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Metronidazole and anaerobic sepsis

SUSANNAH J EYKYN, IAN PHILLIPS

British Medical Journal, 1976, **2**, 1418-1421

Summary

Fifty patients with anaerobic sepsis were treated with intravenous and oral metronidazole. In 26 cases this was combined with other antimicrobial agents. Highly satisfactory clinical results were obtained in most patients, though in many surgical drainage was also essential. No side effects or thrombophlebitis were seen.

Mixed growths of anaerobic and aerobic bacteria were isolated from all except five patients, who produced only anaerobes. Metronidazole is the only available antimicrobial agent providing selective activity against anaerobic organisms. It is effective and safe and is usually the drug of choice for treating severe anaerobic sepsis.

Introduction

Anaerobic bacteria have always been important pathogens, but the "anaerobic renaissance" that has occurred over the past decade may probably be attributed to greatly improved laboratory techniques resulting in increased isolation of these organisms. Although isolating an anaerobe does not inevitably indicate disease, anaerobic bacteria are clearly responsible for a wide variety of infections and are particularly important in sepsis associated with the genital and intestinal tracts. Some patients with anaerobic infection can be satisfactorily treated by surgical intervention alone, but in many appropriate chemotherapy is required. The antimicrobial agents that are active against anaerobic bacteria include chloramphenicol, lincomycin, and clindamycin, each of which may cause potentially serious toxicity. Phillips and Warren concluded that *Bacteroides fragilis* is usually also susceptible to sulphonamides, co-trimoxazole, and spectinomycin but there has been no clinical evaluation of the efficacy of these drugs.[1 2]

Metronidazole has been used as an oral treatment for trichomoniasis for 16 years and has proved highly effective and safe. It has also been used extensively in larger doses for giardiasis and amoebiasis. Metronidazole was first used against anaerobic infections in 1962 by Shinn[3] in the treatment of acute ulcerative gingivitis, but not until 10 years later did Tally *et al* report its use in bacteroides infections.[4] Since then many workers have shown that it is clinically effective[5-9] in several types of anaerobic infection. It has also been used successfully in the chemoprophylaxis of infection after hysterectomy,[6] appendicectomy,[8] and elective colonic surgery.[10] Until recently, however, its therapeutic use in severely ill patients, particularly those undergoing major abdominal surgery, was limited because no parenteral preparation was available, although a rectal preparation has been used successfully in some patients, largely as prophylaxis.[7 8] An intravenous preparation of metronidazole is now avaiable, and we have reviewed the results of treating anaerobic sepsis in severely ill patients with intravenous and oral metronidazole.

Patients and methods

The decision to use metronidazole was made after consultation between clinician and microbiologist. It was mainly given for sepsis associated with the genital or intestinal tracts, gas gangrene, and anaerobic bacteraemia. The presence of putrid pus together with typical peaks of volatile fatty acids on chromatography of the pus provided excellent and immediate evidence of anaerobic sepsis. Blood counts and liver enzyme concentrations were regularly monitored in all patients receiving metronidazole, and whenever possible serum was taken for assay during treatment.

When possible metronidazole was given as the sole antimicrobial, but in many patients other agents were also given, either for co-existing infection of the respiratory tract or because it was considered clinically desirable to cover aerobic as well as anaerobic bacteria. Intravenous metronidazole was administered as a buffered, isotonic 0·5% w/v

Department of Microbiology, St Thomas's Hospital Medical School, London SE1 7EH
SUSANNAH J EYKYN, MB, MRCPATH, senior lecturer in microbiology
IAN PHILLIPS, MD, MRCPATH, professor of microbiology

aqueous solution in 100-ml bottles each containing 500 mg metronidazole and infused every eight hours over about 20 minutes. The only two children treated received 250 mg every eight hours. The decision to discontinue intravenous treatment or to change to oral treatment (adult dose 400 mg every eight hours; children 200 mg every eight hours) was made on clinical grounds and in no case was the drug initially prescribed for a specific time. The dose was not reduced for four patients in renal failure, but three were undergoing haemodialysis and the fourth was recovering from an episode of acute renal failure. The patients on dialysis were treated for a maximum of five days, and the other patient was treated for 10.

BACTERIOLOGICAL METHODS

Specimens of pus or drainage fluids—Whenever possible these were collected in a special "gassed out" container and delivered to the laboratory and cultured with minimum delay. Specimens were held in ultraviolet light to detect red fluorescence, which is said to be specific for *B melaninogenicus*.[11] If enough clinical material was available, gas-liquid chromatography was performed on a Pye Unicam Series 104 Chromatograph. This method of enabling a rapid presumptive diagnosis of anaerobic infection to be made was originally described by Gorbach *et al.*[12] Our method did not involve derivatisation or extraction of the volatile substances from the specimen, which was injected directly on to a column containing Chromosorb 101.[13] The medium used for anaerobic culture was brain-heart infusion agar (Oxoid CM 375) with the following additives: 0·05% cysteine hydrochloride, 1% vitamin K-haemin solution, 0·5% yeast extract, 100 mg/l neomycin, 7·5% whole defibrinated horse blood, and 2·5% lysed (by freezing and thawing) defibrinated horse blood. Plates were incubated in an atmosphere of 10% carbon dioxide in nitrogen in an anaerobic jar. Whenever possible anaerobic isolates were fully identified and their sensitivity to metronidazole assessed with a 2·5-μg disc. Aerobic cultures were set up on appropriate media according to routine laboratory procedure.

Blood cultures—Blood was taken for culture when patients wree thought to be bacteraemic. The method was that used routinely for all blood cultures in the laboratory: 10 ml venous blood was added to each of two bottles containing 10 ml digest broth plus 0·1% glucose and 0·06% Liquoid (sodium polyanethol sulphonate, Southern Group Laboratories). One culture was incubated in an atmosphere of carbon dioxide and the other contained a nail for anaerobiosis. Subcultures were made on blood agar plates after incubation at 37°C for 24 hours, 48 hours, and one to two weeks.

Stool specimens—Stools were obtained from some patients within a week of completion of their course of metronidazole. Specimens were cultured anaerobically as described above. Minimum inhibitory concentrations (MICs) of metronidazole were determined for all anaerobic isolates on solid medium (Oxoid DST agar CM 261) with 6% saponin-lysed defibrinated horse blood added. Inocula were prepared by suspending growth from blood agar plates, incubated overnight in Gas Pak jars (BBL), in nutrient broth (Southern Group Laboratory) containing 6% lysed blood. These suspensions were suitably diluted to give an inoculum of 10^{3-4} colony-forming units when applied with a multiple inoculator. The inoculated plates were incubated in 10% CO_2 for 24 or 48 hours depending on the growth rate of the organisms and MICs read as the concentration of metronidazole that caused complete inhibition of growth.

Microbiological assay of serum concentration—Serum concentration of metronidazole was assayed by the plate method. Lysed blood agar plates were flooded with a suspension of *B fragilis* NCTC 9343, and standard solutions of metronidazole were made up in doubling dilutions of horse serum. Standards and patients' sera were placed in wells cut in the plates, which were then incubated overnight in Gas Pak jars (BBL).

TABLE I—*Type of infections in 50 patients treated with metronidazole*

	No of patients		No of patients
Genital tract:	6	Diverticulitis with abscess	5
Tubo-ovarian abscesses	2	Subphrenic abscess	2
Uterine gas gangrene	1	Other intra-abdominal sepsis	3
Pyometrium	1	Others:	8
Pelvic infection after hysterectomy	1	Thigh abscess	2
Acute salpingitis	1	Osteomyelitis	1
Gastrointestinal tract:	36	Perinephric abscess	1
Sepsis and cellulitis after bowel surgery	11	Pyonephrosis	1
Perforated gangrenous appendix and peritonitis	9	Gas gangrene of leg	1
Faecal peritonitis	6	Cholangitis	1
		Wound infection after renal transplant	1

Results

Fifty patients (29 females and 21 males) were treated with metronidazole. Their ages ranged from 17-80 years, with two children aged 6 and 11 years. Eighteen patients received only the intravenous preparation, 10 patients only the oral preparation, and in 22 intravenous treatment was followed by oral treatment. Twenty-four patients received only metronidazole, but 26 also received other drugs, usually gentamicin or ampicillin, in four cases for concomitant bronchopneumonia and in 22 cases to achieve broad-spectrum therapy. The total dose of metronidazole varied from 1·5 to 29·7 g and the total dose of intravenous metronidazole from 1·5 to 29 g. In half the patients who received the intravenous preparation a total dose of 3 to 8 g was given. In all cases the drug was well tolerated with no associated thrombophlebitis and no observed side effects.

The type of infection in the 50 patients treated is shown in table I. Rational assessment of the clinical response to metronidazole was made difficult in many cases as surgical drainage often formed an essential part of treatment. The patients were considered as two main groups: those treated with metronidazole alone (24 patients) and those treated with other antimicrobial agents in addition to the metronidazole (26 patients). Table II shows the clinical response to treatment for both groups, the types of bacteria isolated, and the surgical procedure, if any, involved in treatment. In the patients treated with metronidazole alone there was a good response in 20 of the 24 cases, but in 13 surgery formed an integral part of their treatment. The clinical improvement in the seven patients who did not undergo essential surgery could be attributed only to the metronidazole. Three of the four patients who failed to respond were severely ill from their underlying disease or bronchopneumonia and two of them died. The fourth patient who failed to respond was given intravenous metronidazole after appendicectomy for a perforated gangrenous appendix with considerable pus in the peritoneum and later developed a pelvic abscess, which was subsequently drained successfully. It was thought that the initial operation had failed to establish adequate drainage.

From four of the 24 patients treated with metronidazole alone only anaerobes were grown on culture: 18 mixed cultures were obtained and in two cases no cultures were performed. Seven patients whose initial cultures grew both anaerobic and aerobic bacteria developed clinical signs of sepsis due to aerobes after treatment with metronidazole alone. In five cases these were wound infections; they were deep-seated in three patients, one of whom had received a recent renal

TABLE II—*Bacteria isolated, surgical drainage, and clinical response in patients treated with metronidazole alone and with other antibacterials*

Bacteria isolated	Treatment with metronidazole alone			Treatment with metronidazole and other antimicrobials		
	Good response		Poor response	Good response		Poor response
	With drainage	Without drainage		With drainage	Without drainage	
Anaerobes alone	3	1		1		
Anaerobes and aerobes	8	6	4	11	4	6
No culture*	2			3	1	
Total patients	13	7	4	15	5	6

*All patients had intra-abdominal sepsis.

TABLE III—*Bacteria isolated from pus and blood in 44 patients*

Aerobic and facultative bacteria			Anaerobic bacteria		
Organism	No of strains		Organism	No of strains	
	Pus	Blood		Pus	Blood
E coli	27		*Bacteroides fragilis* subspecies *fragilis*	27	10
P mirabilis	6	1	*B melaninogenicus*	13	1
P morgani	2		*B fragilis* subspecies *thetaiotaomicron*	4	
Klebsiella spp	2	1	*B fragilis* subspecies *ovatus*	2	
Ent cloacae	1	1	*B pneumosintes*	3	
Ps aeruginosa	2		*Bacteroides* spp	3	
Haemophilus vaginalis	1		*Fusobacterium* spp	2	
Strep milleri	9		*Peptostrep anaerobius*	13	
Strep faecalis	13		*Peptostrep assaccharolyticus*	1	
Strep agalactiae	3	1	*Cl perfringens*	11	2
β-Haemolytic streptococcus C	1		*Clostridium* spp	6	
β-Haemolytic streptococcus G	1		*Cl septicum*	1	
α-Haemolytic streptococcus	2		*Cl paraputrificum*	1	
Staph aureus	3		*Cl cochlearium*	1	
C albicans	1		*Cl ramosum*	1	
			Cl clostridiiforme	1	
			Bifidobacterium infantis	1	

transplant and required specific treatment. The other two patients developed signs of septicaemia with *Escherichia coli* in the blood in one case and *Klebsiella* and *Stretococcus faecalis* in the other.

It was more difficult to assess the outcome in the 26 patients treated with other antimicrobials as well as metronidazole. Six patients died, either from their underlying disease or from bronchopneumonia, or both. The other 20 patients definitely improved, but 15 also underwent surgery. Cultures from all but one of the 22 patients from whom they were obtained grew both anaerobes and aerobes. Only one patient developed a subsequent infection—a superficial wound infection with *Staphylococcus aureus;* he had been receiving ampicillin for a respiratory infection in addition to metronidazole.

Bacteriology—Cultures were performed on 44 of the 50 patients and anaerobic organisms were isolated from 44, alone in five cases and in mixed culture with aerobes in 39. In no case were only aerobic organisms recovered. Details of the isolates from pus and blood are given in table III. Specimens of pus often contained several types of both anaerobic and aerobic bacteria with as many as six organisms being isolated on occasions. All anaerobic isolates were sensitive to metronidazole on disc sensitivity testing.

Red fluorescence in ultraviolet light—Of the 25 specimens of pus investigated, 14 fluoresced bright red in ultraviolet light. *B melaninogenicus* was isolated on culture from 10 of these, in each case as part of a mixed anaerobic and aerobic growth. The four specimens that failed to grow *B melaninogenicus* despite red fluorescence grew other anaerobic bacteria, as did the 11 specimens that did not fluoresce.

Gas-liquid chromatography—This was performed on 20 specimens of pus. Details of the volatile fatty acids detected together with the organisms isolated are shown in table IV.

Stool cultures after metronidazole—Stool specimens for anaerobic culture were obtained from 11 patients. They had received a total dose of 6 to 27 g of metronidazole. Anaerobic bacteria were isolated from only seven patients, five of whom had more than one species. All but one of these patients had been receiving metronidazole alone. Anaerobes were grown from four of the five patients in whom the drug was given intravenously and from three of the six patients in whom the oral as well as the intravenous preparation was given. MICs of metronidazole for the 19 anaerobes investigated ranged from 0·25 to 8 mg/l. Four isolates had MICs of 8 mg/l but only two of them were resistant on disc testing.

Serum concentration of metronidazole—Serum concentrations were monitored in 18 patients receiving intravenous metronidazole, including two receiving haemodialysis and one recovering from acute renal failure. In each case serum was taken before the start of an infusion and 30 minutes after its completion. Serum concentrations were measured within 48 hours of the start of intravenous metronidazole in 10 patients and after 48 hours in eight. The mean trough concentration was 15·5 mg/l (range 6·4-26 mg/l) and the mean peak concentration was 27·4 mg/l (range 11·5-41 mg/l).

The serum concentrations for the three patients in renal failure fell within this range, with trough concentrations of 7·2, 13, and 19 mg/l and peak concentrations of 13, 27, and 28 mg/l.

TABLE IV—*Volatile fatty acids detected on gas-liquid chromatography and organisms isolated from 20 specimens of pus*

Specimen No	Source	Volatile acids detected	Number of organisms isolated			
			Anaerobes			Aerobes
			Bacteroides spp	*Clostridium* spp	*Peptostreptococcus* spp	
1	Abdominal wound	Acetic, propionic, isobutyric, butyric, isovaleric, valeric, lactic, isocaproic, succinic	2	1	1	2
2	Thigh abscess	Acetic, propionic, isovaleric, lactic	1			2
3	Abdominal wound	Acetic, propionic, isobutyric, butyric, isovaleric, isocaproic	2			3
4	Pyonephrosis	Acetic, propionic, isobutyric, butyric, valeric, lactic, isocaproic, caproic	1			2
5	Abdominal wound	Acetic, propionic, lactic	1	1	1	2
6	Gas gangrene in leg	Acetic, propionic, butyric, lactic		1		
7	Perinephric abscess	Acetic, propionic, isobutyric, butyric, isovaleric, valeric, lactic, isocaproic, caproic	2 2	1 1	1 1	3 3
8	Peritonitis	Acetic, propionic, isobutyric, butyric, isovaleric, lactic	2	1		3
9	Pelvic abscess	Acetic, propionic, isobutyric, butyric, isovaleric, lactic, isocaproic, caproic	2	3		2
10	Subphrenic abscess	Acetic, propionic, butyric, succinic	1			2
11	Osteomyelitis in foot	Acetic, propionic, lactic	1			1
12	Groin abscess	Acetic, propionic, isobutyric, butyric, isovaleric, lactic	2		1	1
13	Abdominal wound	Acetic, propionic, butyric, lactic	1	1		1
14	Peritonitis	Acetic, propionic, isobutyric, butyric, valeric, lactic caproic	2	1	1	1
15	Abdominal wound	Acetic, propionic, butyric, isovaleric, valeric, isocaproic	2	1	1	2
16	Abdominal wound	Acetic, propionic, isobutyric, butyric, isovaleric, valeric, lactic, isocaproic, caproic	2	1	1	2
17	Diverticular abscess	Acetic, lactic	1			1
18	Pelvic abscess	Acetic, propionic, isobutyric, butyric, isovaleric, lactic	2	2	1	2
19	Abdominal wound	Acetic, propionic, isobutyric, butyric, isovaleric, valeric, lactic, isocaproic, caproic	1		1	1
20	Peritonitis	Acetic, propionic, isobutyric, lactic	1			1

Discussion

Although metronidazole has been available for many years. its therapeutic potential in anaerobic sepsis has only recently been realised. The encouraging reports of the therapeutic use of the drug have, with one exception,[14] concerned the oral preparation.

We have found intravenous metronidazole to be safe, easy to administer, and well tolerated by patients, with no associated thrombophlebitis. It was often difficult to assess the clinical response in the 50 patients because of essential surgical drainage and the administration of other antimicrobial agents, but only 10 patients failed to improve, and severe underlying disease, often with bronchopneumonia, accounted for apparent therapeutic failure in nine. In the tenth case inadequate drainage was the key factor. Half of the 40 patients showing clinical improvement received no other antimicrobials. In a few patients quite dramatic clinical improvement could be attributed only to metronidazole. Many patients and nurses independently observed that after metronidazole treatment the pus lost its putrid odour.

Anaerobic organisms are most commonly isolated as part of a mixed culture with aerobes, and only in a few cases are they the only pathogens. It is difficult when a mixed culture is obtained to assess whether treatment should be directed only against the anaerobes or against both aerobes and anaerobes. Metronidazole is the only available antimicrobial that can achieve selective anaerobic chemotherapy. Our results suggest that the use of metronidazole alone in mixed infections may achieve excellent results but that further bacteriological cultures during treatment invariably yield aerobic bacteria. Clearly these have the potential to assume a pathogenic role, although this actually happened in only a few cases. More work is needed to establish the efficacy of metronidazole in the treatment of infection with mixtures of aerobic and anaerobic bacteria.

Although Freeman *et al*[15] reported that metronidazole was active in experimental clostridial infection in 1968, there have been no reports of its use in gas gangrene in man. Two of our patients were treated with intravenous metronidazole for gas gangrene; one received other antimicrobials including ampicillin and the rapidly fatal outcome made assessment impossible. The other patient, who was hypersensitive to penicillin, made excellent progress after amputation, one treatment with hyperbaric oxygen, and intravenous metronidazole alone, and *Clostridium perfringens* was not recovered from subsequent cultures. Possibly metronidazole will become a useful alternative to penicillin for the treatment and prophylaxis of gas gangrene.

Gas-liquid chromatography of clinical specimens is a particularly useful guide to the presence of anaerobes and, as has been pointed out by Gorbach[12] and by Phillips *et al*,[16] provides a rapid means of diagnosis. Gorbach[12] reported good correlation between the detection of isobutyric, butyric, and succinic acids and growth of *B fragilis* on culture. The Luton study group[16] found that anaerobes were always isolated in significant numbers from samples of pus in which volatile fatty acids other than acetic acid were detected but that it was not possible specifically to identify the anaerobes present in the specimen from the gas-liquid chromatographic analysis. We found that 13 out of 16 specimens that grew *B fragilis* on culture produced isobutyric or butyric acid, or both, but that in four specimens that also produced these acids no *B fragilis* was isolated. Unpublished results from our laboratory show that specimens of pus that yield only aerobic bacteria invariably produce acetic, propionic, and lactic acids. It is therefore of interest that specimens 5, 11, and 17 (see table IV) also produced only these acids, although *B fragilis* was isolated from each sample on culture. We conclude that in most cases pus produced by anaerobic organisms will give characteristic volatile acid peaks other than those of acetic, propionic, and lactic acids but will not invariably do so.

From the rather limited investigation of the anaerobic flora of stools obtained from patients after metronidazole treatment it is interesting that anaerobic organisms were cultured from only seven of the 11 cases. The MICs of the isolates showed increased resistance in four cases. Reports suggest that most anaerobes are highly susceptible to metronidazole, with MICs less than 8 mg/l and often less than 1 mg/l.[17]

Both peak and trough serum concentrations of metronidazole in our patients considerably exceeded the reported MICs for anaerobic bacteria. Whether it is necessary or desirable to exceed the MIC by a factor of 10 or more is questionable, and it may be possible to achieve effective treatment with lower doses than the ones we gave.

Our results suggest that metronidazole is safe and highly effective for treating severe anaerobic sepsis and that the intravenous preparation is well tolerated and of particular use in seriously ill patients, especially after major surgery.

We thank the many surgeons and physicians whose patients we studied; May and Baker for supplies for intravenous metronidazole; Miss C Warren and Miss E Taylor for careful technical help; and our colleagues Dr Otterburn and Dr Unsworth for their help in collecting information on the patients.

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