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Metronidazole Versus Anaerobes

In Vitro Data and Initial Clinical Observations

FRANCIS P. TALLY, M.D., VERA L. SUTTER, PH.D., AND SYDNEY M. FINEGOLD, M.D., *Los Angeles*

■ *Metronidazole, a systemic trichomonicide which has been used extensively since 1960, has recently been shown to be active against various anaerobic bacteria* in vitro *and in experimental infections. In the present study, metronidazole showed significant activity against virtually all of 54 strains of anaerobic and microaerophilic bacteria tested, including Bacteroides fragilis. Since this organism is the anaerobe most commonly isolated from human infections and has demonstrated significant resistance to many antimicrobial agents, metronidazole may prove to be very useful. Our initial clinical evaluation of metronidazole in human anaerobic infections is presented. Because of metronidazole's* in vitro *activity, its low incidence of toxic reactions, and this initial favorable clinical trial, the drug deserves further evaluation in the management of anaerobic infections.*

METRONIDAZOLE (FLAGYL®) was first introduced for the treatment of infections caused by Trichomonas vaginalis in 1959.[1] Subsequently, Shinn reported that metronidazole was effective in the treatment of Vincent's gingivitis.[2] It is also effective in the treatment of tropical ulcer and has been used in primary and secondary syphilis.[3-5] Powell reported the effectiveness of metronidazole in amoebic dysentery and amoebic liver abscess.[6] The first report of *in vitro* susceptibility of an anaerobic bacterium, Bacteroides necrophorus NCTC 7155, to metronidazole was published in 1964.[7] Several recent reports show that metronidazole is active against many different anaerobic bacteria *in vitro* and that it is effective in experimental anaerobic infections.[8-13]

This report presents (1) additional data on *in vitro* effect of metronidazole against various anaerobic and microaerophilic bacteria, including 13 strains of B. fragilis (previously only one strain had been studied) and several other species not previously tested, and (2) our initial clinical experience with metronidazole in treatment of deep-seated anaerobic infections.

Patients and Methods

Methods

The susceptibility of 54 strains of anaerobic or microaerophilic bacteria to metronidazole was determined by agar dilution technique. The inoculum consisted of a 48-hour culture in fluid thiogly-

From the Medical Service and the Anaerobic Bacteriology Laboratory, Wadsworth Hospital Center, Veterans Administration and the Department of Medicine, University of California, Los Angeles, Center for the Health Sciences.

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Reprint requests to: S. M. Finegold, M.D., Wadsworth Hospital Center, Veterans Administration, Wilshire and Sawtelle Boulevards, Los Angeles, Ca. 90073.

collate medium (BBL) enriched with 25 percent ascitic fluid, diluted to approximately 10^5 to 10^6 organisms per milliliter. This was applied to the blood agar (BBL infusion agar base) plates containing various concentrations of metronidazole by means of a replicating device. The tests were incubated for five days and the minimal inhibitory concentration (MIC) recorded as the highest dilution on which no growth occurred.

Patients

Patients with anaerobic infections who were able to take oral medication were selected for clinical evaluation of metronidazole. The drug was administered in a dose of 250 mg by mouth every eight hours.

Case Reports

First Case

A 25-year-old Mexican-American man was admitted with a two-week history of fever following a brief episode of pharyngitis. He had been treated with ampicillin 250 mg every 6 hours before entering the hospital. On the third hospital day his temperature was 40.6°C (105°F) and the only physical finding was slight liver tenderness to percussion. Leukocytes numbered 20,900 per cu mm. On the fifth hospital day, blood cultures were positive for a pleomorphic Gram-negative rod, later identified as Fusobacterium necrophorum (Sphaerophorus necrophorus). The patient was started on metronidazole and became afebrile in 24 hours. Blood cultures became sterile. (See Chart 1). The minimum inhibitory concentration (MIC) of metronidazole for the identified organism was ≦ 0.1 mcg per ml. The patient was treated for 23 days and at last report had remained symptom-free for two months following therapy.

His hospital course was complicated by nonoliguric renal failure, abnormal liver function tests and moderate anemia. These were evident before treatment was begun and returned to normal during therapy. Extensive evaluation during the stay in hospital failed to reveal a portal of entry for sepsis. Liver scans were negative.

Second Case

A 66-year-old man, an alcoholic who also used barbiturates excessively, was admitted with fever, cough productive of foul-smelling purulent

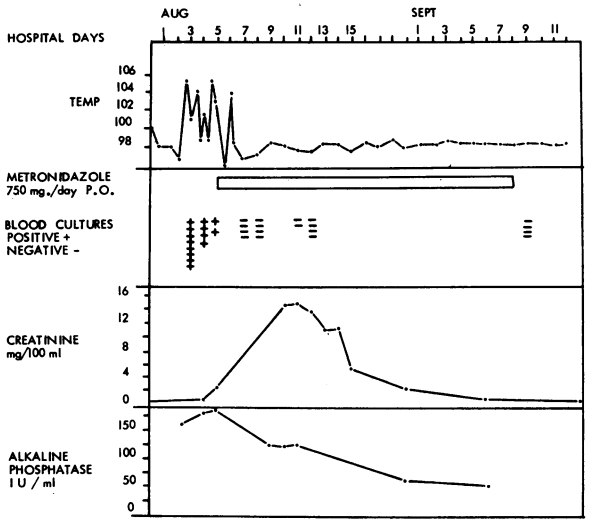

Chart 1.—Clinical Course, First Case.

sputum, a 30-pound loss in weight and a history of several periods of unconsciousness. On examination he appeared chronically ill and lethargic, and there were diffuse rales and rhonchi throughout the left lung. A chest x-ray film showed a diffuse infiltrate in the posterior segment of the left upper lobe and the superior segment of the left lower lobe. Gram stain of the sputum revealed numerous pleomorphic organisms and the patient was started on chloramphenicol for suspected aspiration pneumonia. He was subsequently switched to erythromycin when severe diarrhea developed.

Because the fever and productive cough persisted and an x-ray film showed progression to necrotizing pneumonia despite ten days of erythromycin therapy, percutaneous transtracheal aspiration (TTA) was performed. The purulent, fetid sputum obtained showed pleomorphic Gram-negative and Gram-positive bacilli on stain. Culture yielded Eubacterium lentum and an unidentified non-sporulating Gram-positive anaerobic bacillus.

The patient was started on metronidazole, and there was gradual defervescence and decrease in cough over the first several days. He was treated for a total of 42 days, the pulmonary infiltrates gradually resolving. The hospital course was complicated by normochromic normocytic anemia and a urinary tract infection with Pseudomonas which was treated with a seven-day course of gentamicin. At last report he was afebrile and without cough, two months after com-

TABLE 1.—*Susceptibility of Anaerobic and Microaerophilic Bacteria to Metronidazole*

Organism	Number of Strains	Minimum Inhibitory Concentration (μg/ml)									
		≦ 0.1	0.2	0.4	0.8	1.6	3.1	6.2	12.5	25	>100
Bacteroides fragilis	13			2	4	2	4			1	
B. melaninogenicus	3			1	1	1					
B. oralis	3				1			1	1		
B. corrodens	4				1	1	1	1			
Fusobacterium nucleatum	5	5									
F. necrophorum	1		1								
F. varium	6			6							
F. species	1					1					
Bifidobacterium	4					1		3			
Clostridium sp.	5	1	1		1		2				
Microaerophilic Gram-pos. cocci	6			2	1	2					1
Veillonella	3					2	1				
Total	54										

pletion of therapy, but had decreased pulmonary function with residual scars and pleural thickening observed on x-ray films of the chest.

Third Case

A 53-year-old diabetic man was admitted with a one-month history of dull pain in the left side of the chest, cough productive of "bad tasting" sputum, and low grade fever.

He had been in hospital in October 1970 with a left lower lobe (superior segment) infiltrate, and while he was being evaluated there, focal seizures developed. He proved to have a brain abscess from which Fusobacterium nucleatum was isolated in pure culture. Gram stain of a TTA specimen after the start of therapy showed a similar organism but failed to yield any growth. He was treated initially with chloramphenicol and then with lincomycin for six months. He was left with a residual focal seizure disorder.

On physical examination at the present admission, poor dental hygiene and localized rales in the left upper lobe were noted. Chest x-ray films showed a posterior left upper lobe infiltrate, and dental films revealed periapical abscesses. Gram stain of the TTA specimen revealed slender Gram-negative filamentous rods and culture of the specimen grew Fusobacterium nucleatum in pure culture.

The patient was treated with metronidazole for 90 days with initial prompt defervescence and slow resolution of the pulmonary infiltrate. All remaining teeth were extracted. The hospital course was uncomplicated. When last seen, six weeks following cessation of metronidazole therapy, the patient was asymptomatic and chest x-ray films show only a residual scar.

Results

The MIC's of the 54 strains of anaerobic bacteria are shown in Table 1. Fifty-one strains were sensitive to metronidazole in concentrations of 6.2 μg per ml or less. One microaerophilic coccus strain grew in the presence of 100 μg per ml of metronidazole.

The three anaerobic infections treated with metronidazole were cured. No adverse reactions to metronidazole were noted. In Cases 1 and 2 mild anemia developed during the illness but in both cases the hematocrits were rising while metronidazole was being administered.

Discussion

The importance of anaerobic bacteria in human infections is being recognized increasingly. While most of these organisms are susceptible to several commonly used antimicrobial agents, Bacteroides fragilis (the most commonly encountered anaerobe) is resistant to penicillin and has developed significant resistance to tetracycline in the past few years.[14] Chloramphenicol is the only antibiotic approved in the United States for

TABLE 2.—*Summary of* In Vitro *Data in the Literature on the Susceptibility of Different Strains of Anaerobic Bacteria to Metronidazole*

Organism	References	Range		
		Sensitive (≦6.2 mcg/ml)	*Intermediate* (>6.2 mcg/ml to <50 mcg/ml)	*Resistant* (≧50 mcg/ml)
Clostridium perfringens	8,10,13,19	20*	3	3
Clostridium species	8,10,11,13,18	55	4	0
Fusobacterium species	10,11,18	20	0	0
Sphaerophorus species	7,11,12,13	30	2	0
Bacteroides species	10,11,13	24	3	0
Peptococcus	11,13	11	1	1
Peptostreptococcus	11,13	1	1	7†
Veillonella	9,11,13,18	35	3	1

*Number of strains

†Dr. Ueno (personal communication) indicates that all of these strains became facultative on subculture. They always grew better under anaerobic conditions, although they would grow aerobically. All belong to the species P. evolutus, whereas the strain of Peptostreptococcus with intermediate sensitivity was P. anaerobius.

anaerobic infections which is consistently active against B. fragilis. Clindamycin* shows good activity against almost all anaerobic bacteria *in vitro*, and initial clinical trials are encouraging.[15] These latter two agents are bacteriostatic against B. fragilis. Felner and Dowell,[16] in their discussion of anaerobic endocarditis, pointed out the need for a bactericidal antibiotic against B. fragilis. Our laboratory has been testing several agents *in vitro* which show activity against anaerobes. Metronidazole was unique in showing very good bactericidal activity against 19 strains of B. fragilis tested.[17] The broad activity of metronidazole against anaerobic bacteria is reflected in our results. The *in vitro* data from the literature on the activity of metronidazole against various anaerobic bacteria is summarized in Table 2.

As was noted in the introduction, the list of microorganisms which are susceptible to metronidazole has been expanding over the past 12 years. The common characteristic of these various organisms is that they are all anaerobic, as was pointed out by Prince et al.[18] A large number of strains of many aerobic bacteria and several strains of fungi have been shown to be resistant to metronidazole.[10,18,19] Metronidazole has recently been shown to inhibit electron transport systems in T. vaginalis, which probably explains its selective activity against anaerobic organisms.[20] (This may be why certain microaerophilic bacteria are resistant to metronidazole; some of these organisms are undoubtedly facultative rather than anaerobic).

*Clindamycin has recently been approved by the FDA for use in anaerobic infections.

Metronidazole (1- [2-hydroxyethyl] -2-methyl-5-nitroimidazole) is available only in oral form. Peak serum levels following a single 200 mg oral dose range from 2.6 to 7.0 mcg per ml, and following 200 mg three times a day the range is from 6.1 to 9.8 mcg per ml.[21] With doses of 1 gram four times a day, serum levels range from 15 to 72.5 mcg per ml.[3] The drug is 20 percent bound to plasma proteins, which has little effect on its pharmacokinetics.[22] It is partially metabolized in the liver; the major metabolite results from oxidation of the 2-methyl radical.[23] Approximately 60 to 70 percent is excreted in the urine unchanged.[24] Metronidazole is excreted primarily in the urine, both in active form and as inactive metabolites. The biliary tract is not a major route of excretion, although therapeutic levels may be achieved in bile. Because of metronidazole's excellent absorption and almost total excretion via the urinary tract, only very small amounts reach the large intestine.[25] Preliminary data on one patient currently being treated shows negligible changes in fecal flora as compared with a pre-treatment specimen. If it can be verified that normal bowel flora is not greatly altered by the drug, that would represent a distinct advantage over other drugs such as clindamycin. Metronidazole is known to be taken up rapidly by all tissues in experimental animals and to cross the placental barrier in mice.[13,25] In man, significant levels have been demonstrated in pus from a liver abscess, in breast milk and in cerebrospinal fluid.[3,26,27]

Adverse reactions to metronidazole, which have been infrequent, include gastrointestinal disturbance (vomiting, diarrhea and cramping), metallic taste, urticaria, vaginal and urethral burning and, rarely, darkening of the urine.[28] Metronidazole has been shown to cause a transient neutropenia[29] which is promptly reversible on discontinuation of treatment. Ataxia, vertigo and headache have occurred in humans. A high incidence of psychotic reactions developed in alcoholic patients treated with metronidazole combined with disulfiram.[30] The safety of metronidazole in pregnancy has not been adequately tested. Alcohol is contraindicated while patients are receiving metronidazole therapy.

The *in vitro* evidence of impressive activity of metronidazole against anaerobic bacteria and the good response of our patients indicate that this drug may have a significant role in the treatment of anaerobic infections. The bactericidal activity of metronidazole would make it especially useful in anaerobic endocarditis. Further clinical evaluation of this drug in anaerobic infections of all types is indicated. However, it must be remembered that metronidazole is still experimental for this purpose. Patients should be fully informed and careful pre-treatment and followup bacteriologic studies should be performed.

Addendum

Since preparation of this report, two additional patients with anaerobic infection have been started on metronidazole therapy and are currently still under treatment. One has a necrotizing pneumonia; four anaerobic organisms were recovered from a TTA specimen. One of the four is Bacteroides melaninogenicus, one is probably Fusobacterium nucleatum, one is probably B. fragilis, and one is still unidentified. This patient has been receiving treatment for six weeks. He has shown slow but consistent improvement; there is no longer roentgenographic evidence of excavation, but some infiltrate persists. The second patient has an asymptomatic lung abscess with an anaerobic Gram-negative bacillus (probably a Fusobacterium) recovered in pure culture from a TTA specimen. There has been no change in the size of the abscess cavity after five weeks of metronidazole therapy. Workup for malignant disease, tuberculosis and fungus infection is negative to date.

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