PAPERS AND ORIGINALS

Randomised controlled trial of vancomycin for pseudomembranous colitis and postoperative diarrhoea

M R B KEIGHLEY, D W BURDON, Y ARABI, J ALEXANDER-WILLIAMS, H THOMPSON, DENISE YOUNGS, MARGARET JOHNSON, SANDRA BENTLEY, R H GEORGE, G A G MOGG

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Summary and conclusions

The efficacy of vancomycin in pseudomembranous colitis was assessed in a prospective randomised controlled trial. Forty-four patients with postoperative diarrhoea were allocated to five days' treatment with either 125 mg vancomycin six-hourly or a placebo. Sixteen patients had high titres of the neutralised faecal toxin characteristic of pseudomembranous colitis; nine received vancomycin and seven placebo. At the end of treatment faecal toxins were present in one patient given vancomycin compared with five of the controls. Vancomycin caused the disappearance of Clostridium difficile from the stool in all except one patient, whereas toxicogenic strains of Cl difficile persisted in all but one of the controls. Histological evidence of psuedomembranous colitis had disappeared by the end of treatment in six out of seven patients given vancomycin compared with only one out of five patients given placebo. In patients with faecal toxins bowel habit had returned to normal in seven of the vancomycin group compared with only one of the controls, but there was no significant difference in clinical response among patients without faecal toxins.

The results suggest that vancomycin eliminates toxin-producing Cl difficile from the colon and is associated with rapid clinical and histological improvement in patients with pseudomembranous colitis.

The General Hospital, Birmingham B4 6NH
M R B KEIGHLEY, MS, FRCS, consultant surgeon
D W BURDON, MB, MRCPATH, consultant microbiologist
Y ARABI, FRCS, research registrar
J ALEXANDER-WILLIAMS, CHM, FRCS, consultant surgeon
H THOMPSON, MD, consultant pathologist
DENISE YOUNGS, AIMLS, research technician
MARGARET JOHNSON, FIMLS, research technician
SANDRA BENTLEY, SRN, research nurse
R H GEORGE, MB, MRCPATH, consultant microbiologist
G A G MOGG, FRCS, research registrar

Introduction

Pseudomembranous colitis is potentially lethal; it is usually associated with antibiotic treatment and often occurs after major gastrointestinal surgery.[1-3] Studies from this hospital and at least three other centres have confirmed that it is caused by a toxin produced by *Clostridium difficile*.[4-7] Certain antibiotics may alter the normal bacterial flora of the colon, thereby encouraging the overgrowth of toxicogenic strains of *Cl difficile*.[8] These are usually resistant to many antibiotics, particularly the aminoglycosides and often penicillin, tetracycline, and clindamycin.[9] All strains are sensitive to metronidazole, but this agent is unlikely to be of value in pseudomembranous colitis because it is rapidly absorbed from the small bowel and fails to reach therapeutic concentrations in the colon.[10]

Oral vancomycin, on the other hand, is effective against staphylococcal enterocolitis because it achieves high concentrations in the colon.[11] *Cl difficile* is also highly sensitive to vancomycin,[9] and clinical improvement has occurred with oral vancomycin in nine patients with pseudomembranous colitis.[12] Symptoms may, however, resolve with antidiarrhoeal agents alone.[8] We report the results of a prospective randomised controlled trial of oral vancomycin in patients with postoperative diarrhoea and pseudomembranous colitis.

PRELIMINARY INVESTIGATION

A preliminary investigation was conducted to find the dose of vancomycin necessary to achieve therapeutic concentrations in the stool. Ten patients with postoperative diarrhoea were prescribed six-hourly vancomycin in doses of 500 mg (n=2), 250 mg (n=3), and 125 mg (n=5). The mean daily faecal concentrations of vancomycin in these groups were 714±SD 341, 447±260, and 351±172 mg/l respectively. As all isolates (n=26) of *Cl difficile* from patients with pseudomembranous colitis were inhibited by 16 mg vancomycin/l,[9] we chose a dose of 125 mg six-hourly for use in the trial.

Materials and methods

The study groups comprised all patients with postoperative diarrhoea seen in one surgical ward from 1 March to 31 August 1978. Criteria for entry included three or more liquid stools a day or a colostomy output exceeding 1 litre in 24 hours. Patients with a previous history of pseudomembranous colitis were excluded. Forty-four patients fulfilled the criteria and were identified by a research nurse during a daily ward round. All patients were immediately investigated by sigmoidoscopy, at which three biopsy specimens were obtained, and stools collected for quantitative measurement of faecal toxin and culture of *Cl difficile*. Treatment was then prescribed according to a trial number. Patients received either 125 mg vancomycin six-hourly for five days or an identical-looking placebo, randomisation being based on a code held in the pharmacy. The compounds were added to 50 ml fruit juice and taken slowly over 10 minutes.

Each patient was placed on a fluid-balance chart to record faecal output (volume and frequency) and seen daily by the research nurse or a registrar. We recorded "normal" bowel habit as one solid stool a day. Stool samples were collected on the third and fifth days of treatment and when possible seven days afterwards for measurement of faecal toxins and culture of *Cl difficile*. Faecal samples were also collected on the second and fourth days of treatment for vancomycin assay. Sigmoidoscopy and rectal biopsies were repeated on the last day of treatment.

Faecal toxins were identified by their characteristic appearance on monolayers of HeLa cells and neutralisation by *Cl sordellii* antitoxin. The toxin titre was determined by serial dilutions of faecal suspensions. *Cl difficile* was isolated on lysed blood agar containing kanamycin. Broth cultures of *Cl difficile* were also tested for neutralised toxin on monolayers of HeLa cells. Vancomycin assays were performed by the plate-diffusion method, *Bacillus subtilis* (NCTC 10073) being used as the test organism. Results of rectal biopsies were reported as diagnostic of pseudomembranous colitis only when they conformed to the criteria of Price and Davies.[13]

Results

Forty-four patients were admitted to the trial. Sixteen had evidence of neutralised faecal toxins and toxicogenic strains of *Cl difficile* in the stool (group 1). Five patients had evidence of toxicogenic strains of *Cl difficile* without faecal toxins (group 2), and the remaining 23 patients (group 3) had neither toxins nor *Cl difficile* on stool analysis. Ten of the 16 patients in group 1 and two of the five in group 2 had histological evidence of pseudomembranous colitis (see table I). None of the patients without toxins or *Cl difficile* had histological evidence of pseudomembranous colitis.

When the randomisation code was deciphered equal numbers were found in the two treatment groups (n = 22). The groups were similar with respect to the presence of faecal toxins and isolation of *Cl difficile* from the stool (table I). The distribution of preoperative diagnoses was also similar (table II), and apart from the use of cephalosporins the antibiotics prescribed were similar in the two groups (table III).

TABLE I—*Composition of groups treated with vancomycin and placebo (numbers of patients with histological evidence of pseudomembranous colitis given in parentheses)*

	Group 1	Group 2	Group 3
Vancomycin (n = 22)	9 (6)	3 (1)	10
Placebo (n = 22)	7 (4)	2 (1)	13

TABLE II—*Preoperative diagnoses in patients treated with vancomycin and placebo (numbers of patients with neutralised faecal toxins in stool given in parentheses)*

	Vancomycin (n = 22)	Placebo (n = 22)
Colorectal cancer	12 (2)	14 (4)
Crohn's disease	2	2
Gastric carcinoma	1 (1)	1
Ischaemic enteritis		1 (1)
Gall stones	2 (2)	
Appendicitis	1	1 (1)
Perianal abscess	1 (1)	
Peptic ulcer	1 (1)	1
Rectal prolapse	1 (1)	1 (1)
Fractured femur		1
Infected sebaceous cyst	1 (1)	

TABLE III—*Antimicrobial agents used in vancomycin-treated and placebo-treated groups*

	Vancomycin		Placebo	
	PMC (n = 9)	Others (n = 13)	PMC (n = 7)	Others (n = 15)
Metronidazole	4	10		13
Clindamycin/lincomycin	4	3	6	1
Gentamicin/tobramycin	7	4	6	5
Kanamycin	1	9		8
Ampicillin/penicillin	1	3	2	7
Cephalosporins	3	2		
Co-trimoxazole	3	1	1	1
Tetracycline	1			
Cloxacillin	2		1	1

PMC = Pseudomembranous colitis as characterised by high titres of neutralised faecal toxin.

Faecal concentrations of vancomycin exceeding four times the MIC for *Cl difficile* were recorded in all patients on the second and fourth days of treatment. The values on the second day ranged from 64 to 760 mg/l, with a mean of 351 ± SD 154 mg/l. Similar results were obtained on the fourth day (152-880 mg/l, mean 399 ± 132 mg/l).

Before treatment *Cl difficile* was detected in samples from 10 out of 11 patients in groups 1 and 2 given vancomycin and six of the nine patients in groups 1 and 2 given placebo (table IV). On the third day of treatment *Cl difficile* was recovered from only four patients receiving vancomycin but from all of the controls tested ($P < 0.05$). By the end of treatment only one of the vancomycin-treated patients had *Cl difficile* in the stool compared with eight of the nine patients given placebo ($P < 0.01$).

Before treatment faecal toxins were identified in seven out of eight patients in group 1 given vancomycin compared with four of the seven patients in group 1 given placebo (table IV). On the third day of treatment only three of the nine patients receiving vancomycin had toxins evident in the stools compared with all of the controls tested. This difference, however, was not significant. By the fifth day toxins were identified in only one patient receiving vancomycin compared with five out of six patients given placebo ($P < 0.05$).

In group 1 stool frequency and consistency had returned to normal by the end of treatment in seven of the nine patients given vancomycin but in only one of the seven patients given placebo ($P < 0.02$). There was no significant difference in clinical response in groups 2 and 3 (table V).

At follow-up, apart from one patient with a pelvic abscess who had persistent diarrhoea, none of the patients given vancomycin had further symptoms. In contrast four of the controls had diarrhoea lasting from 14 to 29 days, two of whom were later given a therapeutic course of vancomycin. Two of the controls also developed a faecal fistula from the site of colostomy closure, and both were later treated with vancomycin. There were no deaths from pseudomembranous colitis in either group. During follow-up four of the controls continued to have evidence of faecal toxins and *Cl difficile* in the stool, whereas none of the vancomycin-treated patients had evidence of toxins or *Cl difficile* on stool culture.

Discussion

When designing this trial we thought it was important to include all patients at the onset of diarrhoea rather than await the results of biopsy or determination of faecal toxin titres. We therefore recognised that we would be evaluating the influence of vancomycin in patients with pseudomembranous colitis (group 1) and those with non-specific postoperative diarrhoea (group 3).

Vancomycin had a significant clinical influence in patients with neutralisable faecal toxins but not in patients with post-operative diarrhoea. Clinical response to treatment was based on stool frequency, which was recorded daily by the research

TABLE IV—*Counts of Cl difficile* and faecal toxin titres (logarithmic values) in vancomycin-treated and placebo-treated patients*

Patients' trial numbers	Before treatment		Treatment day 3		Treatment day 5		Convalescence	
	Count	Titre	Count	Titre	Count	Titre	Count	Titre‡
				Vancomycin-treated patients				
Group 1								
1	+	2·4	0	0	0	0	†	†
17	0	0	+ +	3·6	0	0	0	0 (1)
18	†	†	+ +	3·6	0	1·5	†	†
23	+ + +	3·6	+ +	0	+ +	0	0	0 (2)
25	+ + +	4·2	+ +	2·8	0	0	0	0 (2)
28	+ +	2·8	0	0	0	0	0	0 (*Cl difficile in* abscess) (1)
34	+ + +	2·4	0	0	0	0	†	†
38	+ + +	2·7	0	0	0	0	†	†
41	+ + +	4·6	0	0	0	0	†	†
Group 2								
7	+ +	0	0	0	0	0	†	†
8	+ +	0	0	0	0	0	†	†
29	+ +	0	0	0	0	0	†	†
				Placebo-treated patients				
Group 1								
9	+ +	0	+ +	3·2	+ + +	3·8	4·7	2·4 (3)
14	+ +	0	+ + +	2·4	+ +	4·6	0	0 (4)
16	0	4·3	+ + +	2·2	+ +	0	5·5	4·6 (2)
22	+ +	3·8	+ +	4·2	+ +	2·8	6·3	2·8 (1)
24	0	0	†	2·2	+ + +	3·6	†	†
26	+ +	4·6	+ +	†	+ +	†	†	†
30	+ + +	4·6	+ + +	3·3	+ + +	3·6	3·5	2·8 (1)
Group 2								
10	+ +	0	+ + +	0	+ +	0	†	†
13	0	0	+ + +	0	0	0	†	†

* + = <29; + + = 30-59; + + + = 60-89.
†Specimen not taken or mislaid.
‡Figures in parentheses refer to time of specimen in weeks.

TABLE V—*Diarrhoea grades in the three groups at end of five days' treatment with vancomycin and placebo*

Diarrhoea grade	Group 1		Group 2		Group 2	
	Vancomycin	Placebo	Vancomycin	Placebo	Vancomycin	Placebo
Normal stool ..	7*	1*	2	0	6	6
Improved	1	1	1	0	2	2
Same	1	4	0	2	2	5
Worse	0	1	0	0	0	0

*P <0·02.

nurse (SB), rather than volume, which proved difficult to measure in patients passing solid stools.

The only other clinical study was based on collective data from six different American hospitals.[12] Using a much larger dose of vancomycin (2 g daily for seven days), the authors reported similar observations to our own—namely, that vancomycin is associated with a reduction in the titre of faecal toxins. The American study was not designed as a clinical trial, however, and there were no data on the influence of vancomycin on *Cl difficile* in the stool.

Vancomycin is expensive and potentially toxic. We found that 125 mg in 24 hours provided therapeutic concentrations in the colon and eliminated *Cl difficile* from the stool in all except one patient. Furthermore, the relatively small dose used in this study was associated with a significant reduction in faecal toxins in patients with pseudomembranous colitis. There was no biochemical or clinical evidence of nephrotoxicity in the vancomycin-treated patients, and none developed impaired auditory or vestibular function.

We acknowledge the difficulties in confirming pseudomembranous colitis by rectal biopsy.[14] Four of the six patients with neutralised faecal toxins without histological evidence of pseudomembranous colitis had a colostomy from which biopsy specimens were obtained, and the remaining two did not have evidence of a membrane on sigmoidoscopy. Histological diagnosis of pseudomembranous colitis is unreliable unless specimens are obtained from an area of colon with a visible membrane.[13] Furthermore, the characteristic membrane in pseudomembranous colitis is often patchy, and rectal sparing is also recognised from postmortem and colonoscopic inspection of the entire large bowel.[8 15]

Our results confirm that pseudomembranous colitis is due to overgrowth of a toxin-producing clostridium in the colon. Rapid improvement in symptoms occurred only when *Cl difficile* and the toxin elaborated by this organism were no longer present in the colon. Of the seven vancomycin-treated patients with histological evidence of pseudomembranous colitis, all except one were normal on rectal biopsy at the end of treatment. On the other hand, histological features of pseudomembranous colitis were still apparent in four of the five patients given placebo. These data confirm the aetiological implications of our previous findings and indicate that *Cl difficile* is responsible for this potentially lethal complication of antibiotic treatment.

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