Reciprocal antimicrobial synergism between *Escherichia coli* and *Bacteroides fragilis* in the presence of metronidazole

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SUMMARY We have studied the antimicrobial action of metronidazole against *Bacteroides fragilis* and *Escherichia coli* both in pure cultures and in combination. Concentrations of metronidazole above the minimal inhibitory concentration (MIC) produced a marked bactericidal effect on *B fragilis* at six hours. *E coli* however showed only a slight and transitory decrease in population when exposed to concentrations of metronidazole close to its MIC. Kinetic studies of this drug's effect on mixed cultures of both organisms showed mutual bactericidal synergy between *E coli* and metronidazole against *B fragilis* and between *B fragilis* and metronidazole against *E coli*. These in vitro findings agree with the results obtained in vivo by other authors.

It has been shown that metronidazole reduces mortality from experimental peritonitis induced in rats by inoculation of caecal content or mixtures of facultative and anaerobic bacteria from the gut.¹ It has been suggested that metronidazole may possess an antimicrobial effect not only against anaerobic bacteria, especially *Bacteroides fragilis*, but also against aerobic and facultative organisms, although the results published are to a certain extent contradictory.¹⁻⁵ While it has been shown that certain strains of *Escherichia coli* are, in specific experimental conditions, susceptible to metronidazole,³ there is much doubt as to interaction between this drug and the mixed flora of the gut.

The antimicrobial action of metronidazole against anaerobes and possibly aerobic and facultative bacteria also is an important feature of this drug and one that is not shared by others chemically unrelated to it but highly active against certain anaerobes like clindamycin.¹

Most of the literature on metronidazole, *E coli* and *B fragilis* interaction has been mainly concerned with the drug's effect on *E coli*. This paper studies the kinetic effect of metronidazole on both *E coli* and *B fragilis* as well as the interaction of these three variables in an in vitro system.

Material and methods

BACTERIAL STRAINS

One freshly isolated strain of *B fragilis* and another of *E coli* were studied. Their anaerobic minimal inhibitory concentration (MIC) were 1 and 64 µg/ml of metronidazole, respectively.

CULTURE MEDIUM

An EBB medium was used. This consisted of brucella broth (Difco) enriched with sodium bicarbonate (11.9 mmol/l), hemin (Sigma) (7.67 µmol/l), and menadione (Sigma) (1.16 µmol/l). The final pH was 7.3.

INOCULUM

The bacteria were inoculated in anaerobiosis using the GasPak (BBL) system at 35°C for 24 h. One drop of Pasteur pipette from a 24 hour-culture was added to a tube containing 10 ml of EBB medium without drug, and to three others containing respectively 4, 16 and 64 µg/ml of metronidazole (Rhodia Ibérica) in the same medium. The bacteria were placed in pure culture and, in another experiment, mixed equally in order to obtain 10⁶ to 10⁷ CFU/ml in every case.

KINETIC STUDY

Colony forming units of the two organisms were counted at 0, 6, 24 and 52 h using dilutions of their

Effect of metronidazole on pure and mixed cultures of *Escherichia coli* and *Bacteroides fragilis*

Culture, time (h)	Metronidazole concentration ($\mu\text{g/ml}$)							
	0		4		16		64	
	<i>E coli</i> (log ₁₀ CFU/ml)	<i>B fragilis</i>	<i>E coli</i> (log ₁₀ CFU/ml)	<i>B fragilis</i>	<i>E coli</i> (log ₁₀ CFU/ml)	<i>B fragilis</i>	<i>E coli</i> (log ₁₀ CFU/ml)	<i>B fragilis</i>
<i>E coli</i>								
0	7.2	—	6.8	—	6.8	—	6.8	—
6	9	—	8	—	7.7	—	6.6	—
24	9.2	—	>8	—	7.5	—	6.1	—
52	9.1	—	>8	—	>8	—	7.6	—
<i>B fragilis</i>								
0	—	7.3	—	6.8	—	6.8	—	6.8
6	—	8.1	—	6.8	—	5.8	—	6.4
24	—	9.6	—	<3	—	<3	—	<3
52	—	9.2	—	<3	—	<3	—	<3
<i>E coli</i> + <i>B fragilis</i>								
0	6.9	7.2	6.3	6.6	6.3	6.6	6.3	6.6
6	9	8.1	>8	<3	8.2	<3	7	<3
24	9.2	9.3	>8	<3	7.4	<3	5.2	<3
52	9.1	8.9	>8	<3	7.6	<3	7.6	<3

respective broths, subcultures with calibrated loop on blood agar and blood-amikacin agar plates for differential colony-counts analysis. All experiments were repeated three times.

Results

The Table shows the number of colony forming units (log₁₀) per ml of the medium and the growth of *E coli* and *B fragilis* in pure and mixed cultures, both with and without metronidazole.

The first column gives the growth of the two bacteria without metronidazole in pure and mixed cultures. Growth is exponential, reaching a maximum at 24 h. Growth within the same system of the two microorganisms showed no interference and in both cases the rate was similar to that shown when they grew in pure culture.

The second, third and fourth columns give the results obtained when the cultures, isolated and combined were exposed to 4, 16, and 64 $\mu\text{g/ml}$ of metronidazole. The growth rate of *E coli* did not change when it was exposed to 4 $\mu\text{g/ml}$ of the drug either in pure culture or in combination with *B fragilis*. The latter, however, was killed when exposed to 4 $\mu\text{g/ml}$ of the drug after six hours but much more rapidly when exposed in combination with *E coli*.

Concentrations of 16 $\mu\text{g/ml}$ of metronidazole produced, at 24 h a very slow increase in the *E coli* population growing in pure culture as compared with the control unexposed to the drug while *B fragilis* already showed a slight drop at six hours and a much greater one at 24 h. The same concentration of metronidazole proved much more bactericidally effective against *B fragilis* when this organism was mixed with *E coli* than when in pure culture.

Finally, 64 $\mu\text{g/ml}$ of metronidazole was markedly bactericidal against *B fragilis* but only very slightly against *E coli* growing in pure cultures. This effect of metronidazole was observed after six hours' exposition with peak action at 24 h. However, when the drug was added to a mixed culture of *B fragilis* and *E coli*, rapid and intense bactericidal action was observed at the sixth hour in the case of *B fragilis* and at 24 h against *E coli*, the population of the latter dropping from 10^7 to 1.5×10^5 CFU/ml between the sixth and twenty-fourth hour of exposition. *E coli* was later able to resume growth, since at 52 h, bacterial density was twenty times higher than that of the initial inoculum. This population was not re-exposed to metronidazole to discover whether it had acquired resistance to this drug.

Metronidazole showed intense bactericidal activity against *B fragilis* at the twenty-fourth hour of exposition at all the concentrations (higher than the MIC) tested. *E coli* showed a population decrease only when subjected to high concentrations of metronidazole, 16 and especially 64 $\mu\text{g/ml}$ (study in anaerobiosis showed the latter figure to be its MIC).

Metronidazole was found to be not only more intensely but also more rapidly bactericidal, both against *B fragilis* and *E coli* in mixed than in pure cultures.

Discussion

E coli and *B fragilis* and metronidazole have been seen to be strongly interactive. *E coli*-metronidazole synergy against *B fragilis* has been little studied and the published data present a somewhat contradictory picture.^{1-3 6 7}

Our study shows that the strain of *E coli* used was susceptible in vitro to high concentrations of met-

ronidazole (16 and particularly 64 $\mu\text{g/ml}$) in anaerobiosis. This effect was only moderate and transitory since, after 24 h, the bacteria resumed growth. These results agree with the finding of other authors³ and *E coli* may be susceptible to the drug in specific experimental conditions.

Analysis of the interaction of *E coli*, *B fragilis* and metronidazole showed mutual synergy between *E coli* and metronidazole against *B fragilis* and between *B fragilis* and metronidazole against *E coli*.

The bactericidal effect of metronidazole on *B fragilis* combined with *E coli* took place before six hours, much earlier therefore, than when the drug acted upon a pure culture of the anaerobe. It has been shown that in certain experimental conditions *E coli* is capable of raising the levels of biologically active metronidazole, but that in the presence of *B fragilis* the increase is slight and slow,¹ which may be due to overconsumption of the drug. These results contrast with the findings of other authors who have observed the action of metronidazole against *Entamoeba histolytica*² and *Trichomonas vaginalis*⁵ to be reduced when bacteria are present in the same system.

The antimicrobial action of metronidazole against *E coli* combined with *B fragilis* was greater than when the former was exposed to the drug in pure culture. The effect also depended on the concentration of metronidazole used and was greatest with 64 $\mu\text{g/ml}$ in the presence of *B fragilis*. Although it has been said that *E coli* is resistant to metronidazole,^{2,4-6} in certain experimental conditions, as when anaerobes are present in the same system, this microorganism may be much more sus-

ceptible than it is when exposed in pure culture. Our in vitro findings largely agree with the results obtained in vivo by other authors.¹

Addendum

After this paper was submitted for publication, we have noted work by Ingham *et al* (*J Antimicrob Chemother* 1981;8:475-9) showing that *E coli* reduces the PO_2 of the medium, thereby enhancing the activity of metronidazole against *B fragilis*.

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