoconazole appeared to augment the effectiveness of topical natamycin. The antifungal activity of natamycin was enhanced by adding oral ketoconazole alone (P < .01) or by adding both oral and topical ketoconazole (P < .05) to the treatment regimen. The effectiveness of topical natamycin in conjunction with oral ketoconazole could not be further enhanced by adding topical ketoconazole. The two of us who clinically scored the infection in each cornea on the fifth day of treatment correctly predicted the eye with the greater number of fungi in 69% and 75% of the cases.

**Experiment 2**—The second experiment compared topical arachis oil to three treatments chosen on the basis of their demonstrated statistical effectiveness in the first experiment. In this second experiment, all three treatment groups (topical natamycin, oral ketoconazole and topical natamycin, and both oral and topical ketoconazole with topical natamycin) significantly reduced colony counts per cornea when compared to the control group (P < .01 for each group). Treatment groups combining ketoconazole with topical natamycin had the lowest colony counts in this
Mean colony counts of *Aspergillus fumigatus* per cornea in experiment 1. A, control (topical benzalkonium chloride); B, control (topical arachis oil); C, oral ketoconazole; D, topical ketoconazole; E, oral and topical ketoconazole; F, oral ketoconazole and topical natamycin; G, oral and topical ketoconazole and topical natamycin; H, topical natamycin.

**Experiment** (Table). However, this decrease in colony counts was not statistically different from those with topical natamycin alone.

The smaller numbers of organisms per cornea in the second experiment were probably the result of serial passages of the fungus in vitro.

**Comparison of experiments 1 and 2**—In both experiments, natamycin alone, natamycin with oral ketoconazole, and natamycin with both oral and topical ketoconazole produced a statistically significant reduction in colony counts when compared to the control.

In the first experiment, there was a statistically significant reduction in colony counts when ketoconazole was added to natamycin compared to the colony counts attained with natamycin alone. However, in the second experiment the lower colony counts achieved with combinations of ketoconazole and natamycin were not statistically significant compared with natamycin therapy alone.

**Discussion**

Oral ketoconazole has recently been used to treat several patients with fungal keratitis. Foster treated a woman who had chronic *Candida* corneal ulcers with topical and subconjunctival miconazole. After two days of this therapy, the patient underwent lamellar keratoplasty. The miconazole therapy was slowly decreased and discontinued four weeks after surgery. Oral ketoconazole at a dose of 200 mg/day was then initiated on the 14th postoperative day and continued for six months. The patient has been free of *Candida* infection since that time.
Aspergillus fumigatus Keratitis

Searl and associates described a patient with A. fumigatus keratitis who was treated with multiple antifungal agents, including oral ketoconazole. The infection recurred after a penetrating keratoplasty was performed despite treatment with 5-flucytosine, ketoconazole, and miconazole. The eye was subsequently enucleated.

Ishibashi described two patients with fungal keratitis whom he successfully treated with oral ketoconazole alone. The first patient, infected with an unidentified fungus, received oral ketoconazole for 27 days. The second patient, who had Fusarium solani keratitis, received more than 47 days of oral ketoconazole therapy. No topical antifungal therapy was administered. These two patients prompted the recommendation that ketoconazole might be used to treat fungal keratitis.

By using a fungus moderately sensitive to ketoconazole, we demonstrated that oral or topical ketoconazole alone, or the combination of the two, was no better than placebo in reducing colony counts of A. fumigatus keratitis in rabbits. This model of keratitis was chosen to give quantitative results to an infection that is clinically difficult to assess.

The results of our first experiment indicated that oral ketoconazole may have augmented topical natamycin therapy. However, the second experiment failed to confirm this initial finding of in vivo synergy. This may well have been the result of a decrease in virulence after serial passages in vitro as has been described with other organisms. Ketoconazole penetretes into the cornea and aqueous humor well, and this ocular penetration may explain the augmentation of topical natamycin therapy, a drug that penetrates the cornea only superficially.

Oji reported that A. flavus keratitis in rabbits responded to topical ketoconazole. He determined the minimum inhibitory concentrations of ketoconazole for several isolates of A. fumigatus and F. solani and found them to be resistant.

Jones and associates found ketoconazole to be effective in the treatment of endogenous Candida albicans endophthalmitis in rabbits after 28 days of treatment compared to placebo but this difference was not apparent after only five days of therapy. Oral ketoconazole may be useful in augmenting topical therapy with natamycin in treatment of fungal keratitis but this effect may take several weeks before becoming apparent. Because our protocol used a shorter treatment duration, we could not demonstrate this benefit conclusively.

Based on our studies, topical or oral ketoconazole is not well suited for treatment of A. fumigatus keratitis, a common cause of human keratomycosis. Its role in treatment of Candida keratitis, an organism more predictably sensitive to ketoconazole, remains to be examined. Oral ketoconazole may prove to be useful in preventing recurrent fungal keratitis in patients previously treated with intensive topical antifungal therapy. This may be particularly important in patients requiring lamellar or penetrating keratoplasty for fungal keratitis. However, additional randomized, placebo-controlled trials should be performed before ketoconazole is adopted for widespread clinical treatment of fungal keratitis.

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References