Natamycin Treatment of Experimental *Candida albicans* induced Keratomycosis in Rabbits

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ABSTRACT

Objective: *The efficacy of topical natamycin 5% was studied using a reproducible model of keratomycosis produced by* Candida albicans *in the rabbits.*

Method: Candida albicans *was isolated from infected human eye and 4 x 10^5 cells of the* Candida albicans *was injected into the corneal stroma of the eyes of 15 rabbits. All eyes developed a corneal ulcer without pretreatment with immunosuppressive agents. Forty-eight hours after inoculation, the animals were divided into two groups: test group I, 10 eyes receiving natamycin drops in a 5% suspension; control group II, five eyes receiving 0.9% normal saline solution. The rabbits' corneas were removed for* Candida albicans *recovery and placed in 1 ml of sterile 0.9% normal saline solution, minced within two hours with scalpel and thoroughly homogenized with a piston and mortar. Serial dilutions of this corneal solution from 10^{-1} – 10^{-4} were made in 0.9% sterile saline solution and 100 µl aliquots were plated onto tryptic soy agar. All cultures of cornea from the treated eyes were negative after seven days of inoculation while five cultures were still positive in the control eyes at the end of the experiment.*

Result: *It was found that 5% natamycin was effective in treating experimental* Candida albicans *induced keratomycosis in rabbits.*

Conclusion*: It is concluded that natamycin has a significant effect ($p < 0.01$) against* Candida albicans *in treating experimental keratomycosis.*

Tratamiento con Natamicina en un Experimento de Queratomicosis Inducida Mediante *Candida albicans* en Conejos

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RESUMEN

Objetivo: *La eficacia de la natamicina tópica al 5% fue estudiada usando un modelo reproducible de queratomicosis producida por* Candida albicans *en conejos.*

Método: Candida albicans *fue aislada de una infección ocular humana y 4 x 10^5 células de* Candida albicans *fueron inyectadas en el estroma córneo de los ojos de 15 conejos. Todos los ojos desarrollaron una úlcera córnea sin pre-tratamiento con agentes inmunosupresores. Cuarenta y ocho horas después de la inoculación, los animales fueron divididos en dos grupos: un grupo experimental I, en el que diez (10) ojos recibieron gotas de natamicina en suspensión al 5%; y un grupo control II, en el que cinco (5) ojos recibieron solución salina normal al 0.9%. Las córneas de los conejos fueron extraídas para recuperar* Candida albicans *y colocadas en 1 ml de solución salina normal estéril, para ser luego desmenuzadas a las dos horas con un escalpelo, y homogeneizadas completamente con un mortero. Se hicieron diluciones seriadas de esta solución córnea de 10^{-10} -10^{-4} en solución salina al 0.9% y 100 ml de alícuotas fueron colocadas en placas con agar de soya tríptico. Todos los cultivos de corneas de los ojos tratados, resultaron negativos luego de siete días de inoculación, mientras que 5 cultivos eran todavía positivos al final del experimento en los ojos del control.*

Resultado: *Se halló que la natamicina al 5% era efectiva en el tratamiento de la queratomicosis inducida experimentalmente mediante* Candida albicans *en conejos.*

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Conclusión: *Se concluyó que la natamicina surte efecto (*p < *0.01) sobre* Candida albicans *en el tratamiento experimental de la queratomicosis.*

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INTRODUCTION

Keratitis is a major cause of blindness in the developing countries of the world. This situation is especially true in areas where trachoma, onchocerciasis, leprosy and other infectious causes of ocular diseases are endemic (1). Studies from several developing countries have reported the incidence of fungal pathogens isolated from ulcerated corneas (2–3). The incidence of fungal keratitis has increased over the last four decades worldwide (4–8) but the available therapies are limited. Nystatin was the first polyene antibiotic that was recommended for topical ocular use but corneal toxicity and poor ocular penetration limited its value (6). Topical miconazole (1%) and amphotercin B (1%) exhibit minimal toxicity characterized by punctate epithelial corneal erosions (9–10). The increasing use of broad-spectrum antibacterial, corticosteroid and other immunosuppressive drugs over long periods of time is also associated with a rapidly rising incidence of fungal keratitis.

Natamycin (Pimaricin), a board-spectrum anti-fungal agent with low ocular toxicity, became the first anti-fungal agent approved for ocular use in 1975 in the United States of America (USA). The aim of this study is to evaluate the effectiveness of 5% topical natamycin against experimental *Candida albicans* keratitis in rabbits.

SUBJECTS AND METHODS

Micro-organisms

Candida albicans was isolated from infected human eye at Akhtar Eye Hospital, Karachi (a postgraduate teaching hospital for ocular diseases). The *Candida albicans* was transported to the laboratory in cotton swab (commercially available Stuart transport medium, Oxide, UK).

One day before inoculation, the *Candida albicans* was plated onto Sabourad dextrose agar and incubated at 37°C for 24 hours. The *C albicans* culture was diluted in normal (0.9%) saline solution to yield a concentration of 4 x 10^5 cfu/ml (colony-forming unit/ml).

Experimental Model

White healthy rabbits, averaging 1.5–2 kg in weight and having disease free corneas were obtained from the animal house of the Pakistan Council of Scientific Industrial Research (PCSIR) Laboratories Complex, Karachi. Rabbits were kept under observation for 72 hours to exclude any local or systemic diseases. They were categorized into a test group and a control group. The test group comprised ten rabbits while the control group had five.

The study was carried out in accordance with the guideline published by the Association for Research in Vision and Ophthalmology Research on the use of Animals in Research.

Before producing intraocular infection, general anaesthesia was induced in the rabbits by an intramuscular injection of 2 ml of phenobarbitone sodium (4 mg/ml). After topical anaesthesia was achieved with proparacaine HCl 0.5% (Alcane) then the eye was gently exposed with an eye dilator. An 8.5 mm trephine blade was used to create a circular corneal incision approximately 0.1 mm in depth in the right eye of each rabbit. A 10 µl aliquot of the inoculums, containing 4 x 10^5 organisms was injected into the circular corneal incision in both rabbit groups.

Topical studies

All eyes without pretreatment with immunosuppressive agents developed corneal ulcers within 48 hours. Natamycin ophthalmic suspension (Ophth-natamycin 5%) was purchased from the local market and five drops (50 µl/drop) loading dose of natamycin was instilled in the inferior conjunctival sac, half hourly for the first three hours, then hourly for five days in test animals. The natamycin drops were instilled during the office hours (8 am to 3 pm). Control rabbits were treated with a loading dose of 0.9% saline drops similar to natamycin dosing. The rabbits eyes were examined daily at random.

Colony Count Determination

The rabbits were killed at the end of the seven-day treatment. Corneas were removed for isolates recovery and placed in 1 ml of sterile 0.9% normal saline solution, minced within two hours by scalpel and thoroughly homogenized with a piston and mortar. Serial dilutions of this corneal solution from 10^{-1} – 10^{-4} were made in 0.9% sterile saline solution and 100 µl aliquots were plated onto tryptic soy agar. Each dilution was plated in duplicate. Plates were incubated for seven days at 37°C and the number of cfu/cornea was counted using the last two countable plates. All cultures of cornea from the treated eyes were negative up to the seventh day after inoculation, while five cultures were still positive at the end of the experiment (seven days) in the control eyes. Finally, cornea from one randomly assigned rabbit was taken, thick section made and stained with Haematoxylin Eosin and Giemsa.

Statistical analysis

The colony counts were compared by means of a two tailed *t- test*.

RESULTS

After seven days of treatment, all animals were clinically evaluated and both the extension and the depths of the corneal infiltrates were graded. The pretreatment grade for all animals was 1.4 (mean clinical slit lamp score). In control animals receiving saline eye drops, the combined score for the extension and the depth of the corneal infiltrate increased significantly during treatment to a score of 2.6. In contrast, no significant change was observed in animals treated with 5% natamycin. After seven days treatment, C albicans was recovered from culture of five corneas of the control group. In contrast, 10 corneas of the test group treated with 5% natamycin showed no growth ($p < 0.01$). The comparative isolates recovered from both groups are given in the Table.

Table: Recovery of *C albicans* from control and test group

Control group *vs* Test group		
Control group		
Rabbit No	Eye	No of organisms/ ml
1	R	$7 \times 10^4 \pm 0.69$
2	R	$6.4 \times 10^4 \pm 0.73$
3	R	$6 \times 10^4 \pm 0.75$
4	R	$4 \times 10^5 \pm 1.15$
5	L	$3.8 \times 10^5 \pm 1.03$
Mean		1.948×10^5
Test group		
6	R	NG
7	R	NG
8	R	NG
9	R	NG
10	L	NG
11	L	NG
12	R	NG
13	R	NG
14	L	NG
15	L	NG

R = right eye; L = left eye; NG = No growth
Results are means ± SD (standard deviation)

DISCUSSION

In treating patients with fungal keratitis, ophthalmologists are confronted with three major difficulties: the limited selection of antifungal agents available, the poor intraocular penetration of many of the available agents and the toxicity of these agents. Behrens *et al* (11) compared standard amphotericin B with natamycin drops 1% and 2.5% using a reproducible model of keratomycosis from *Candida albicans* in the rabbit and concluded that natamycin is inferior to amphotericin B and not effective in controlling experimental keratomycosis. But in the present study, 5% natamycin drops was effective in keratomycosis. In therapeutic review, Mishima *et al* (12) reported that *Candida albicans* is less sensitive to 5% natamycin.

A standardized model of *Candida albicans* keratitis was developed by O' Day *et al* (13) in rabbits to evaluate the disease at intervals throughout the study. The efficacy of five antifungal agents was compared. Amphotericin B in concentrations of 0.5% to 0.075%, was superior to all other agents tested. Natamycin (5%) ranked next, followed by 1% flucytosine and 1% miconazole. Ketoconazole 1% was ineffective. Although our results are not exactly comparable with that of O'Day *et al* noted above, they are not too dissimilar.

In another study, O'Day *et al* (14) compared the efficacy of 0.15% amphotericin B and 5% natamycin in two models of *Candida albicans* infection. Amphotericin B treatment was begun immediately after inoculation while natamycin was delayed for 24 hours. A significant therapeutic effect was found for amphotericin B in both models. However, delayed treatment with natamycin was ineffective. But in the present study, we found that 5% natamycin was effective even after delayed treatment of 24 hours.

Oji *et al* (15) investigated corneal infection caused by an ocular pathogenic *Candida albicans* on the New Zealand white male rabbits. Cornea was treated with 3% mycolase II, 5% natamycin and a combination of 3% mycolase II and 5% pimaricin respectively and there was a significant result against *Candida albicans*. In the present study, we also found 5% natamycin effective against *Candida albicans*.

There are many reports which have shown that other available antifungals are toxic. Pleyer (16) *et al* described that amphotericin B and natamycin are the most effective agents for the treatment of keratomycosis. Natamycin has gained favour as an antifungal agent because of its broad spectrum effects and relatively low ocular toxicity.

Topical natamycin is well tolerated. Corneal toxicity usually in the form of punctate keratitis is rare; although a low-grade inflammation may develop with prolong use (13). In an animal induce keratomycosis study, natamycin did not retard the healing of corneal epithelial defects (17).

It is concluded that natamycin is effective in treating experimental *Candida albicans* induce keratomycosis.

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