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Susceptibility of Trichomonas vaginalis to metronidazole: A survey of 76 random isolates

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Introduction

Since there is no unique standardized method used in testing the susceptibility of T. vaginalis isolates to metronidazole, results are subject to large variations depending on the methods used. Laboratory-internal reference values are therefore necessary for the interpretation of data obtained from drug assays.

The aim of the reported study was to evaluate the range of susceptibility in a well-defined assay in vitro of isolates from 76 consecutive patients suffering from vaginal trichomoniasis. For comparison of in vitro and in vivo data the susceptibility to metronidazole of 15 isolates has also been evaluated in an animal model.

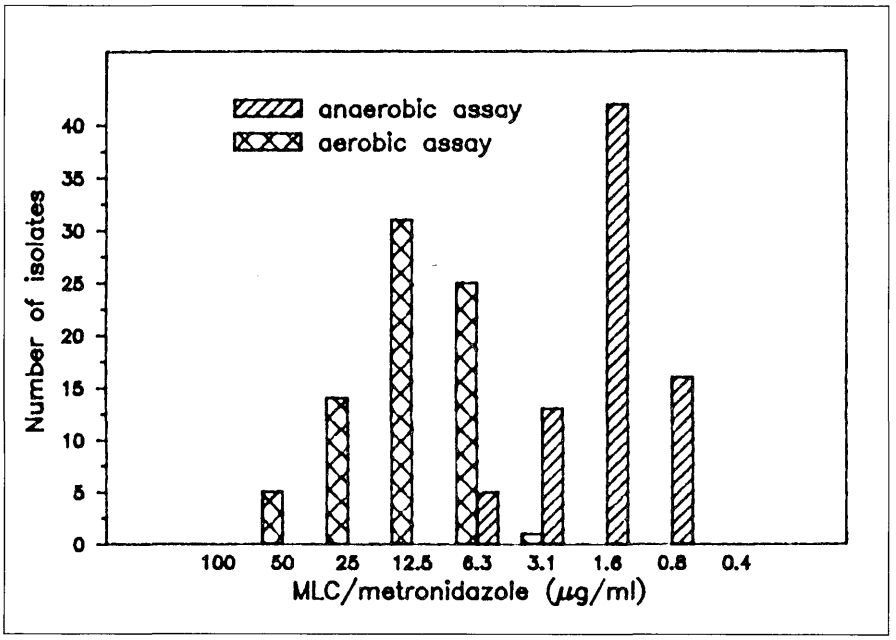
Material and Methods

Isolates were cultured in an TYM-medium (MEINGASSNER and HEYWORTH 1982) containing antibiotics for inhibition of bacterial and fungal growth. Inhibitors were omitted from the growth medium when the cultures proved to be free from contaminants. Drug testing on the isolates was usually performed within 10 passages after isolation. The laboratory procedure for susceptibility testing to metronidazole in microtitration plates has been previously reported in detail (MEINGASSNER and THURNER 1979), but differed in the use of TYM-medium instead of CPLM-medium. For the assay, stock cultures of the isolates were prepared by two successive transfers of 5×10^5 to 10 ml medium at 24 hr intervals. The cell density was finally adjusted to 5×10^4 cells per ml by addition of fresh medium. 150 µl of this suspension were pipetted into 50 µl drug solution per well in the plates. The minimal lethal concentration (MLC), taken as the highest dilution in which no flagellar motility could be observed after exposure to the drug for 48 hr, was evaluated under aerobic and anaerobic test conditions. Individual isolates were tested in a mouse assay (MEINGASSNER and THURNER 1979). Mice were infected ectopically by two subcutaneous injections of 0.1 ml inoculum and an intraperitoneal dose of 0.3 ml. The inoculum (4×10^6 cells ml^{-1}) prepared from a 24 hr culture in TYM-medium plus 0.5% agar. The seed culture was also 24 hr old. Survival in the subcutis and in the peritoneal fluid was tested after oral treatment with 3 doses of 25, 50 or 100 mg kg^{-1} each. 8–12 mice were used per dose group.

In 29 trichomoniasis patients the treatment outcome, confirmed by a reisolation test, could be compared with the sensitivity of the organisms.

Results

Under aerobic conditions the MLC ranged from 3.1 to 50 μg ml^{-1} with the peak at 12.5 μg lm^{-1}. Anaerobically most strains (55%) were inhibited by 1.6 μg ml^{-1} with limits at 6.3 and 0.8 μg ml^{-1} (Fig.).



The aerobic and anaerobic susceptibilities to metronidazole differed and varied by up to 4 serial twofold dilution steps in some isolates. The MLC of metronidazole was 50 μl ml^{-1} for isolates from 5 patients, only three of whom were available for a follow-up study. Two of these were cured by standard doses, while the third did not respond. The isolate from this patient showed a relative resistance to metronidazole when compared with the other isolates in the mouse model. After 3 oral doses of 50 mg kg^{-1} the parasites were eliminated from the subcutis and the peritoneal cavity in only 11 and 33% of the infected mice respectively. At this dose level infections were usually eradicated in 80% and 95% from the respective infection sites. Doses of 3 × 100 mg kg^{-1}, normally 100% curative, failed to achieve cure in all animals infected with that isolate. The patient responded neither to two doses of 1.5 g M, given with an interval of 12 hr, nor to 2 × 1 g tinidazole, administered with the same interval. Cure was finally achieved by a combined oral (1 × 1.5 g ornidazole) and intravaginal treatment (0.5 g M daily for 10 days).

The patient's history is in conformity with the animal data, indicating a decrease in the sensitivity to metronidazole.

The other 26 follow-up patients with trichomonads of various sensitivity (MLC: 6.3 to 25 μg ml^{-1}) were cured with standard dose regimens.

Conclusions

The tests used revealed varying sensitivity of T. vaginalis-isolates to metronidazole. This range is usually covered by standard oral treatment with metronidazole, but failures due to a decreased sensitivity of the organisms do occur.

According to the tests described, MLC values of >25 μg ml^{-1} indicate decreased sensitivity. Resistance is proven by a poor dose response in animal tests. In the model used, cure rates in less than 50% of the infected mice after three oral doses of 3×50 mg kg^{-1} metronidazole are abnormal.

Summary

Isolates from 76 patients with vaginal trichomoniasis were tested for sensitivity to metronidazole (M) in vitro. The MLC ranged from 3.1 to 50 μg ml^{-1} M under aerobic conditions (mean 12.5 μg ml^{-1}). Under anaerobic conditions the isolates were inhibited by MLCs of 6.3 to 0.8 (mean 1.6 μg ml^{-1}). A follow-up study was possible in 29 patients. 28 patients were cured with two oral doses of 1.5 g M, with an interval of 12 hrs between doses. The isolates of these patients were inhibited aerobically by least concentrations ranging from 6.3 to 50 μg ml^{-1} M. In one patient, standard oral treatment with M and tinidazole failed. The isolate showed decreased sensitivity both in vitro (MLC: 50 μg ml^{-1}) and in an animal model infection. Complete cure was not achieved in infected mice by three oral doses of 100 mg kg^{-1} M.

Zusammenfassung

Untersuchungen zur Metronidazol-Empfindlichkeit von T. vaginalis-Isolaten

Die minimale letale Konzentration (MLK) von Metronidazol betrug für Isolate von 76 Patientinnen mit vaginaler Trichomoniasis unter aeroben Testbedingungen in vitro 3,1 bis 50 μg/ML (Mittel: 12,5). Unter anaeroben Verhältnissen wurden die Isolate durch Konzentrationen von 0,8 bis 6,3 μg/ml abgetötet. Bei 29 Patientinnen war eine mikrobielle Therapiekontrolle möglich. 28 Frauen wurden durch 2 Dosen von 1,5 g Metronidazol, im Abstand von 12 Stunden, geheilt. Mindestkonzentrationen von 6,3 bis 50 μg/ml Metronidazol waren für deren Isolate bei aerober Testung letal. Bei einer Patientin führten Standarddosierungen von Metronidazol und Tinidazol jedoch nicht zur Elimination der Erreger. Das Isolat erwies sich sowohl bei der aeroben Testung in vitro mit einer MLK von 50 μg/ml wie auch im Tierversuch als relativ unempfindlich. Von einem Kollektiv infizierter Mäuse wurden nicht alle Tiere durch dreimalige orale Gaben von 100 mg/kg KG Metronidazol geheilt.

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