J. Hyg., Camb. (1974), **73**, 205
Printed in Great Britain

Elimination of bacteria from dogs with antibiotics*

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(*Received* 5 *March* 1974)

SUMMARY

The effect of oral administration of neomycin cephalothin or kanamycin cephalothin on the aerobic intestinal bacterial flora, was studied in dogs maintained under isolation conditions in a conventional animal room. The dogs were successfully freed of aerobic bacteria with both combinations within two to seven days after the start of antibiotic treatment, and were maintained bacteria free for up to 21 days. Decontamination was attained more rapidly in dogs that were bathed in hexachlorophene surgical soap before and during the first and third days of antibiotic treatment. There was no evidence of toxicity from either of the antibiotic combinations. These results indicate that, as with mice and monkeys, decontamination of dogs with oral antibiotics is feasible. The technique is of potential value in preventing endogenous bacterial infections in canine experimental studies involving use of immunosuppressive agents.

INTRODUCTION

Bacterial infections are a principal cause of morbidity and mortality in conventional experimental animals that have been subjected to immunosuppression (Abaza, Nolan, Watt & Woodruff, 1966). Similarly, after receiving immunosuppressive agents in conjunction with organ transplants or cancer therapy, patients are also highly susceptible to endogenous infections from potentially pathogenic micro-organisms in their microflora (Rifkind, Marchioro, Waddell & Starzl, 1964; Remington, 1972). Protection of patients and experimental animals against such infections is best provided by selectively eliminating the causative bacteria with antibiotics, combined with simultaneous strict isolation to prevent recontamination (van der Waaij & Andreas, 1969; Vossen & van der Waaij, 1972). Protective isolation systems for this purpose are available and are being used increasingly in experimental medicine and medical practice (Levitan & Perry,

* This work was aided in part by grants from the Animal Resources Branch, DRR, NIH (RR 00200, RR 05001).

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1968; McGarrity *et al.* 1969; Barnes & Tuffrey, 1968; Levitan, Schulte, Strong & Perry, 1967; Dietrich, Fliedner & Krieger, 1973). However, the techniques for selective elimination of potentially pathogenic bacteria with antibiotics are complex; they have been well studied in only a relatively few animal species. Furthermore, the toxicity of certain of the antibiotics could be a limiting factor in their use for the extended periods required to achieve decontamination.

Oral, nonabsorbable antibiotics have been used successfully in mice (van der Waaij & Sturm, 1968; Heit, Wilson, Fliedner & Kohne, 1973) and in monkeys (van der Waaij, de Vries & Lekkerkerk, 1970) to eliminate bacteria from the digestive tract. In mice, coincident with the decontamination of the digestive tract, the skin flora disappeared in the first 4 weeks of antibiotic therapy except for a few bacillus species (van der Waaij & Sturm, 1971). In monkeys, the skin microflora largely disappeared in 48 hours after antibiotic therapy was started (van der Waaij *et al.* 1970). This study was undertaken to determine whether dogs can be similarly decontaminated, and whether specific antibiotics can be administered without toxic effects for the time required to eliminate the aerobic intestinal microflora.

MATERIALS AND METHODS

Animals, animal care, and housing

Ten healthy, mongrel, male and female dogs, estimated to be 6–36 months of age, and weighing 4–14·5 kg. were used. Each dog was isolated and caged individually in a separate conventional animal room. A freshly washed stainless-steel cage, equipped with a sterilized feeder and waterer was provided daily. Sterilized food and water also were provided daily. The principal investigator was the only human contact; a sterile gown and gloves and a face mask were worn whenever the dogs were handled. Three of the ten dogs were bathed with hexachlorophene surgical soap before treatment with antibiotics, and during the first and third days of treatment. They were scrubbed vigorously with a soft-bristle surgical scrub brush for 10 min. and rinsed with tap water.

Antibiotics

Combinations of neomycin-cephalothin, and kanamycin-cephalothin were used. The minimal effective dose of each antibiotic to achieve decontamination of the gastrointestinal tract was determined by a two-phase sensitivity test identical with that described previously for use in monkeys (van der Waaij *et al.* 1970).

The calculated dose of antibiotics was administered orally three times daily in a suspension, in small amounts, over a 10 min. period. In addition, 500,000 units of nystatin (USP) were administered three times daily to control fungi. Nystatin treatment was started 3–4 days before antibiotic treatment was initiated. Previous experience indicated that elimination of yeasts and fungi from the gastrointestinal tract could be accomplished more readily in the presence of the enteric flora. On the first day of treatment, oropharyngeal cultures were taken at 30 min., 60 min., and hourly thereafter for 6 hr. after the antibiotics were administered to determine whether the combination was effective.

Test samples

During the decontamination period, cultures were obtained daily from the skin of the back, feet, perirectum, and chest; from the oral cavity, and from the rectum. Cotton-tipped swabs sterilized in tubes containing 10 ml. brain heart infusion broth were used. After incubation for 24 hr. at 37° C, the cultures were subinoculated on Staphylococcus 110, buffered azide glucose glycerol agar (BAGG), and Endo selective media. The subcultures were incubated for 24 hr. at 37° C. and the results recorded.

Blood and fresh faeces were collected weekly. Blood and serum were examined for changes indicative of antibiotic toxicity. Fifteen ml. of heparinized blood was collected weekly for determination of white blood cells (WBC), red blood cells (RBC), haemoglobin, and blood urea nitrogen (BUN). Total protein, serum glutamic oxalacetic transaminase (SGOT), and alkaline phosphatase were also measured (Table 1). Samples of serum and faeces were frozen at −20° C. for subsequent measurement of antibiotic concentration. Twenty-four-hour bacterial cultures were prepared in final dilutions of 10^5 *Escherichia coli* organisms/ml. in tryptose broth containing tetrazolium (Goss & Cimijotti, 1968). Two strains of *E. coli* were used; a cephalothin resistant-neomycin sensitive strain, and a cephalothin sensitive-neomycin resistant strain. This made it possible to determine the concentration of the antibiotic components separately. Stock dilutions of neomycin and cephalothin were made in concentrations of 100, 50 and 25 mg./ml., and each of these concentrations was serially diluted four steps in 9 ml. of brain heart infusion broth. The bacterial cultures were tested for susceptibility to the antibiotics as follows: 0·05 ml. tryptose broth was added to each well of the microtiter plate; 0·05 ml. of each antibiotic concentration (12 concentrations) was added to two wells in column 1 of the plates; these wells were mixed by to-and-fro rotation of the microdiluters; the microdiluters then were transferred to the well in column 2 and mixed. With the same technique, serial dilutions were made from column 2 through 11; 0·05 ml. of the 10^5 bacterial culture was added to each well in columns 1–11. Column 12 was a media control. The plates were sealed with cellophane tape, incubated aerobically for 24 hr., and examined. The first clear cell in each row was considered the minimum inhibitory concentration (MIC). The results were plotted on a semilog scale and used as the standard for reading the MIC for the test.

The determination of the antibiotic concentration in the faeces and serum was carried out after the samples were thawed. The faecal samples were diluted 1/10 in tryptose broth; the serum was not prediluted. The tests were performed as described above, substituting 0·05 ml. of sample for the antibiotic.

RESULTS

Eight of the ten dogs used in this study were successfully freed of bacteria that could be demonstrated by standard aerobic culture techniques (Table 2). Complete decontamination was achieved 2–7 days after the start of antibiotic treatment. Thereafter, with continuous administration of antibiotics, the dogs were maintained free of bacteria for up to 21 days, except for an occasional contaminant

Table 1. *Haematological and blood chemistry values during antibiotic decontamination*

Dog no.	Days after start of treatment	WBC (10^3/mm.^{3})	RBC (10^6/ mm.^{3})	HB (g./100 ml.)	BUN (mg./ 100 ml.)	Total protein (g./ 100 ml.)	SGOT (I.U.)	Alk. phos-phatase (I.U.)
1	0	18·5	7·6	16·0	15	6·2		
	7	15·2	8·8	16·5	15	6·1	21	28
	14	13·5	7·9	17·0	10	6·2	32	36
2	0	12·8	8·0	17·0	16	7·0		
	7	13·3	8·5	18·0	10	6·2		
	14	13·8	7·9	17·0	15	5·8	28	33
3	0	32·0*	8·1	18·0	15	5·0	40	41
	7	18·7	8·0	17·0	18	6·1		
	14	18·4	8·5	15·6	20	6·2	33	34
4†	0	11·3	7·9	17·0	10	6·2	29	14
	7	18·4	6·8	17·4	15	5·8	26	13
	14	7·6	6·8	15·0	10	6·4	30	34
5	0	11·1	6·1	15·5	15	5·1		
	7	9·8	7·5	11·9	19	4·7		
	14	12·1	5·6	14·0	16	7·3		
6†	0	20·0	8·4	14·5	10	7·2		
	7	21·5	8·4	17·2		7·0	61	75
	14	20·8	7·4	16·4	17·5	6·2	50	147
7	0	12·5	7·7	17·9	3·6	5·6	35	25
	7	13·3	8·0	14·4	9	5·8	78	59
	14	NA‡	NA	NA	NA	NA	NA	NA
8	0	21·6	8·0	16·5	19·8	6·1	50	162
	7	9·9	6·7	15·7	11·2	6·3	52	29
	14	NA	NA	NA	NA	NA	NA	NA
9†	0	6·4	6·8	15·0	17·2	5·4	38	30
	7	6·6	7·1	16·5	16·4	5·3	35	25
	14	5·9	6·7	15·2	28·8	5·1	45	37
10	0	9·9	9·2	17·4	13·0	6·5	25	62
	7	16·0	8·2	17·5	13·4	6·4	21	54
	14	9·8	9·8	17·9	15·8	6·7	31	71

* No clinical evidence of disease or infection was noted in this dog.
† Bathed with hexachlorophene surgical soap.
‡ Not available.

from the room or one introduced by faulty technique. Two dogs remained positive for bacteria throughout the treatment period; one developed resistance to the antibiotic combination, and another inadvertently received less than the calculated dose of antibiotics (Table 2). Age and sex had no apparent effect on decontamination. Weight was a major factor in determining the dose of antibiotic but did not otherwise affect the decontamination procedure.

Decontamination of the three dogs that were bathed with hexachlorophene surgical soap was attained more rapidly than was decontamination of unbathed dogs; two to four days were required but unbathed dogs required from four to seven days to become decontaminated (Table 2). Serum antibiotic concentrations

Table 2. *Decontamination of dogs with antibiotics*

Dog no.	Est. a (mont	Weight (kg.)	Antibiotic combination	Dose of each antibiotic (g. TID)	Total antibiotic concentration		Decontamination status		Days quired
					Faeces (μg./g.)	Serum (μg./ml.)	GI tract	Skin	
1	12	9·0	Neomycin-cephalothin	1·0	150	5	C	C	5
2	6	6·0	Kanamycin-cephalothin	0·5	300	8	C	C	4
3	36	14·5	Neomycin-cephalothin	1·0	80	5	I	I	I
4*	12	4·0	Neomycin-cephalothin	0·5	150	5	C	C	4
5	9	6·5	Neomycin-cephalothin	1·5	90	5	I	I	I
6*	36	11·0	Neomycin-cephalothin	2·0	150	8	C	C	2
7	12	9·0	Neomycin-cephalothin	1·5	650	8	C	C	7
8	18	11·0	Kanamycin-cephalothin	1·0	300	5	C	C	6
9*	15	4·5	Neomycin-cephalothin	0·5	150	8	C	C	3
10	12	11·0	Neomycin-cephalothin	1·0	300	5	C	C	7

C = Complete decontamination.
I = Incomplete decontamination.
* Bathed with hexachlorophene surgical soap.

ranged from 5 to 8 μg./ml. Faecal antibiotic concentrations ranged between 150 and 650 μg./g. in the eight dogs in which decontamination was achieved.

A direct relation was found between decontamination of the gastro-intestinal tract and elimination of bacteria from the skin. The bacteria isolated from the skin after treatment had begun were identical with those isolated from the gastro-intestinal tract. Evidence of contamination of the gastro-intestinal tract with normal skin flora such as staphylococci was not found. No changes in blood or clinical chemistry values, suggestive of antibiotic toxicity, were found (Table 1).

DISCUSSION

These results indicate that decontamination of dogs with orally administered antibiotics is feasible. Housing the animals in conventional rooms posed no particular problems when strict hygienic practices were followed. Strict isolation of the animal was deemed essential from the time of sampling the faeces through the decontamination period, to prevent bacterial colonization of the gastrointestinal tract with a contaminant having a different sensitivity pattern. Hexachlorophene surgical soap baths reduced the time for decontamination while the methods of strict isolation and careful handling reduced the chances of recontaminating the skin and hair coat with faecal bacteria.

Neomycin and cephalothin, although classified as nonabsorbable, are minimally absorbed in the dog, as they are in man and monkeys (van der Waaij *et al.* 1970). Neomycin was used to control Gram-positive and certain Gram-negative organisms; cephalothin was used for its broader spectrum of bactercidal activity. Gentamycin, bacitracin and several other antibiotics also are minimally absorbed, but they are more expensive. Oral antibiotics that are nonabsorbed or are only minimally absorbed are preferred, to limit the possibility of immunosuppression stemming from the antibiotic therapy itself.

The faecal antibiotic concentration should be measured at least twice weekly, to provide an index of the minimal concentration needed to inhibit bacterial growth. A continuing concentration of antibiotics in the faeces of at least 100 μg./g. is essential (van der Waaij *et al.* 1970). The dose of antibiotics can be reduced as the faecal bacterial population is reduced, provided the faecal antibiotic concentration does not fall below 100 μg./g.

The study of antibiotic decontamination not only involves bacteriological feasibility, but also its effect on organ functions and possible toxicity. If antibiotic decontamination is applied in organ transplantation, toxic effects of the antibiotics used must be avoided because transplanted organs such as the liver or the kidneys are particular target organs of these antibiotics. It is known from studies in man (Vossen, Dooren & van der Waaij, 1973) and in monkeys (Hendriks, personal communication) that, although so-called nonabsorbable antibiotics are employed for decontamination, toxic alterations in tissue and organ enzymes or in serum proteins may follow oral administration. These alterations can sometimes be measured in serum, and, when properly evaluated, may be an important aid to diagnosis of toxic effects. The antibiotic concentration in serum should be measured

at least weekly because such measurements may give the first indications of approaching toxicity, which should be avoided. Serum concentrations observed during our investigation were between 0 and 8 μg./ml. The exact toxic concentration for dogs needs further investigation; however, values above 15 μg./ml. for neomycin, 25 μg./ml. for kanamycin (Cohn, 1958) and 50 μg./ml. or higher for cephalothin (Venuto, Stein & Ferris, 1972) are usually considered as approaching the toxic range and should be avoided. Toxicity may result from reduced organ function, as in the case of an antibiotic that is normally detoxified by that organ. It may also indicate a dose above the threshold for that organ, or increased absorption from the gastrointestinal tract (Loomis, 1968).

The erythrocyte count and blood chemistry values remained relatively stable in the dogs during antibiotic decontamination, with no consistent changes in any of the values (Table 1). Our observations on the total WBC count show a similar pattern during decontamination to that described for monkeys; total WBC counts in monkeys were shown to decrease slightly after an initial increase, during the first few days of antibiotic treatment (van der Waaij *et al.* 1970). These changes were accompanied by a transitory rise in lymphocytes and a fall in granulocytes. If dog 3 (Table 1) is excluded because of his abnormally high initial WBC count, which we cannot explain, and dogs 7 and 8 are excluded because of the unavailability of WBC counts for day 14, the average counts of the seven other animals at days 0, 7 and 14 also show a peak at day 7. Data about the ratio between lymphocytes and granulocytes during antibiotic decontamination are not available. It seems likely, however, that the higher elevation of the WBC in the first week in the dogs can also be attributed to a rise in the lymphocyte count.

Behavioural and environmental factors can significantly influence the decontamination process. Dogs that walk or rest in their faeces can readily recontaminate themselves as long as bacteria are present in the faeces. Dogs that salivate profusely may dilute and expel the antibiotic before it reaches the base of the oral cavity; others may not permit the antibiotic to remain in the oral cavity for an adequate time. In dealing with this problem we found it helpful to make a suspension of the drugs and to administer the suspensions in small quantities over longer intervals until the calculated dose was given. Dogs requiring longer periods for decontamination were believed to have had less than an adequate dose of antibiotics or less than adequate contact time with the antibiotic.

The feasibility of this technique suggests that it could usefully be applied in preventing endogenous bacterial infections such as may occur in experimental studies involving immunosuppression (Abaza *et al.* 1966).

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