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Effect of twelve antimicrobial drugs on the colonization resistance of the digestive tract of mice and on endogenous potentially pathogenic bacteria

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SUMMARY

Twelve antimicrobial drugs were studied for their effect on the endogenous aerobic potentially pathogenic bacteria (*Enterobacteriaceae*, *Streptococcus faecalis*) in the intestines and on the colonization resistance (CR) of the digestive tract. Three subclasses of antimicrobial drugs could be recognized: (1) those which suppress the CR following low oral doses (rifamycin, penicillin V, cloxacillin, fenethicillin); (2) those in which the CR is suppressed only following relatively high oral doses (amoxycillin); and (3) those in which no obvious suppression of the CR was noticed even following substantial oral doses (nalidixic acid, cinoxacin, co-trimoxazole, oral cephalosporins, piv-mecillinam and doxycyclin). Some of the drugs in the third category were found to suppress endogenous *Enterobacteriaceae* (nalidixic acid, co-trimoxazole, piv-mecillinam and doxycyclin) and *S. faecalis* (doxycyclin) at dose levels at which they did not decrease CR.

INTRODUCTION

In man, systemic antibiotic therapy often leads to 'bacterial overgrowth' in the digestive tract by resistant bacteria (Louria & Kaminski, 1962; Selden *et al.* 1971; Pollack *et al.* 1972; Hirsch, Burton & Blenden, 1974; Holzman *et al.* 1974). This unfortunate shift in the bacterial population appears to be the result of a decrease in the 'Colonization Resistance' (CR) of the digestive tract (Van der Waaij, Berghuis-de Vries & Lekkerkerk-Van der Wees, 1971). Colonization resistance is a mechanism in which host and anaerobic microflora co-operate in limiting the colonization of the digestive tract by potentially pathogenic micro-organisms. The quality of the CR can be measured by experimental oral contamination with potentially pathogenic bacteria and be expressed in the $\log_{10}$ of the oral dose of bacteria which results in colonization for a minimum of 2 weeks in 50% of a group of individuals (men or animals).

Anaerobic micro-organisms are susceptible to many antimicrobial drugs. Antibiotic treatment of infection with oral, partially absorbed antibiotics or with antibiotics excreted with the bile, may therefore lead to a decrease in CR. A low

CR permits yeasts and resistant bacteria to grow in the oropharynx and to high concentrations in the intestines; often to well over 10^8 bacteria/g faeces (Van der Waaij *et al.* 1971). This in turn may favour the transfer of R-factors between related species (Kasuya, 1964; Reed, Sieckmann & Georgi, 1969; Jones & Curtiss, 1970). Furthermore, a decrease in CR lowers the 'threshold contamination dose' for colonization of the digestive tract, which means that small numbers of resistant bacteria can colonize the intestines following oral contamination.

The objectives of this study were to investigate to what extent and at what dose levels a number of antimicrobial drugs decrease the colonization resistance of the digestive tract of mice, in other words cause a significant increase of the concentration of resistant micro-organisms in the faeces.

Previous experiments (Van der Waaij *et al.* 1971, 1972) have indicated that the mouse provides an ideal experimental model for the study of the influence of antibiotics on the CR; in the mouse a correlation has been shown between CR and the concentration of a resistant bacterium in the faeces, the relative weight of caecum (Koopman, Janssen & Druten, 1977), and the concentration of a dipeptide beta-aspartylglycine (Welling, 1979).

MATERIALS AND METHODS

Mice

Conventional female Swiss mice of 8–12 weeks with a mean body weight of 28 g were used. The animals were housed four per cage. Food pellets and water were supplied *ad libitum*, and during the antimicrobial treatment period the respective drugs were added to the drinking water. The daily water intake was determined every other day in each cage.

Antibiotic treatment

The following drugs were used: nalidixic acid, cinoxacin, co-trimoxazole, ryfamicin SV, doxycyclin, cephalexin, cefaclor, amoxycillin, fenethicillin, penicillin V, cloxacillin and piv-mecillinam. For each drug tested (except for nalidixic acid and cinoxacin, in which case more animals were used) 20 mice were subdivided in 5 subgroups of four animals. During the first two weeks the animals were not treated, only monitored bacteriologically. In the following 4 or 5 weeks, the drugs were supplied in the drinking water. Different doses were given to each of the four subgroups (nos. 1, 2, 3 and 4). One subgroup remained untreated to serve as a control. The various doses applied are presented in Table 1. To estimate the mean daily drug intake per mouse the water intake was determined every other day per cage. Because piv-mecillinam is unstable in solution the suspensions were prepared fresh twice daily.

Oral contamination

The mice were contaminated on the first day of the fifth week of the experiment (third week of treatment) with 0·2 ml of an overnight broth culture of a resistant strain of *Escherichia coli* containing approximately 10^9 bacteria per ml. If the contaminant did not 'take', this procedure was repeated twice with other resistant strains at intervals of one week.

Caecal weight

At the end of the experiment, i.e. after four (occasionally five) weeks of treatment the body weight as well as the wet weight of the caecum with its contents were determined.

Bacteriological procedures

Fresh faeces were collected three times a week from each mouse. The faeces of each individual animal was suspended in Brain Heart Infusion (BHI) broth (Difco) in the proportion of 1 unit to 10 volumes of suspension. These suspensions were subsequently serially diluted (1:10) in duplicate for eight – if necessary twelve – steps in BHI broth. After overnight incubation at 37 °C each dilution was subcultured on MacConkey agar for the isolation and enumeration of *Enterobacteriaceae*. Inoculation on aesculin-azide agar provided information concerning the concentration of *S. faecalis*. From the results, the mean faecal concentrations of the endogenous *Enterobacteriaceae* and *S. faecalis* could be calculated per group of four animals in each week. The procedure for isolation of *Enterobacteriaceae* and their subsequent (bio)typing for inventory, has been described previously (Van der Waaij *et al.* 1972).

RESULTS

The effect of different doses of the antimicrobial drugs tested on the concentration of the *Enterobacteriaceae* and *S. faecalis* in the faeces, are presented in Tables 1, 2 and 3. In Table 1 an example is given of the results obtained with antimicrobial drugs that do not affect the CR in mice, even following treatment with high doses. In Table 2 the results are shown of an antibiotic which only decreases the CR following moderate and high daily doses, while in Table 3 a representative example is given of those drugs which were found to decrease the CR even following low dose treatment. Full data of all twelve drugs investigated are deposited with the editors of this Journal.

An influence of the drugs on the colonization resistance of the digestive tract can be deduced from an increase of the relative caecal weight, from the concentration of a resistant *E. coli* strain used for oral contamination and from the concentration of *S. faecalis* in the faeces in weeks 3 and 4 of the experiment when drugs were used for which enterococci are naturally resistant.

Oral administration of nalidixic acid reduced the concentration of endogenous *Enterobacteriaceae* in the faeces when it was given in daily doses of more than 1·6 mg/mouse. In the second week of oral treatment no *Enterobacteriaceae* could be cultured from faeces of mice which were given a daily dose of 3·8 mg/mouse or more. Nalidixic acid did not influence the concentration of *S. faecalis* at any dose level, the resistant *E. coli* did not grow out to high concentrations and the relative caecal weight did not increase. In one treatment group the mean relative caecal weight (daily drug dose 14 mg/mouse) was slightly higher than in the control groups.

Cinoxacin also reduced the concentration of endogenous *Enterobacteriaceae* at a

Table 1. *The influence of various antimicrobial drugs (Group I) on the endogenous flora during oral administration*

	Trimethoprim-Sulfamethoxazole (co-trimoxazole) Daily dose administered orally in mg/mouse					
	0/0	0·11/0·58	0·24/1·26	0·35/1·84	0·52/2·70	1·10/*5·80
Mean log conc.† (SD) of endogenous *Enterobacteriacae* in week 1+2	4·0 (1·2)	4·1 (1·0)	4·2 (1·0)	5·0 (1·4)	3·8 (1·1)	0·8 (1·1)
Mean log conc. (SD) of endogenous *Enterobacteriacae* in week 3+4	4·8 (1·2)	2·9 (1·3)	2·2 (1·7)	2·3 (1·2)	0·9 (1·3)	0 (0)
Mean log conc. (SD) of the resistant *Enterobacteriacae* in week 5	1·7 (1·7)	3·1 (1·9)	3·4 (1·6)	1·9 (2·0)	3·1 (2·4)	3·2 (2·0)
Mean log conc. (SD) of *Streptococcus faecalis* in week 1+2	4·3 (0·7)	4·5 (1·0)	4·5 (0·9)	4·8 (0·7)	4·6 (1·1)	4·7 (0·8)
Mean log conc. (SD) of *Streptococcus faecalis* in week 3+4	5·1 (1·1)	4·9 (0·4)	4·7 (1·0)	4·5 (0·6)	5·0 (1·1)	5·1 (1·0)
Mean log conc. (SD) of *Streptococcus faecalis* in week 5	6·0 (1·4)	6·6 (1·1)	5·9 (1·2)	5·8 (1·3)	5·7 (1·1)	5·9 (1·3)
Mean (SD) body weight	27·2 (4·5)	29·7 (2·7)	28·8 (1·0)	28·6 (3·2)	28·6 (2·0)	28·5 (2·2)
Mean relative caecal weight (SD)	1·8 (0·2)	1·8 (0·1)	1·9 (0·2)	1·7 (0·2)	2·1 (0·3)	

* Trimethoprim/sulfamethoxazole.
† Concentration = number/g faeces.

daily dose of more than 1·6 mg/mouse, but *Enterobacteriaceae* could still be detected in the faeces at the end of the second treatment week. Only when a dose of 15·2 mg/mouse was applied did the endogenous gram-negative bacteria disappear from the faeces. The concentrations of *S. faecalis* and the relative caecal weights were not affected by the cinoxacin treatment at any dose level; the resistant *E. coli* did not differ in concentration in comparison with the control group at any dose level.

The application of co-trimoxazole decreased the concentration of endogenous *Enterobacteriaceae* at all doses, notwithstanding the fact that the daily doses appeared to be much smaller than desired, due to the poor solubility of trimethoprim in water. In the fourth week *Enterobacteriaceae* were still detectable in the low treatment groups. Co-trimoxazole did not change the concentration of *S. faecalis* in the faeces, the resistant *E. coli* did not show evidence of overgrowth and the relative caecal weight of the treated mice did not differ from that of the untreated control animals (Table 1).

Cefaclor reduced the concentration of endogenous *Enterobacteriaceae* only slightly in mice given the two highest doeses (2·28 and 4·56 mg/mouse). This reduction was not significant and also occurred in the untreated mice. The concentration of *S. faecalis* was not influenced by cefaclor treatment and resistant *E. coli* did not 'take'. There was also no influence on the relative caecal weight.

Cephalexin had neither an effect on the endogenous *Enterobacteriaceae* nor on the *S. faecalis* concentrations. The resistant *E. coli* did not 'take'. The relative caecal weight was not changed after treatment.

Piv-mecillinam significantly decreased the concentration of endogenous *Enterobacteriaceae* in mice treated with 2·0 and 2·5 mg/mouse/day, but did not influence the *S. faecalis* concentrations. The resistant *E. coli* did not grow to high concentrations and was isolated in the same concentrations as in the control group. The relative caecal weight did not increase, even in animals treated with high doses.

Doxycyclin reduced the concentration of endogenous *Enterobacteriaceae* from $10^{4\cdot5}$ to $10^{1\cdot8}$ per 0·1 g faeces when a daily dose of 0·34 mg/mouse or more was given; at doses of 0·7 and 1·2 mg/mouse *Enterobacteriaceae* were completely suppressed in the second week of treatment. The concentration of *S. faecalis* was reduced to an equal extent in the faeces of mice that were treated with 0·34 mg/mouse and it was absent at the end of the third week of treatment (week 5). However, the resistant *E. coli* strain colonized the intestine more easily than in the control mice; unlike the control group it was still present in the faeces two weeks after oral contamination in all treated mice, but not in high concentrations. The relative caecal weight was not significantly increased even during treatment with the highest dose (1·2 mg/mouse).

The oral administration of amoxycillin did not affect the concentration of endogenous *Enterobacteriaceae* and *S. faecalis* at any dose level. Its effect on the anaerobic bacteria was apparently stronger since in mice treated with 1·6 mg/mouse or more the relative caecal weight was found to be significantly increased and the amoxycillin-resistant *E. coli* used for experimental contamination was recovered from the faeces in high concentrations (Table 2).

Oral administration of penicillin V resulted in increased concentrations of

Table 2. *The influence of various antimicrobial drugs (Group II) on the endogenous flora during oral administration*

	Amoxycillin Daily dose administered orally in mg/mouse					
	0	0·41	0·82	1·60	2·50	3·00
Mean log conc.* × (SD) of endogenous *Enterobacteriacae* in week 1 + 2	4·7 (0·6)	4·6 (0·7)	4·6 (0·7)	4·6 (0·7)	4·3 (0·6)	4·3 (0·7)
Mean log conc. (SD) of endogenous *Enterobacteriacae* in week 3 + 4	4·6 (0·8)	4·6 (0·9)	5·6 (2·2)	4·9 (2·6)	4·6 (3·4)	3·7 (3·7)
Mean log conc. (SD) of the resistant *Enterobacteriacae* in week 5	1·7 (1·6)	1·9 (1·6)	5·5 (2·1)	7·2 (2·0)	7·4 (1·1)	8·3 (0·6)
Mean log conc. (SD) of *Streptococcus faecalis* in week 1 + 2	6·0 (1·0)	6·0 (0·7)	5·8 (0·9)	5·6 (0·8)	6·0 (0·5)	5·2 (0·7)
Mean log conc. (SD) of *Streptococcus faecalis* in week 3 + 4	5·6 (0·9)	5·5 (1·1)	4·3 (1·5)	4·7 (2·5)	2·9 (2·0)	3·3 (3·5)
Mean log conc. (SD) of *Streptococcus faecalis* in week 5	6·2 (1·1)	3·4 (1·4)	2·2 (1·7)	5·3 (1·5)	5·3 (1·7)	5·7 (1·3)
Mean (SD) body weight	26·5 (1·7)	27·4 (1·2)	25·4 (0·7)	26·6 (2·4)	27·6 (1·5)	28·2 (2·4)
Mean relative caecal weight (SD)	1·4 (0·2)	1·6 (0·3)	2·2 (1·1)	2·0 (0·5)	2·9 (0·2)	2·8 (0·5)

* Concentration = number/g faeces.

endogenous *Enterobacteriaceae* when daily doses of 0·5 and 1·0 mg/mouse were applied. At the same dose level, however, the *S. faecalis* concentrations decreased. The resistant *E. coli* persisted only at relatively high concentrations in the faeces of mice treated with the highest doses used, i.e. 0·5 and 1·0 mg/day. In addition the relative caecal weight of mice treated with a daily dose of 0·5 and 1·0 mg/mouse was significantly higher than in the control animals.

Treatment with cloxacillin resulted in a strong increase in the endogenous *Enterobacteriaceae* concentration when the mice received 0·6 and 2·6 mg/day, while treatment with doses of 4·9 and 9·6 mg/mouse caused an obvious decrease. The same occurred with the *S. faecalis* concentrations. In the third week of treatment the concentration of *S. faecalis* was significantly increased in comparison with initial values in all treatment groups. The oral contaminant *E. coli* was found in high concentration in all treated mice and the relative caecal weight was higher than in the untreated animals (Table 3).

Fenethicillin treatment significantly increased the concentration of endogenous *Enterobacteriaceae* at a daily dose level of 0·7 mg/mouse or more. The *S. faecalis* concentration was not influenced by this treatment. The resistant *E. coli* grew to high concentrations in animals given 0·7, 1·3 and 2·5 mg daily, and the relative caecal weight was also slightly higher in these groups although the difference was only significant in animals given the highest doses.

Rifamycin SV slightly increased the concentration of endogenous *Enterobacteriaceae* in the faeces of the mice which received 0·08 and 0·15 mg/day. The mean concentration of *Enterobacteriaceae* decreased in the group receiving a daily dose of 0·6 mg/mouse. However, in two mice of this group this concentration was increased in the first and second week of treatment. In mice given the highest daily dose (1·2 mg/mouse) endogenous *Enterobacteriaceae* soon disappeared. The *S. faecalis* concentrations in mice receiving a daily dose of 0·6 mg/mouse or more were strongly decreased in the first and second week. In the third week of treatment this concentration had increased although not back to normal values. The resistant *E. coli* strain grew to relatively high concentrations at dose levels at or above 0·15 mg/mouse. The relative caecal weight was significantly increased in all treatment groups receiving a dose of 0·15 mg/mouse or more.

DISCUSSION

The results of this study support a conclusion drawn in an earlier report (Van der Waaij, 1979), namely that antimicrobial drugs can apparently be grouped in three major classes as far as their influence on the colonization resistance of the digestive tract is concerned. On the basis of the present results we can distinguish:

(1) antimicrobials which do not affect CR even during treatment with excessively high oral doses (Table 1);

(2) antimicrobial drugs which do not affect CR during treatment with low doses, but in which evidence was obtained of a decrease in CR following high doses (Table 2);

(3) antimicrobial drugs which decrease the CR even when given at low oral doses of 0·5 mg/mouse/day (Table 3).

Table 3. *The influence of various antimicrobial drugs (Group III) on the endogenous flora during oral administration*

	Cloxacillin Daily dose administered orally in mg/mouse				
	0	0·66	2·60	4·90	9·60
Mean log conc.* (SD) of endogenous *Enterobacteriaceae* in week 1+2	3·5 (0·9)	3·8 (0·9)	3·6 (0·8)	3·6 (1·1)	3·6 (1·1)
Mean log conc. (SD) of endogenous *Enterobacteriaceae* in week 3+4	3·7 (0·7)	5·7 (2·1)	6·1 (2·6)	1·7 (2·6)	1·4 (2·3)
Mean log conc. (SD) of the resistant *Enterobacteriaceae* in week 5	1·5 (1·1)	6·2 (2·0)	7·2 (0·6)	7·2 (0·9)	7·1 (0·9)
Mean log conc. (SD) of *Streptococcus faecalis* in week 1+2	5·4 (0·8)	4·4 (0·7)	5·3 (0·7)	3·9 (0·9)	5·5 (0·8)
Mean log conc. (SD) of *Streptococcus faecalis* in week 3+4	5·7 (0·6)	6·2 (2·0)	4·5 (2·7)	2·0 (1·4)	2·7 (1·5)
Mean log conc. (SD) of *Streptococcus faecalis* in week 5	5·9 (0·7)	7·6 (0·6)	7·6 (0·6)	8·0 (0·8)	6·1 (1·9)
Mean (SD) body weight	27·9 (1·2)	28·1 (1·1)	28·3 (2·7)	27·8 (3·2)	26·6 (1·3)
Mean relative caecal weight (SD)	2·0 (0·5)	2·9 (0·4)	2·8 (0·4)	3·2 (0·3)	2·7 (0·3)

* Concentration = number/g faeces.

Rifamycin and mainly oral penicillins were found to belong to the last group. The endogenous *Enterobacteriaceae* were only suppressed during high-dose rifamycin treatment. Endogenous *S. faecalis* was suppressed during treatment with high doses of penicillin V, cloxacillin and rifamycin. Both oral cephalosporins, cephalexin and cefaclor acted in the same way as cephradine in a previous study (Thijm & Van der Waaij, 1979). They did not influence CR and were not suppressive to endogenous (sensitive) enterobacteria. Absorption in the small intestines is apperently more or less complete, which prevents active concentrations being established during treatment, even during high-dose oral treatment.

Piv-mecillinam apparently reached the colon in suppressive concentrations, since even following oral doses of 0·5 mg/mouse/day the endogenous *Enterobacteriaceae* were suppressed; oral treatment with 4 to 5 times higher doses made this suppression statistically significant. *S. faecalis* concentration in the faeces did not change during piv-mecillinam treatment. This finding is in accordance with the M.I.C. of *S. faecalis* for this drug ($> 25\ \mu g/ml$) whereas the M.I.C.s of the Gram-negative bacteria were lower than $5\ \mu g/ml$. Consequently, during piv-mecillinam treatment concentrations of less than $25\ \mu g/g$ intestinal contents may have existed, which were apparently *not* suppressive to the anaerobic bacteria responsible for CR. Doxycyclin was suppressive to the endogenous Gram-negatives and to *S. faecalis* and yet did not decrease CR. In man this drug has been reported to promote acquisition of resistant bacteria following doses of 200 mg per day (Hinton, 1970). Perhaps this reflects the fact that the susceptibility of the mouse anaerobic flora – which differs in composition from that of man – for doxycyclin is lower than that of man.

Co-trimoxazole was only suppressive to the Gram-negative bacilli; *S. faecalis* did not respond to the doses given. No evidence of any effect on CR was noticed following the highest oral dose given to mice. This suggests that this drug, like nalidixic acid and perhaps piv-mecillinam and doxycyclin can be considered for selective decontamination of the digestive tract of mice (Van der Waaij *et al.* 1974). Selective decontamination has appeared feasible as prophylactic treatment in experiments in which the resistance of animals to infections is severely decreased (Heidt, 1979). Its efficiency in clearing the digestive tract of sensitive, aerobic, potentially pathogenic micro-organisms depends on the difference in susceptibility between the latter and the anaerobes responsible for CR (Emmelot & Van der Waaij, 1980).

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