Efficacy of Antifungal Agents in the Cornea

II. Influence of Corticosteroids

Denis M. O'Day, Wayne A. Ray, Richard Robinson, and W. Steven Head

The influence of topical corticosteroid on the efficacy of five topical antifungal agents was evaluated in a standardized rabbit model of Candida keratitis using a quantitative mycologic technique. Topical 1% prednisolone acetate worsened the disease when given alone and adversely influenced the efficacy of 5% natamycin, 1% flucytosine, and 1% miconazole when given in combination. The efficacy of amphotericin B appeared unaffected when the antifungal agent was administered in concentrations of 0.5% and 0.15%. The adverse effect of the topical corticosteroid appeared to be inversely related to the efficacy of the antifungal agent in vivo. Invest Ophthalmol Vis Sci 25:331-335, 1984

The use of corticosteroid in the management of infectious corneal disease continues to be controversial.1-4 Locally applied, these drugs offer a singular opportunity to protect the structure and integrity of the eyeball from the damaging effects of the host inflammatory response, yet their safety in the presence of active microbial infection is of major concern.3 This concern is particularly appropriate in the case of fungal infection because of the well-known predisposing effect of corticosteroids.1 In addition, the low level of efficacy of most available antifungal agents would appear to argue against the concept of combining antifungal therapy with topical corticosteroid administration.

In this study, we have examined the effect of combining corticosteroid with antifungal therapy in a quantitative rabbit model of Candida keratitis to evaluate the influence of corticosteroid therapy on the efficacy of various antifungal agents.

Materials and Methods

A standardized infection with Candida albicans was established in the corneas of pigmented adult outbred rabbits 1.5–3 kg in weight.5 In this model, eight wells 1 mm in diameter and one half-stromal thickness in depth were trephined in a regular pattern on the surface of the cornea and inoculated with yeast.

Inoculum

Two-day-old cultures of C. albicans (strain LV) grown on trypticase soy agar with 5% sheep blood (BBL) were used as the inoculum. A suspension in normal saline in a concentration of 5 × 10⁶ CFU per ml was prepared and stored at 4 C overnight before use. A modification of the method of Shadomy was used to determine the in vitro susceptibility of this strain (personal communication, Dan B. Jones, 1980).6 Results are shown in Table 1.

Treatment Protocols

Thirty minutes after inoculation, treatment was begun with topical antifungal agents, with and without concomitant prednisolone acetate, using an untreated group as a control. In initial experiments to provide data on the effect of corticosteroid on fungal replication, a group treated with corticosteroid alone was included. Animals were assigned randomly to the various treatment groups with both eyes of each animal receiving the same treatment. Three rabbits were exposed to each treatment regimen. The antifungal agents were administered 10 times daily at hourly intervals while 1% prednisolone acetate (Pred Forte 1%, Allergan Pharmaceuticals, Irving, CA) was administered four times each day during the same period.

The antifungal agents selected for study were: (1) polyenes—amphotericin B 0.5%, 0.15%, and 0.075% in distilled water (Fungizone®, E. R. Squibb & Son, Princeton, NJ), Natamycin 5% suspension (Alcon Laboratories, Forth Worth, TX); (2) imidazoles: miconazole 1% in Cremaphor (Janssen Pharmaceuticals, New Brunswick, NJ), Ketoconazole 1% in polyethylene glycol 400 (Janssen); and (3) pyrimidine: flucytosine...
Table 1. Candida Albicans, Strain LV: In Vitro susceptibility (µg/ml)

<table>
<thead>
<tr>
<th>Drug</th>
<th>MIC</th>
<th>MFC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphotericin B</td>
<td>0.20</td>
<td>0.39</td>
</tr>
<tr>
<td>Natamycin</td>
<td>6.25</td>
<td>&gt;50</td>
</tr>
<tr>
<td>Ketoconazole</td>
<td>&gt;50</td>
<td>&gt;50</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>0.39</td>
<td>&gt;50</td>
</tr>
<tr>
<td>Miconazole</td>
<td>12.50</td>
<td>&gt;50</td>
</tr>
</tbody>
</table>

MIC, minimal inhibitory concentration.  
MFC, minimal fungicidal concentration.

Isolate Recovery

Eighteen hours after the conclusion of treatment, the animals were sacrificed with commercially prepared euthanasia solution, T-61 (Taylor Pharmacal, Decatur, IL). The whole cornea was removed by excision at the limbus, placed in a sterile petri dish, and cut into small pieces. These were then ground in a tissue grinder (Ultraturax Model SDT) for three 10-second intervals in 3 ml sterile saline. Ten and 100 µl aliquots of each corneal suspension were plated in triplicate on trypticase soy agar with 5% sheep blood (BBL). After 48 hrs incubation at 25 C, the colony forming units (CFU) were counted and the number of colony forming units per whole cornea was derived based on a total volume of 3 ml.

Statistical Analysis

Two types of experimental design were employed. In the first, there were four experimental groups: control; steroid only; drug only; and drug and steroid. In the second, the steroid only group was omitted, leaving three experimental groups. For both designs, three rabbits were analyzed per experimental group. Thus, findings were based on totals of 9 or 12 rabbits.

The measure of response to treatment was the natural logarithm of CFU. This transformation is required because the effect of treatment is proportional to pretreatment CFU. Because zero CFU counts were observed in some treatment groups, the actual response measure was log(1 + CFU). The rationale for the log transformation is discussed extensively by Snedecor and Cochran,7 and Steel and Torrie.8

The data were analyzed using analysis of variance with subsampling.7,8 This technique recognizes that the rabbit is the basic experimental unit; all conclusions are based on the number of rabbits, not the number of eyes. However, it utilizes the additional information from the two-eye measurements to produce (1) a more precise measurement of the effect of the treatment upon a single rabbit; and (2) an estimate of the intrarabbit, intereye variability. This technique is described in detail in elementary statistical texts.7,8

Each hypothesis of interest was tested using a single degree of freedom contrast,7,8 which compared the particular experimental groups. The error term for a contrast was the between-rabbits mean square from the analysis of variance with subsampling. This accounted for the fact that rabbits, not eyes, were the basic experimental unit.

Results

In this model, as anticipated, the administration of corticosteroid alone worsened the disease compared with untreated controls (Fig. 1). This effect was significant at 48 hr. The addition of corticosteroid to the antifungal therapy produced varied results, depending on the agent involved.

Amphotericin B

The excellent therapeutic effect of 0.5% and 0.15% amphotericin B was not adversely affected by corticosteroid administration in this model (Fig. 2). When the concentration of the drug was reduced to 0.075%, steroid administration at 48 hr caused a slight increase in the isolate recovery rate, although it still remained far below that of the untreated control group.
Natamycin

Natamycin administered alone had a highly significant effect on the isolate recovery rate, producing a mean CFU/cornea at 48 hr of 1090, compared with 22,900 for untreated controls. The concurrent administration of corticosteroid had an adverse effect on the efficacy of this agent (Fig. 2). At both sampling times this effect was highly significant. At 48 hr, the mean CFU/cornea recovered from eyes treated with corticosteroid and natamycin was 6600, significantly less than the untreated controls but significantly greater than the natamycin-only group (Fig. 2).

Miconazole

The disease was significantly worsened at both sampling times by the concurrent administration of corticosteroid. After 48 hr, untreated control eyes yielded a mean CFU/cornea of 21,500, miconazole-treated eyes a mean of 7790, and miconazole-and-steroid-treated eyes a mean of 15,750 CFU/cornea (Fig. 2). There was no significant difference between the untreated and the steroid/miconazole treated eyes ($P = 0.1$).

Ketoconazole

After 48 hr, eyes treated with both steroid and ketoconazole yielded significantly higher CFU/cornea compared with the ketoconazole-treated group and the untreated control eyes.

Flucytosine

After 48-hr treatment with flucytosine, treated eyes yielded 3,200 CFU/cornea compared with 14,000 CFU/cornea in untreated control eyes (Fig. 3).
concurrent administration of corticosteroid and 1% flucytosine resulted in a significantly higher isolate recovery rate (14,700 CFU/cornea) compared with eyes treated with flucytosine alone and abolished the therapeutic effect of flucytosine (Fig. 3). When treatment with topical steroid was delayed 24 hr to allow pretreatment with 1% flucytosine, there was no detectable adverse steroid effect; the drug-plus-steroid group had significantly lower isolate recovery rates than the control group ($P = 0.005$).

Discussion

These experiments are part of an overall examination of factors that might influence antifungal therapy. We have previously used this model of Candida keratitis to judge relative efficacy of a group of topical antifungal agents in terms of quantitative isolate recovery after a standard period of treatment. Ranked in this way, the polyenes were superior to both miconazole and flucytosine. Ketoconazole, an experimental antifungal agent, was apparently ineffective.

In the present study, we investigated the effect of concomitantly administered corticosteroid on the efficacy of these agents. The model and the strain used were the same as those used in the previous experiments. Miconazole, flucytosine, and natamycin were used in concentrations largely dictated by solubility or toxicity. Amphotericin B is customarily prepared for clinical use at a concentration of 0.5%, in these experiments it was also diluted to 0.15% and 0.075% because we found an antifungal effect at these concentrations that was superior to the other agents. We were interested to learn whether corticosteroid administration would adversely affect this activity. A 1% preparation of ketoconazole was used because it is well tolerated by the cornea.

The influence of corticosteroid was readily apparent although it depended on the agent with which it was combined. Thus, with ketoconazole, a drug we found completely ineffective, the concomitant administration of steroid led to a worsening of the disease. When steroid was given in combination with moderately efficacious agents, such as miconazole and flucytosine, the result was an ablation of the antifungal effect. Even with the polyenes (more efficacious drugs), amphotericin B, and natamycin, an adverse effect followed steroid administration. With amphotericin B this was apparent only at the 0.075% concentration. Concomitant corticosteroid administration did not influence the antifungal activity of this drug at the higher concentrations. However, when given with 5% natamycin, corticosteroid diminished the antifungal activity at both sampling times.

In the case of flucytosine, this adverse influence of corticosteroid could be mitigated by pretreatment with the antifungal agent. In this study the subsequent period of evaluation was only 24 hr. For this reason, the success of this strategy with prolonged administration remains to be determined.

These studies clearly suggest that the adverse effect of corticosteroid administration is inversely related to the in vivo efficacy of the antifungal agents. It is of interest that amphotericin B, the only agent not associated with a deterioration of its antifungal activity when combined with corticosteroid, is also the only one presumed to exert its antifungal activity through a fungicidal mechanism. The remainder of the agents, all of which were adversely affected to varying degrees by corticosteroid, are thought to be fungistatic at the levels likely to be achieved in the cornea.

The principal mechanism by which corticosteroids impair the efficacy of antifungal agents is presumed to be interference with host response mechanisms. However, there is also evidence that these hormones may interact directly with the fungus to block antifungal agent activity. Recent in vitro studies with yeasts using a Warburg assay suggest that the antifungal activity of the imidazoles is impaired in the presence of high concentrations of glucocorticoids. The significance of this finding is at present unknown, but it implies a complex relationship between the host, the invading organism, and the antifungal agent.

In the eye, although the deleterious effect of corticosteroid administration on antifungal agent activity has been widely assumed, the subject has not been investigated in depth. Corticosteroid has been used to help to establish keratomycoses in animals otherwise resistant to infection. Newmark and associates examined the effect of combined pimaricin (natamycin) and dexamethasone therapy in an animal model of fungal keratitis caused by Aspergillus fumigatus. They concluded that this combination could be employed safely if dilute concentrations of steroid were used. However, at no stage in this study was an attempt made to isolate fungus; the observations were confined to a clinical evaluation of the disease. Thus, the crucial question of the effect of corticosteroid on fungal replication was not directly addressed. In animal models of fungal infection in which innate resistance leads to a natural resolution of the infection in the absence of treatment, clinical signs may not be a reliable guide to the status of the infecting organism. Quantitative techniques, such as those we used in this study, offer a more objective way of assessing response to an antimicrobial agent.

The recent introduction of new and potentially more effective antifungal agents has raised the possibility of
greater latitude in the use of corticosteroids in managing fungal corneal disease. However, on the basis of our studies, this optimism may be presumptive. As shown for the LV strain, a human ocular isolate, the efficacy of these agents is fragile and is easily reduced by factors that disturb host defense mechanisms. Studies with additional strains of *C. albicans* and filamentous fungi with different antifungal agent susceptibilities are needed to explore further the efficacy of these agents and the effects that corticosteroid may exert. For the moment, it would seem advisable to continue to exercise great caution when combined therapy with corticosteroid and an antifungal agent is contemplated.

**Key words:** *Candida albicans*, fungal corneal infection, antifungal agents, amphotericin B, natamycin, miconazole, ketoconazole, flucytosine, corticosteroid, prednisolone acetate

**Acknowledgment**

The authors acknowledge with gratitude the assistance of Luke O'Day in the performance of these experiments.

**References**