Effect of disturbance of the gastrointestinal microflora on the faecal excretion of *Fusobacterium necrophorum* biovar A

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(Accepted 8 October 1992)

SUMMARY

Oral pretreatment of mice with either a mixture of kanamycin and erythromycin or metronidazole to modify the gut microflora greatly enhanced the faecal excretion of *Fusobacterium necrophorum* biovar A given by mouth. This lends support to the suggestion that disturbance of the gastrointestinal microflora in animals such as cattle, which often carry the organism in the rumen, may lead to intestinal multiplication and faecal excretion, thereby providing a source of infection that may lead to necrobacillosis of the body surface.

INTRODUCTION

Faecal excretion of *Fusobacterium necrophorum* biovar A is believed to provide the primary source of infection in necrobacillosis of the body surface of animals, for example bovine foot rot. However, the overall prevalence of biovar A in the faeces of cattle, deer and wallabies, animals in which the disease is well known, was found to be low, though the organism was commonly present in the rumen of young beef cattle; a predisposing factor of some kind, possibly disturbance of the gastrointestinal microflora, appeared to play an important role in faecal excretion [1].

Oral antibiotic treatment may, by suppressing the competing microflora of the gut, encourage the multiplication of intestinal bacteria whose growth is normally held in check. Thus pseudomembranous colitis in man results from the overgrowth of antibiotic-resistant *Clostridium difficile* after antimicrobial treatment [2]. Experimentally, antibiotics given by mouth lowered dramatically the intestinal-colonization resistance of mice to orally introduced *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* [3, 4] and to *Clostridium botulinum* [5, 6].

This report describes experiments in mice designed to discover whether modification of the gut microflora by oral antibiotic pretreatment would enhance the faecal excretion of biovar A organisms given by mouth.

MATERIALS AND METHODS

Culture media, anaerobic methods and viable count technique were essentially as already described [7].

Mice

Female Swiss white mice weighing *c.* 20 g were obtained from an outbred closed colony.

Organism

F. necrophorum strain A42, a biovar A isolate from a wallaby (*Macropus rufogriseus*) with necrobacillosis, has been described previously and used extensively in laboratory experiments [8].

Pretreatment and infection of mice

Oral pretreatment with a mixture of kanamycin and erythromycin or with metronidazole, to disturb the gastrointestinal microflora, was based on the methods used by Burr and Sugiyama [5, 6]. Mice were dosed twice a day for 2 days with either (a) 0.5 ml of an aqueous solution/suspension containing kanamycin monosulphate 4 mg and erythromycin 5 mg (Sigma Chemical Co.), or (b) 0.5 ml of a 0.5% solution of metronidazole (Torgyl; May & Baker Ltd). The doses were administered with a 1 ml syringe and curved animal feeding needle (18-gauge, 2 inch; Harvard Apparatus Ltd, Edenbridge, Kent). Control mice received no pretreatment. After an interval of 24 h the mice were infected orally with 0.5 ml of an appropriate dilution of a 24 h BM broth [9] culture of *F. necrophorum*. Additional control mice were pretreated with antibiotic but received sterile broth instead of culture.

Detection of F. necrophorum in mouse faeces

At a chosen time after infection groups of mice, all of which remained apparently healthy throughout, were killed and dissected. Faeces removed from the rectum of each animal was then examined by an *in vivo* method already published [10] and referred to later as the 'improved' method [1].

RESULTS

Effect of antibiotic pretreatment on the faecal excretion of F. necrophorum at intervals after oral infection

Table 1 shows the experimental design and the results. One day after oral infection with 5×10^6 *F. necrophorum* all mice (kanamycin-erythromycin pretreated, metronidazole pretreated, and non-pretreated) were excreting the organism in their faeces. Thereafter (4 and 7 days after infection) antibiotic pretreatment increased the number of mice with 'positive' faeces, control animals not pretreated in this way giving almost always negative results. There was no obvious difference between the effect of metronidazole and that of the kanamycin-erythromycin mixture. Appropriate additional controls proved that the organism excreted was that administered orally, and not a normal inhabitant of the mouse gut.

Effect of antibiotic pretreatment on the faecal excretion of F. necrophorum 4 days after oral infection with graded doses

Table 2 shows the combined results of two experiments, each made with a series of decimally diluted doses of *F. necrophorum*. Oral doses ranging from 78750 to 37

Table 1. *Faecal excretion of F. necrophorum at intervals after oral infection in mice whose gut microflora had been disturbed by oral antibiotic pretreatment*

Days after oral dose (5×10^6) of FN	Mice excreting FN, in groups of eight pretreated with		
	KE	M	Nothing (controls)
1	7*	8	8
4	4*	6	0
7	2	4	1

* Seven mice only in group.

FN, *F. necrophorum*; KE, kanamycin and erythromycin; M, metronidazole.

Mice (additional controls) in 9 further groups of 8, treated as above but given sterile broth instead of FN, all had 'negative' faeces.

Table 2. *Faecal excretion of F. necrophorum 4 days after oral infection with graded doses in mice whose gut microflora had been disturbed by oral antibiotic pretreatment*

Experiment	Oral dose of FN	Mice excreting FN in groups of six pretreated with		
		KE	M	Nothing (controls)
1	78750	6	5	—
	7875	6	5	—
	787	6	5*	—
	78	6	3*	—
2	37	6	0	0
	4	5	2	0
	c. 0	1	2	0

* Five mice only in group.

Abbreviations as in Table 1.

Mice (additional controls) in 3 further groups of 6, pretreated as above but given broth instead of FN, all had 'negative' faeces.

fusobacteria gave rise to faecal excretion of the organism 4 days after infection in all mice pretreated with the kanamycin-erythromycin mixture, and even a dose of 4 fusobacteria produced positive faeces in 5 of 6 animals. Similar though somewhat less striking results were obtained with metronidazole-pretreated mice. Antibiotic pretreatment gave rise to a few positive results (3 of 12 animals) when the oral dose of *F. necrophorum* was theoretically nil but undoubtedly contained at least one organism.

It should be borne in mind that in the previous experiment (Table 1) a dose of *F. necrophorum* > 60 times greater than the largest in the present experiment failed to give rise to faecal excretion 4 days after infection unless the mice had been pretreated with antibiotic. The inclusion of appropriate controls in this as in the previous experiment proved that the antibiotic pretreatment was responsible for enhanced faecal excretion of organisms derived from the infecting *F. necrophorum* strain. The positive results produced by minute infecting doses (≤ 37 fusobacteria) strongly suggested that the disturbance of the gut microflora brought about by

antibiotic pretreatment [5, 6] encouraged intestinal multiplication of the organism.

DISCUSSION

The two forms of oral antibiotic pretreatment were based on those used in studies on infant botulism by Burr and Sugiyama [5, 6], who found that dosing with a mixture of kanamycin and erythromycin greatly reduced the variety and total population of bacteria in the gut and that dosing with metronidazole, an agent selectively active against anaerobic bacteria, increased the number of total aerobes, Enterobacteriaceae and enterococci.

Faecal excretion of *F. necrophorum* biovar A was short-lived in normal mice given a single large dose of the organism by mouth but prolonged in mice pretreated with both antibiotic regimens (Table 1). In mice infected with graded doses by mouth (Table 2), antibiotic pretreatment by either method led to greatly enhanced faecal excretion 4 days later, almost certainly as a result of intestinal multiplication of the organism. Presumably this multiplication is explained by a reduction in the bacterial competition exerted by an undisturbed gut microflora.

This work lends support to the suggestion that in animals such as cattle, which are known to carry *F. necrophorum* biovar A in the rumen, disturbance of the gastrointestinal microflora may lead to fusobacterial multiplication in the intestine and consequent faecal excretion. Necrobacillosis is particularly well known in cattle, deer, antelope and macropods [11]. Experimental attempts should be made to induce faecal excretion of biovar A organisms in such animals by deliberately disturbing the gut microflora.

ACKNOWLEDGEMENTS

Thanks are due to Miss L. M. Wallace and Miss S. A. Barton for technical assistance.

REFERENCES

1. Smith GR, Thornton EA. The prevalence of *Fusobacterium necrophorum* biovar A in animal faeces. *Epidemiol Infect* 1993; **110**: 327-31.
2. Willis AT, Smith GR. Gas gangrene and other clostridial infections of man and animals. In: Topley and Wilson's Principles of bacteriology, virology and immunity, 8th ed. Vol. 3, Bacterial diseases. General eds MT Parker, LM Collier, vol. eds GR Smith, CSF Easmon. London: Edward Arnold, 1990; 308-29.
3. van der Waaij D, Berghuis-de Vries JM, Lekkerkerk-van der Wees JEC. Colonization resistance of the digestive tract in conventional and antibiotic-treated mice. *J Hyg* 1971; **69**: 405-11.
4. van der Waaij D, Berghuis-de Vries JM, Lekkerkerk-van der Wees JEC. Colonization resistance of the digestive tract and the spread of bacteria to the lymphatic organs in mice. *J Hyg* 1972; **70**: 335-42.
5. Burr DH, Sugiyama H. Susceptibility to enteric botulinum colonization of antibiotic-treated adult mice. *Infect Immun* 1982; **36**: 103-6.
6. Burr DH, Sugiyama H. Metronidazole-induced susceptibility of adult mice to intestinal *Clostridium botulinum* colonization. *Curr Microbiol* 1983; **9**: 275-8.
7. Smith GR, Oliphant JC, Parsons R. The pathogenic properties of *Fusobacterium* and *Bacteroides* species from wallabies and other sources. *J Hyg* 1984; **92**: 165-75.
8. Smith GR, Barton SA, Wallace LM. Further observations on enhancement of the infectivity of *Fusobacterium necrophorum* by other bacteria. *Epidemiol Infect* 1991; **106**: 305-10.

9. Deacon AG, Duerden BI, Holbrook WP. Gas-liquid chromatographic analysis of metabolic products in the identification of Bacteroidaceae of clinical interest. *J Med Microbiol* 1978; **11**: 81-99.
10. Smith GR, Barton SA, Wallace LM. A sensitive method for isolating *Fusobacterium necrophorum* from faeces. *Epidemiol Infect* 1991; **106**: 311-7.
11. Smith GR. Anaerobic bacteria as pathogens in wild and captive animals. *Symp Zool Soc Lond* 1988; No. 60: 159-73.