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The role of econazole in the management of oculomycosis

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Abstract

The possible role of econazole in the management of fungal eye infections is presented in this study. An animal model of oculomycosis was used to demonstrate the efficacy of econazole in an in vivo system both as a prophylactic and as a powerful therapeutic agent against sensitive fungal pathogens. In vitro tests on 114 ocular pathogenic fungal isolates exemplified the superiority of econazole to other available imidazoles.

Introduction

Econazole is one of the newer substitute phenethylalcohol imidazoles (Fig. 1). As a nitrate, it is irritant to the eye in aqueous suspensions but as a base, a 1% solution of econazole in arachis oil was found to be non-toxic to both human and animal eyes (4, 5).

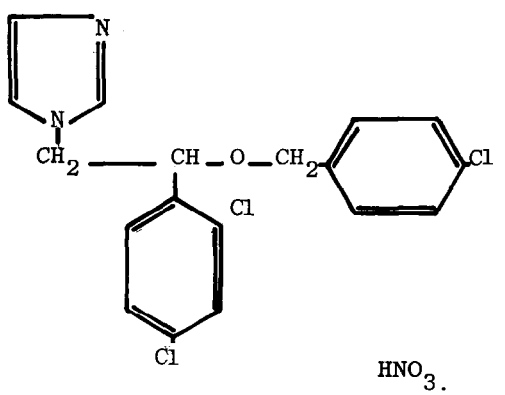

Fig. 1. The chemical structure of econazole.

The evidence for the efficacy of the various new imidazole compounds in fungal infections of the eye is anecdotal (4) and in vivo controlled trials although not possible in humans, can be undertaken in animals to demonstrate the usefulness of these new antimicrobials in a controlled system.

The present study illustrates the usefulness of econazole in an in vivo system against corneal infections caused by *Candida albicans* and *Fusarium solani* fungi in the New Zealand White (NZW) and Dutch male rabbits. The former organism is by far the commonest cause of fungal keratitis in Europe at the present time (3), while the latter organism is emerging as the most frequent cause of keratomycosis in the humid tropical world. (2, 1).

In this study we have also examined the geometric mean (GM) of minimal inhibitory concentrations (MIC) of various ocular fungal isolates to econazole and compared these values to those of clotrimazole and miconazole to illustrate a general principle in the use of these imidazoles for the management of fungal eye infections.

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Materials and methods

Six New Zealand White (NZW) male rabbits weighing 2.5 to 3.5 kg each were used to study candida keratitis while six male Dutch rabbits weighing 2 to 2.5 kg were used to study *Fusarium solani* keratitis. In both animal models, 1% econazole in arachis oil was used to determine the prophylactic and therapeutic effects of econazole.

Corneal microtrephination

Seventeen corneal microtrephinations, half corneal thickness (Fig. 2) were made in each cornea of the rabbits using a sterile glass trephine 1.5 mm diameter and 100 mm long. All surgery was done with the Zeiss Operating Microscope Mark 1 at 10× to 15× magnification. Anaesthesia of the animals was with althesin (alphaxalone and alphadolone); 0.5 ml per kilogram body weight, (6 mg total steroids/kg) or with intravenous sagatal (pentobarbitone sodium) 15 mg/kg).

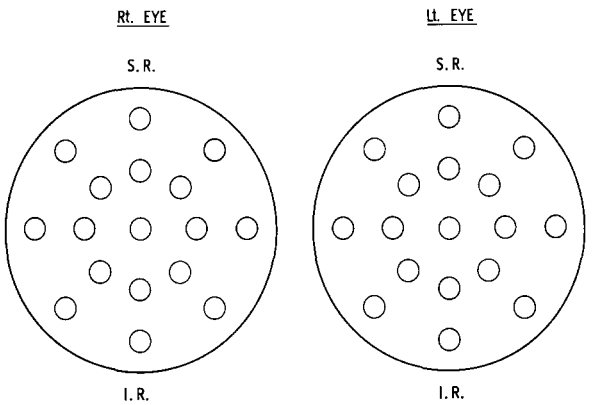

Fig. 2. Configuration of the seventeen corneal microtrephination on each cornea.

Fungus inoculum

The seventeen corneal microtrephine sites were inoculated with a 100% corneal infective dose of the fungi used in this study; *Candida albicans* for the NZW rabbits at a concentration of 4×10^4 yeasts per ml, and *Fusarium solani* at a dose of 10^5 spores per ml in normal saline. These doses were determined by graded experiments described by one of the authors. (5).

Method of scoring

For scoring the infection rates of the various organisms, each circle of trephination was divided into four quadrants and scored one if only one quadrant was infected and four if all quadrants were infected, (Fig. 3). Fig. 4 illustrates a diagramatic score of 65% infectivity and Fig. 5 shows a diagramatic score of 100% infectivity. All readings were done 48 hours after inoculation by one of the authors (E.O.O.) using the Zeiss Op. Ml. Microscope.

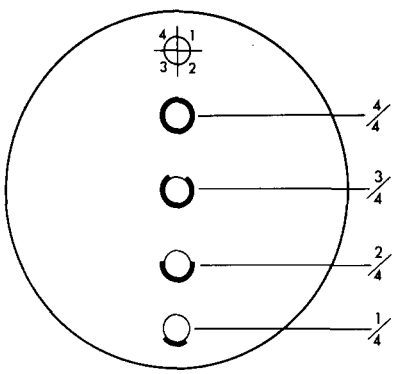

Fig. 3. Method of scoring corneal infective lesions.

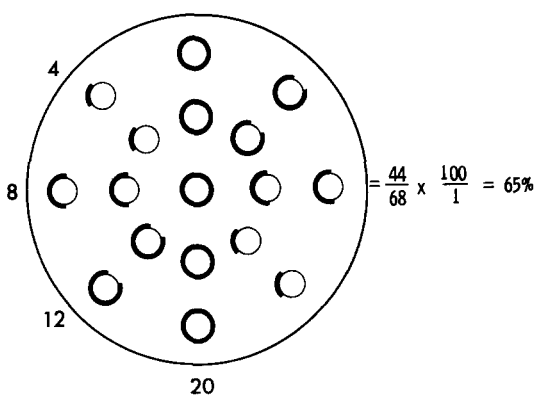

Fig. 4. Diagramatic representation of 65% corneal infectivity.

Econazole prophylaxis and therapy

To study the prophylactic effect of econazole, two pre-inoculation drops of econazole were applied topically to the test eyes of the rabbits for two consecutive hours before the eyes were inoculated with the respective pathogens. The control eyes received drops of the vehicle for the drug only (arachis oil). After 48 hours, the infectivity rates of the test and control eyes were read and compared to determine the rate of inhibition of infection produced by prior challenge with econazole (Prophylaxis). When 100% infection had been established in each cornea, the eyes were then treated with hourly drops of econazole for six to ten consecutive hours daily until resolution of all corneal lesions occured, (Therapy). The control and test eyes in each group of rabbits were randomly allocated. Two rabbits (4 eyes) in each group were left untreated after achieving 100% corneal infection.

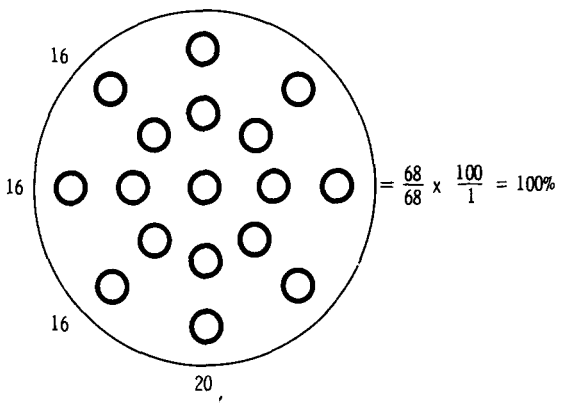

Fig. 5. Diagramatic representation of 100% corneal infectivity.

Mycology

The minimum inhibitory concentrations (MIC) of econazole against the *Candida albicans* and *Fusarium solani* strains used in this study and the MIC's against 114 other ocular pathogenic fungal isolates for econazole, clotrimazole and miconazole were done by one of the authors (Y.M.C.) using a yeast-Nitrogen base medium, (6). Swabs were taken from inoculated eyes 48 hours after inoculation and at one and two weeks after the initiation of intensive topical treatment for culture studies.

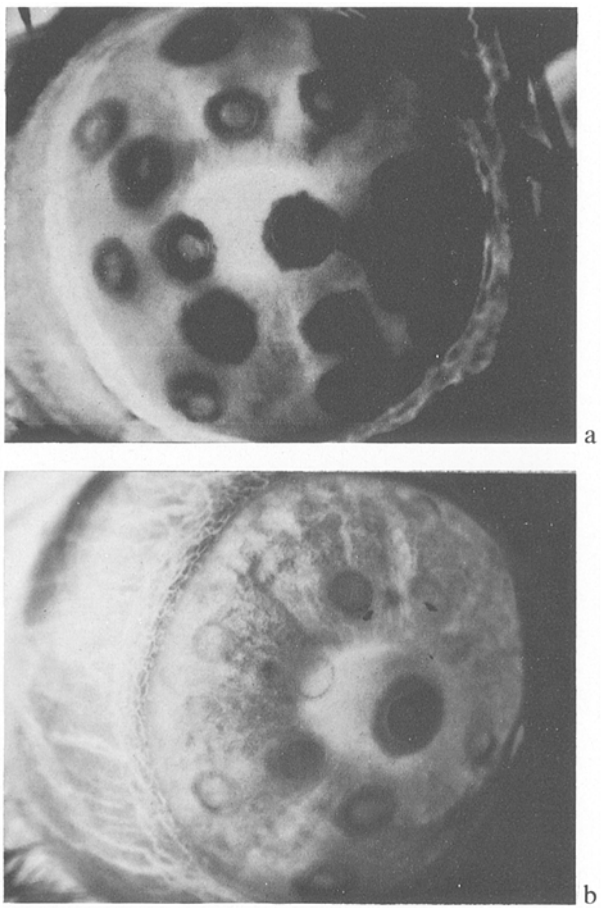

Fig. 6.a. Corneal macrophotograph of a 100% corneal infectivity on a NZW rabbit 48 hours after inoculation with *Candida albicans* 4×10^4 yeasts/ml. (Control eye). b. Corneal macrophotograph of a 72% corneal infectivity on the same NZW rabbit as 6a, 48 hours after inoculation with *Candida albicans* 4×10^4 yeasts/ml. (Test eye).

Results

Prophylaxis; corneal lesion inhibition-assay (CLIA)

The table shows the mean infection rates and the standard errors for the six NZW rabbits infected with *Candida albicans* and the six Dutch rabbits infected with *Fusarium solani*. Forty-eight hours after inoculation of their corneas with the respective pathogens there is a difference in the mean infection

rates between the test and the control eyes for both the *Candida albicans* group (18%, and the *Fusarium solani* group (19%). In both groups of animals the prophylactic effect of econazole in the inhibition of lesion development is statistically significant, ($P < 0.01$) using the matched pair t-test.

Fig. 6a is a corneal macrophotograph of a control eye scored 100% infected and Fig. 6b is the macrophotography of the test eye of the same rabbit, scored 72% infected. Both photographs were taken at the same time; 48 hours after inoculation with *Candida albicans*.

Therapy; corneal lesion reduction assay (CLRA)

Econazole as a 1% solution in arachis oil when applied hourly for ten consecutive hours daily to well established candida keratitis in the rabbit, took 2 to 3 weeks to resolve the corneal lesions completely. For *Fusarium solani* keratitis it took 12 to 18 days to resolve well established lesions on a similar regimen of treatment. All the eyes which were untreated progressed to severe endophthalmitis and perforated within two weeks.

The MIC of econazole to the *Candida albicans* used in this study was 3.1 mg/l while for the *Fusarium solani* it was 3.0 mg/l.

Fig. 7 shows the cumulative percentage sensitivity curves of 114 various filamentous pathogenic ocular fungi to econazole, miconazole and clotrimazole. The horizontal ogive at 70% of the organisms tested illustrates the superiority of econazole over miconazole and clotrimazole when matched against organisms sensitive to all three imidazoles; MIC for econazole at the above ogive is 1.1 mg/l, that of miconazole is 2.7 mg/l while it is 4.7 mg/l for clotrimazole (Fig. 7).

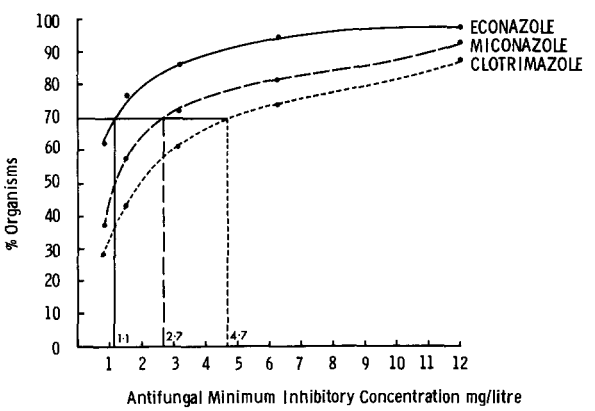

Fig. 7. Cumulative percentage sensitivity curves for 114 various filamentous pathogenic ocular fungi to econazole, miconazole and clotrimazole.

The geometric mean MIC for about 90% of these filamentous fungi tested for econazole was 1.29 mg/l, for miconazole it was 2.23 mg/l while that of clotrimazole was 2.95 mg/l.

Cultures from swabs taken 48 hours after inoculation of the pathogens grew the appropriate fungus. However, one week after the start of intensive therapy with hourly 1% econazole, 90% of the eyes from the *Candida albicans* infected rabbits and 100% of the *Fusarium solani* group gave negative cultures. Two weeks after initiation of treatments swabs from all eyes from the two groups of rabbits gave negative cultures for fungi. All the eyes which were untreated showed the respective pathogens both in the smear stained and in saboroud's culture medium.

Discussion

To get easily reproducible results from the model described above it is essential to choose suitable animals and suitable pathogenic fungal isolates. Because of the large number of sites used for the assay of drug action in each cornea, it is possible to obtain sufficient quantitative data for precise statistical analysis in a few animals.

However, considerable practice and meticulous care are needed to ensure consistent results. With the above model, econazole has been shown to possess a prophylactic potential and if the effect observed in the rabbit could be applied to humans, it could become a new approach to the problem of oculomycosis in man. This is a new concept in the field of oculomycosis.

This model also demonstrates the efficacy of econazole as a powerful therapeutic agent in these rabbits. This quality is already witnessed in the few clinical cases treated with econazole at the Moorfields Eye Hospital, London.

In vitro studies comparing various imidazoles against ocular fungal pathogens show that econa-

Table. Infectivity rates, means and standard error of means (SEM) for 12 rabbits: six of which were infected with *Candida albicans* and six with *Fusarium solani.*

Rabbit No	Inoculum Dilution	% Infectivity on test eyes 48 hours after inoculation	% Infectivity on control eyes 48 hours after inoculation
1.	*Candida albicans* 4×10^4/ml.	94.12	100.0
2.	*Candida albicans* 4×10^4/ml.	88.24	100.0
3.	*Candida albicans* 4×10^4/ml.	82.35	100.0
4.	*Candida albicans* 4×10^4/ml.	73.53	100.0
5.	*Candida albicans* 4×10^4/ml.	72.06	100.0
6.	*Candida albicans* 4×10^4/ml.	80.88	100.0
		Mean: 81.86	100.0
		SEM: 3.45	0.0
7.	*Fusarium solani* 10^5/ml	63.24	88.24
8.	*Fusarium solani* 10^5/ml	94.12	97.06
9.	*Fusarium solani* 10^5/ml	72.06	86.79
10.	*Fusarium solani* 10^5/ml	64.00	94.12
11.	*Fusarium solani* 10^5/ml	72.06	88.24
12.	*Fusarium solani* 10^5/ml	72.06	97.06
		Mean: 72.92	91.91
		SEM: 4.55	1.93

zole is more effective than the other imidazoles tested for the majority of the organisms. The above animal model did not indicate the reason for the superiority of econazole over the other imidazoles tested, however, it was not designed to do so any way. However, subsequent work with this model would soon show whether this superiority which econazole showed over the other two imidazoles in vitro could apply in an in vivo system.

The prophylactic effect observed with econazole in these animals opens yet another avenue for research in the prevention of oculomycosis and in conclusion it is suggested that two possible roles exist for econazole and other newer antifungal antibiotic agents in fungal eye infections; a prophylactic and a therapeutic role.

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