Failure of Treatment for Chancroid in Rwanda Is Not Related to Human Immunodeficiency Virus Infection: In Vitro Resistance of *Haemophilus ducreyi* to Trimethoprim-Sulfamethoxazole

Jos Bogaerts, Luc Kestens, Waldina Martinez Tello, Jeannette Akingeneye, Véronique Mukantabana, Jan Verhaegen, Eddy Van Dyck, and Peter Piot*

From the Laboratory of Microbiology (Centre Hospitalier de Kigali), Belgo-Rwandan Medical Cooperation, and Centre Médico Social de Bilyogo, Kigali, Rwanda; the Department of Infection and Immunity, Institute of Tropical Medicine, Antwerp, Belgium; and the Laboratory of Microbiology, Catholic University of Leuven, Belgium

A comparative open study was performed to evaluate the efficacy of single doses of ciprofloxacin (500 mg) and trimethoprim-sulfamethoxazole (TMP-SMZ; 640 mg/3,200 mg) for the treatment of culture-proven chancroid. Clinical cure or improvement was observed 7 days after treatment in 32 (76.2%) of the 42 patients who received ciprofloxacin and 21 (52.5%) of the 40 patients who received TMP-SMZ ($P = .04$). Cultures for one (4.5%) of 22 patients not cured with ciprofloxacin and 16 (59.3%) of 27 patients not cured with TMP-SMZ were still positive for *Haemophilus ducreyi* 7 days after treatment ($P < .001$). Although 77 (71.3%) of the 108 patients tested were seropositive for HIV-1 antibody, HIV infection and the degree of $CD4^+$ lymphocyte depletion had no effect on clinical and bacteriologic outcome. All isolates of *H. ducreyi* were highly susceptible to ciprofloxacin (MIC, 0.004–0.06 mg/L). In contrast, resistance to TMP-SMZ (MIC, $\geq 4/76$ μg/mL) was observed in 48.9% of isolates (22 of 45) and was significantly associated with treatment failure. Therefore, the administration of TMP-SMZ, in single or multiple doses, is no longer indicated for the treatment of chancroid in Rwanda.

Genital ulcer disease is a common sexually transmitted disease in many parts of the developing world and constitutes a major risk factor for the heterosexual transmission of HIV [1]. Its early treatment has now become a prime means of intervention against HIV infection in populations where chancroid is endemic. Chancroid is an important cause of genital ulcer disease in Kigali, the capital of Rwanda, Central Africa. During a survey conducted in 1986 at the Centre Médico Social de Bilyogo, Nyamirambo, Kigali, *Haemophilus ducreyi*, the causal agent of chancroid, was recovered from 23.6% of men and 12.0% of women with genital ulcers; 59% of the men and women were also HIV-positive [2].

Multidose therapy with erythromycin for 7 days and single-dose treatment regimens with azithromycin and ceftriaxone are now recommended as the treatments of choice for chancroid [3]. In the past, single-dose therapy with four double-strength tablets of trimethoprim with a sulfonamide and therapy with single oral doses (500 mg or 1,000 mg) of ciprofloxacin have been reported to be as effective as multiple-dose regimens [4–10]. More recently, in vitro resistance to trimethoprim associated with high failure rates following administration of single doses of trimethoprim-sulfonamide have been observed in Kenya and Thailand [11, 12]. There are some indications that single-dose therapy for chancroid may be ineffective in patients with HIV infection, and since a substantial proportion of patients with chancroid are also infected with HIV, it is currently uncertain whether single-dose regimens can still be recommended as therapy for chancroid [13, 14]. The aim of this study was to compare two single-dose regimens for the treatment of chancroid (ciprofloxacin [500 mg] and trimethoprim-sulfamethoxazole [TMP-SMZ; 640/3,200 mg) and to examine the effect of HIV infection and in vitro antimicrobial susceptibility of *H. ducreyi* on treatment outcome.

Materials and Methods

Enrollment of Patients

This study was performed at the Centre Médico Social de Bilyogo in Nyamirambo and at the laboratory of the Centre Hospitalier de Kigali in Kigali, Rwanda. The Centre Médico Social de Bilyogo, a primary health care center that serves the lower socioeconomic strata of Kigali, is situated ~4 km from the laboratory. Four-hundred and five consecutive patients (243 men and 162 women) presenting with genital ulcers between September 1990 and June 1992 on the first 3 days of each week were considered for enrollment in the study. Demographic, epidemiological, and clinical information was obtained in standard interviews, and patients were asked

Received 19 May 1994; revised 16 September 1994.

* Present affiliation: World Health Organization, Global Program on AIDS, Geneva, Switzerland.

Reprints or correspondence: Prof. Dr. Sc. L. Kestens, Department of Infection and Immunity, Institute of Tropical Medicine, Nationalestraat 155, B-2000 Antwerpen 1, Belgium.

Clinical Infectious Diseases 1995;20:924–30

1058-4838/95/2004-0029$02.00

about their previous use of antibiotics. A physical examination of the external genitals and the inguinal regions was performed. Buboes were defined as tender inguinal lymph nodes enlarged to >2 cm. Ulcers were characterized as to number, site, shape, purulence, and invasiveness. Only patients with culture-proven *H. ducreyi* infection were eligible for evaluation in the study.

Diagnostic Procedures

Isolation of H. ducreyi and in vitro susceptibility testing. After the ulcer was cleaned with a sterile gauze, a cotton swab was rolled in the ulcer base and immediately plated onto two media: (1) gonococcal agar base (Difco Laboratories, Detroit) supplemented with 1% bovine hemoglobin (Difco), 5% sterile fetal calf serum (Gibco, Paisley, Scotland), factors X and V (Bacto Supplement VX; Difco), and 3 μg of vancomycin per mL and (2) Mueller-Hinton II agar (bioMérieux, Marcy l'Etoile, France) supplemented with the same ingredients. Culture plates were incubated at 33°C in a candle-extinction jar and read after 2–7 days. Presumptive identification of *H. ducreyi* was based on results of gram staining, typical colonial morphology, a negative catalase test, and a positive oxidase test with tetramethyl-*p*-aminophenylene diamine. All isolates were tested for the presence of a β-lactamase with a chromogenic cephalosporin test (Nitrocefin; Oxoid, Basingstoke, England). Isolates were stored in 10% skim milk (Difco) at −80°C and sent to the Institute of Tropical Medicine in Antwerp, Belgium, where a final identification was made by means of recommended techniques [15].

MICs of azithromycin, ceftriaxone, ciprofloxacin, erythromycin, trimethoprim, and a combination of TMP-SMZ (1:19) were determined with use of an agar dilution technique. Serial twofold dilutions of the antibiotics were added to the testing media, which consisted of gonococcal agar (Difco), 1% hemoglobin (Becton Dickinson, Cockeysville, MD), 5% fetal bovine serum, 0.1% glucose, 0.01% L-glutamine, and 0.025% L-cysteine hydrochloride. The 48-hour growth in cultures on gonococcal agar enriched with 1% hemoglobin, 1% IsoVitaleX (Becton Dickinson), and 5% fetal bovine serum was suspended in Mueller-Hinton broth (Difco), centrifuged, and allowed to sediment for 20 minutes. The supernatant was transferred to another tube and adjusted to a concentration of 10^8 bacteria per mL by comparison with the 0.5 McFarland standard. With a multipoint replicator, inocula of 10^5 cfu were spotted on the test media. MIC testing results were read after 48-hour incubation of the test plates at 35°C in a 5% CO_2 atmosphere; the MIC was defined as the lowest concentration of the antimicrobial agent that allowed no visible growth.

Other diagnostic procedures. Specimens for isolation of herpes simplex virus (HSV) were obtained with a nontoxic cotton swab (Dacroswab type 1; Spectrum Laboratories, Houston), stored in Hanks' balanced salt solution for 1 month at −80°C, and shipped on dry ice to the Institute of Tropical Medicine, where HSV was detected by its cytopathic effect on a monolayer of Vero cells. For all patients, whether a discharge was noted or not, a culture for *Neisseria gonorrhoeae* was performed. For men, a swab was introduced up to the fossa navicularis urethrae and specimens were immediately plated onto modified Thayer Martin medium. For women, only a vaginal specimen was cultured, as the introduction of a speculum was very painful (or even impossible) in most cases. Culture plates were incubated for 3 days in a candle-extinction jar at 37°C. Isolates of *N. gonorrhoeae* were tested for the presence of a β-lactamase with the chromogenic cephalosporin test and stocked in 10% skim milk at −80°C until transport to the Institute of Tropical Medicine. All *H. ducreyi* culture plates were also visually screened for *N. gonorrhoeae*, and after accurate identification of the germ, colony growth was considered to be evidence of the presence of *N. gonorrhoeae* in the ulcers. Each patient was tested for syphilis with the rapid plasma reagin test (RPR; Becton Dickinson) at a 1:2 serum dilution. Serum specimens were shipped to the Institute of Tropical Medicine, where further serological tests were performed, including a quantitative RPR and a *Treponema pallidum* hemagglutination assay (Fujirebio, Tokyo). An RPR-determined titer of ≥ 1:2 in association with a positive *T. pallidum* hemagglutination assay was considered compatible with a diagnosis of active syphilis. Antibodies to HIV-1 were detected by an ELISA (Vironostika HIV-mixt; Organon Teknika, Oss, the Netherlands). All positive sera were retested by an indirect immunofluorescence test and by a western blotting technique (Dupont de Nemours, Wilmington, DE).

Lymphocyte subset analysis. Five mL of blood was collected into EDTA by venipuncture and transported to the laboratory within 1 hour. Absolute white and red blood cell counts were determined with a Coulter Counter (model T540; Coulter Electronics, Bedfordshire, UK) for 246 patients and with a manual technique for 159 patients. Differential white blood cell counts were determined microscopically after May-Grünwald Giemsa staining. $CD4^+$ and $CD8^+$ lymphocyte subsets were stained in Kigali with anti-CD4 and anti-CD8 monoclonal antibodies (Leu3-PE, Leu2a-FITC; Becton Dickinson, Erembodegem, Belgium) and fixed in 0.5% paraformaldehyde. Counting was done at the Institute of Tropical Medicine by flow cytometry analysis. The procedure was performed as described elsewhere [16].

Treatment Regimens

Patients with clinical ulcers were alternately, assigned to treatment with a single oral dose of either ciprofloxacin (500 mg) or TMP-SMZ (640/3,200 mg) before culture results were available. Pregnant women did not receive ciprofloxacin. Irrespective of the clinical diagnosis, all patients received

an intramuscular dose of 2.4 million units of penicillin G benzathine. Patients whose RPR was reactive received two additional doses during the second and third visit, as recommended by the World Health Organization for the case management of genital ulcer disease [17]. On day 7, all patients who were not completely cured following ciprofloxacin treatment received a single dose of TMP-SMZ (640/3,200 mg), and all those who were not completely cured following TMP-SMZ treatment received a single dose of ciprofloxacin (500 mg). Patients whose conditions had not improved by day 14 were treated with a 7-day course of TMP-SMZ (160/800 mg b.i.d.). Single-dose treatments were always administered under direct supervision of a nurse.

Evaluation and Follow-up

All patients were asked to return for follow-up on days 7, 14, 21, and 28. Ulcers were considered (1) cured if epithelialization was complete, (2) improved if they had decreased in size, (3) unchanged if no improvement was observed, or (4) worse if they were larger. For patients whose ulcers were not completely healed, cultures were performed until epithelialization was complete. A negative or positive culture for *H. ducreyi* was considered proof of bacteriologic cure or failure, respectively. Clinical relapse (or reinfection) was defined by the reappearance of the ulcer by day 14 (in initially cured or improved cases); bacteriologic relapse (or reinfection) was diagnosed when a culture performed on day 14 became positive for *H. ducreyi*.

Statistical Analysis

The Yates' corrected χ^2 test and (when appropriate) the two-tailed Fisher's exact test were applied to assess differences in proportions for statistical significance. The Mann-Whitney U test was performed to compare results of two patient groups. The OR was used for measuring associations. Statistical analysis was performed with use of Epi-Info software, Version 5 (Centers for Disease Control and Prevention).

Results

Baseline Data

Twenty-eight percent of the examined patients with genital ulcers (112 of 405) had culture-proven chancroid. Although 35 (31%) of the 112 culture-positive patients had taken some form of self-medication in the month before the first visit, the rate of positive cultures was similar among patients who self-medicated ($P = .34$). *H. ducreyi* tended to be recovered more frequently from men (74 of 243, or 30.5%) than from women (38 of 162, or 23.5%) ($P = .15$). Significant differences with respect to demographic, epidemiological, and clinical characteristics were not observed between the treatment groups (table 1). The relative number of HIV antibody–positive subjects, the mean CD4 lymphocyte count of the HIV-positive patients, and the relative number of patients with other sexually transmitted diseases like *N. gonorrhoeae* infection, HSV infection, or syphilis were similar in the two treatment groups (table 1).

Results of Treatment

The treatment outcome noted on day 7 is presented in table 1. The proportion of patients who initially were cured or whose conditions improved and who returned for follow-up on day 14 tended to be higher in the TMP-SMZ group than in the ciprofloxacin group ($P = .052$).

In the ciprofloxacin treatment group, 20 (62.5%) of 32 initially cured or improved patients presented for follow-up on day 14. Nineteen were completely cured and one had relapsed or was reinfected and was culture-positive for both *H. ducreyi* and HSV. Ten patients whose ulcers were unchanged or worse on day 7 then received crossover treatment with TMP-SMZ. When six of these patients presented for follow-up on day 14, four were cured or improved. Both patients for whom the crossover treatment with TMP-SMZ failed were culture-negative for *H. ducreyi*.

In the TMP-SMZ treatment group, 19 (90.5%) of 21 initially cured or improved patients presented for follow-up on day 14. Sixteen were completely cured and three had relapsed or were reinfected; one was culture-positive for HSV and the other two for *H. ducreyi*. Nineteen patients whose ulcers were unchanged or worse on day 7 received crossover treatment with ciprofloxacin. Of 13 of these patients who presented for follow-up on day 14, eight were cured or improved. Cultures for one of five patients who did not respond to crossover treatment with ciprofloxacin were positive for *H. ducreyi*.

Overall, the percentage of cured or improved patients on day 14, after exclusion of patients lost to follow-up, was 88.5% (23 of 26) in the original ciprofloxacin group and 75% (24 of 32) in the original TMP-SMZ group ($P = .31$).

For investigation of the influence of other, concomitant sexually transmitted diseases on the outcome of treatment for *H. ducreyi* infection, the significance of a positive syphilis serology and the presence of *N. gonorrhoeae* or HSV were monitored at the start of the therapy for chancroid. Seven days after treatment, the rate of cure or improvement among patients for whom syphilis serology was positive was similar to that among patients for whom it was negative (17 of 23 [73.9%] vs. 36 of 59 [61%]; $P = .27$). Four (40%) of the 10 patients whose ulcers contained *N. gonorrhoeae* were cured or improved after 7 days, a rate similar to that among patients whose *N. gonorrhoeae* cultures were negative (29 of 72, or 40%). Of six patients with mixed *H. ducreyi*/HSV infection who were examined on day 7, three (50%) were cured or improved. A similar proportion of cure or improvement was

Table 1. Characteristics of and treatment outcome for 112 patients with culture-proven chancroid who were given single doses of ciprofloxacin or TMP-SM2.

Variable	Data per treatment group		
	Ciprofloxacin (n = 62)	TMP-SMZ (n = 50)	P value
Characteristic			
Age (y)	25.1 ± 4.97	25.7 ± 4.55	.29
Weight (kg)	55.0 ± 7.52	57.0 ± 5.91	.16
Men/women	41/21	33/17	.85
Circumcised (men)	2 (4.9)	3 (9.1)	.65
Ulcer(s) present			
Duration, >4 w	8 (12.9)	11 (22.0)	.31
Invasive	41 (66.1)	31 (62)	.65
>1 ulcer	33 (53.2)	34 (68.0)	.26
Bubo(es) present	13 (21.0)	9 (18.0)	.69
Previous antimicrobial treatment	19 (30.6)	16 (32.0)	.88
RPR-proven titer of antibody to *T. pallidum*, ≥1:2	15 (24.2)	14 (28.0)	.65
HSV isolated	2 (3.2)	5 (10.0)	.24
N. gonorrhoeae in urethra/vagina	14 (22.6)	9 (18.0)	.71
N. gonorrhoeae in ulcer	8 (12.9)	3 (6.0)	.34
Seropositive for HIV-1 antibody	39/60 (65.0)	38/48 (79.2)	.16
T lymphocytes, in HIV+ patients*			
CD4+, percentage	20 ± 11	19 ± 10	.54
<20%	20 (54.1)	23 (60.5)	.57
CD4+, no. per mm^3	706 ± 473	572 ± 299	.39
<400/mm^3	13 (35.1)	14 (36.8)	.87
Treatment outcome			
Day 7			
Clinical cure or improvement	32/42 (76)†	21/40 (52.5)†	.04
New cultures again yield *H. ducreyi*‡	1/22 (4.5)	16/27 (59.3)	<.001
Day 14			
Bacteriologic relapse or reinfection	1/20§ (5)	2/19§ (10.5)	.60

NOTE. Data are given as mean ± SD or the number (%) of patients.

* Of the HIV-seropositive patients, 37 in the ciprofloxacin group and 38 in the TMP-SMZ group had relative and absolute CD4$^+$ lymphocyte counts determined.

† Proportion of patients who presented for follow-up on day 7; OR = 2.9.

‡ Cultures were performed again on day 7 for 12 ciprofloxacin-treated and eight TMP-SMZ-treated patients whose conditions had improved and all patients for whom treatment failed; OR = 30.5.

§ The denominator is the number of patients with clinical cure or improvement who presented for follow-up on day 14.

seen among patients without concomitant HSV infection (50 of 67 [74.5%]; P = .73).

Fewer patients with an ulcer that had been present for >4 weeks were cured or improved on day 7 than were patients with an ulcer that had been present for ≤4 weeks (7 of 17 [41%] vs. 46 of 65 [71%]; OR = 3.46; P = .04). Other factors associated with delayed healing of an ulcer were not identified.

Effect of HIV and Degree of CD4$^+$ Lymphocyte Depletion on Treatment Outcome

HIV infection had an effect on neither the treatment outcome noted on day 7 nor the reisolation of *H. ducreyi* in cultures performed on day 7 (table 2). An association between the degree of CD4$^+$ T cell depletion and therapeutic outcome was not observed (table 3). Among HIV-positive patients, the mean CD4$^+$ lymphocyte count was comparable in the cured or improved patient group and in patients whose ulcers were unchanged or worse on day 7. Of 75 HIV-seropositive patients for whom CD4$^+$ lymphocyte counts were available, 12 (16%) had less than a 10% proportion of CD4$^+$ lymphocytes and 4 (5.3%) had <200 CD4$^+$ lymphocytes per mm^3.

Antimicrobial Susceptibility of *H. ducreyi* and Correlation with Treatment Outcome

All isolates of *H. ducreyi* were β-lactamase producing and therefore completely resistant to penicillin G and ampicillin. MICs were available for 20 and 18 pretreatment isolates of the ciprofloxacin and TMP-SMZ groups, respectively, and

Table 2. Effect of HIV on treatment outcome for patients with chancroid who received one dose of ciprofloxacin or TMP-SMZ.

	Ciprofloxacin			TMP-SMZ		
Treatment outcome on day 7	HIV+ (n = 27)	HIV− (n = 15)	P value	HIV+ (n = 32)	HIV− (n = 7)	P value
Clinical cure or improvement	20 (74.1)	12 (80)	1	19 (59.4)	2 (28.6)	.21
H. ducreyi reisolated*	1/12 (8.3)	0/10	1	11/20 (55.0)	5/6 (83.3)	.35

NOTE. Data are presented as number (%) of patients; n = number presenting for follow-up on day 7.
* Denominator is number of patients for whom cultures were performed again on day 7 (relapsing patients not included).

for 12 posttreatment isolates of the TMP-SMZ group. Posttreatment MICs were not available for the two isolates recovered after ciprofloxacin treatment failure.

The MICs of TMP-SMZ for pretreatment and posttreatment isolates from four patients whose treatment failed all were ≥4/76 μg/mL, indicating in vitro resistance. For one patient, the MIC of TMP-SMZ was the same for both the pretreatment and posttreatment isolate: 0.125/2.375 μg/mL. Seven patients had only a posttreatment isolate tested. Patients whose ulcers were unchanged or worse following treatment with TMP-SMZ more frequently harbored organisms for which MICs of TMP-SMZ were ≥4/76 μg/mL than did patients who were cured or improved (10 of 12 [83.3%] vs. 4 of 13 [30.7%]; OR = 11.25; P = .02). It is interesting that MICs of trimethoprim of ≥4 μg/mL were equally frequent in both groups (table 4). All isolates were highly susceptible to ciprofloxacin (MIC, 0.004–0.06 μg/mL), erythromycin (MIC, 0.008–0.25 μg/mL), ceftriaxone (MIC, 0.001–0.06 μg/mL), and azithromycin (MIC, 0.002–0.125 μg/mL).

Discussion

Since genital ulcer disease constitutes a major risk for the transmission of HIV, efficient treatment of chancroid also contributes to the reduction of HIV transmission. The treatment currently recommended by the Centers for Disease Control and Prevention consists of a multiple doses of erythromycin [3]. One major drawback of this regimen, however, is that it may lead to incomplete cure and relapse when patient compliance is poor, as it often is in developing countries. On the other hand, the cost of treatment with single doses of azithromycin and ceftriaxone (the single-dose treatment of choice for chancroid) [3] is relatively high; as a consequence, these drugs are practically inaccessible to health care centers in developing countries. Therefore, we compared the efficacy of two single-dose regimens of ciprofloxacin and TMP-SMZ for the treatment of chancroid in Rwanda, where HIV is highly endemic. Several reports have indicated that single-dose treatments with these antibiotics are as effective as multiple-dose regimens [4–10].

This study, however, shows that single-dose regimens of ciprofloxacin and TMP-SMZ for chancroid treatment are associated with a significant failure rate in Rwanda. Even when treatment for patients who were lost to follow-up was considered a success, only 83.9% and 62.0%, respectively, of patients in the ciprofloxacin and TMP-SMZ groups were clinically cured or improved. Although all isolates were highly susceptible to ciprofloxacin, a single dose of 500 mg did not sterilize the ulcer of at least one (4.5%) of 22 patients. Since levels of drug in the blood must exceed the MIC of the drug for *H. ducreyi* for 36–48 hours to eradicate the pathogen at the infected site, blood levels after a single 500-mg oral dose of ciprofloxacin may be insufficient when intestinal absorption is incomplete [18].

Table 3. Effect of CD4+ lymphocyte depletion on treatment outcome for HIV-positive patients who received one dose of ciprofloxacin or TMP-SMZ.

	Proportion of $CD4^+$ lymphocytes			$CD4^+$ lymphocyte count per mm^3		
Treatment outcome on day 7	<20% (n = 33)	≥20% (n = 25)	P value	<400 (n = 22)	≥400 (n = 36)	P value
Clinical cure or improvement	20 (60.6)	18 (72.0)	.53	14 (63.6)	24 (66.7)	.96
H. ducreyi reisolated*	7/20 (35.0)	5/12 (41.6)	.72	6/13 (46.2)	6/19 (31.6)	.47

NOTE. Data are presented as number (%) of patients; n = number of patients presenting for follow-up on day 7.
* Denominator is number of patients for whom cultures were performed again on day 7 (relapsing patients not included).

Table 4. In vitro susceptibility of *H. ducreyi* to TMP-SMZ and trimethoprim, as related to treatment with ciprofloxacin or TMP-SMZ and clinical outcome on day 7.

	Ciprofloxacin			TMP-SMZ		
Antimicrobial, MIC	Cure or improvement (n = 14)	No change or worsening (n = 6)	P value	Cure or improvement (n = 13)*	No change or worsening (n = 12)†	P value
TMP-SMZ, ≥4/76 μg/mL	4 (28.6)	4 (66.7)	.16	4 (30.8)	10 (83.3)	.02
Trimethoprim, ≥4 μg/mL	6 (42.9)	4 (66.7)	.63	8 (61.5)	10 (83.3)	.38

NOTE. Data are presented as number (%) of patients.
* For two of these patients, only posttreatment isolates were tested.
† For five of these patients, only posttreatment isolates were tested.

Although HIV infection, which was highly frequent among our patients, may alter intestinal absorption and lead to low blood levels of the drug, differences in treatment effect between HIV-positive and HIV-negative patients were not observed. The apparent contradiction between failure of ciprofloxacin treatment and the high in vitro susceptibility of all *H. ducreyi* isolates to ciprofloxacin should be interpreted with caution, however. Only one dosage of ciprofloxacin was evaluated, and *H. ducreyi* was isolated from only a single patient after treatment with the drug. For this patient no data (such as sex partner's *H. ducreyi* status or any other potential cause of reinfection) were available that would help to distinguish treatment failure from reinfection.

After 1986, *H. ducreyi* became increasingly resistant to TMP-SMZ and to trimethoprim in Rwanda, a phenomenon unknown at the start of the present study [19]. All strains were tested simultaneously in 1993, and results indicated the resistance to TMP-SMZ of 48% of strains isolated in 1991 but none of the strains isolated in 1986. Since a good correlation exists between in vitro susceptibility and clinical outcome, the administration of TMP-SMZ as single- or multiple-dose therapy can no longer be recommended for the treatment of chancroid, neither in Rwanda nor (probably) in any other part of Central Africa.

The results of this study indicate that the proportion of *H. ducreyi*–infected patients who are positive for HIV has increased from 59% in 1986 to 71% in 1992, a finding confirming earlier statements that chancroid constitutes a major risk factor for transmission of HIV [2, 1]. With regard to treatment outcome, we were not able to demonstrate any relationship between therapeutic response and HIV infection or the degree of $CD4^+$ lymphocyte depletion. These findings agree with those of investigators in South Africa [20] but conflict with those in reports published by others in Kenya [12–14]. The reason for this discrepancy is not clear but may be related to the degree of patients' immune deficiency. In our study and in the study in South Africa, only a few patients had <200 $CD4^+$ lymphocytes per mm^3, a finding indicating that the majority of the HIV-seropositive patients had a light or moderate rather than advanced degree of immune deficiency.

Rapid bacterial eradication and early epithelialization of the ulcer remain the primary objectives of treatment, but the fact that treatment stops the transmission of *H. ducreyi* and reduces the spread of HIV cannot be ignored. Single-dose regimens, even of agents highly active in vitro, should not be recommended as first-line therapy for chancroid unless their clinical and bacteriologic efficacy have been proven. Since the antimicrobial resistance of *H. ducreyi* may change over time, periodic surveillance of the clinical efficacy of treatment regimens and of the in vitro susceptibility of the pathogen is mandatory for administrators of programs for controlling sexually transmitted diseases. Empirical treatment regimens for genital ulcers, particularly with single doses, should be based on the clinical and bacteriologic efficacy of such regimens.

Acknowledgments

Ciprofloxacin and TMP-SMZ (for treatment) were donated by Bayer and Roche Pharmaceuticals (Brussels), respectively. The authors appreciate the secretarial assistance of Ms. Greet Verhulst in the preparation of the manuscript.

References

1. Laga M, Nzila N, Goeman J. The interrelationship of sexually transmitted diseases and HIV infection: implications for the control of both epidemics in Africa. AIDS 1991;5(suppl 1):S55–63.
2. Bogaerts J, Ricart CA, Van Dyck E, Piot P. The etiology of genital ulceration in Rwanda. Sex Transm Dis 1989;16:123–6.
3. Centers for Disease Control and Prevention. 1993 Sexually transmitted diseases treatment guidelines. MMWR 1993;42(RR-14):1–102.
4. Fast MV, Nsanze H, D'Costa LJ, et al. Antimicrobial therapy of chancroid: an evaluation of five treatment regimens correlated with in vitro sensitivity. Sex Transm Dis 1983;10:1–6.
5. Taylor DN, Pitarangsi C, Echeverria P, Panikabutra K, Suvongse C. Comparative study of ceftriaxone and trimethoprim-sulfamethoxazole for the treatment of chancroid in Thailand. J Infect Dis 1985;152:1002–6.

6. Bowmer MI, Nsanze H, D'Costa LJ, et al. Single-dose ceftriaxone for chancroid. Antimicrob Agents Chemother **1987**;31:67–9.
7. Plummer F, Nsanze H, D'Costa LJ, et al. Single-dose therapy of chancroid with trimethoprim-sulfametrole. N Engl J Med **1983**; 309:67–71.
8. Ballard RC, Duncan MO, Fehler HG, Dangor Y, Da Lexposto F, Latif AS. Treating chancroid: summary of studies in southern Africa. Genitourin Med **1989**;65:54–7.
9. Naamara W, Plummer F, Greenblatt RM, D'Costa LJ, Ronald AR. Treatment of chancroid with ciprofloxacin: a prospective, randomised clinical trial. Am J Med **1987**;82(suppl 4A):317–20.
10. Bodhidatta L, Taylor DN, Chitwarakorn A, Kuvanont K, Echeverria P. Evaluation of 500- and 1000-mg doses of ciprofloxacin for the treatment of chancroid. Antimicrob Agents Chemother **1988**;32:723–5.
11. Plourde PJ, D'Costa LJ, Agoki E, et al. A randomized, double-blind study of the efficacy of fleroxacin versus trimethoprim-sulfamethoxazole in men with culture-proven chancroid. J Infect Dis **1992**;165:949–52.
12. MacDonald KS, Cameron DW, D'Costa LJ, Ndinya-Achola JO, Plummer FA, Ronald AR. Evaluation of fleroxacin (RO 23-6240) as a single oral dose therapy of culture-proven chancroid in Nairobi, Kenya. Antimicrob Agents Chemother **1989**;33:612–4.
13. Tyndall M, Plourde PJ, Agoki F, et al. Fleroxacin in the treatment of chancroid: an open study in men seropositive or seronegative for the human immunodeficiency virus type 1. Am J Med **1993**;94:85S–8S.
14. Tyndall M, Malisa M, Plummer FA, Ombetti J, Ndinya-Achola JO, Ronald AR. Ceftriaxone no longer predictably cures chancroid in Kenya. J Infect Dis **1993**;167:469–71.
15. Morello JA, Janda WM, Doern GV. *Neisseria* and *Branhamella*. In: Balows A, Hausler WJ Jr, Herrmann KL, Isenberg HD, Shadomy HJ, eds. Manual of clinical microbiology. 5th ed. Washington, DC: American Society for Microbiology, **1991**:258–76.
16. Kestens L, Brattegaard K, Adjorlolo G, et al. Immunological comparison of HIV-1-, HIV-2- and dually-reactive women delivering in Abidjan, Côte d'Ivoire. AIDS **1992**;6:803–7.
17. World Health Organization. Recommendations for the management of sexually transmitted diseases. Geneva: World Health Organization, **1994**; publication no. WHO/GPA/STD 93.1.
18. Dylewski J, Nsanze H, D'Costa L, Slaney L, Ronald AR. Trimethoprim-sulfamethoxazole in the treatment of chancroid: comparison of two single-dose treatment regimens with a five-day regimen. J Antimicrob Chemother **1985**;16:103–9.
19. Van Dyck E, Bogaerts J, Tello WM, Mukantabana V, Smet H, Piot P. Emergence of *Haemophilus ducreyi* resistance to trimethoprim-sulfamethoxazole in Rwanda. Antimicrob Agents Chemother **1994**; 38:1647–8.
20. Ballard RC, Radebe F, Mampuru M, Dangor Y, Fehler HG. A comparison of ofloxacin and ciprofloxacin in the treatment of chancroid [abstract no 116]. In: Program and abstracts of the 10th International Meeting of the International Society for STD Research. Helsinki: Painatuskeskus Oy, **1993**.