

# ACTIVITY OF ORAL ANTIPROTOZOAL AGENTS AGAINST SERIALY SUBCULTURED STRAINS OF TRICHOMONAS VAGINALIS AND THEIR SYNERGIC EFFECTS

Nobuo KAWAMURA

From the Department of Urology, Tokai University, School of Medicine, Isehara, Japan

(Director: Prof. Masaaki Ohkoshi)

## INTRODUCTION

While local treatment with vaginal tablets and vaginal douches as well as systemic medication are practicable in *Trichomonas vaginalis* infection of the vagina, only oral antiprotozoal preparations can be used for the treatment of *T. vaginalis* infection of the male genitourinary tract and, therefore, the drugs available for this purpose are eventually limited. Usefulness of oral antiprotozoal preparations are largely influenced by the degree of their absorption from the gastrointestinal tract and excretion, and, frequently, there have been reports describing the possibility that drugs possessing antiprotozoal activity tend to evoke damage to the spermatogenetic system.

Meanwhile, *T. vaginalis* clinical isolates reported to be markedly resistant to common antiprotozoal agents as yet are rare but it is quite conceivable that such highly resistant organisms may emerge sooner or later<sup>1,2)</sup>. The present study was undertaken to explore, the possibility of attaining potentiation of antiprotozoal actions against *T. vaginalis* by combined use of two out of the currently available drugs which, we believe, is of value from the aforementioned viewpoint.

## MATERIALS AND METHODS

### Experiment 1

Assays of antiprotozoal activity of metronidazol (MZ), tinidazole (TZ) and nitro-

furantoin (NF) in vitro against *Trichomonas vaginalis* were carried out. The organisms were 48 strains of *T. vaginalis* which had been maintained in over twenty consecutive serial subcultures at this laboratory after isolation from clinical specimens from males with trichomoniasis of the genitourinary tract. Cultures of all these strains were bacteria- and fungus- free as used in the present investigation. The so-called Asami's medium<sup>1)</sup> (broth supplemented with human-serum and cysteine) was used for both isolation and subcultures.

The assays of antiprotozoal activity of the drugs against *T. vaginalis* were performed in tubes of MSF (Modified Shaffer-Frye) medium<sup>2)</sup>. Trichomonads grown in Asami's medium were collected by centrifugation, resuspended in MSF medium and adjusted to a concentration of  $2 \times 10^5$  protozoa per tube after counting with a Fuchs-Rosenthal hemocytometer. Triplicate tubes of trichomonad suspension in MSF medium were set up for each drug at graded concentrations. All these assays were conducted for the three drugs in parallel.

Tubes of MSF medium seeded with trichomonads, to which then specified concentrations of a drug were added, were incubated at 37°C. The cultures were read for degree of protozoal growth and motility in comparison with drug-free cultures of the organism, and the trichomonad count of each culture was made. On the third or fourth days of incubation aliquots of these cultures were transferred

to tubes of Asami's medium and incubated to detect viable protozoa and to assess their capacity of growth.

The drugs, MZ, TZ and NF, were added in graded concentrations to tubes of MSF medium after trichomonas seeding as described above. For this purpose, MZ and TZ were diluted in MSF medium

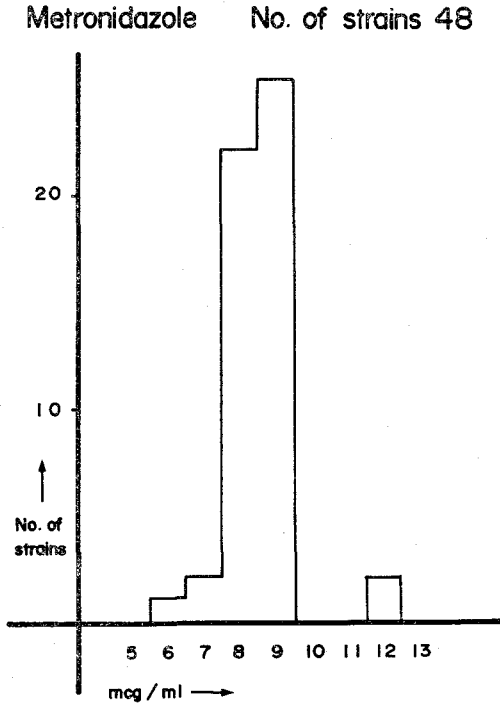


Fig. 1. MIC of metronidazole.

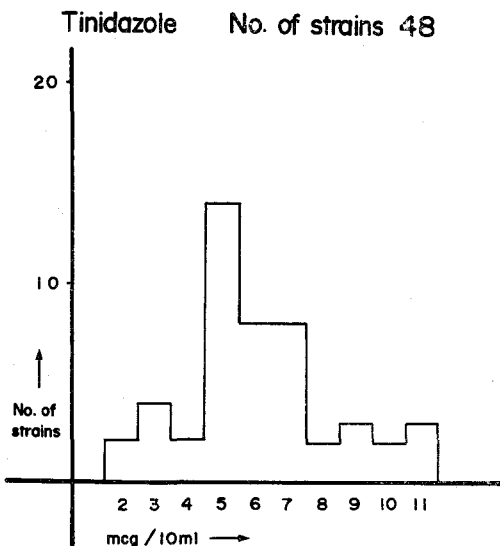


Fig. 2. MIC of tinidazole.

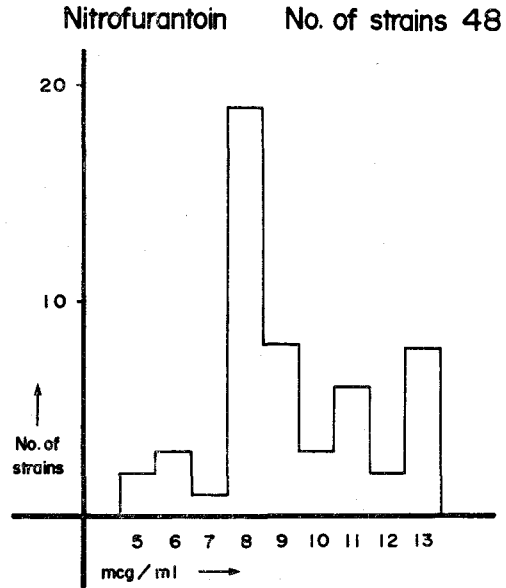


Fig. 3. MIC of nitrofurantoin.

whereas NF which is sparingly soluble in water was dissolved in a minimal quantity of dimethylformamide and then diluted in MSF medium. The drug-free controls contained the same quantity of dimethylformamide as that in the tubes containing the highest concentration of NF.

The graded concentrations of NF ranged from 1 to 15 mcg/ml whilst those of MZ or TZ ranged from 1 to 15 mcg/10 ml. The endpoint for minimal inhibitory concentration (MIC) was the lowest concentration of drug at which no viable trichomonads could be demonstrated (Fig. 1~3).

**Experiment 2**

Experiments were performed to investigate synergistic antiprotozoal actions of trichomycin (TR), TZ, MZ and NF.

For each of combinations of drugs, three strains of *T. vaginalis* were used. All the strains employed had been maintained in over 20 consecutive serial subcultures at this laboratory. The organisms used were not consistently of the same strains and of the same conditions in all experiments since the study did not permit simultaneous multiple experimental systems.

Trichomonads grown in Asami's medium were collected by centrifugation and inoculated  $2 \times 10^5$  per tube as in Experiment 1

followed by addition of two combined drugs at graded concentrations. The cultures were incubated at 37°C, and transferred after 3~4 days of incubation to tubes of fresh medium to detect viable protozoa. The results were recorded as (+) when any of the three strains was found to show viable protozoa in the subculture, or as (-) when no viable protozoa could be demonstrated with any of the three strains tested.

		TRICHOMYCIN → mcg/ml					
		0.25	0.5	1.0	1.5	2.0	3.0
NITROFURANTOIN ↓	3	+	+	+	-	-	-
	4	+	+	+	-	-	-
	5	+	+	+	-	-	-
	6	+	+	+	-	-	-
	7	+	+	+	-	-	-
	8	+	+	+	-	-	-
	9	-	-	-	-	-	-
	10	-	-	-	-	-	-

Fig. 4. Synergic effect between trichomycin and nitrofurantoin.

		TRICHOMYCIN → mcg/ml					
		0.25	0.5	1.0	1.5	2.0	3.0
TRINIDAZOLE ↓	3	+	+	+	-	-	-
	4	+	+	+	-	-	-
	5	+	+	+	-	-	-
	6	+	+	+	-	-	-
	7	+	+	+	-	-	-
	8	+	+	+	-	-	-
	9	-	-	-	-	-	-
	10	-	-	-	-	-	-

Fig. 5. Synergic effect between trichomycin and trinidazole.

		TRICHOMYCIN → mcg/ml					
		0.25	0.5	1.0	1.5	2.0	3.0
METRONIDAZOLE ↓	5	+	+	+	-	-	-
	6	+	+	+	-	-	-
	7	+	+	+	-	-	-
	8	+	+	+	-	-	-
	9	+	+	+	-	-	-
	10	+	+	+	-	-	-
	11	-	-	-	-	-	-
	12	-	-	-	-	-	-

Fig. 6. Synergic effect between trichomycin and metronidazole.

		Metronidazole → mcg/10ml						
		5	6	7	8	9	10	11
NITROFURANTOIN ↓	NF 3	+	+	+	+	+	+	-
	4	+	+	+	+	+	+	-
	5	+	+	+	+	+	+	-
	6	+	+	+	+	+	+	-
	7	+	+	+	+	+	-	-
	8	+	+	+	+	-	-	-
	9	-	-	-	-	-	-	-
	10	-	-	-	-	-	-	-

Fig. 7. Synergic effect between metronidazole and nitrofurantoin.

		Tinidazole → mcg/10ml					
		5	6	7	8	9	10
NITROFURANTOIN ↓	Tinidazole 3	+	+	+	+	-	-
	4	+	+	+	+	-	-
	5	+	+	+	+	-	-
	6	+	+	+	+	-	-
	7	+	+	+	+	-	-
	8	+	+	+	-	-	-
	9	-	-	-	-	-	-
	10	-	-	-	-	-	-

Fig. 8. Synergic effect between nitrofurantoin and trinidazole.

Five different combinations of drugs, i.e. TR-NF, TR-TZ, TR-MZ, MZ-NF and TZ-NF, were assessed for synergistic effects against *T. vaginalis*, excluding combination of MZ and TZ which are drugs of the same series (Fig. 4~8).

RESULTS

The distributions of *Trichomonas* strains of MIC for MZ, TZ and NF observed in Experiment 1 are presented in Figures 1, 2 and 3.

The data indicate that the MIC is estimated to be from 8~10 mcg/ml for NF, from 0.8~0.9 mcg/ml for MZ and from 0.3~0.9 mcg/ml for TZ, respectively.

The results of Experiment 2 are shown in Figures 4 to 8. Synergic actions, though modest, were observed with the combination of MZ and NF and with that of TZ and NF while there was no evidence of such augmentation of antiprotozoal effect in the tests with combinations of TR and three other drugs.

## DISCUSSION

Treatment with oral antiprotozoal agents is usually successful in cases of *T. vaginalis* infection of the male genitourinary tract<sup>3)</sup>. However, oral medication with NF is ineffective in some cases as we previously reported and such instances where combined therapy regimens with other drugs should be considered may arise. The present study was undertaken to find bases for effective combinations of antiprotozoal agents which would be employed in such circumstances. Because of its poor absorption from the gastrointestinal tract, oral preparation of TR has been virtually abandoned recently and the data heretofore obtained in experimental studies also indicate that it is not much meaningful to use this drug in combination with other drugs while combined therapy regimens of NF and MZ or TZ might be of some value.

The data reported herein may also be useful not only in selecting effective combinations of oral antiprotozoal drugs but in choosing drugs for combined therapy regimens consisting of an oral preparation and an intravaginal tablet preparation or of two intravaginal tablet preparations as well.

It is generally advisable to use antiprotozoal agents against *T. vaginalis* in as low doses and for as short a duration as practicable since impairment of spermatogenic function, adverse effects on the fetus and carcinogenesis associated with their use have been purported. A maximum duration of 7 days has been advised recently for therapy with MZ according to re-evaluation published by the Drug Evaluation Committee of the American Medical Association.<sup>4)</sup> It would not be fruitless, there-

fore, to explore the possibility of reducing dosage of drugs by their combined use.

This paper was presented in part at the 22nd Congress of the Japanese Society of Chemotherapy.

## SUMMARY

Four antiprotozoal agents, metronidazole, nitrofurantoin and trichomycin were tested about their activity and synergic effects against trichomonas vaginalis in vitro.

MIC is estimated to be from 8~10 mcg/ml for nitrofurantoin, from 0.8~0.9 mcg/ml for metronidazole and from 0.3~0.9 mcg/ml for tinidazole, respectively. Synergic actions were observed with the combination of metronidazole and nitrofurantoin and with that of tinidazole and nitrofurantoin slightly.

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(Accepted for rapid publication, April 27, 1979)

## 和文抄録

## 継代培養脛トリコモナスに対する経口抗脛トリコモナス剤の抗力と相乗効果

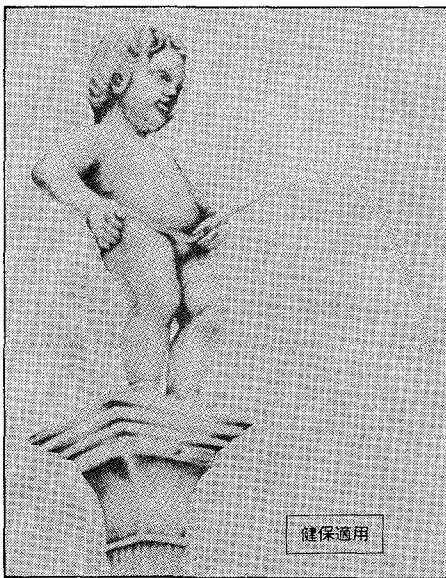
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3種の経口的に使用できる抗脛トリコモナス剤について、男子尿性器由来保存脛トリコモナス株48株を用いてMIC分布を測定した。ニトロフラントインは8~10  $\gamma$ /ml, メニトロニダゾールは0.8~0.9  $\gamma$ /ml, チニダゾールは0.3~0.9  $\gamma$ /mlのMICが得られた。相

乗効果について4種の薬剤の組合わせで検討したところ、メトロニダゾールとニトロフラントイン、チニダゾールとニトロフラントインの組合わせで軽度のみとめられた。

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