RESEARCH ARTICLE

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Bacteriological conversion in twenty urinary tuberculosis patients treated with ofloxacin, rifampin and isoniazid: a 10-year follow-up study

Abstract Twenty patients with urinary tuberculosis were treated with ofloxacin (200 mg/day, 6 months), rifampin (600 mg/day, 3 months) and isoniazid (300 mg/day, 3 months) between 1989 and 1990. All patients were new cases, diagnosed by observation and/or isolation of *Mycobacterium tuberculosis* in one of the three morning urine samples. Bacteriological culture conversion (negativization) was assessed as a clinical guide of efficacy, comparing it, as the only parameter, against a control group (150 patients) with urinary tuberculosis who received conventional therapy. Bacteriological follow-up studies were performed in both groups monthly for 6 months, then again 6 months later and then every year for 10 years after completion of treatment. In the 20 patients, the initial culture was positive with over 100 colonies per culture ($>50\%$); the smear was positive in 45% of the patients (most were 2 +). All strains were susceptible to rifampin, isoniazid and ofloxacin. Two patients discontinued treatment. Beginning with the first month of treatment, the bacteriological conversion was 100%, 89.5% and 100% in the remaining controls. In the control group, which received conventional treatment, the conversion was: 90%, 87%, 93% and 100% in the remaining controls. Treatment with ofloxacin resulted in a bacteriological conversion similar to that following conventional treatment ($p > 0.05$, Fisher's exact test). After 10 years of patient follow-up, we conclude that ofloxacin, in combination with rifampin and isoniazid (both for 3 months only is effective against *M. tuberculosis*, providing satisfactory bacteriological and clinical efficacy.

Keywords Tuberculosis treatment · Urinary tuberculosis · Ofloxacin · Tuberculosis conversion

Introduction

The incidence of tuberculosis, in all its forms of clinical presentation, remains high [11]. Although treatment guidelines are well defined, this is a field of continuous research, the objectives of which are: (1) to reduce treatment duration without losing efficacy, (2) to decrease the side effects of the drugs used, and (3) to encourage adequate patient cooperation [22]. Currently, treatment duration is either 6 months (short treatment) or 9 months for both pulmonary and extrapulmonary sites [3, 14, 16, 21]. Both treatments combine isoniazid and rifampin, adding pyrazinamide in the short regimen. However, although the efficacy of these drugs has been shown, significant side effects occur, and thus new therapeutic possibilities must be investigated [13].

The fluoroquinolones, with activity against *Mycobacterium tuberculosis*, are of interest in the field of tuberculosis treatment as they combine a favorable pharmacokinetics and low toxicity [6, 9, 20, 23]. Ofloxacin, a fluoroquinolone which acts on DNA gyrase, has high bioavailability and reaches high blood concentrations, with renal excretion greater than 90% during the first 24 h after its administration. Furthermore, previous studies have demonstrated the efficacy of ofloxacin as a monotherapy [24] and in combination with other antituberculosis drugs in the treatment of primary tuberculosis. Thus, in 1989, we began to investigate the efficacy of ofloxacin in combination with other antituberculosis drugs in the treatment of urinary tuberculosis (UTB) [1].

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The other antituberculosis drugs chosen were isoniazid and rifampin, which show significant activity in sterilizing tuberculous lesions [8] and do not cause antagonism or cross-resistance with ofloxacin [10].

The bacteriological conversion rate of the urinary samples studied as well as the follow-up performed over the last 10 years for the 20 patients included within the study were considered as markers of the efficacy of the treatment as assessed by the detection of relapse or treatment failure. This study was compared with a control group of 150 patients treated with the short 6-month treatment regimen.

Materials and methods

Patients: inclusion criteria

Patients treated with ofloxacin:

Twenty patients with confirmed UTB were admitted into this study between April 1989 and March 1990. Pregnant women, nursing mothers and children under 15 years of age were excluded (Table 1). UTB was diagnosed by observation and/or isolation of *M. tuberculosis* in one of three morning urine samples sent to the laboratory.

Control group:

The controls consisted of 150 patients with UTB. All of the patients were new cases and had not been previously diagnosed with or treated for tuberculosis. Informed consent, according to the established guidelines, was obtained from all patients.

Definitions

New case:

Patient in whom there was no knowledge of tuberculosis or previous antituberculosis treatment.

Table 1. Patients included in the protocol. *F* Female, *M* male

Patient	Age	Sex	Symptoms
1	50	M	Cystitis
2	29	F	Nephritic colic
3	60	M	Epididymitis
4	50	F	Lumbar pain
5	64	M	Prostatitis
6	45	M	Lumbar pain
7	16	F	Cystitis
8	64	F	Cystitis
9	64	F	Cystitis, general disorders
10	67	M	Cystitis
11	58	M	Cystitis
12	63	F	Lumbar pain
13	62	M	Lumbar pain
14	48	M	Bilateral epididymitis
15	46	F	Cystitis, lumbar pain
16	56	F	Lumbar pain
17	60	M	Epididymitis, prostatitis
18	54	F	Pyelonephritis, incontinence
19	56	M	Lumbar pain
20	35	F	Lumbar pain

Treatment failure:

No bacteriological conversion was produced after 3 months of treatment.

Relapse:

Finding of a positive smear and/or culture of *M. tuberculosis* in patients who completed the 6 months of treatment.

Probable cure:

Patient who, once the 6 months of treatment was completed, did not suffer any relapse or treatment failure.

Cure:

Patient in whom no relapse was observed once the treatment had been completed and after 12 months of further observation.

Microbiological investigation and follow-up

M. tuberculosis was identified by sample decontamination using 6% sulfuric acid [15], examination by fluorescence microscopy, and culture in Löwenstein-Jensen and pyruvate medium, incubating the cultures at 35 °C for up to 8 weeks. Once growth was observed in solid media, *M. tuberculosis* complex probes (Accuprobe, Gen-Probe, now bioMérieux) were used for identification.

Antibiotic treatment and follow-up

Ofloxacin group Ofloxacin was administered orally (200 mg/day) for 6 months. During the first 3 months, oral rifampin (600 mg/day) and oral isoniazid (300 mg/day) were added.
Control group Oral rifampin (600 mg/day) and oral isoniazid (300 mg/day) were administered during the 6-month treatment period. During the first 2 months, pyrazinamide (25 mg/kg/day, orally) and ethambutol (25 mg/kg/day, orally) were added.

Microbiological follow-up

During the treatment period, microbiological follow-up consisted of a monthly study of three morning urine samples. This was repeated after 6 month and every year after the completion of the treatment for 10 years.

Clinical controls

The baseline studies included a complete blood profile, renal and hepatic function tests, a chest X-ray, and an endovenous urography. The blood tests, as well as the renal and hepatic function tests, were repeated every month. The intravenous urography was repeated after 3 and 6 months of treatment.

Determination of *M. tuberculosis* susceptibility

To ofloxacin

Ofloxacin (DL 8280 Hoechst-Roussel, now Aventis) susceptibility was tested in Mycobacteria 7H11 agar medium (Difco, Detroit, Mich,, USA) with Middlebrook OADC enrichment (Difco). First, a mother solution was prepared with 10 mg of ofloxacin dissolved in 0.3 ml of 0.1 N NaOH; 9.4 ml of H_2O were then added and finally 0.3 ml of 0.1 N HCl, resulting in a 1,000 µg/ml solution, pH 5.8. This solution was diluted appropriately with water and added aseptically to previously sterilized medium to obtain the final concentrations of 1 and 4 µg/ml. The medium, with the antibiotics, was added to screw-cap tubes and solidified in a tilted position. Inoculi were prepared by diluting each strain to a turbidity equal to

a McFarland no. 1 standard, followed by a dilution to 1:100 [9]. Once the inoculum was added, strains were considered susceptible if they were inhibited by 1 μg ofloxacin/ml. Strains able to grow in the presence of either 1 μg ofloxacin/ml or 4 μg ofloxacin/ml were considered resistant.

To rifampin and isoniazid

Susceptibility rifampin and isoniazid was tested using the Canetti's proportions method [4]. A strain was considered resistant when the proportion of growth on drug-containing medium was equal or more to 1% of the growth observed on drug-free medium.

Statistical study

The differences in the results between the therapeutic regimens were compared using the EPI INFO, version 6.0, statistical package (USD Incorporated, Stone Mountain, Ga., USA). Proportions were compared by χ^2 test or Fisher's exact test when appropriate; means were calculated using Student's t test. Negativization of the culture served as endpoint. The difference between the therapeutic regimens was considered to be statistically significant for values of $p = < 0.05$.

Results

Patient population

Two patients withdrew from the protocol, one of them due to pregnancy 4 months after initiation of treatment. Her clinical, analytical and microbiological evolution during this period had been satisfactory and both her pregnancy and childbirth developed normally. The other patient suffered from acute hepatopathy at the onset of the protocol and a decision was made to exclude him. One patient with left epididymitis presented discomfort that could be attributed to the testis; the pain gradually subsided after completion of the protocol and he did not require an epididymectomy. The evolution of the

Table 2. Effect on the smear and on *Mycobacterium tuberculosis* culture of the administration of ofloxacin, rifampin, and isoniazid to 20 patients. −, 1+, 2+ Microscopic observation according to CDC guidelines, x confluent growth, *Arabic numbers* number of colonies in the culture

Patient	Initial bacteriology	Follow-up							
		1 month	2 months	3 months	4 months	5 months	6 months	12 months	10 years
1	Smear: 2+	2+	−	−	−	−	−	−	−
	Culture: x	−	−	−	−	−	−	−	−
2	Smear: +	−	−	−	−	Withdrawal			
	Culture: 10	−	−	−	−				
3	Smear: −	−	−	−	−	−	−	−	−
	Culture: 20	−	−	−	−	−	−	−	−
4	Smear: 2+	−	−	−	−	−	−	−	−
	Culture: x	−	−	−	−	−	−	−	−
5	Smear: −	−	−	−	−	−	−	−	−
	Culture: x	−	−	−	−	−	−	−	−
6	Smear: 2+	1+	1	−	−	−	−	−	−
	Culture: x	−	1	−	−	−	−	−	−
7	Smear: 2+	−	−	−	−	−	−	−	−
	Culture: x	−	−	−	−	−	−	−	−
8	Smear: −	Withdrawal							
	Culture: 8								
9	Smear: −	−	−	−	−	−	−	−	−
	Culture: 4	−	−	−	−	−	−	−	−
10	Smear: 3+	−	−	−	−	−	−	−	−
	Culture: x	−	−	−	−	−	−	−	−
11	Smear: 2+	−	−	−	−	−	−	−	−
	Culture: x	−	−	−	−	−	−	−	−
12	Smear: 1+	−	−	−	−	−	−	−	−
	Culture: 20	−	−	−	−	−	−	−	−
13	Smear: −	−	30	−	−	−	−	−	−
	Culture: 30	−	30	−	−	−	−	−	−
14	Smear: 1+	−	−	−	−	−	−	−	−
	Culture: 100	−	−	−	−	−	−	−	−
15	Smear: −	−	−	−	−	−	−	−	−
	Culture: 6	−	−	−	−	−	−	−	−
16	Smear: −	−	−	−	−	−	−	−	−
	Culture: 10	−	−	−	−	−	−	−	−
17	Smear: −	−	−	−	−	−	−	−	−
	Culture: 100	−	−	−	−	−	−	−	−
18	Smear: −	−	−	−	−	−	−	−	−
	Culture: 1	−	−	−	−	−	−	−	−
19	Smear: −	−	−	−	−	−	−	−	−
	Culture: x	−	−	−	−	−	−	−	−
20	Smear: −	−	−	−	−	−	−	−	−
	Culture: 100	−	−	−	−	−	−	−	−

Table 3. Evolution of urographic findings

Patient	Pre-treatment	3 months	6 months
1	Chronic pyelonephritis of left kidney; left renal function poor; large bladder filling defect	Nonfunctioning left kidney with calcifications; bladder normal	Similar to previous findings
2	Small papillary lesion in right kidney	Normal	Normal
3	Normal	Normal	Normal
4	Cavitations in left renal parenchyma; diverticulum in left upper calyx; stenosis in left distal ureter	Nonfunctioning left kidney	Similar to findings at first evaluation
5	Normal	Normal	Normal
6	Right renal function poor; stenosis in upper calyx of right kidney	Nonfunctioning right kidney	Similar to previous findings
7	Right kidney with chronic pyelonephritis; right renal function poor	Nonfunctioning right kidney	Similar to previous findings
8	NR		
9	Dilated upper calyx of left kidney	Similar to previous findings	Similar to previous findings
10	Dilated left renal cavities; left renal function poor	Size of left kidney smaller; left renal function poor	Similar to previous findings
11	Chronic pyelonephritis of left kidney; left renal function poor	Nonfunctioning left kidney	Similar to previous findings
12	Necrosis of upper pole of left kidney	Similar to previous findings	Similar to previous findings
13	NR		
14	Stenosis in left distal ureter; enlarged prostate with cavitations inside	Ureteral stenosis disappeared	Normal
15	Scars in right renal parenchyma	Similar to previous findings	Similar to previous findings
16	Chronic pyelonephritis of left kidney; left renal function poor	Nonfunctioning left kidney	Similar to previous findings
17	Small renal scars	Similar to previous findings	Similar to previous findings
18	Normal	Normal	Normal
19	Left urinary tract slightly dilated	Normal	Normal
20	Right urinary tract slightly dilated; right renal cavities	Normal	Normal

remaining patients was satisfactory, the symptoms subsiding once the treatment began. Patient collaboration was very good, and no patient complained that he or she could not tolerate treatment.

Microbiological studies and follow-up

A microbiological diagnosis was required by the protocol. Cultures were positive in the 20 patients; in more than half, the number of colonies was greater than 100. The staining was positive in nine patients (45%), and most were 2+ (Table 2). All of the strains of *M. tuberculosis* isolated were susceptible to the antituberculosis antibiotics studied. Positive cultures were observed in the second month only in patients 6 and 13, with one and 30 colonies respectively. Acid-fast bacilli were observed in the first month of treatment in two patients (1 and 6); the subsequent examinations were negative.

Clinical controls

No changes were observed in the hemogram or in renal function during follow-up. The transaminase levels of four patients increased during the first 3 months of treatment but liver function returned to normal when rifampin and isoniacid were withdrawn. Seven patients had an elevated ESR before treatment which returned to the normal range in five patients after 4 months of treatment. Of the patients whose ESR remained persistently high, one had rheumatoid arthritis and the other, with no previous history, still undergoes control evaluation. Up to now, this patient has not presented with disease recurrence.

Seventeen patients had radiological evidence of changes in the renal parenchyma which remained unchanged at the end of treatment in ten patients and improved in two patients. The function of five kidneys deteriorated during treatment. These were all previously damaged kidneys. A percutaneous nephrostomy was left indwelling for 2 months in two patients but failed to improve renal function. Nephrectomy was not performed and the patients with nonfunctioning kidneys still undergo control evaluation regularly. Patient number 13 was allergic to iodine and was therefore evaluated by ultrasound.

Changes in the calices or ureters were observed in 12 patients. Four improved radiologically and eight remained unchanged during treatment. None of these patients were considered for conservative surgical treatment. One patient presented bladder involvement which markedly improved within the first few months of treatment. Two patients who presented with prostatic involvement were monitored by a pelvic CT scan and both improved, showing a normal morphology of the prostate at the end of treatment. The radiological evolution of the patients is described in Table 3.

Table 4. Percentage of bacteriological culture conversions in the two series

Follow–up month	Ofloxacin group (20 patients)	Control group (150 patients)
1	100	90
2	89.5	87
3	100	93
4	100	100
5	100	100
6	100	100
12	100	100

Bacteriological conversion and comparison between two groups

Once the treatment was initiated, the bacteriological conversion of the cultures (negativization) in the ofloxacin group was 100% in the first month, 89.5% in the second and 100% in the remaining months. In the 150 patients of the control group, who also had urinary tuberculosis, the following bacteriological conversion rates of the cultures were observed month by month: 90%, 87%, 93%, and 100% in the remaining months. The overall evolution of the cultures in both series is described in Table 4. No statistically significant differences were found between the two groups.

Discussion

All of the strains of *M. tuberculosis* were susceptible to isoniazid and rifampin. In addition, the MICs observed in response to ofloxacin were less than 1 μg/ml, similar to those reported by other authors [17, 18, 26]. The dosage of 200 mg ofloxacin every 12 h, together with the favorable pharmacokinetics, make it possible to reach mean concentrations in urine that widely exceeds the MICs. The absence of cross- resistance and the possibility of a synergistic effect of ofloxacin with the antituberculosis agents used suggest promising results with this drug. Nonetheless, we believe that the use of isoniazid and rifampin in the first 3 months of treatment was fundamental to successful treatment of UTB, due to their rapid sterilization of the lesions, eliminating both the extracellular population as well as that present in the closed lesions and in the macrophages [8]. The treatment regimen described in this study, which was aimed at alleviating some of the inconveniences observed with the established regimens without diminishing therapeutic efficacy, is based on the properties of the quinolone ofloxacin [2, 5, 7].

The clinical results measured in the follow-up studies were good; all patients recovered, and no relapses were observed. Only one patient had to withdraw from the study due to significant liver toxicity, which, based on the patient's symptoms, we believe may have been due to rifampicin and isoniazid. Others patients had increased transaminase levels which returned to normal when these drugs were discontinued. Therefore, there are no objective reasons to attribute hepatic, renal, or hematological toxicity or teratogenic effects to ofloxacin in patients who were withdrawn from the protocol.

Although the negativization of the sputum of patients with pulmonary tuberculosis is generally achieved within 2 months when the therapeutic regimen incorporates isoniazid and rifampicin, no specific data are available for genitourinary tuberculosis. Our results with a treatment regimen that includes ofloxacin indicate that 100% negativization is reached after the third month of therapy; in the second month of treatment, there were positive cultures from two patients, with 1 and 30 colonies.

The strict follow-up of the patients during the last 10 years, during which time no abnormalities occurred, shows the adequacy of the therapeutic regimen described here. Thus, it is not surprising that no significant differences are observed when these patients were compared to those in the control group.

However, while the potential role of the fluoroquinolones has been studied [19], larger studies are needed aimed at evaluating new therapeutic alternatives which, with equal efficacy, can improve treatment while avoiding the adverse side effects and strengthening the degree of patient cooperation. In addition, it should be noted that a single-drug treatment was used in the middle of our therapeutic regimen. If the sterilizing power of the two antituberculosis drugs– rifampicin and isoniazid– is fundamental at the onset of the treatment, the activity of ofloxacin can later culminate therapy, assuming, as stated by Yew [26], that "it is not a superfluous association." We believe that this approach opens up a new therapeutic perspective, which, although novel with respect to the current literature, nonetheless bases its efficacy on a careful follow-up over 10 years. Finally, in agreement with Jacobs [12], we feel that the fluorquinolones present a "considerable potential if they are used sensibly" and, although controversies remain [25], the bacteriological conversion of the cultures of *M. tuberculosis* is proof of their therapeutic efficacy.

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