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Treating chancroid with enoxacin

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SUMMARY Increasing resistance of *Haemophilus ducreyi* to antimicrobials necessitates further trials of new antimicrobial agents for treating chancroid. Enoxacin has excellent in vitro activity against *H ducreyi*, and a randomised clinical trial of three doses of enoxacin 400 mg at intervals of 12 hours compared with a single dose of trimethoprim/sulphametrole (TMP/SMT) 640/3200 mg was therefore conducted. Of 169 men enrolled in the study, 86 received enoxacin and 83 received TMP/SMT. Ulcers were improved or cured in 65/73 men treated with enoxacin and 57/70 men treated with TMP/SMT. This difference was not significant. At 72 hours after treatment, *H ducryei* was eradicated from ulcers of 72/77 men treated with enoxacin and of 67/74 of those treated with TMP/SMT. Patients with buboes responded equally well to both treatments. Of 100 *H ducreyi* strains tested, all were susceptible to both 0·25 mg/l enoxacin and the combination of 0·25 mg/l TMP and 5 mg/l SMT. Although most men treated with either regimen were cured, neither regimen appeared to be the optimum treatment for chancroid. This study shows the efficacy of enoxacin for a soft tissue infection caused by Gram negative organisms.

Haemophilus ducreyi is the most common cause of genital ulcers in both men and women in Kenya.[1,2] It is endemic throughout the tropics. Chancroid is not a problem exclusive to developing countries, however, as witnessed by the recent epidemic on Orange County, California, United States of America.[3] Because of increasing resistance of *H ducreyi* to antimicrobials, optimum treatment of chancroid requires further study.[4,5] Sulphonamides were once the mainstay of treatment, but resistance, both plasmid and non-plasmid mediated, is now prevalent.[4] Plasmid mediated resistance to ampicillin of strains producing β lactamase is also common.[5] A single dose of trimethoprim/sulphametrole (TMP/SMT), has been shown to be effective treatment for chancroid and is now the standard regimen used in Nairobi.[6] Because *H ducreyi* was resistant to trimethoprim, however, the same regimen was ineffective in Thailand.[7] New treatment regimens must therefore be evaluated to meet the continuing challenge presented by the evolution of resistant strains.

Enoxacin is a new quinolone antimicrobial agent, which inhibits microbial replication by blocking a DNA gyrase. It has excellent in vitro activity against *H ducreyi*, and all strains we tested were inhibited at a concentration of 0·25 mg/l. In addition, chancroid is an infection that can serve as a model for assessing the efficacy of antimicrobial agents in Gram negative soft tissue infections. In one previous uncontrolled study, seven men with chancroid were successfully treated with enoxacin prescribed for seven to 12 days in a dose of 400 mg twice daily.[8] We compared the in vivo efficacy of enoxacin for treating chancroid with the standard regimen of single dose TMP/SMT in a randomised clinical trial.

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Patients, materials, and methods

We enrolled 169 men in the study. Men aged between 18 and 50 presenting to the special treatment clinic in Nairobi, Kenya, complaining of a genital ulcer were considered for entry into the trial. They were eligible if they yielded cultures of *H ducreyi* (men were recruited

on the basis of a clinical diagnosis of chancroid and later excluded from analysis if the culture was negative); had not taken antimicrobials in the previous five days; dark field examination showed no spirochaetes; had no history of allergy to sulphonamides, trimethoprim, or quinolone antimicrobial agents; and agreed to return for follow up visits.

Informed verbal consent was obtained at enrollment, and a standardised interview and genital examination were performed. Ulcers were measured in two dimensions, and tenderness, purulence, and induration were assessed. Buboes, defined arbitrarily as inguinal lymphadenitis more than 1 cm in size, were measured in two dimensions, and graded for fluctuance, tenderness, and matting. The chancroid was classified as severe if an ulcer was larger than 2 cm or if there were four or more ulcers and one was larger than 1 cm or if a bubo was larger than 3 cm. Patients were then assigned by a computer generated randomisation table to receive TMP/SMT 640/3200 mg as a single oral dose or three doses of enoxacin 400 mg at intervals of 12 hours. We estimate that serum concentrations of antimicrobial agents in vivo must exceed the minimum inhibitory concentration (MIC) for *H ducreyi* for 36 hours to be effective. We chose the three dose regimen of enoxacin because it has a half life of six hours. Patients with severe chancroid were randomised separately from those with less severe infection to ensure comparability of the treatment groups.

Participants were followed up on days 3, 7, 10, 14, and 28. At each visit they were clinically reassessed, and any ulcer that had not epithelialised was cultured again. Patients who were not followed up to the study end point are included in the analysis for all visits attended.

Clinical outcomes were assessed as follows: a cure was defined by epithelialisation of the ulcer at any time up to day 28; improvement was defined by an objective decrease in the size of the ulcer from previous visits, and failure of the patient to return subsequently; treatment failure was any patient with an ulcer showing no objective improvement at day 7 or worsening at any follow up; and relapse referred to healing of the ulcer with subsequent reappearance at the same site. Treatment failures were then given an alternative treatment and excluded from further analysis, although followed up to cure.

Bacteriological cure was defined as culture negativity by day 3 and for all subsequent cultures. Twelve patients clinically cured at their first follow up visit were also identified as being bacteriologically cured. Persistence referred to culture positivity on day 3. Relapse was defined by a negative culture on day 3 with culture positivity at a later visit.

Cultures for *H ducreyi* were performed as described previously.[9] Briefly, swabs were inoculated directly on two selective media; gonococcal agar base with 1% bovine haemoglobin and 5% fetal bovine serum or Mueller-Hinton agar base with 5% chocolatised equine blood, each supplemented with 1% CVA (cofactors, vitamins, and amino acids) and vancomycin 3 mg/ml. Plates were transported to the laboratory at room temperature within three hours, incubated in a candle extinction jar at a mean (SEM) of 33°C (1°)C, and read at two days and then daily for a further five days. *H ducreyi* was identified by colony morphology, appearance on Gram staining, oxidase reactivity, and a requirement for X but not V factor.[9]

ANALYSIS OF DATA

We used the χ^2 test with Yates' correction (when applicable) to compare sample proportions and t tests to compare sample means. Because of a high loss to follow up, we calculated the maximum probability of cure on the assumption that all defaulters were cured, and the minimum probability of cure on the assumption that all defaulters were not cured.

Results

Of the 169 men enrolled, 86 were treated with enoxacin, and 83 with TMP/SMT. The characteristics of each group are shown in table 1. They were similar in all respects, including the proportion classified as having severe infection, duration of the ulcer before treatment, and the mean area of the largest ulcer.

Table 2 shows clinical outcome in 143 men. The remaining 26 patients failed to return for follow up and were not clinically evaluable. Cure and improvement were seen in 65/73 patients treated with enoxacin and 57/70 of those treated with TMP/SMT, which was not an appreciable difference. The one relapse seen in the enoxacin group was excluded from the treatment failures. Also excluded were two patients, one in each treatment group, whose ulcers were cured but who had persistent bubos that were culture positive for *H ducreyi*. The maximum probably

Table 1 *Demographic and clinical findings of men with chancroid treated with trimethoprim/sulphametrole (TMP/SMT) or enoxacin*

	TMP/SMT (*n* = 83)	*Enoxacin* (n = 86)
No followed to study end point	44	48
Mean (SEM) age (years)	26·3 (6·1)	26·0 (8·5)
Mean (SEM) duration of ulcer (days)	9·5 (6·5)	9·5 (4·9)
Mean (SEM) area of largest ulcer (cm²)	1·1 (0·9)	0·9 (0·6)
No (%) with concomitant urethral discharge	3 (4)	4 (5)
No (%) with concomitant inguinal lymphadenitis (> 1 cm)	36 (43)	28 (32)
No with severe/less severe infection	44/39	52/34

Table 2 *Clinical outcome of treatment with enoxacin or trimethoprim/sulphametrole (TMP/SMT) of 143 men*

Treatment	*No*	*Cure*	*Improvement**	*Failure*	*Relapse*
Enoxacin	73	41	24	7	1
TMP/SMT	70	31	26	13	0

*Patients improving at last recorded visit, but not followed up to cure.

cured were 78/86 with enoxacin and 70/83 with TMP/SMT if all defaulters were cured, and the minimum probably cured were 41/86 with enoxacin and 31/83 with TMP/SMT if all defaulters were treatment failures.

The mean numbers of days to cure were 9·4 in those treated with enoxacin and 9·5 in those treated with TMP/SMT. A second analysis by ulcer severity, however, showed mean days to healing of 10·6 (enoxacin) and 10·6 (TMP/SMT) in the severely infected group versus 8·6 (enoxacin) and 7·8 (TMP/SMT) in the less severely infected group. The figure shows the mean initial size of the largest ulcer related to the time to complete healing in each of the 72 patients cured. The excellent correlation between ulcer size and time to complete healing was not unexpected as eradication of the organism probably occurs in the first 36 hours, and healing is then a process of epithelialisation, depending on ulcer size and healing ability of the host.

Table 3 outlines the bacteriological results. At 72 hours *H ducreyi* was eradicated from 72/77 men treated with enoxacin compared with 67/74 of those receiving TMP/SMT. As no bacteriological relapses occurred in patients negative at day 3 and followed up for a longer time, we concluded that all patients with negative culture results at day 3 were bacteriologically cured.

Bacteriological persistence at day 3 predicted clinical failure. Of the 12 patients with positive cultures at day 3, eight failed clinically. The other four improved, but we failed to document complete healing during follow up; one of them did not return after day 3, one did not return after day 7, and the other two were followed up to day 14 without their ulcers healing and were then lost to follow up.

Table 4 shows the response to treatment of buboes, which resolved equally rapidly in both treatment arms.

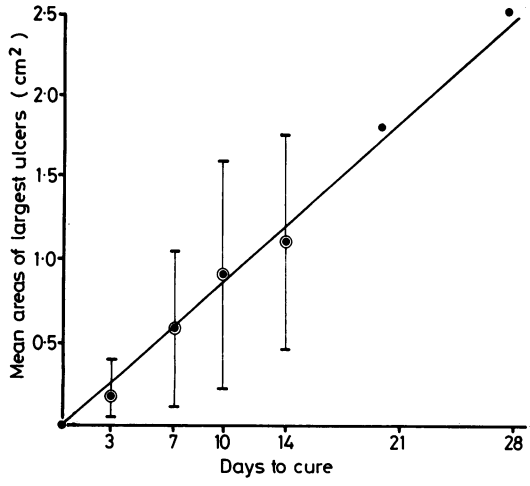

Figure *Relation between area of largest ulcer and mean days to epithelialisation after treatment of 72 patients cured. (Bars represent SEM.) (Points at days 21 and 28 represent single patients.)*

SUSCEPTIBILITY TO ANTIMICROBIALS

Table 5 shows the results of antimicrobial susceptibility testing of 110 of the isolates from patients in the study. All isolates were susceptible to enoxacin 0·25 mg/l and to the combination of TMP/SMT at concentrations of 0·25/5·0 mg/l. The MIC of enoxacin was estimated for six of nine strains isolated from patients who were classified as treatment failures. It was 0·25 mg/l for five and 0·12 mg/l for the other strain. Although these strains were less susceptible to enoxacin than those cultured from men who were cured, the difference was not significant.

Table 3 *Bacteriological eradication of Haemophilus ducreyi from ulcers of 151 men receiving enoxacin or trimethoprim/sulphametrole (TMP/SMT)*

	No	*Eradicated*	*Persisting*
Enoxacin	77	72	5
TMP/SMT	74	67	7

*Most cultures obtained at 72 hours.

Table 4 *Response of buboes to treatment with enoxacin or trimethoprim/sulphametrole (TMP/SMT)*

		No (%) resolving by:				
	No	*Day 3*	*Day 7*	*Day 10*	*Day 14*	*Day 28*
Enoxacin	23	6 (26)	8 (35)	12 (52)	18 (78)	20 (87)
TMP/SMT	22	5 (23)	8 (36)	9 (41)	15 (68)	18 (82)

Table 5 *Susceptibility of Haemophilus ducreyi to enoxacin and trimethoprim/sulphametrole (TMP/SMT)*

	Cumulative per cent inhibited by concentration (mg/l) of:																
	0·015	*0·03*	*0·06*	*0·12*	*0·25*	*0·5*	*<0·03/0·6*	*0·03/0·6*	*0·06/1·2*	*0·12/2·5*	*0·25/2·5*	*2·5*	*5*	*10*	*20*	*40*	*80*	*160*
Enoxacin	1	4	30	65	100													
TMP			6	8	73	100												
SMT												3	21	65	86	86	86	87
TMP/SMT							35	64	87	87	100							

Discussion

In this study we have shown that a three dose regimen of enoxacin was as effective as a single dose of TMP/SMT for treating chancroid, though neither was effective enough to be considered to be an optimum oral regimen for chancroid. Severe manifestations of chancroid, such as large ulcers and buboes, responded equally well to both regimens. We are concerned that the failure rate for TMP/SMT was higher than we previously experienced,[6] even allowing for the high dropout rate. From previous experience we assumed that most, if not all, patients who had a poor response to treatment would return for follow up. Thus a high rate of patient dropout would bias the study towards a higher apparent failure rate. The 94% bacteriological cure achieved with enoxacin also led us to expect an excellent clinical outcome. An alternative explanation for TMP/SMT failures is that resistance is developing to this combination. This seems possible as recent *H ducreyi* isolates from Thailand have been resistant to trimethoprim.[7] The susceptibility of these *H ducreyi* isolates to both trimethoprim and sulphametrole, however, was identical to that previously observed.[6]

Our observation that healing time is proportional to the size of the ulcers is consistent with our hypothesis that cure is effected with eradication of the organism in the first 72 hours, and healing is then an independent event.[11] In fact, culture positivity at 72 hours was a good predictor of clinical treatment failure. No patients culture positive at day 3 went on to cure during follow up. This is analogous to the untreated patient, as by day 3 antibacterial activity is presumably absent in the ulcer exudate and outcome can be predicted based on the natural course of the disease.

Reasons for the relatively limited activity of a three dose treatment course of enoxacin, despite its excellent in vitro activity, are not clear. Although no enoxacin pharmacokinetic studies were performed in this group of men, previous studies suggest that blood, and presumably tissue, concentrations should exceed the MIC for *H ducreyi* for four to eight hours after oral ingestion of 400 mg. The most likely explanation for treatment failure may be that tissue concentrations must exceed the MIC for the organism for at least 36 hours.[12] A slightly longer duration of treatment, perhaps four doses, would probably be more effective and may be an optimum regimen with enoxacin. The fact that two of the three oral doses of enoxacin were unsupervised may also have led to reduced compliance and a higher failure rate.

Human immunodeficiency virus infection is present in about 15% of patients with chancroid in Nairobi.[13] Studies are under way to assess whether clinical response to or bacteriological outcome of treatment are altered by concomitant infection with this virus.

This study showed that enoxacin is effective in skin and soft tissue infection. This is an important feature as nalidixic acid, the parent compound of the quinolones, is considered to be ineffective for infections outside the urinary tract.

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