ANTIMICROBIAL THERAPY OF CHANCROID: EFFECTIVENESS OF ERYTHROMYCIN*

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RUNNING HEAD: ERYTHROMYCIN TREATMENT OF CHANCROID.

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ABSTRACT

Chancroid is a prevalent sexually transmitted disease in developing nations. The emergence of Haemophilus ducreyi resistant to multiple antibiotics has limited the effectiveness of sulfonamides and tetracycline for the therapy of chancroid. We conducted a randomized double blind comparison of 10 day courses of erythromycin base 500 mg and rosaramicin 250 mg, each four times daily for the treatment of males with chancroid in Nairobi, Kenya. Of 99 evaluable patients, 84 were culture positive for H. ducreyi. H. ducreyi positive genital ulcers in men treated with either drug resolved with mean healing times of 10.8 ± 5.1 days for erythromycin and 10.7 ± 5.5 for rosaramicin. H. ducreyi was eradicated from all ulcers by day 7. There were no clinical or bacteriologic failures with either agent. Fifteen men with H. ducreyi negative genital ulcers for whom no other etiology could be determined also responded rapidly to treatment with either agent. Both erythromycin and rosaramicin are highly effective in the treatment of chancroid.
Chancroid or "soft sore", although a clinical curiosity in industrialized nations, is endemic in many tropical countries. In Nairobi, approximately 5,000 cases of clinical chancroid are seen yearly at the City Council Special Treatment Clinic; chancroid being the most common diagnosis given to men with genital ulcers presenting to the clinic. *Haemophilus ducreyi* has been implicated as the cause of chancroid since 1889 (1), but some still doubt its role as a primary pathogen. We have been able to consistently isolate *H. ducreyi* from more than 70% of men with clinical chancroid (2,3,4) in Nairobi and have demonstrated a correlation between clinical response, microbiologic response and antimicrobial susceptibility of *H. ducreyi* (4).

Tetracyclines and sulfonamides have long been the standard therapy for chancroid. However, multiresistant strains of *H. ducreyi* are very prevalent in Kenya. Virtually all strains isolated in Kenya produce TEM-1 type β-lactamase (4,5), the production of which is plasmid mediated (6). Many of the Kenyan strains show in vitro resistance to sulfonamides and 90% are resistant to tetracycline (3). The obvious implications of these data for therapy have been confirmed in clinical studies in Kenya, where over 40% of patients treated with sulfonamides and over 70% of patients treated with tetracycline fail therapy (3). For ulcers initially positive for *H. ducreyi*, clinical failure is reflected by persistence of *H. ducreyi* in the ulcers (4). Studies from Vietnam suggest a similar situation exists in Asia (7,8). Thus, improved therapy for this painful and debilitating sexually transmitted infection is needed.

Isolates of *H. ducreyi* from Kenya, Canada, the United States and Europe have been shown to be susceptible to less than .06 mg/L of
erythromycin or rosaramicin (9), a new macrolide antibiotic. Carpenter et al. (10), and Codero (11) have reported successful therapy of chancroid with erythromycin in a limited number of patients. For these reasons we conducted a double blind, randomized, clinical trial in Nairobi, comparing erythromycin with rosaramicin for the treatment of chancroid.

METHODS

Recruitment and Evaluation of Patients

One hundred and nine males presenting to the Nairobi City Council Special Treatment Clinic with genital ulcers, presumed to be chancroid, were enrolled in the study. Patients with genital ulcers were screened by the clinic staff and, if suitable for study, referred to the study group for further evaluation. Patients were eligible for study if they were between the ages of 18 and 60, otherwise healthy, willing to return for follow-up visits and darkfield negative on microscopic examination.

Patients were usually enrolled before results of culture for H. ducreyi were known. Informed consent was obtained from all patients.

At the time of enrollment a standard history and examination were performed. Ulcers were counted, measured in two dimensions with calipers and the associated clinical features of tenderness, purulence and induration noted. Ulcers were arbitrarily categorized as severe if a single ulcer larger than 2 cm was present or if four or more ulcers were present with the largest being 1 cm or greater in diameter. Bubbles (defined as tender inguinal or femoral lymph nodes larger than 1 cm), if present, were examined for tenderness, fluctuance, consistency and size. Ulcers were cultured for H. ducreyi, N. gonorrhoeae and other aerobic bacteria on a selective media previously described (2). Specimens for Herpes simplex
virus were obtained. Buboes, when fluctuant, were aspirated and cultured for _H. ducreyi_. One or more darkfield examinations were performed on material aspirated from the surface of ulcers. Serum and blood were obtained for syphilis serology, biochemistry and complete blood count (CBC).

Following evaluation, patients were randomly assigned to receive erythromycin base 500 mg or roxacillin 250 mg each given orally four times a day for 10 days, according to a preconstructed random sequence. Three days supply of medication was dispensed at the initial visit.

Follow-up visits were scheduled for days 3 or 4, 7, 10, 14 and 28. At each follow-up visit patients were questioned about ulcer symptoms, sexual contact, therapeutic compliance and adverse effects of therapy. Ulcers and buboes were re-evaluated clinically and unhealed ulcers were cultured for _H. ducreyi_. Ulcers were considered cured when completely epithelialized, improved it less tender and/or smaller in diameter, no change if no improvement was discernible and worse if larger in diameter. Failure was defined as no improvement or progression of ulcers by the day 7 visit. Buboes were considered cured when no longer tender to palpation or fluctuant. On days 3 and 7 a further supply of medication was dispensed and instructions on taking the medication were reinforced. On days 10 and 28 repeat specimens for syphilis serology, biochemistry and CBC were obtained.

**Laboratory Procedures**

Swabs from ulcers were directly inoculated on biplates of an enriched selective chocolate agar medium (2) and immediately incubated at 35 ± 1°C. After 2 to 3 hours, plates were transported to the laboratory, streaked for isolation of single colonies and incubated in humidified candle extinction
jars at 35 ± 1°C. Cultures were examined at 48, 72 and 96 hours. _H. ducreyi_ was identified by typical colonial morphology, Gram stain, oxidase reaction and by testing requirements for X and V factors (12). _Beta lactamase_ production was tested using the chromogenic cephalosporin assay (13). Isolates of _H. ducreyi_ were obtained in pure culture and stocked in skim milk and glycerol at -70°C.

Minimal inhibitory concentrations of _H. ducreyi_ strains to erythromycin and rosaramicin were tested using an agar dilution technique (9).

Specimens for Herpes simplex culture were obtained using calcium alginate swabs and held in transport media at -70°C until cultured. Specimens were inoculated on human amnion cells and examined for typical cytopathic effect.

Rapid plasma reagin (RPR, Wyman, Wescott and Dunnin, Baltimore, Maryland) and Macrohemagglutination Treponema pallidum (MHA-TP, Ames Laboratories, Rexdale, Ontario) test on serum were performed using commercially available kits.

Analysis

Statistical analysis of sample proportions were performed using chi-square or Fischer's exact test and of sample means with Student's t-test.

RESULTS

One hundred and nine men were enrolled in the study. Ten patients are excluded from analysis - six subsequently were diagnosed as primary syphilis, two later admitted to receiving antibiotics prior to the study period, only a pure growth of _Neisseria gonorrhoeae_ was isolated from the ulcer of one patient and one culture was contaminated by a swarming Proteus.
H. ducreyi Culture Positive Genital Ulcers.

Haemophilus ducreyi was isolated from 84 of the remaining 99 patients (85%). Forty-five were treated with erythromycin and 39 with rosmaricin. As shown in table 1, the two groups were comparable in age and ulcer severity. More patients receiving erythromycin were circumcised. The mean number of ulcers present and the number of patients with multiple ulcers were not significantly different between the two treatment groups.

Thirty-three of 45 erythromycin treated patients (73%) and 26 of 39 (67%) rosmaricin treated patients attended all follow-up visits. Thirty-eight of 45 (84%) and 31 of 39 (80%) patients in the erythromycin and rosmaricin group, respectively, were followed until their ulcers were completely healed. Patients who were cared when last seen but did not complete all follow-up visits are tabulated as cures in the calculation of response rates.

Table 2 shows the clinical response of all H. ducreyi positive ulcers to therapy. Both erythromycin and rosmaricin treated patients healed rapidly. In almost 70% of patients, in either group, ulcers were completely epithelialized by day 10, and at least 90% were healed at day 14. All ulcers were healed by day 28. Although more rosmaricin treated patients were healed at day 7, the difference is not statistically significant. The mean healing time for erythromycin was 10.3 ± 5.1 days and for rosmaricin was 10.7 ± 5.5 days. There were no failures in either group.

One patient in the erythromycin group had a recurrence of ulcers on day 28 and denied further sexual exposure. Culture of the recurrent ulcer was negative for H. ducreyi. Although this case is considered here as a relapse, reinfection cannot be ruled out.
Eleven ulcers were classified as severe. Five received erythromycin and six roxaramicin. There was no difference in the rate of healing or the eventual cure rates between the two treatment groups. Seven of 16 severe ulcers were healed by day 10 and all were healed at day 28.

Erythromycin and roxaramicin were very effective in eradicating H. ducreyi from ulcers. All but one patient in each group were culture negative at day 4. Both these patients were culture negative for H. ducreyi at subsequent visits and healed uneventfully.

Buboes were present in 16 of 45 patients in the erythromycin group and 19 of 39 patients in the roxaramicin group. Buboes responded equally well to therapy with either agent. The response of buboes to treatment paralleled that of ulcers; more than 50% of buboes were healed at day 10 and all but one patient in each group were healed by day 14. These two had resolved at the day 28 visit. Four buboes in each treatment group progressed to fluctuance and required aspiration; all were culture negative for H. ducreyi. One patient receiving roxaramicin developed a bubo while on therapy which resolved uneventfully, without requiring additional antimicrobial therapy.

Twenty-nine of 84 patients with H. ducreyi positive ulcers demonstrated reactivity in one or more serologic tests for syphilis. Five patients had both a positive RPR and a positive MHA-TP, in two RPR was reactive only on undiluted serum and the other titres were 1:8, 1:8 and 1:16. These five may represent coincident infections or incidental latent syphilis. Twenty-four had a positive MHA-TP with a negative RPR. These patients were treated with penicillin therapy appropriate for syphilis of undetermined status after the study regimen was completed and ulcers had healed. All 84 cultures for Herpes simplex were negative.
H. ducreyi Culture Negative Genital Ulcers.

Fifteen patients were culture negative for H. ducreyi and Herpes simplex and seronegative for syphilis. Table 3 compares epidemiologic and clinical features of H. ducreyi positive with H. ducreyi negative ulcers. No significant differences in incubation period, severity of ulcers, sites of ulcers and presence of buboes between the two groups were detected. Ten patients received rosaramicin and five patients received erythromycin. All ulcers were healed by day 14 with no apparent differences between the two treatment groups.

Adverse Effects

Fifty-two patients treated with erythromycin and 49 treated with rosaramicin were evaluable for adverse symptoms occurring during therapy. Thirteen erythromycin treated patients experienced gastrointestinal upset and four experienced other symptoms (dizziness, one; joint pain, one; chest pain, two). Of rosaramicin treated patients, 11 complained of gastrointestinal upset and three complained of other symptoms (headache, one; dizziness, one; joint pain, one). Adverse symptoms were all minor and did not require alteration in therapy in any case. Serial complete blood counts were evaluable in 36 erythromycin treated patients and 35 rosaramicin treated patients. Six patients receiving rosaramicin had a fall in hemoglobin concentration of 1.0 g/dl or greater. The mean fall was 2.6 g/dl (range 1.2 - 6.0 g/dl). No further investigation of these findings was possible and thus no mechanism for the fall in hemoglobin was discovered. One erythromycin treated patient had a drop in hemoglobin of
3.7 g/dl. Serial serum biochemical tests were performed in 33 erythromycin treated patients and in 27 rosaramicin treated patients. Elevation of serum alkaline phosphatase and aspartate transaminase activity, occurring during therapy, were detected in two patients who received erythromycin and three who received rosaramicin. In three patients, the enzyme elevations were transient, resolving spontaneously within two weeks. The remaining two patients did not return for further follow-up.

**Antimicrobial Susceptibility of H. ducreyi.**

Minimum inhibitory concentrations (MIC) of erythromycin and rosaramicin were determined for 47 H. ducreyi isolates. Thirty-three isolates were from patients included in the present study; twelve others were also from Kenya. All strains tested were inhibited by 0.06 mg/L or less of erythromycin or rosaramicin. The median MIC's of erythromycin and rosaramicin were .030 mg/L and .015 mg/L, respectively.

**DISCUSSION**

In this randomized single blinded study a ten-day course of either erythromycin or rosaramicin proved to be extremely effective for H. ducreyi culture positive chancroid. All but the largest ulcers were healed after fourteen days; two giant ulcers (greater than 2 cm) required 28 days to heal. There were no treatment failures. This represents a considerable shortening of the median healing times of untreated chancroid of 25 to 30 days (14,15) and is superior to the recent experience with tetracycline and sulfonamides for the treatment of chancroid (2,7,8,16). H. ducreyi was uniformly very susceptible to both erythromycin and rosaramicin thus they compare favourable in activity to other antimicrobial agents against Kenyan H. ducreyi (17).
Buboes also responded rapidly to treatment with either agent; most buboes were healed by day 14. We noted, as has Asin (18), that buboes may progress to suppuration in the face of adequate antimicrobial therapy. Progression to fluctuance does not seem to be related to failure to eradicate _H. ducreyi_ from buboes, since all bubo aspirates were culture negative for _H. ducreyi_ and buboes healed without further antimicrobial therapy. However, in our experience it is uncommon to isolate _H. ducreyi_ from buboes even prior to the initiation of therapy.

_H. ducreyi_ negative ulcers also responded well to either erythromycin or rosaramicin. No microbial pathogens could be implicated as responsible for this group of ulcers and the similarity of epidemiologic and clinical characteristics between _H. ducreyi_ negative and positive ulcers suggests these may represent falsely culture negative chancreoid. These ulcers might be caused by strains of _H. ducreyi_ which elude detection because of special nutritional characteristics. Alternatively, patients may have received antibiotic therapy prior to enrolling in the study, masking an infection with _H. ducreyi_. A third possibility is that one or more as yet unidentified microbial agents are responsible for these ulcers.

In contrast to the North American and European experience, we did not encounter significantly more gastrointestinal adverse effects with rosaramicin compared to erythromycin. Although 25% and 22% of patients receiving erythromycin or rosaramicin, respectively, experienced gastrointestinal upset, it was mild and in no case required discontinuation of the study drug. The fall in hemoglobin and hematocrit associated with rosaramicin has not been previously reported and is disconcerting.
As plans to market rosaramicin have been suspended by the parent pharmaceutical firm, at this time it seems unlikely that rosaramicin will have an important role in the treatment of chancroid. However, erythromycin should prove an effective therapy for chancroid worldwide, offering high efficacy and moderate expense, an important consideration in most chancroid endemic areas where health budgets are small. Further studies to determine the optimal duration of erythromycin therapy and the relative efficacies to other antimicrobial agents such as trimethoprim-sulfamethoxazole (3,17) are required.

Erythromycin may be the agent of choice for the treatment of genital ulcer disease in areas of the developing world where laboratory facilities for the diagnosis of sexually transmitted diseases are minimal or nonexistent. As the clinical diagnosis of genital ulcers is inaccurate (19) and dual infections are not infrequent (2), a single therapy effective for both chancroid and syphilis is desirable. Although less effective than penicillin and perhaps tetracycline, 14 days of erythromycin therapy cures early syphilis (20) and thus such a regimen could prove suitable for the treatment of genital ulcers in areas of the world where laboratory support is absent. Erythromycin is an excellent agent for the treatment of chancroid in pregnant women.
REFERENCES

1. Ducrey, A. Experimentelle untersuchungen über den ansteck ungsstoff
des wachen schankers und über die bubonen. Montash F. wPrakt.

Genital ulcers in Kenya: A clinical and laboratory study of 97

Ronald, A.R. Antimicrobial therapy of chancroid: An
evaluation of five treatment regimens correlated with in vitro

clovulanic acid with amoxicillin in patients with β-lactamase

5. Maclean, I.W, Bowden, CH, Albritton, WL. TEM-type β-lactamase
production in Haemophilus ducreyi. Antimicrob. Agents

mediated ampicillin resistance in Haemophilus ducreyi.


8. Hart, G. Venereal disease in a war environment. Incidence and

activity of rosaramicin, erythromycin, clindamycin, metronidazole
and ornidazole against H. ducreyi including β-lactamase producing


<table>
<thead>
<tr>
<th></th>
<th>Erythromycin (n=45)</th>
<th>Rosaramicin (n=39)</th>
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<tbody>
<tr>
<td>Age (yrs, Mean ± S.D.)</td>
<td>25.1 ± 10.2</td>
<td>26.6 ± 12.5</td>
</tr>
<tr>
<td>Circumcised</td>
<td>36 (80)</td>
<td>20 (51)</td>
</tr>
<tr>
<td>Number of ulcers (Mean ± S.D.)</td>
<td>1.8 ± 1.5</td>
<td>2.1 ± 1.6</td>
</tr>
<tr>
<td>Multiple Ulcers</td>
<td>17 (38)</td>
<td>21 (54)</td>
</tr>
<tr>
<td>Severe Ulcers</td>
<td>5 (11)</td>
<td>6 (15)</td>
</tr>
<tr>
<td>Completed Follow-up</td>
<td>33 (73)</td>
<td>26 (67)</td>
</tr>
<tr>
<td>Followed to Cure</td>
<td>38 (84)</td>
<td>31 (79)</td>
</tr>
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TABLE 1

CHARACTERISTICS OF PATIENTS WITH H. DUCREYI POSITIVE GENITAL ULCERS TREATED WITH ERYTHROMYCIN OR ROSARAMICIN.
<table>
<thead>
<tr>
<th>Day</th>
<th>Treatment</th>
<th>Cured</th>
<th>Improved</th>
<th>No Improvement</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>Erythromycin (43)*</td>
<td>2</td>
<td>35</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Rosaramicin (37)</td>
<td>2</td>
<td>29</td>
<td>6</td>
</tr>
<tr>
<td>7</td>
<td>Erythromycin (38)</td>
<td>14</td>
<td>22</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Rosaramicin</td>
<td>13</td>
<td>18</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>Erythromycin (37)</td>
<td>25</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Rosaramicin (31)</td>
<td>21</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>14</td>
<td>Erythromycin (38)</td>
<td>36</td>
<td>2</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Rosaramicin (32)</td>
<td>29</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>28</td>
<td>Erythromycin (38)</td>
<td>38</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Rosaramicin (31)</td>
<td>31</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Numbers in parentheses indicate the number of patients evaluated at each visit.
### TABLE 3

#### COMPARISON OF FEATURES OF HAEMOPHILUS DUCREYI CULTURE POSITIVE AND CULTURE NEGATIVE ULCERS

<table>
<thead>
<tr>
<th></th>
<th>Culture Positive Ulcers (n=84)</th>
<th>Culture Negative Ulcers (n=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Incubation Period (Mean ± S.D.)</strong></td>
<td>7.6 ± 7.4 days</td>
<td>10.5 ± 8.9 days</td>
</tr>
<tr>
<td><strong>Duration of Ulcers (Mean ± S.D.)</strong></td>
<td>9.3 ± 6.0 days</td>
<td>7.7 ± 4.1 days</td>
</tr>
<tr>
<td><strong>Circumcised</strong></td>
<td>56 (67)*</td>
<td>11 (73)</td>
</tr>
<tr>
<td><strong>Multiple Ulcers</strong></td>
<td>38 (45)</td>
<td>9 (60)</td>
</tr>
<tr>
<td><strong>Mean Area Largest Ulcer</strong></td>
<td>233.2 ± 199.8 mm²</td>
<td>268.8 ± 270.3 mm²</td>
</tr>
<tr>
<td><strong>Buboes</strong></td>
<td>35 (42)</td>
<td>6 (40)</td>
</tr>
</tbody>
</table>

**Ulcer Characteristics:**

- Tenderness alone: 61 (73) / 8 (53)
- Induration alone: 2 (2) / 2 (13)
- Tenderness and Induration: 17 (20) / 3 (20)
- No Tenderness or Induration: 4 (5) / 2 (13)

**Distribution of Ulcer on Penis:**

- Glans: 6 (5) / 1 (6)
- Sulcus: 16 (14) / 2 (11)
- Frenulum: 11 (10) / 0
- Prepuce: 10 (9) / 5 (28)
- Shaft: 48 (42) / 6 (33)
- Base: 7 (6) / 4 (22)

*Figures in parentheses indicate percentage*