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Binding to and antibacterial effect of aztreonam, temocillin, gentamicin and tobramycin on human faeces

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SUMMARY

Aztreonam, temocillin, gentamicin and tobramycin were studied for their effect on the human faecal flora *in vitro* and for their usefulness for selective decontamination (SD) of the gastrointestinal tract. The sensitivities of the obligately anaerobic flora and the Gram-negative facultatively anaerobic bacteria were determined and the ratio was expressed as SD factor. The high SD factor of the flora from most subjects for aztreonam and tobramycin indicates that the drugs are useful for SD in contrast to temocillin and gentamicin. Binding to and subsequent release of tobramycin from faeces are presumed to facilitate the maintenance of adequate concentrations in the intestine despite the discontinuous intake.

INTRODUCTION

Selective decontamination (SD) of the immune-compromised host to prevent infections with micro-organisms from the indigenous flora is increasingly applied (van der Waaij, 1979; Young, 1983; Guiot & van Furth, 1984). SD aims to eliminate life-threatening Gram-negative facultatively anaerobic bacteria in the gut by antimicrobial agents. In this respect non-absorbable antibiotics are of special interest because they will reach the large bowel. In animal studies (Emmelot & van der Waaij, 1980; van der Waaij *et al.* 1982; Hazenberg *et al.* 1983*a*) as well as in clinical trials (Sleijfer *et al.* 1980; de Vries-Hospers *et al.* 1981; Guiot, van der Meer & van Furth, 1981; Guiot *et al.* 1983; Kurrle *et al.* 1983) low doses of neomycin and particularly polymyxin B proved to be very useful. *In vitro* studies on the human intestinal flora showed that the selective effect was associated with a relative insusceptibility of the obligately anaerobic flora as compared with the Gram-negative facultatively anaerobic rods (Hazenberg *et al.* 1983*b*, 1984). It was supposed that the binding and release by faeces maintained a rather constant concentration in the intestine (Hazenberg *et al.* 1984). In this study the effect of four non-absorbable antibiotics, aztreonam (Swabb, Sugerman & Stern, 1983), temocillin (Slocombe *et al.* 1981), gentamicin and tobramycin, on the human faecal flora *in vitro* was investigated and binding to and release from human faeces was determined.

MATERIALS AND METHODS

Human faeces

Faecal samples from eight healthy laboratory workers were used.

Intestinal flora

Anaerobic culture. Faeces were cultured within 1 h of passage as described previously (Hazenberg, Bakker & Verschoor-Burggraaf, 1981) on non-selective medium (Schaedler broth, Oxoid, Basingstoke, UK) solidified with 2% agar.

Aerobic culture. Dilutions of faecal samples were plated on blood-agar in triplicate and incubated for 24 h at 37 °C. Gram stains of all colony types were made and Gram-negative rods were subcultured. Gram-negative rods were identified with the API-20 system for Enterobacteriaceae (API Benelux B.V., The Netherlands).

Antibiotics

The following antibiotics were used: aztreonam (SQ 26,776, Squibb, Princeton, USA), temocillin (BRL 17421, Beecham, Betchworth, UK), gentamicin (Schering, Kenilworth, USA) and tobramycin (Eli Lilly, St Cloud, France). Antibiotic solutions were sterilized by membrane filtration (disposable filters, pore size 0·2 μm, Schleicher and Schüll, Dassel, Federal Republic of Germany).

Minimal inhibitory concentrations

Cultures of Enterobacteriaceae were diluted and aliquots containing 10^5/ml bacteria were incubated in triplicate in Schaedler broth with antibiotic concentrations ranging from 0·125 to 128 mg/l in twofold dilution steps. The minimal inhibitory concentration (MIC) was defined as the lowest concentration of the antibiotic that inhibited growth during 24 h at 37 °C.

Inhibition of the human faecal flora by antibiotics

Inhibition of the obligately anaerobic flora by antibiotics was tested by comparing numbers of colonies on anaerobic media without antibiotics with those on media with increasing concentrations of antibiotics. Using twofold dilution steps, antibiotic concentrations ranged as follows: aztreonam 8–1024, temocillin 4–512, gentamicin 2–56 and tobramycin 8–1024 mg/l. Colonies were counted after incubation of the samples for 48 h at 37 °C. Inhibition was expressed as a percentage according to the formula $100\% - (A/W \times 100\%)$, where A = the number of colonies on medium with antibiotic and W = the mean number of colonies on two media without antibiotic. Inhibition was only calculated if a minimum of 100 colonies of anaerobes was counted on each of the media without antibiotic.

SD factor

The magnitude of the difference in sensitivity of the obligately and facultatively anaerobic flora is expressed as the quotient of the concentration giving 50% inhibition of obligately anaerobic bacteria (calculated from the regression lines of Fig. 1) and the MIC for Enterobacteriaceae and is further referred to as the SD factor.

Antibiotic assay

Concentrations of antibiotics in faecal supernatant (see below) were determined with the plate-agar diffusion technique (Bennet *et al.* 1966). Indicator organisms were *Escherichia coli* (MIC value of aztreonam and temocillin 0·125 and 2 mg/l) and *Staphylococcus aureus* (MIC value of gentamicin and tobramycin 0·25 and 0·125 mg/l). Petri dishes (diameter 14 cm) with 50 ml DST agar (Oxoid) were covered with 10 ml of a diluted (*S. aureus*, 100 × ; *E. coli*, 1000 ×) overnight culture of the indicator organism. After some minutes the fluid was removed and the plates were dried. Three to six cells (diameter 6 mm) were filled with 50 μl of supernatant of pre-mixed faeces (see below, binding of antibiotic to faeces) and six with various known concentrations of antibiotics dissolved in faecal supernatant. The supernatant of faeces was obtained by centrifugation (10000 ***g***) of faeces suspended in 9 vols. of water. Inhibition zones were measured after incubation for 16 h at 37 °C.

Binding of antibiotic to faeces

The differences between concentrations of antibiotics in supernatant before and after incubation with faecal samples is referred to as the binding of antibiotic to faeces. For determination, 1 vol. of the antibiotic dissolved in water was added to 9 vols. of suspended faeces (1 g in 8 vols. water). After incubation for 1 h at 37 °C the suspension was centrifuged (10000 ***g***) and the antibiotic concentration in the supernatant determined. The binding of antibiotics to the solid part of faecal suspensions was determined with concentrations in the range 40–5120 mg/l, using twofold dilution steps. The antibiotics were found not to be destroyed or inactivated by the supernatant fluid during 1 h at 37 °C.

Release of antibiotic from faeces

Tenfold diluted faeces were incubated at 37 °C with gentamicin or tobramycin. After 1 h the concentration in the supernatant was determined (see above) and the amount of antibiotic bound to the solid fraction calculated. The removed supernatant (65%, v/v) was replaced by fresh faecal supernatant and the pellet was resuspended. After 1 h at 37 °C the suspension was centrifuged and the antibiotic concentration in the supernatant was determined. This procedure was done nine times at hourly intervals and the amount of antibiotic released calculated.

Statistical methods

Regression lines (least-square method) and coefficients of determination (r^2) were calculated, either with the original data or with their logarithmic values. Probability values (P) were derived from two-tailed tests.

RESULTS

Effect of antibiotics on the faecal flora

The median total number ($\log_{10}$) of obligately anaerobic bacteria cultured per gram faeces was 10·25 (range 10·10–10·60). The inhibitory effect of antibiotics in the medium on the numbers of anaerobic bacteria from the flora is shown in Fig. 1.

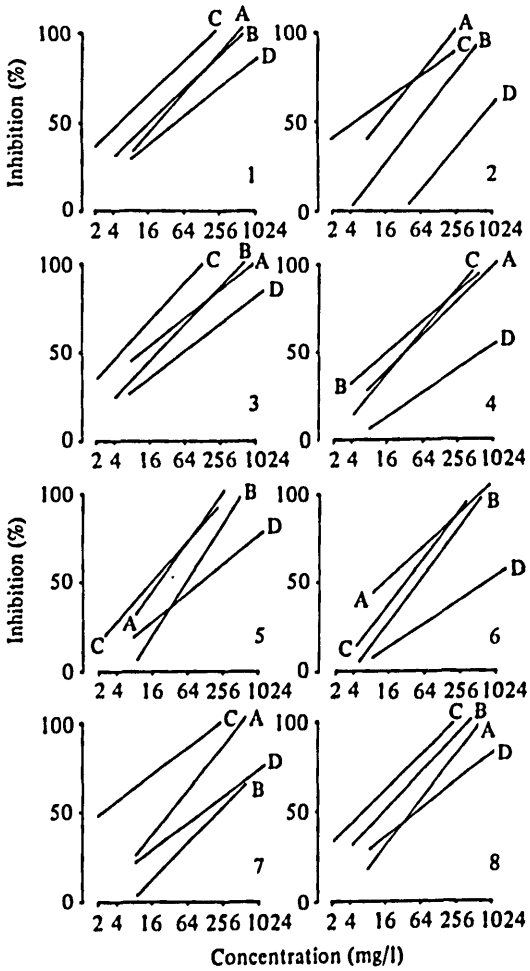

Fig. 1. Inhibitory effect of aztreonam (A), temocillin (B), gentamicin (C) and tobramycin (D) in anaerobic media (mg/l) on numbers of obligate anaerobes cultured from the human faecal flora of eight healthy subjects (1–8). Concentrations are given on a logarithmic scale. For each antibiotic the regression line was determined from at least six data, $r^2 > 0{\cdot}90$, $P < 0{\cdot}002$.

Differences were seen between the inhibitory effects of the four antibiotics on individual floras as well as between the effect of a particular antibiotic on the eight floras. In general, tobramycin had the least (except subject 7) and gentamicin (except subjects 4 and 6) the most effect on total numbers of obligately anaerobic bacteria.

Fig. 2 shows that the concentrations of aztreonam, temocillin (except for subject 7) and gentamicin giving 50 % inhibition of the obligately anaerobic flora of eight healthy subjects were similar. The anaerobic flora of subjects 2, 4 and 6, however, were much less sensitive to tobramycin than the flora of subjects 1, 3, 5 and 8 (see also Fig. 1).

The Gram-negative facultatively anaerobic rods isolated from the faecal flora were identified as *E. coli* and *Enterobacter hafniae*. Table 1 shows that the MIC of the antibiotics varied widely.

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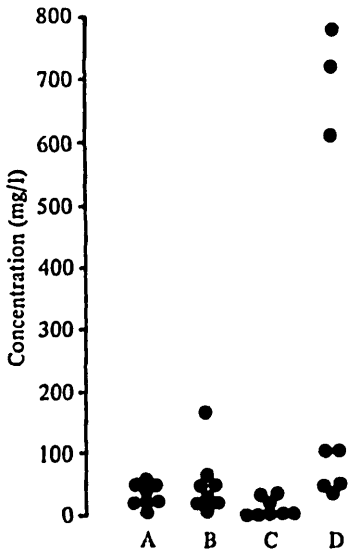

Fig. 2. Concentrations of aztreonam (A), temocillin (B), gentamicin (C) and tobramycin (D) giving 50% inhibition of the obligately anaerobic flora of eight healthy subjects.

The data given in Fig. 1 and the MIC of Table 1 were used to calculate the SD factor of an individual faecal flora for an antibiotic. The results show that the highest SD factors (Table 1) of the faecal floras were for aztreonam or tobramycin.

Binding of antibiotics to faeces

The results presented in Fig. 3 show a linear correlation between the concentrations of aztreonam and temocillin before and after incubation with faecal suspensions. The slope of the regression lines varied between 43 and 50° and crossed the abscissa between −12 and +12 mg/l. It is concluded that aztreonam and temocillin were not bound by faecal suspensions.

The regression lines of gentamicin and tobramycin show that both were bound by the solid part of faeces. The binding capacity of faecal suspensions was dependent on the concentration of the added antibiotic and varied between individuals.

Release of gentamicin and tobramycin bound to faeces

Diluted faeces of subject 1 was incubated with 500 mg/l gentamicin or tobramycin. After 1 h incubation, 3·9 and 3·7 mg were bound to 1 g of faeces (wet wt.). Hereafter, the release of the bound antibiotics was determined. Fig. 4 shows that in 9 h more than 60% of tobramycin and more than 40% of gentamicin were released. From the results it is concluded that the binding of gentamicin and tobramycin is a reversible process.

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Table 1. *Minimal inhibitory concentrations (MIC) of antibiotics for Enterobacteriaceae and the SD factor of the human faecal flora of eight healthy subjects*

		MIC (mg/l)			
		E. coli†			
Subject	Antibiotic	1	2	*E. hafniae*	SD factors*
1	Aztreonam	0·125	—	—	192
	Temocillin	4	—	—	5·75
	Gentamicin	2	—	—	3·00
	Tobramycin	1	—	—	48·0
2	Aztreonam	0·125	—	0·125	104
	Temocillin	16	—	16	3·50
	Gentamicin	1	—	0·25	5·00
	Tobramycin	0·5	—	0·125	1222
3	Aztreonam	0·125	—	—	64·0
	Temocillin	16	—	—	1·31
	Gentamicin	2	—	—	2·50
	Tobramycin	4	—	—	13·5
4	Aztreonam	0·125	—	—	272
	Temocillin	8	—	—	2·25
	Gentamicin	2	—	—	15·5
	Tobramycin	4	—	—	193
5	Aztreonam	>128	—	0·125	<0·16
	Temocillin	>128	—	16	<0·48
	Gentamicin	0·125	—	4	3·75
	Tobramycin	0·25	—	4	26·5
6	Aztreonam	0·125	0·125	—	112
	Temocillin	1	2	—	26·0
	Gentamicin	2	16	—	1·94
	Tobramycin	2	4	—	182
7	Aztreonam	0·125	—	—	248
	Temocillin	16	—	—	9·75
	Gentamicin	0·25	—	—	8·00
	Tobramycin	4	—	—	26·5
8	Aztreonam	0·125	—	—	368
	Temocillin	16	—	—	0·88
	Gentamicin	2	—	—	3·50
	Tobramycin	2	—	—	29·0

* SD factor is the quotient of the concentration giving 50% inhibition of the obligately anaerobic flora and the MIC for Enterobacteriaceae.

† From faeces of subject 6 a non-haemolytic (1) and a haemolytic (2) strain of *E. coli* were isolated.

DISCUSSION

The success of SD of the digestive tract may be expected to depend on different susceptibilities for antibiotics of the Gram-negative facultatively anaerobic rods that are to be eliminated and the obligately anaerobic bacteria that must remain resident. In a previous study on the influence of neomycin and polymyxin B on

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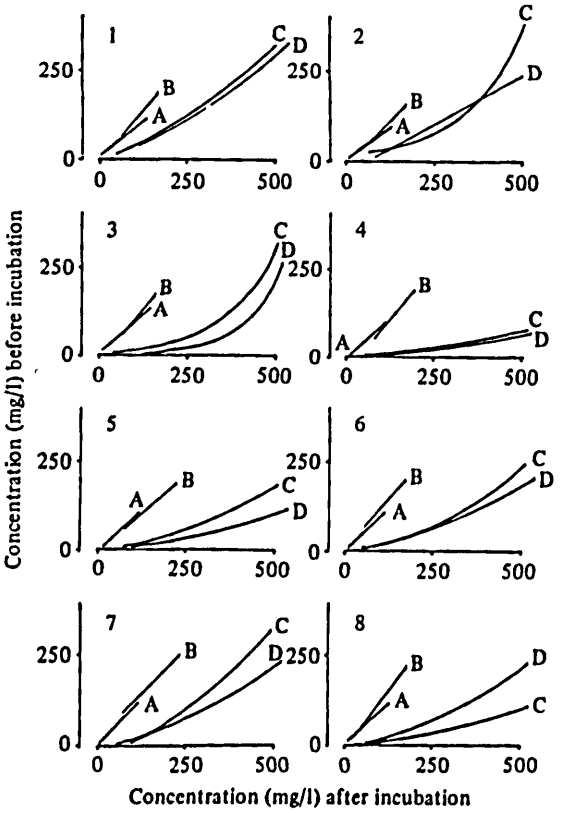

Fig. 3. Effect of incubation with diluted faeces of eight healthy subjects (1–8) on concentrations of aztreonam (A), temocillin (B), gentamicin (C) and tobramycin (D). For each antibiotic the regression line was determined from at least six data, $r^2 > 0{\cdot}90$, $P < 0{\cdot}002$.

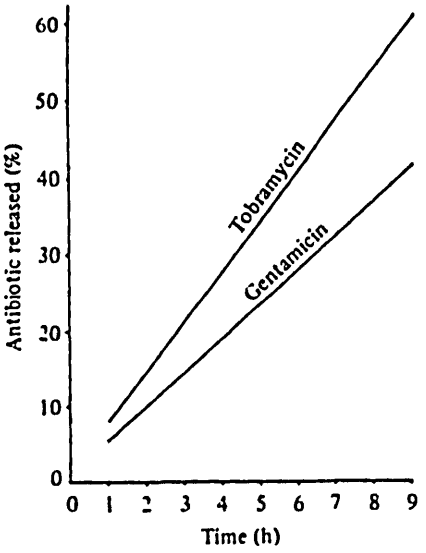

Fig. 4. Release of antibiotics bound to the solid part of faecal suspensions. Release is expressed as a percentage of the amount initially bound. For each antibiotic the regression line was determined from nine data, $r^2 > 0{\cdot}95$, $P < 0{\cdot}0005$.

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the intestinal flora of mice harbouring a human flora (Hazenberg *et al.* 1983*a*) we showed that this difference really existed and that the animals were selectively decontaminated. For estimation of the oral dose, however, binding of neomycin and polymyxin B to faeces had to be taken into account. A study with the faecal flora of eight subjects showed that both the susceptibility of the flora for neomycin and polymyxin B as well as the binding of antibiotics to faeces varied markedly between individuals (Hazenberg *et al.* 1984).

The present study shows that the obligately anaerobic floras of eight subjects were moderately sensitive to gentamicin, aztreonam and temocillin. The anaerobic flora of three subjects was very insusceptible to tobramycin. The results indicate that it is essential to determine individually the sensitivity of the obligately anaerobic flora for antibiotics used for SD.

The SD factor is introduced as an index of the difference in susceptibility of the obligate anaerobic flora and the Enterobacteriaceae for aztreonam, temocillin, gentamicin or tobramycin. The magnitude of the SD factor parallels the usefulness of the antibiotic for SD. A value of 1 indicates that the lowest concentration eliminating the Enterobacteriaceae also will eradicate 50% of the obligately anaerobic bacteria. If the SD factor exceeds 32 (considering an average slope of the regression lines of 45°) an antibiotic concentration can be chosen that does not affect the obligately anaerobic flora but will eliminate the Enterobacteriaceae. The SD factors of aztreonam and tobramycin meet this criterion and may therefore be used for SD. The usefulness of tobramycin for SD is in line with results of van der Waaij *et al.* (1982), who showed that tobramycin could be used for SD of mice in contrast to gentamicin. Another study by this group (de Vries-Hospers *et al.* 1984) showed that the administration of aztreonam (300 mg/day) to 10 healthy subjects resulted in the elimination of the Enterobacteriaceae in 8 persons. On the basis of microscopic bacterial counts in faeces the anaerobic flora was not appreciably influenced.

A strain of *E. coli* isolated from subject 5 was found to be resistant to aztreonam and temocillin. This resistance may be due to enzymic inactivation of both antibiotics or to altered receptors in the cell wall and needs further study.

Our study shows that gentamicin and tobramycin were bound by the solid part of faeces. The binding was not due to inactivation of the drugs since a time-dependent linear release was observed that could only be explained by reversible binding to the solid part of faeces. The binding and release of gentamicin after incubation with canine faeces was described by Wagman, Bailey & Weinstein (1974). We assume that the binding of antibiotics to the solid part of intestinal contents is an advantage for SD. The solid parts absorb and liberate antibiotics, thus maintaining a rather constant concentration of free antibiotic in the large bowel despite discontinuous intake.

We conclude that aztreonam and tobramycin are powerful tools for SD of the immune-compromised host and can be used like polymyxin B, the value of which was described in a previous study (Hazenberg *et al.* 1984).

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