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Role of 0.02% Polyhexamethylene Biguanide and 1% Povidone Iodine in Experimental *Aspergillus* Keratitis

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Purpose. To determine the efficacy of 0.02% polyhexamethylene biguanide and 1% povidone iodine in experimental *Aspergillus* keratitis. **Methods.** *Aspergillus fumigatus* keratitis was induced by corneal intrastromal injection of spores in 24 healthy rabbits that were randomly divided into four groups of six rabbits each. Drugs used were 5% natamycin (standard antifungal), 0.02% polyhexamethylene biguanide (PHMB) (test drug), 1% povidone iodine (test drug), and 0.5% hydroxypropylmethyl cellulose (HPMC) (control). **Results.** The average healing times of the ulcer were 21.5 ± 3.08 days with 5% natamycin, 27.8 ± 2.28 days with 0.02% PHMB, 36.4 ± 2.57 days with 1% povidone iodine, and 38.2 ± 4.74 days with 0.5% HPMC. While no corneal perforations occurred with natamycin treatment, one perforation was noted with PHMB, three perforations were noted with povidone iodine, and five perforations were noted with controls. **Conclusion.** Polyhexamethylene biguanide (0.02%) is a moderately effective drug for experimental *Aspergillus* keratitis, but 1% povidone iodine is not effective.

Key Words: Polyhexamethylene biguanide—Povidone iodine—Natamycin—Hydroxypropylmethyl cellulose—Experimental *Aspergillus* keratitis—Hypopyon—Epithelial defect.

Fungal keratitis is a chronic smoldering infection of great significance in tropical countries like India. *Aspergillus* sp. is the most common (36–40%) cause of fungal corneal ulcers in India,[1–3] whereas Dunlop et al.[4] reported *Aspergillus* in 37% cases of fungal keratitis from Bangladesh. Worldwide, McDonnell et al.[5] reported that 20% to 30% of ulcers were due to fungi, and Rosa et al.[6] reported that *Fusarium* sp. was the most common organism in South Florida. There is a paucity of antifungal agents available for use against this sight-threatening condition, unlike bacterial keratitis, for which there is a battery of chemotherapeutic agents. Moreover, these drugs are not widely available and are relatively expensive for the patients in rural agricultural communities where fungal keratitis is prevalent.

Thus, in a quest for an inexpensive and readily available alternative drug for the therapy of fungal keratitis, we selected 0.02% polyhexamethylene biguanide (PHMB) and 1% povidone iodine for our study. Both drugs are relatively nontoxic at therapeutic concentrations, have good in vitro efficacy against common agents causing fungal keratitis, are relatively inexpensive, and have widespread availability in large quantities.

Polyhexamethylene biguanide, a polymeric biguanide, is widely used as a swimming pool disinfectant and as a biocide in commercial contact lens solutions.[7] In vitro activity of 0.02% PHMB against fungi has been extensively tested[8,9] and has shown good in vivo efficacy against *Fusarium* keratomycosis.[10] Polyhexamethylene biguanide (0.02%) has been successfully used for the treatment of *Acanthamoeba* keratitis without significant corneal toxicity.[11,12]

Povidone iodine has a broad spectrum of action, is cost effective, and has been used for preoperative antibiotic prophylaxis.[13] Its efficacy against *Aspergillus* has been recognized both in vitro[14] as well as in vivo[15] at a concentration of 1%. White et al.[15] reported that 1% povidone iodine led to a significant reduction in the duration of experimentally induced fungal contamination with *Aspergillus niger* in the conjunctival fornices of rabbits without notable irritation. This experimental study was undertaken to find out the relative efficacy of 0.02% PHMB and 1% povidone iodine in the management of *Aspergillus* keratitis.

MATERIALS AND METHODS

A prospective, randomized, case-control study was carried out in a rabbit model. Prior permission for the experimental study was obtained from the institutional animal ethics committee, and the experimental rules set forth in the ARVO resolution on animal experimentation were strictly adhered to. Twenty-four rabbit corneas were inoculated with a human ocular isolate of *Aspergillus fumigatus* obtained from the Department of Ocular Microbiology of our center.

Production of the Corneal Ulcer

All eyes received prior 0.3% ciprofloxacin ophthalmic ointment twice daily for 2 days to minimize the risk of secondary bacterial infections. Topical 4% Xylocaine drops were instilled three times

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TABLE 1. *Perforations in experimental* Aspergillus *keratitis (n = 24)*

Group	Perforation	
	No. of eyes	Range (d)
A (5% natamycin)	0/6	—
B (0.02% PHMB)	1/6	20
C (1% povidone iodine)	3/6	19–22
D (0.5% HPMC)	5/6	19–23

PHMB, polyhexamethylene biguanide; HPMC, hydroxypropylmethyl cellulose.

at 2-minute intervals to the inferior cul de sac 5 minutes prior to the inoculation. The inoculation was carried out under an operating microscope (Wild Heerbrugg, Switzerland). Rabbits were held per the guidelines prepared by University of Edinburgh.[16] The lids were separated using a self-retaining wire speculum. A 5-mm diameter corneal trephine was used to mark the central cornea. The enclosed area was infiltrated with intrastromal injection of *A. fumigatus* suspension (0.03 mL, 5.5×10^4 spores/mL) by a tuberculin syringe with 26-gauge needle. The animals were examined daily under a slit lamp (Carl Zeiss GmbH, West Germany). When ulcers appeared, corneal scrapings were taken from the leading edge of the ulcer and inoculated into blood agar and Sabouraud's dextrose agar media. In addition, smears were taken, stained with Gram and Giemsa stains, and evaluated under light microscopy by the ocular microbiologist (G.S.). Only culture-proven eyes (growth in either media) were selected for the study. Eyes with smears showing evidence of bacterial contamination were excluded from the study despite fungal culture positivity. Care was taken to initiate the topical drugs only when the ulcer size was 4 mm. After the initial cultures to confirm the existence of *Aspergillus* in the cornea, a standard protocol of weekly cultures was followed for each rabbit. The anterior chamber was perforated during intrastromal inoculation of spores in three eyes, and these were removed from the study. Four eyes had bacterial infections and thus were excluded from the study. To fulfill the target of 24 cases, fresh rabbits were selected and the procedures were repeated.

The rabbits with a 4-mm ulcer were randomly distributed into four groups (groups A, B, C, and D) of six eyes each. Group A received commercially available 5% natamycin suspension (Pimafucin, Elder Pharmaceutical Ltd., India). Group B received PHMB (Cosmocil CQ 20%, Avecia Biocides, U.K.) diluted to 0.02% eyedrops at our ocular pharmacy under sterile conditions. Group C received a sterile ophthalmic solution of povidone iodine (Ocudone 5%, FDC Ltd., India) diluted to 1% eyedrops, and group D received 0.5% hydroxypropylmethyl cellulose (HPMC) eyedrops prepared by the Ocular Pharmacy Section of Rajendra Prasad Center.

The drugs were coded as A, B, C, and D by an ocular pharmacologist (N.R.B.) and instilled by a trained nonmedical staff member at hourly intervals from 8:00 A.M. to 8:00 P.M. Besides the coded drug, one drop of 1% atropine sulfate eyedrops was also administered in each case of fungal keratitis. During the therapy, all the eyes were evaluated daily by two independent evaluators (R.A., S.K.) separately, and the findings were noted until healing occurred. At the initiation of the healing, the nonmedical staff member was advised to reduce the frequency of the drugs to every 2, 3, 4, and 6 hours, then twice daily, and then totally stopped at healing. The signs of resolution considered in this study included rounding of the ulcer margin, decreased hypopyon, decreased infiltrates, decreased corneal edema, resolution of epithelial defect, and average healing time. At the end of the study, the code was broken, and the data obtained were evaluated statistically using Wilcoxon's signed rank sum test.

RESULTS

The average time to initiation of the ulcer was 3.62 ± 1.87 days and to attaining 4-mm size was 5.4 ± 2.24 days. None of the ulcers perforated in group A, while there was one perforation in group B, three in group C, and five perforations in group D during the course of therapy (Table 1). None of the eyes developed endophthalmitis. There was no significant variability noted between the two examiners.

The average times to rounding of the ulcer margin, decrease in size of the hypopyon, decrease in infiltrates, decrease in corneal edema, and resolution of epithelial defects are depicted in Table 2. The average healing times for the four groups are shown in Table 3. The difference between groups A and B was statistically significant ($p = 0.012$). Similarly, differences between groups A and C and groups A and D were highly significant ($p < 0.001$) (Table 3). All corneas healed with scarring, and those with perforation healed with the formation of adherent leucoma. Scarring was noted to be least with natamycin (group A). It was surprising to note that

TABLE 2. *Comparison of PHMB, povidone iodine, and HPMC (control) with natamycin in experimental* Aspergillus *keratitis*

Group	Rounding of ulcer margin (d)	Decreased hypopyon (d)	Decreased infiltrates (d)	Decreased corneal edema (d)	Resolution of epithelial defect (d)
A (n = 6)	8.16 ± 1.47	12.83 ± 2.23	9.0 ± 1.41	10.5 ± 1.37	21.5 ± 3.08
B (n = 6)	16.83 ± 3.31	20.8 ± 4.54	18.8 ± 3.7	20.8 ± 4.54	27.8 ± 2.28
C (n = 6)	23.6 ± 2.42	24.9 ± 2.85	25.3 ± 2.91	26.2 ± 2.51	36.4 ± 2.57
D (n = 6)	26.3 ± 2.06	26.8 ± 2.82	26.2 ± 2.52	27.1 ± 3.15	38.2 ± 4.74
A vs. B	$p = 0.002$	$p = 0.01$	$p = 0.004$	$p = 0.004$	$p = 0.012$
A vs. C	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$
A vs. D	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$
B vs. C	$p = 0.004$	$p = 0.01$	$p = 0.007$	$p = 0.006$	$p = 0.02$
B vs. D	$p = 0.006$	$p = 0.02$	$p = 0.008$	$p = 0.014$	$p = 0.016$
C vs. D	$p = 0.466$	$p = 0.491$	$p = 0.579$	$p = 0.596$	$p = 0.64$

Values are expressed as mean ± SD (n = 24).

Group A, 5% natamycin; group B, 0.02% PHMB; group C, 1% povidone iodine; group D, 0.5% HPMC.

PHMB, polyhexamethylene biguanide; HPMC, hydroxypropylmethyl cellulose.

TABLE 3. *Average healing time (mean ± SD) in experimental* Aspergillus *keratitis (n = 24)*

Groups	Healing in days (range)	Significance
A	21.5 ± 3.08 (19–26)	A vs. B, $p = 0.012$ (S) A vs. C, $p < 0.001$ (HS) A vs. D, $p < 0.0001$ (HS)
B	27.8 ± 2.28 (25–36)	B vs. C, $p = 0.02$ (S) B vs. D, $p = 0.016$ (S)
C	36.4 ± 2.57 (33–42)	C vs. D, $p = 0.64$ (NS)
D	38.2 ± 4.74 (36–45)	

Group A, 5% natamycin; group B, 0.02% polyhexamethylene biguanide; group C, 1% povidone iodine; group D, 0.5% hydroxypropylmethyl cellulose; HS, highly significant; NS, not significant; S, significant.

in the control group (group D), one eye also healed. Natamycin (5%) performed better in all the clinical categories than PHMB.

DISCUSSION

Fungal corneal ulcers are one of the leading causes of blindness in developing countries along with other ocular infections and cataract. In these countries, ocular trauma, aggravated by injudicious use of topical antibiotics and corticosteroids leads to a high incidence of fungal ulcers. Fungal keratitis is different from other types of keratitis since the fungal hyphae penetrate deep into the corneal stroma where the organisms are inaccessible to the usual diagnostic and therapeutic measures.[17,18]

An infectious corneal ulcer is defined as a breach in the epithelium accompanied by infiltration and necrosis of corneal tissue. For our study, the ulcer was defined when slit-lamp evidence of such signs was present along with fluorescein staining. Healing time in our study was defined as the absence of inflammation of the cornea and reduction of the epithelial defect as tested by fluorescein staining.

The current rabbit model of *Aspergillus* keratitis is based on the study by Mohan et al.[19] and Biswas et al.[20] who performed intrastromal injection of infective agents. Data in the current study are consistent with those of their study and confirm that fungal corneal ulcers are easily produced in rabbit eyes and progress to perforation in untreated cases. The time for initiation of fungal ulcer in all the rabbits was 3 to 4 days, and all the eyes were started on drug therapy only after a 4-mm ulcer was achieved. Healing was observed in the eyes that perforated and that subsequently healed with adherent leucoma formation. The presence of an iris provides factors for fighting the infection and healing the cornea. These eyes had a denser and full thickness scar.

The results of our study showed that although PHMB provided a good protective effect, its efficacy was not absolute compared with that of the standard antifungal (natamycin). Our report is in concurrence with that of Messick et al.[8] who found that the MIC of PHMB for *Aspergillus* was 6.1 μg/mL. Thus, it seems that the drug may require supplementation of a standard antifungal drug. On the basis of our results and those reported in the literature, PHMB is likely to have a supplementary role in the therapy of *Aspergillus* keratitis. However, a drug trial using a large number of eyes with a single drug and in combination with natamycin will provide further support for its use in clinical practice.

The lack of efficacy of 1% povidone iodine against *Aspergillus* keratitis as revealed in the current study is discouraging. The discrepancy between in vitro and in vivo studies is again highlighted. The lack of in vivo efficacy of 1% povidone iodine could be explained by poor corneal penetration. Higher concentrations of povidone iodine may be more effective.

The strengths of this study were that it was a double-masked, prospective, randomized, case-control study in which multiple drugs were tested in a controlled environment and cases were seen by two clinicians masked as to which drug each rabbit was receiving. The weaknesses of this study were that higher concentrations of povidone iodine should have been tested and monkeys would have been a more ideal host because of the similarity between monkey and human corneas. A randomized, controlled trial[21] was already conducted using 0.2% chlorhexidine in mycotic keratitis, which showed its efficacy; hence, we felt no need to repeat the studies in an experimental setting.

This study showed that natamycin is superior to the antiseptic agents used in our study for the treatment of *Aspergillus* keratitis. In India, despite the high prevalence of *Aspergillus* keratitis and the high cost of drugs, natamycin still remains the drug of choice over less expensive antiseptic agents. However, the antiseptic agents may be tried as an adjuvant therapy in *Aspergillus* keratitis. Worldwide, there is a need for further research on the role of less expensive and more readily available drugs that are effective against ocular fungal infection. Thus, we may conclude that 0.02% PHMB is a moderately effective drug for *Aspergillus* keratitis, which needs further assessment for regular use. However, 1% povidone iodine is not an effective drug for topical use against *Aspergillus* keratitis. Further assessment with higher concentrations within tolerable limits is warranted.

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