Clinical and microbiological study of non-gonococcal urethritis with particular reference to non-chlamydial disease

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SUMMARY A double-blind placebo-controlled study of minocycline in 221 men with non-gonococcal urethritis (NGU) was undertaken. Techniques were used which enabled diagnoses of chlamydial and mycoplasmal infections to be made within 24 hours of a patient attending a clinic. All patients from whom *Chlamydia trachomatis* was isolated were treated with minocycline, while patients from whom *Ureaplasma urealyticum* or *Mycoplasma hominis* was isolated, or from whom no micro-organisms were isolated, were treated on a double-blind basis with either minocycline or placebo.

Chlamydia were isolated from 77 (35%) patients and were eradicated by minocycline from 76 (99%). Ureaplasmas were isolated initially from 96 (43%) patients. Treatment with minocycline eradicated them from 43 of 52 (83%) patients, and they disappeared from six of 31 (19%) patients who were treated with placebo. After one week significantly more patients had responded clinically to minocycline than to placebo.

The response to minocycline was not influenced by the microbiological status of the patients, which suggests that ureaplasmas are playing a similar role to chlamydia in the pathogenesis of the disease and that an antibiotic-sensitive micro-organism may be producing disease in the isolate-negative group. An immunological approach is required to resolve the problem of the persistent urethral inflammation which occurred despite eradication of the micro-organisms.

Introduction

In the last 30 years it has been demonstrated convincingly that non-gonococcal urethritis (NGU) responds better to tetracyclines and erythromycin than to penicillin derivatives or placebos,^{1,2} thus suggesting a microbial aetiology. Further studies have sought an organism or organisms responding to the same range of antimicrobial agents that are effective in treating the disease. While there is convincing evidence that *Chlamydia trachomatis* is involved,³ and some evidence for the role of *Ureaplasma urealyticum*,⁴ the extent to which these micro-organisms act as primary pathogens in acute and recurrent NGU is unclear.

We present the short-term results of a double-blind placebo-controlled study of minocycline in patients with NGU attending two West London clinics. By using cycloheximide-treated McCoy cells combined with indirect immunofluorescent antibody staining⁵ we were able to isolate chlamydia within 24 hours and so allot patients to particular treatment groups based on microbiological findings. In this way it was possible to focus attention on the chlamydia-negative patients in order to elucidate the role of mycoplasmas, in particular ureaplasmas, in the disease and to investigate the group of patients from whom micro-organisms were not isolated.

Patients and methods

SELECTION OF PATIENTS

Men attending two venereal disease clinics in West London during specified morning sessions were con-

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sidered for inclusion in the study if the following criteria were fulfilled: (1) a Gram-stained smear of urethral exudate or scraping contained 15 or more polymorphonuclear leucocytes per high-power microscope field ($\times 800$ magnification) (PMNL/hpf) but no Gram-negative diplococci presumptively identified as *N gonorrhoeae*; (2) antibiotics had not been taken during the seven days before attendance; (3) the patient was prepared to reattend on at least three subsequent occasions; and (4) informed consent was given to participate in the study.

DESIGN OF THE STUDY

The study was approved by the ethics committees of the participating hospitals. Patients were informed that the purpose of the study was to identify possible causative organisms of NGU. Specimens were taken from patients at the first visit and they were asked to reattend the following day, when the results of tests would be available and appropriate treatment given.

Chlamydia-positive patients were treated with minocycline (Minocin, Lederle) 1.3 g in divided doses over a six-day period (a loading dose of 200 mg and twice daily doses of 100 mg thereafter) because we considered it to be unethical to withhold antibiotic treatment from patients infected with a micro-organism widely believed to be a pathogen. Patients who were chlamydia-negative but mycoplasma-positive (either *U urealyticum* or *M hominis*) were treated on a double-blind basis with either minocycline or a placebo (lactose tablets). Patients who were isolation-negative were treated also on a double-blind basis with either minocycline or placebo. Patients were instructed to complete the course of treatment, to abstain from alcohol and sexual intercourse, and to reattend one week later. Written instructions were issued.

At each follow-up visit clinical and microbiological assessments were undertaken and appropriate treatment prescribed for those patients who were not considered to be cured. Follow-up was continued until the patient was regarded as cured on the basis of tests on two consecutive visits at least one week apart. Those who admitted to sexual intercourse were not excluded so that it was possible to consider the role that reinfection might play in recurrent disease. Sexual partners were seen when possible and were treated empirically with tetracyclines.

MANAGEMENT AT FIRST ATTENDANCE

A full medical history was taken. The genital area was examined and a smear made from the urethral exudate or, in the absence of exudate, from a scraping obtained from the anterior urethra with a bacteriological loop; the smear was Gram-stained and examined for PMNL and Gram-negative

diplococci. A second specimen, taken similarly, was plated directly on to a non-selective medium consisting of GC agar base (Difco) containing 2% v/v Isovitalex (BBL), which was incubated at 37°C in 5% CO₂ in air. After 24 hours the medium was examined for typical colonies of *N gonorrhoeae*. Suspicious colonies were subcultured and identified by Gram-staining and an oxidase test. Patients who were suspected or proved to have gonorrhoea were excluded from the study. An endourethral specimen was then taken for the isolation of *C trachomatis* as described.⁶ A second nasopharyngeal swab was inserted 3 cm into the urethra and expressed in mycoplasma standard liquid medium. Urine was also collected for the isolation of mycoplasmas.

The specimens were kept at room temperature until titration in urea-containing and arginine-containing media within five hours of collection.⁷ Organisms producing a colour change in urea-containing medium were regarded presumptively as *U urealyticum* and those which hydrolysed arginine as *M hominis*.

A specimen of blood was taken when possible for serological tests for syphilis and for chlamydial antibody studies. Chlamydial IgM and IgG antibody to a range of serotypes was sought by a micro-immunofluorescence method.⁸

ASSESSMENT AT FOLLOW-UP ATTENDANCES

Each patient was questioned about the nature of any residual symptoms, and a smear or anterior urethral scraping was obtained. Swabs were taken for isolation of chlamydia and mycoplasmas. Blood was taken for chlamydial serology approximately 14 days after the initial attendance and in some cases on subsequent occasions. Patients who had residual symptoms or whose urethral smear showed more than 5 PMNL/hpf were retreated with a full course of minocycline. Patients were discharged from the study if they were asymptomatic and a urethral smear showed ≤ 5 PMNL/hpf at two visits at least one week apart. Patients were seen at weekly intervals until they were discharged or until they defaulted. Appropriate antibiotic therapy was instituted for those who failed to respond to minocycline.

DIAGNOSIS OF CHLAMYDIAL INFECTION

Patients from whom *C trachomatis* was isolated are designated C(I)+, chlamydia-(isolation)-positive. For this study, serological evidence of current chlamydial infection is defined as either: (1) the presence of serum IgM antibody at a titre of $\geq 1/8$ within 28 days of the first attendance; or (2) seroconversion or a four-fold rise in the titre of serum IgG antibody within 28 days of the initial attendance. On this basis, 19 patients were isolation-

negative but had serological evidence of current chlamydial infection. Patients who were isolation-positive or seropositive or both are designated C(IS)+.

DESIGNATION OF MYCOPLASMAL INFECTION

Mycoplasmal infections with *U urealyticum* or *M hominis* are designated M+; M- indicates the absence of both mycoplasmas. U+ indicates the presence of ureaplasmas and MH+ the presence of *M hominis* specifically.

DIAGNOSIS OF CURE

For analysis a patient was regarded as cured if he was asymptomatic and had <15 PMNL/hpf in a urethral smear or scrape. These criteria differed from those used to determine the need for retreatment during the study when it was felt that clinical judgment must override defined research criteria. Cure rates were calculated from the proportion of all patients (excluding defaulters) who were known to be cured.

STATISTICAL METHODS

Groups were compared by χ^2 tests, using Yates's correction for 2×2 tables. The χ^2 test for trend was used to analyse variables with ordered categories.

Results

MICRO-ORGANISMS ISOLATED INITIALLY

The study group consisted of 241 patients, of whom 20 were excluded because they were shown to have gonorrhoea or because of technical problems in the isolation of chlamydia. Of the remaining 221 patients, *C trachomatis* was isolated from 77 (35%), ureaplasmas from 96 (43%), and *M hominis* from 29 (13%).

The patients were categorised according to whether or not chlamydia and mycoplasmas had been isolated (C(I)+M+, C(I)+M-, C(I)-M+, C(I)-M-). The distribution of patients in the four groups shown in table I is based on chlamydial infec-

TABLE I Distribution of patients in four microbiological categories based on chlamydial isolation only or chlamydial isolation and serology

Category	Isolation only		Category	Isolation and serology	
	No	%		No	%
C(I)+M+	31	14	C(IS)+M+	39	18
C(I)+M-	46	21	C(IS)+M-	57	26
C(I)-M+	67	30	C(IS)-M+	59	27
C(I)-M-	77	35	C(IS)-M-	66	30

+ Positive; - negative; C(I)=chlamydial isolation; C(IS)=chlamydial isolation and serology; M=mycoplasmas.

tion diagnosed by isolation only and by isolation combined with serology. In approximately one-third of patients these micro-organisms could not be detected.

BACKGROUND FACTORS AND MICROBIOLOGICAL DIAGNOSIS

The isolation of chlamydia and mycoplasmas from patients in different ethnic groups is shown in table II. Chlamydia were isolated less frequently from Arabs than from other men, although this might be a chance finding related to the small sample size. Mycoplasmas were, however, isolated significantly less frequently ($\chi^2 = 11.6$; $P < 0.001$) from Caucasians (excluding Arabs) in a large sample than from all the other men except Asians.

TABLE II Distribution of patients of various ethnic groups in relation to isolation results

Ethnic group	No of patients	Isolation results			
		C(I)+		M+	
		No	%	No	%
British, Irish, and others of West European origin	139	52	37	49	35
Arab	25	5	20	16	64
West Indian	38	12	32	24	63
African Negro	12	6	50	8	67
Asian	7	2	29	1	14
Total	221	77		98	

C(I)+ = chlamydia-(isolation)-positive; M+ = mycoplasma-positive

Age, marital state, sexual orientation, history of sexually transmitted diseases, a remote or recent history of NGU or post-gonococcal urethritis (PGU), the apparent incubation period, a history of casual sexual contact, and the nature and duration of symptoms were considered in relation to microbiological findings. For each of these factors, the proportion of patients in each of the four microbiological categories was similar except that a history of treatment of NGU or PGU in the previous three months was associated with the failure to isolate *C trachomatis* ($P < 0.001$).

CLINICAL RESPONSE TO TREATMENT

Minocycline was given to 163 patients and the placebo to 58. The response of patients treated with minocycline was significantly better at the first follow-up visit (usually at one week) than that of those given the placebo ($\chi^2 = 7.3$; $P < 0.01$) (table III). The proportion of patients cured after one week's treatment was largest (67%) in the C(IS)-M+ group treated with minocycline. The results of treatment were little influenced by choosing to classify the

TABLE III Clinical outcome at the first follow-up visit in relation to microbiological category based on isolation and serology

Microbiological category	No of patients	Treatment	No of patients			Cure rate (%)
			Cured	Not cured	Defaulted	
C(IS) + M +	39	Minocycline	17	13	9	57
C(IS) + M -	57	Minocycline	30	23	4	57
C(IS) - M +	29	Minocycline	16	8	5	67
C(IS) - M +	30	Placebo	9	18	3	33
C(IS) - M -	38	Minocycline	17	19	2	47
C(IS) - M -	28	Placebo	8	17	3	32
Total	221		97	98	26	50*

+ Positive; - negative; C(IS) = chlamydial isolation and serology; C(I) = chlamydial isolation; M = mycoplasmas

*Mean figure

19 seropositive patients who were C(I) - as having chlamydial infections.

The response to minocycline treatment was not influenced by microbiological status. When all the C(IS) + patients were compared with all the C(IS) - patients who were treated with minocycline there was no statistically demonstrable difference in clinical outcome ($\chi^2 = 0.01$; $P > 0.9$). Likewise, when M + and M - patients treated with minocycline were compared no difference in outcome could be shown ($\chi^2 = 0.6$; $P > 0.4$).

CLINICAL RESPONSE AND MICROBIOLOGICAL SEVERITY OF THE INITIAL INFECTION

The proportion of patients cured by treatment with minocycline was unrelated to the number of inclusions produced by specimens when inoculated into McCoy cell monolayers or to the microbiological severity of infection with *U. urealyticum* as measured in colour-changing units (ccu/ml) (table IV). The numbers of patients infected with *M. hominis* were too small to draw any valid conclusions.

FACTORS AFFECTING DISEASE OUTCOME IN MINOCYCLINE-TREATED PATIENTS

Age, marital state, time since the last treatment for NGU, the apparent incubation period, a history of casual sexual contact, and the nature and duration of symptoms were unrelated to the outcome of the disease. The cure rate (44%) for West Indians, however, was lower than that for all other groups (58-80%). While a larger proportion of homosexual/bisexual patients (68%) appeared to be cured in comparison with heterosexuals (54%), the number of patients was too small to draw any conclusions. When the study group was analysed in terms of the number of previous episodes of NGU there appeared to be a trend suggesting that the clinical outcome was worse with increasing numbers of episodes; the cure rate was 59% for patients with a first episode and

40% for those experiencing their fifth or subsequent episode. This trend was not, however, statistically significant ($\chi^2 = 1.3$; $P > 0.2$).

MICROBIOLOGICAL RESPONSE TO TREATMENT

C. trachomatis was isolated from only one of 68 initially C(I) + patients who were treated with minocycline and who returned for follow-up and was not isolated from any patient at the second or subsequent follow-up visit. The one patient who failed to respond to minocycline was retreated with a second course of the drug and had a complete clinical and microbiological recovery. Although the putative pathogen was eradicated from 99% of patients clinical cure was achieved in only 54%. *C. trachomatis* was not isolated from any initially C(I) - patients (including those seropositive patients treated with placebo) at any follow-up visit.

U. urealyticum was isolated from nine of 52 (17%) patients initially U + after treatment with minocycline. Two of these patients admitted the

TABLE IV Relation of disease outcome to the microbiological severity of the initial infection in minocycline-treated patients

No of organisms isolated	No of patients	Cure rate (%)
<i>C. trachomatis</i>		
0	86	57
Low ($1-9$)*	43	55
High (≥ 10)*	34	53
<i>U. urealyticum</i> (swab)		
0	99	53
Low (10^1-10^3 ccu/ml)	45	63
High (10^4-10^6 ccu/ml)	19	58
<i>U. urealyticum</i> (urine)		
0	106	53
Low (10^1-10^3 ccu/ml)	42	65
High (10^4-10^6 ccu/ml)	15	60

ccu = colour-changing units

*Mean number of inclusions in 30 hpfs

possibility of reinfection, and ureaplasmas isolated from four others were resistant to minocycline *in vitro*.⁹ The reason for treatment failure in the remaining three patients was unexplained.

U urealyticum was reisolated from 25 of 31 (81%) U+ patients who were given the placebo. Of these, 21 were retreated with minocycline and attended for follow-up; 11 were cured clinically and microbiologically, ureaplasmas persisted in three without clinical disease and in one with urethritis, and symptoms or signs persisted in six patients in the absence of ureaplasmas. The clinical cure rate of 56% (14 out of 25) for these patients is of the same order as that for U+ patients who were treated initially with minocycline (63%). *U urealyticum* was not isolated at the first follow-up visit from any patients who were initially U-, irrespective of whether they were treated with minocycline or placebo.

M hominis was isolated from three (21%) of 14 patients initially MH+ who were treated with minocycline. Despite eradication of the micro-organism from 11 patients symptoms or signs of disease or both persisted in seven (64%).

M hominis was isolated from four of 10 (40%) patients initially MH+ who were given the placebo. After treatment with minocycline one patient defaulted, one was cured clinically and microbiologically, and signs persisted in two patients in the absence of *M hominis*. Surprisingly, *M hominis* was no longer isolated from six patients who had been given placebo, but five of them had persistent disease.

M hominis was isolated from one patient in whom it was not demonstrated at the first visit.

Discussion

Although there have been some dissenting voices,¹⁰ the role of *C trachomatis* as a primary pathogen in a proportion of cases of NGU is now well established, and this study was designed to focus attention on non-chlamydial NGU. The clinical outcomes for all microbiological groups in both the short-term and long-term (P E Munday, unpublished data) were equally bad. The cure rate of about 60% for all groups at the completion of one week's treatment with minocycline was worse than in most other published series, in which a cure rate of about 80% has been obtained after a week's course of tetracycline.

There are several possible explanations for the poor short-term clinical outcome in this study. (1) The population was different from that studied previously; this is unlikely because the patients were

drawn from two clinics where previous studies have been undertaken.^{11 12} (2) Patients were reinfected; although patients who admitted resuming sexual intercourse were not excluded, we feel this is an unlikely explanation because the first assessment was carried out usually within two days of completion of treatment and only nine patients admitted re-exposure. (3) Treatment was inadequate or inappropriate; Willcox *et al.*,¹³ however, using the same regimen, claimed a failure rate at three months of only 10.4%. In a previous study,¹² in which the same regimen was used but with less strict criteria for cure, we obtained a 10.8% failure rate. (4) The criteria for cure are more stringent than those used previously. We included in our "failure" group patients who had continuing urethritis and also those with subjective complaints. Our insistence on a scraping of the anterior urethra in the absence of any expressible discharge and the frequent finding of asymptomatic urethritis suggests that it might have been overlooked in previous studies.^{12 13 14} The interpretation of subjective complaints in the absence of evidence of urethritis is controversial. It is widely believed that these symptoms are psychogenic. Many patients with such complaints and without sexual re-exposure, however, return within hours or days with a frank urethritis suggesting that signs of the disease are intermittent. It would, therefore, be unrealistic to dismiss such complaints without repeated investigation and wrong to regard the patient as "cured". (5) A low default rate (12%) occurred. Asymptomatic patients who considered themselves cured were persuaded to reattend, and many were found to have residual urethritis. If 30% of the patients had defaulted and had been excluded, as usually occurs, it would have required only 56 of an initial 100 patients to reattend cured to achieve an 80% cure rate (that is 56/70). While we have complied with the usual method of determining the cure rate, an alternative approach is to express it as a ratio of the number of patients who reattend cured to the total number in the study (that is, 56/100 in the above example). Similarly, a failure rate (14/100) would comprise that proportion of the total number of patients who failed to respond to treatment. The cure rate, failure rate, and default rate would total 100%. In our study, these rates for minocycline-treated patients would be 49%, 39%, and 12%. Of these five possibilities for a relatively poor response to treatment, we believe that the last two are the most likely.

Although chlamydial urethritis was not the principal focus of this study, one aspect is worthy of note. The clinical outcome for patients with chlamydial urethritis was found not to differ from that for those with non-chlamydial urethritis, which

is in agreement with the finding of Oriel *et al.*¹⁵ This is also in keeping with the short-term results of Handsfield *et al.*,¹⁶ although they reported that patients with non-chlamydial urethritis had a poorer long-term response to treatment. If chlamydial and non-chlamydial NGU respond in a similar way in both the short-term and long-term it seems unlikely that NGU is caused by a wide spectrum of different micro-organisms unless they act indirectly to trigger an underlying inflammatory process. An alternative proposition, that all cases of NGU are caused by chlamydia, is unlikely for reasons to be discussed.

The assessment of the role of *U urealyticum* in NGU is made more difficult by the poor clinical response of the C+ patients. The fact that U+ minocycline-treated patients responded equally as well as C+ minocycline-treated patients suggests that ureaplasmas are playing a similar role to chlamydia in the pathological process. This view is strengthened by the response of U+ placebo-treated patients to subsequent minocycline. As with chlamydial infections there was no evidence that the more severe infections, as assessed microbiologically, responded better or worse to treatment.

Ureaplasmas were reisolated from 14% of minocycline-treated patients and 81% of placebo-treated patients. The former must indicate treatment failure or reinfection and the latter spontaneous clearance of organisms in 19% of these patients. As the failure rate for U+ minocycline-treated patients was greater than 14% and the cure rate for placebo-treated patients greater than 19%, it is evident that the correlation between clearance of ureaplasmas and clinical cure was poor. This situation is similar, however, to that seen in chlamydial urethritis where clearance of the micro-organism was not always associated with clinical cure.

It should also be noted that cure rates were related to certain background factors which were also related to isolation of specific micro-organisms. For example, fewer West Indian patients were cured than Caucasians and mycoplasmas were isolated more frequently from West Indians than from Caucasians. Thus it might be argued that had West Indians not been overrepresented in the M+ groups, the cure rates for these groups might have been better. This is unlikely, however, since more than two-thirds of M+ patients were C- and, as it is believed that C- patients do worse than¹⁶ or as well as¹⁵ C+ patients, it would be surprising if M+ patients had a higher rate of cure than M- patients. Conversely, chlamydia were isolated less frequently from those patients who had been treated for NGU in the preceding three months although cure rates for these patients were almost identical to those for patients who had not been so treated. This again throws some

doubt on the overriding importance of chlamydia as pathogens in NGU.

The clinical response to minocycline in the isolation-negative group of patients suggests that a tetracycline-sensitive organism is involved. It seems unlikely that all isolation-negative patients have an undiagnosed chlamydial infection because many have no serological evidence of a current or past chlamydial infection despite the testing of appropriately timed serum specimens. Although a small proportion (<10%; B J Thomas, unpublished data) of C(I)+ patients have no detectable serum chlamydial antibody, it is unlikely that isolation should fail more frequently in patients without antibody than in those with a normal antibody response. Studies of local antibody, although difficult in male patients, might help to resolve this point. Chlamydia were not found in second specimens from placebo-treated patients suggesting that the organisms had not been missed at the first attempt, although isolation probably failed in a few patients who had serological evidence of a current infection. It is also unlikely that isolation-negative patients may have undiagnosed ureaplasma infections. Although there is no serological evidence to substantiate this point, repeated isolation attempts during five months on untreated women attending an antenatal clinic have not succeeded in identifying ureaplasmas in those who were originally ureaplasma-negative (P M Furr, personal communication). Although it has been shown that the chlamydial isolation rate is related to the experience and technique of the person taking the specimen,¹⁷ there appears to be no difficulty in obtaining ureaplasmas from expressed discharge. If most isolation-negative patients do not have undiagnosed chlamydial or ureaplasma infections, the identity of another tetracycline-sensitive micro-organism should be sought. The possibility that it is *M hominis* has been discussed,⁴ and it now seems unlikely that this organism has any role in NGU. In this study, its eradication was not correlated with clinical improvement nor its persistence with continuing disease. It may be that the isolation-negative group of patients does not have disease of uniform aetiology and that a proportion of them had, for example, a chronic antibiotic-resistant urethritis. Factors other than infection may also be involved in the aetiology of isolation-negative NGU. The history of many patients suggests that exogenous infection is unlikely, particularly when there is no history of recent sexual exposure or when the only sexual relationship is with a partner who has been examined to exclude other transmissible infections and who has been treated with tetracyclines. In these situations, the urethritis must be either a relapse stimulated by an endogenous

source of infection or an inflammatory response to a non-microbial agent. While chlamydial infections exhibit latency,¹⁸ it is unlikely for the reasons already discussed that endogenous relapse of chlamydial infections accounts for the isolation-negative seronegative cases of NGU. An inflammatory response to a non-microbial agent would explain the failure of many patients to respond to antibiotic therapy and also the recognised relationship of the onset of urethritis to the taking of alcohol. One might, therefore, hypothesise that a microbial infection—chlamydial, ureaplasma or unidentified—initiates an inflammatory reaction. Antibiotic therapy eradicates the initiating organism but in many patients the inflammation persists in a chronic form and can be reactivated by many factors both microbial and non-microbial. Whether the inflammatory process persists or not is likely to be a manifestation of the host response and it is possible that immunological studies might be fruitful in elucidating the aetiology of the recurrences in this disease.

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