NOTES

Chlorhexidine as an Effective Agent Against *Chlamydia trachomatis* In Vitro and In Vivo

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In a small field trial, chlorhexidine (50 μg/ml) was as effective against clinical trachoma as topical tetracyclines or oral co-trimoxazole; in vitro, 2.5 μg/ml was chlamydicidal.

There has been considerable controversy about the most effective treatment for trachoma (2, 4, 7, 9). One reason for this is that the various trials have been conducted by using different treatment regimens on different populations in different cultural and socioeconomic situations. The social culture of outback Australian aborigines and the environment in which they live are quite unlike those of other peoples in the world. In the population studied here, the point prevalence rates of trachoma were high (manuscript in preparation), and the standards of hygiene were low.

It is not easy to conduct field trials in the remote areas of Australia in which aborigines live, and it is particularly difficult to get large numbers of patients in one place. Despite these problems, it was thought worthwhile to conduct a small field trial in conjunction with the Department of Health, Northern Territory to compare the effectiveness of topical tetracycline with oral co-trimoxazole for treatment of trachoma among these people.

Aboriginals at Libanangu (Wave Hill) were graded for trachoma according to World Health Organization criteria (16). Children with trachoma follicles were divided into three groups of 25, randomized by age, sex, and stage of trachoma.

Treatment began 2 months later was administered twice daily for 21 consecutive days by nursing sisters. The treatments used in the three groups of patients were (i) ocular oxytetracycline-hydrochloride (0.5%) ointment containing polymyxin B (10,000 U/g; 6 mm per eye); (ii) oral co-trimoxazole suspension, consisting of Septrin (Burroughs Wellcome Co.), trimethoprim, (40 mg/5 ml), and sulfamethoxazole, (200 mg/5 ml) (children under 3 years of age received 2.5 ml, those 4 to 6 years received 5 ml, and those over 7 years received 10 ml); and (iii) a placebo, planned to be innocuous, known as "soothing eye drops," a preparation widely used by adult aborigines, 2 drops per eye. No untreated control group was included, because it was considered unethical.

At examinations 3 and 9 months after treatment, the percentage of children with trachoma follicles in each group had decreased (Table 1). Of the original children, 20 were not seen on all 3 occasions and 6 did not receive 17 or more full days of treatment so were not included in the results.

Using an arcsin transformation (12) and a χ² test, differences between treatments were not significant—any present may have been masked by the small numbers in each group. When the data from each examination were pooled, there was a significant decrease (P < 0.05) in the number of children with trachoma follicles between the first examination and each of the two subsequent examinations.

These findings seem to indicate that the soothing eye drops were as effective as tetracycline or oral co-trimoxazole. However, since there was no untreated group, it could not be determined whether factors other than treatment could have contributed to the improvements detected. No obvious change in standards of living occurred during this period as had occurred during treatment trials among American Indian children in boarding schools (3, 4, 9) in which improvement was found in placebo groups.

After the trial it was found that the placebo was 0.85% NaCl containing 50 μg of chlorhexidine per ml. Therefore, since chlorhexidine is a known antibacterial agent both in vivo and in vitro (10, 11) it seemed likely that the effect of

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the "placebo" was due to the presence of this agent. Therefore, the effect of chlorhexidine on the infective agent of trachoma, Chlamydia trachomatis (C. trachomatis), was investigated in vitro.

Suspensions of four strains of C. trachomatis grown in HeLa 229 cells, at initial concentrations of $0.3 \times 10^5$ to $1.2 \times 10^5$ inclusion-forming units per ml, were tested: TRIC/B/AUS-WA/MU-14/OT (5); TRIC/D/USA-WASH/UW-3/Cx, LGV/L2/AUS-VIC/MU-17/LN (6); and HAR-2f (13), serotype L2.

Suspensions were incubated in growth medium (GM) in the presence or absence of chlorhexidine digluconate (Hibitane; Imperial Chemical Industries, Melbourne) in concentrations ranging from 2.5 to 500 $\mu$g/ml. At intervals, samples were diluted in GM containing 0.2% normal yolk sac to prevent further action of chlorhexidine (1) and then titrated for infectivity in HeLa 229 cells (8).

At initial concentrations greater than 100 $\mu$g/ml, chlorhexidine caused extensive cell damage. At 100 $\mu$g/ml, damage was minimal, and a 100-fold reduction in inclusion-forming units was detected after 5 min of incubation; 10 $\mu$g/ml also caused a rapid decrease in inclusion-forming units (Fig. 1). The effect of 5 and 2.5 $\mu$g/ml was less marked. All four strains were equally susceptible. The results are similar to and extend those of Richmond (14), who found strong antichlamydial activity against a genital strain with 5.6 $\mu$g/ml but only a marginal effect with 2.8 $\mu$g/ml.

To determine the effect of chlorhexidine on the infectivity of elementary bodies inside intact inclusions, HeLa 229 cell monolayers infected with UW-3 were incubated for 68 h at 35°C, after which the maintenance medium replaced with maintenance medium containing 0, 10, 20, or 50 $\mu$g of chlorhexidine per ml. After 20 min at 35°C, monolayers were washed once with 0.5 ml of GM. Cover slips from two vials were stained, and inclusions were counted. Cells from the remaining three vials in each group were stripped into 1.0 ml of fresh GM and pooled. Suspensions were shaken with glass beads to disrupt inclusions and then titrated in fresh monolayers. The yield of infectious particles per inclusion was calculated.

Chlorhexidine (50 $\mu$g/ml) caused extensive cell damage. Concentrations of 20 or 10 $\mu$g/ml caused no damage and produced no difference in the yield of infectious particles per inclusion between treated and untreated monolayers (Table 2), although both these concentrations have strong antichlamydial action on free elementary bodies. This finding is consistent with evidence that chlorhexidine is unable to penetrate mammalian cells (A. Hutchinson, ICI, England, personal communication). Therefore, any effect that chlorhexidine may have exerted in the small group of patients described herein was probably due to inactivation of free elementary bodies in the eye.

Chlorhexidine is considerably less expensive than other drugs used for treatments of trachoma; it has low toxicity toward ocular and

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. per group</th>
<th>% of children with follicles 2 months before treatment</th>
<th>% of children with follicles 3 months after treatment</th>
<th>% of children with follicles 9 months after treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>15</td>
<td>100</td>
<td>73</td>
<td>80</td>
</tr>
<tr>
<td>(containing chlorhexidine)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Topical tetracycline</td>
<td>18</td>
<td>100</td>
<td>78</td>
<td>61</td>
</tr>
<tr>
<td>Oral cotrimoxazole</td>
<td>16</td>
<td>100</td>
<td>88</td>
<td>75</td>
</tr>
<tr>
<td>Overall</td>
<td>49</td>
<td>100</td>
<td>80</td>
<td>71</td>
</tr>
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TABLE 1. Effect of treatment on the point prevalence rates of trachoma follicles in children

![Fig. 1. Rate of inactivation of infectious C. trachomatis by chlorhexidine at concentrations of (A) 10 $\mu$g/ml, (B) 5 $\mu$g/ml, and (C) 2.5 $\mu$g/ml. Symbols: MU-14 ( ), HAR-2f ( ), UW-3 ( ), MU-17 ( ).](http://aac.asm.org/)

<table>
<thead>
<tr>
<th>Concen. of chlorhexidine (pg/ml)</th>
<th>UW-3 (IFU/inclusion)$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>46 ± 6$^b$</td>
</tr>
<tr>
<td>10</td>
<td>48 ± 9$^b$</td>
</tr>
<tr>
<td>20</td>
<td>53 ± 6$^b$</td>
</tr>
</tbody>
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$^a$ IFU, Inclusion-forming unit.

$^b$ Mean ± standard deviation of 12 replicates.
mucous membranes even during long periods of treatment (10, 15); it is active against a wide range of bacteria, and resistant strains have not been detected in clinical practice (11). In soothing eye drops, it has an immediate soothing effect in contrast to the temporary stinging produced by tetracycline drops.

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LITERATURE CITED


