THERAPY OF ACANTHAMOEBA KERATITIS - AN EXPERIMENTAL STUDY

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(Received on January 10, 2000)

Abstract: The study was conducted to determine the efficacy of various drugs/agents in the treatment of experimentally induced Acanthamoeba Keratitis (A. keratitis). Thirty rabbits were divided into 5 equal treatment groups and polyhexamethylene biguanide (PHMB) 0.02%, fluconazole 0.2%, povidone iodine 5%, aprotinin 40 IU/ml and normal saline were administered in a double blind manner, at a dose of one drop, 5 times/day. Neomycin 1700 U/ml was used as a common drug in all groups, with same frequency. Post therapy subsidence of lid oedema, mucopurulent discharge, conjunctival congestion, corneal epithelial defect and anterior chamber reactions were noted. Average healing time in days (Mean ± S.D.) were 13.67 ± 1.21, 22 ± 1.41, 23.5 ± 1.05, 17.0 ± 1.41 and 28.0 ± 1.90 for PHMB, fluconazole, povidone iodine, aprotinin and normal saline respectively. Lid oedema, mucopurulent discharge and conjunctival congestion improved faster in PHMB and aprotinin treated eyes. The results of this study suggest that PHMB is a better drug for A. keratitis, while aprotinin may be used as an adjuvant drug in the management of A. keratitis.

Key words: acanthamoeba polyhexamethylene biguanide
povidone iodine keratitis fluconazole
aprotinin

INTRODUCTION

Acanthamoeba keratitis (A. keratitis) poses a serious challenge to the clinicians because of problems in its management. Though various anti-acanthamoebal drugs are presently available, but, no conclusive reports are available in the literature regarding their efficacy (1-9).

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animal ethics committee was obtained. Before starting the experimental procedure, ocular examinations like conditions of the lids, eye lashes, conjunctiva, cornea, iris and pupil as well as intraocular pressure measurements were carried out. Systemic examinations included condition of skin of the rabbits, their movement, alertness etc. to exclude any obvious disease process.

**Production of acanthamoeba keratitis in rabbits**

Right eye of each rabbit was selected for the production of A. keratitis. Lids were separated with the use of speculum and anaesthetized by topical 4% lidocaine hydrochloride. A trephine of 5mm diameter was placed over the central cornea and, area included in the trephine mark was debrided of corneal epithelium.

Acanthamoeba suspension (0.1 ml) containing Acanthamoeba culbertsoni cysts 

\[(52 \times 10^4 \text{ organisms/ml})\]

was injected intra-stromally into the central cornea, already debrided of epithelium. All the rabbits were observed daily with slit lamp examination, for the initiation of ulcers. Once the ulcer was produced, the extent of lid oedema, conjunctival discharge, degree of corneal epithelial defect, extent of anterior chamber (AC) reactions and onset of vascularisation were determined (10). From all eyes, specimens were obtained by corneal biopsy from the leading edge of the ulcer and inoculated to non-nutrient agar medium lawn ed with E.coli for growth of Acanthamoeba and to blood agar plate to isolate growth of any superadded infection.

Criteria used for initiation of therapy included ulcer size of at least 3 mm and culture positivity for Acanthamoeba (Fig. 1). Neomycin eye drop (1700 U/ml) 5 times daily and atropine ointment (.1%) twice daily were used as the common drug in all groups.

Thirty rabbits were divided into 5 groups (Group I-V), 6 in each. Various drugs/agents used in these groups included: PHMB 0.02%, fluconazole 0.2%, povidone iodine 5%, aprotinin 40 IU/ml and normal saline. All these agents were water soluble and delivered in amber colored vials to make them indistinguishable from outside and were blinded for the investigators. The frequency of drug administrations in all groups was 5 times/day. Following initiation of therapy all the eyes were evaluated daily by slit lamp examination for signs of healing which included reduction in size and depth of ulcer, decrease in the area of infiltration and decrease in the extent of hypopyon, if any. At the end of the study the coded drugs were decoded and evaluated for their efficacy.

**Statistical analysis**

Data on subsidence of lid oedema, disappearance of discharge, corneal epithelial defect, subsidence of AC reaction, onset of vascularisation and healing time were entered and analysed in STATA 6.0 inter cooled version. All these parameters were summarized by mean and standard deviation. These parameters amongst five groups were compared by one-way analysis of variance (Kruskal Wallis test-Non parametric). Multiple range test was applied to detect which pair/pairs of groups mean were statistically different (11).
RESULTS

All the eyes, inoculated with Acanthamoeba strain, developed culture proven Acanthamoeba keratitis. In 4 specimens, superadded bacterial keratitis were diagnosed by microbiological evaluation and these were excluded from the study. To maintain the similar number in all groups for comparison, 4 more rabbits were inoculated for the formation of Acanthamoeba keratitis by the similar technique. All the specimens had shown growth of Acanthamoeba within 48 hours. The times needed for lid oedema to subside, discharge to disappear, corneal epithelial defects to subside, anterior chamber reactions like flare to subside and time needed for vascularisation to start in all these five groups are shown in Table I.

![Photograph showing the active A. keratitis ulcer.](image1)

![Photograph showing healed stage of A. keratitis ulcer.](image2)

**TABLE I: Healing of acanthamoebal corneal ulcer in rabbit eye.**

<table>
<thead>
<tr>
<th>Subsidence of lid oedema</th>
<th>Disappearance of discharge</th>
<th>Corneal epithelial defect healing</th>
<th>Subsidence of AC reaction</th>
<th>Onset of vascularisation</th>
<th>Onset of healing time</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>8±1.41*</td>
<td>13±2.10*</td>
<td>9±1.40*</td>
<td>17±3.16*</td>
<td>8±2.10*</td>
</tr>
<tr>
<td>B</td>
<td>13±1.41*</td>
<td>22±2.61*</td>
<td>18±2.00*</td>
<td>23±4.05</td>
<td>13±2.45**</td>
</tr>
<tr>
<td>C</td>
<td>16±2.53***</td>
<td>24±2.00***</td>
<td>23±2.61***</td>
<td>24±4.69</td>
<td>20±3.03</td>
</tr>
<tr>
<td>D</td>
<td>10±2.45*</td>
<td>19±2.60*</td>
<td>14±2.37***</td>
<td>20±2.97**</td>
<td>11±2.09*</td>
</tr>
<tr>
<td>E</td>
<td>21±3.74</td>
<td>29±2.19</td>
<td>29±3.79</td>
<td>30±4.00</td>
<td>20±3.85</td>
</tr>
</tbody>
</table>

*P<0.001 compared to normal saline
**P<0.01
***P<0.05
A : Polyhexamethylene biguanide (PHMB) 0.02%
B : Fluconazole 0.2%
C : Povidone Iodine 5.0%
D : Aprotinin 40 IU/ml
E : Normal saline

Each value represents mean ± S.D. of days
Number of eyes in each group = 6
one-way ANOVA (Kruskal Wallis test)
Average healing times taking all the above parameters in days (Mean ± S.D.) were 13.67 ± 1.21, 22 ± 1.41, 23.5 ± 1.05, 17.0 ± 1.41, 28 ± 1.90 for PHMB, fluconazole, povidone iodine, aprotinin and normal saline respectively (Table I). At the end of treatment, cornea remained scarred in all the cases which was the sign of healing (Fig. 2).

**DISCUSSION**

In this experimental study, rabbits were used because it is relatively easy to induce experimental Acanthamoeba keratitis in rabbits (12). Acanthamoeba culbertsoni strain is one of the pathogenic strain that can cause A. keratitis (13) and has been used.

Neomycin, the common drug in this experimental study is one of the common drugs, used in Acanthamoeba and has a reported efficacy rate as high as 50% (14). It is also freely available though it is known to have poor cysticidal activity but, is a good trophozoicidal agent (4). As good cysticidal activity seems to be an important factor for antiamoebic effect in the treatment of clinical disease, neomycin ideally should be used in combination with agents having good cysticidal activity (4).

Keeping this in mind neomycin was added to an already established antiacanthamoebal agent i.e. PHMB having both cysticidal and trophozoicidal effects, to know their efficacy and to compare with other combination therapies. In 1991, Larkin et al (1), first described the role of PHMB, as an effective anti-acanthamoebal agent. At a concentration of 0.02% it was found to be both trophozoicidal and cysticidal in vitro and was tolerated by the corneal and conjunctival epithelium. Its toxicity was found to be less than neomycin and propramidine (15). Our study also proved that PHMB in combination with neomycin produced the best antiacanthamoebal effect, as compared to other combinations (Table I).

Various antifungal agents like itraconazole, miconazole and ketoconazole have been tried with varying success rates in A. keratitis (12). Savani DV et al 1987 (16), demonstrated that fluconazole had a good corneal penetration. Considering its safety, better penetration and effectiveness as an antimiycotic agent, we selected fluconazole to evaluate its antiacanthamoebal role. However, as evidenced in the present study, this is not a very effective drug against acanthamoeba.

Povidone Iodine 5% an antiseptic agent, has been tried for various infective conditions including that of corneal ulcer (17). Considering its broad spectrum of activity, cost effectiveness and easy availability, this agent was tried in this study for its possible antiacanthamoebal role. However, in our study, we did not find any promising role of povidone Iodine in A. keratitis.

Stuart JC et al (18), reported that aprotinin, an antiplasmin agent had the ability to neutralize the enzymes responsible for corneal degradation in a bacteria free filtered Pseudomonas aeruginosa supernatant. Thus, aprotinin was used as an adjuvant drug along with neomycin eye drop in this study. The healing process was
although not comparable to that of PHMB-neomycin group, still the healing rate was better than other combinations suggesting that it may have some role in A. keratitis.

In conclusion it can be said that, PHMB is one of the best anti-acanthamoebal agent, presently available and should be used all cases of acanthamoeba keratitis along with neomycin. Regarding aprotinin, it may be suggested that it can be used as an adjuvant drug in the management regimen of A. keratitis. However, further clinical study is required to strengthen the findings of this experimental study.

REFERENCES