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Metronidazole-resistant *Trichomonas vaginalis*

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SUMMARY A 36-year-old woman with symptomatic metronidazole-resistant trichomonal vaginitis for 10 years had a total of 22 courses of treatment with either metronidazole or tinidazole according to different schedules. The minimum trichomonicidal concentration of metronidazole for the strain of *Trichomonas vaginalis* isolated from the patient was 160 μg/ml compared with 1·25-10 μg/ml for other freshly isolated strains. The former strain also showed a definitely decreased sensitivity to ornidazole and tinidazole (80 μg/ml). The mechanisms behind the appearance of resistance in this clinical isolate are at present unknown and require further study from the theoretical as well as the therapeutic viewpoint.

Introduction

The treatment of *Trichomonas vaginalis* infections was significantly improved by the discovery of metronidazole in 1959 (Cosar and Julou, 1959). Since that time other antiprotozoal agents among the nitroimidazole derivatives have been used; of these tinidazole and ornidazole are the most powerful (Howes *et al.*, 1970; Sköld *et al.*, 1977). Metronidazole seems to have maintained its efficacy since it was introduced almost 20 years ago, and similar results have been reported for tinidazole (Wallin and Forsgren, 1974). Recently, however, a strain with decreased sensitivity to metronidazole has been isolated (Thurner and Meingassner, 1978). To our knowledge, no previous evidence of the emergence of metronidazole-resistant strains has been reported (Korner and Jensen, 1976; Josey, 1978). This paper describes treatment failure with metronidazole and tinidazole in a patient infected with a metronidazole-resistant strain of *T. vaginalis*.

Case report

A female patient, born in 1943, first visited this clinic because of vaginal trichomoniasis in 1968. Since then she has been seen repeatedly by different doctors in our outpatient department. Trichomonads were found in her vaginal secretions on wet smear examination at most of her visits. Since 1968 she has had 11 courses of metronidazole at the standard dose of 200 mg three times a day for seven days. On two occasions treatment was given for two or three weeks and on one occasion she had 400 mg three times daily for two weeks. Tinidazole at the standard dose of 2 g as a single dose was given six times; on one occasion a dose of tinidazole 4 g was given. In 1969 nifuratel 200 mg was given three times daily for one week. A cyst of the left Bartholin's gland was excised in 1976, but even after removal of this possible focus of infection her trichomoniasis persisted.

Her husband, who was a ship's mate and went to sea for long periods, also received antitrichomonal treatment at the same time and at the same dosage. The patient denied extramarital sexual contact. In February and in October 1978 her strain (BO) of *T. vaginalis* was analysed for its susceptibility to imidazole derivatives. In June 1978 the patient was given a single dose of tinidazole 2 g and the serum concentration was regularly estimated.

LABORATORY METHODS

Minimum trichomonicidal concentrations were determined (Forsgren, 1972) for the strain (BO) of *T. vaginalis* from the patient and for freshly isolated strains of *T. vaginalis* taken at random from patients' specimens.

The micro-organisms were grown at 37°C in Diamond's medium (Diamond, 1957) containing streptomycin 100 mg, penicillin 1 megaunit, and doxycycline 10 mg/100 ml medium. A suspension of 8×10^4 *T. vaginalis* organisms in 1 ml thioglycollate medium was added to metronidazole, ornidazole, or

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tinidazole in serial twofold dilutions in 1 ml saline giving a final concentration of 4×10^4 viable organisms per ml. After incubation for three days at 37°C, 0·2 ml from each test tube was transferred to 4 ml Diamond's medium and the subculture was incubated for five days. Evaluation of the trichomonicidal activity was based on the final result of the subculture and was defined as a concentration (μg/ml) in which no viable organisms could be detected by subculture. The determinations were always carried out in duplicate.

The serum concentrations of tinidazole were determined before and at four, eight, 24, and 48 hours after ingestion of tinidazole 2 g on an empty stomach. The sera were heat-inactivated (Forsgren, 1972), diluted in saline, and tested against the sensitive strain of *T. vaginalis* in thioglycollate medium; finally subcultures were performed in Diamond's medium as described above. The serum concentration was calculated by reference to a standard series of tubes containing known concentrations of tinidazole.

Results

The sensitivity to metronidazole of fresh clinical isolates of *T. vaginalis* taken at random from patients' specimens in the routine laboratory is shown in Table 1. The trichomonicidal concentration was 1·25-10 μg/ml with a mean value of 3·75 μg/ml, the same concentration as detected with the same technique for clinical isolates in 1972 (Forsgren and Wallin, 1974). The difference in sensitivity for the *T. vaginalis* strain BO was striking (Table 2). The concentration required to obtain a trichomonicidal effect for this strain was 16-128 times higher than for the fresh isolates from patients with therapeutically uncomplicated trichomonal infections.

The serum concentration of tinidazole in the patient after ingestion of tinidazole 2 g showed that absorption of the drug was satisfactory. Serum concentrations of 40 μg/ml and 2·5 μg/ml were detected at four and 48 hours after ingestion respectively.

Table 1 *Minimum trichomonicidal concentration (μg/ml) of metronidazole for freshly isolated strains of T. vaginalis*

Strain	*Minimum trichomonicidal concentration (μg/ml)*
1	1·25
2	10
3	5
4	2·5
5	5
6	2·5
7	1·25
8	10
Mean value	3.75

Table 2 *Minimum trichomonicidal concentration (μg/ml) of three imidazole derivatives for resistant T. vaginalis strain BO (I = strain isolated February 1978; II = strain isolated November 1978.)*

T. vaginalis strain	*Minimum trichomonicidal concentration (μg/ml)*					
	Metronidazole		*Ornidazole*		*Tinidazole*	
	Mean	*Range*	*Mean*	*Range*	*Mean*	*Range*
BO I	160	80-320	80	40-160	80	40-160
BO II	160		80		80	

Discussion

Metronidazole, which has been widely used during the last 20 years for treatment of *T. vaginalis* infections, is still generally accepted as being active against all clinical strains of this protozoon (Josey, 1978). Clinical failures in the treatment of trichomoniasis by standard doses of metronidazole have been reported. In most cases, however, these failures were possibly due either to poor absorption of the compound (Kane *et al.*, 1961) or to inactivation of the compound by the vaginal flora (Nicol *et al.*, 1966; McFadzean *et al.*, 1969). In our case, however, no impairment of absorption was evident from study of the serum concentrations after ingestion of a standard dose of tinidazole. The addition of three different antibiotics to the culture medium most probably excluded the possibility of growth *in vitro* of organisms which might have inactivated imidazole derivatives. In addition, no contaminating organisms were seen microscopically.

Some authors have suggested that resistant clinical strains might have appeared (Aure and Gjonnaess, 1959; Arnold, 1966; Thurner and Meingassner, 1978), but in only the last of these reports were microbiological confirmatory tests performed. Successful *in-vitro* and *in-vivo* experiments to induce metronidazole resistance in *T. vaginalis* strains, however, have been performed. Carneri *et al.* (1969) and Carneri and Gionnane (1971) observed that after passages of *T. vaginalis* on media containing increasing concentrations of metronidazole the minimum trichomonicidal concentration of metronidazole for the original strain had risen from 0·23 μg/ml to 80 μg/ml. On the other hand, in five strains injected into a mouse treated with suboptimal doses of metronidazole and then enriched in culture in the absence of the drug and subjected to 20 of these alternated passages the *in-vivo* resistance was increased by only 4·5 to 14·5 times. Results which agreed with those of Carneri (Carneri *et al.*, 1969; Carneri and Gionnane, 1971) were obtained by Benazet and Guillaume (1971). Resistance to metronidazole could also be induced in *Trichomonas fetus* strains in hamsters infected intravaginally (Actor *et al.*, 1969).

The mechanisms behind the appearance of resistance in the clinical isolate of *T. vaginalis* in this study are not known. It is tempting to speculate, however, that suboptimal doses of metronidazole in vaginal secretions (Paredes and Hawkins, 1973) caused the resistance in the same manner as described above for experimental induction of imidazole resistance. If so, it would seem sensible to give single, high doses of imidazole derivatives (Csonka, 1971; Wallin and Forsgren, 1974; Sköld *et al.*, 1977). It is of interest that eight months after the resistant organisms were first isolated from the patient imidazole-resistant *T. vaginalis* organisms could again be isolated. The woman had in the meantime received one single dose of tinidazole 2 g. Furthermore, the *T. vaginalis* strain showed almost the same high degree of resistance to all the imidazole derivatives tested. The problem of resistance to *T. vaginalis* is worthy of further study, both from the theoretical and the therapeutic viewpoint.

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