Enterobacteriaceae and neonatal necrotising enterocolitis

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Abstract

A comparative study of bowel colonisation and incidence of necrotising enterocolitis in neonates admitted to an intensive care unit is reported. Neonates of less than 33 weeks gestational age requiring mechanical ventilation for respiratory distress syndrome were randomised during the first week of life to receive either vancomycin and aztreonam or vancomycin and gentamicin for episodes of suspected sepsis after the first week of life. A higher proportion of neonates who received vancomycin and gentamicin had faecal colonisation with enterobacteriaceae at the end of the second, third, and fourth weeks of life. Treatment with vancomycin and aztreonam was associated with a rapid quantitative reduction in faecal colonisation with enterobacteriaceae, whereas there was no quantitative reduction in colonisation with enterobacteriaceae associated with treatment with vancomycin and gentamicin. There were no differences between the two groups in faecal colonisation with anaerobes, *Enterococcus* sp, *Staphylococcus* sp, or yeasts. Six (14·6%) of 41 who received vancomycin and gentamicin compared with 0 of 40 who received vancomycin and aztreonam subsequently developed necrotising enterocolitis.

Neonatal necrotising enterocolitis is the most common abdominal emergency in neonatal intensive care units. Clustering of cases of necrotising enterocolitis in time and place has led to the hypothesis that it has an infective aetiology. Many studies have suggested a link between enterobacteriaceae and necrotising enterocolitis.[1–5] In a previous study we reported changes in the faecal flora preceding the clinical onset of necrotising enterocolitis by up to 72 hours and these changes included a quantitative increase in enterobacteriaceae.[6]

Aztreonam has been shown to be comparable in efficacy to aminoglycosides for the treatment of Gram negative infections in adults[7] and neonates.[8] Preliminary studies in this neonatal unit suggested that treatment of neonates with intravenous aztreonam reduced bowel colonisation with enterobacteriaceae.

In this study we compared the changes in bowel flora and incidence of necrotising enterocolitis in neonates treated with vancomycin and gentamicin or vancomycin and aztreonam for episodes of suspected sepsis after the first week of life.

Patients and methods

Neonates of less than 33 week's gestational age admitted to the Peter Congdon Regional Neonatal Unit in Leeds between February 1989 and April 1990 were randomised during the first week of life to receive intravenous vancomycin (22 mg/kg every 12 hours) with gentamicin (3 mg/kg every 12 hours) or vancomycin with aztreonam (15 mg/kg every 12 hours) for episodes of suspected bacterial infection occurring after the first week of life. The diagnosis of suspected infection was made clinically and the clinical signs included apnoea, bradycardia, metabolic acidosis, hypotension, unstable temperature, and poor peripheral perfusion. Antibiotic treatment after the first week of life was modified if indicated by laboratory results or the clinical condition of the neonate. Neonates with suspected necrotising enterocolitis were treated with benzylpenicillin or ampicillin, gentamicin, and metronidazole.

Samples of faeces were collected each day (when available) from all neonates during the first month of postnatal life, weighed and stored at −70°C in glycerol citrate broths as previously described.[6] Faecal bacteriology was performed within six months of sample storage. Samples were thawed at room temperature and vial contents serially diluted 10-fold in brain heart infusion broth (Oxoid CM225). An aliquot of 100 μl of each dilution was spread onto a range of selective and non-selective media,[6] which were incubated for seven days. Aztreonam concentrations were determined in stored samples using a plate diffusion assay. Gentamicin was assayed using both a plate assay[9] and an immunoassay method (Abbot TDx). Minimum inhibitory concentrations (MIC) of antibiotics for bacterial isolates were determined using a plate dilution method.[9] Resistance was defined by an MIC exceeding 1 mg/l. The proportions of neonates at 7, 14, 21, and 28 days of postnatal age colonised by enterobacteriaceae, enterococci, yeasts, anaerobes, *Staphylococcus aureus,* and coagulase negative staphylococci were determined by examining the sample collected from each neonate nearest to, but within 72 hours, of 7, 14, 21, and 28 days of postnatal age.

The diagnosis of necrotising enterocolitis was suspected if an infant developed illness associated with specific signs of gastrointestinal dysfunction including abdominal distension, blood in the stool, gastric retention, and abdominal wall erythema. A plain radiograph was taken in all infants with suspected necrotising enterocolitis. The radiographs were reviewed by a paediatric radiologist unaware of the clinical signs. Definite necrotising enterocolitis was diagnosed if there were radiological signs of intramural or intrahepatic gas, otherwise the episodes were considered as possible necrotising enterocolitis.

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Local ethics committee approval was obtained for this study.

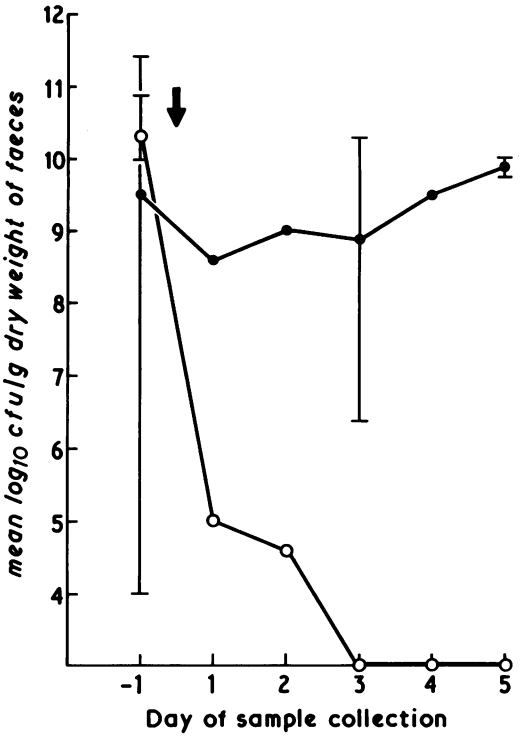
Statistical analysis was carried out using logistic regression with the EGRET software package. Two sided significance tests were used.

Results

Over the 15 month study period 143 neonates were randomised to receive vancomycin and gentamicin or vancomycin and aztreonam for episodes of suspected sepsis after the first week of life. Forty one neonates received vancomycin and gentamicin and 40 received vancomycin and aztreonam after the first week of life. The median duration of antibiotic treatment in the two groups was five days. The clinical features of the two groups are shown in table 1. The two groups of neonates had similar clinical features during the first week of life. Two neonates to whom vancomycin and aztreonam were administered died before enteral feeding was started. The median postnatal age at which enteral feeding was started was 9 days in the group which received vancomycin and gentamicin and 10 days in the group which received vancomycin and aztreonam. If tolerated, then feed volumes were gradually increased from 10 ml/kg/24 hours to full feed volumes over seven days. Fourteen (35%) of the group which received vancomycin and aztreonam compared with 15 (36·6%) of the group which received vancomycin and gentamicin were partially or fully enterally fed with maternal breast milk.

There were eight episodes (involving eight neonates) in the group which received vancomycin and aztreonam and 11 episodes (involving 11 neonates) in the group which received vancomycin and gentamicin where enterobacteriaceae were present at the start of treatment and in which treatment was continued for more than 72 hours. Enterobacteriaceae were present at the end of the treatment in none of the eight episodes in the group that received vancomycin and aztreonam and in all of the 11 episodes in the group that received vancomycin and gentamicin. None of the strains of enterobacteriaceae present before or after treatment were resistant to aztreonam or gentamicin (MIC more than 1 mg/l). There were seven episodes in the group which received vancomycin and aztreonam and eight episodes in the group which received vancomycin and gentamicin for which at least three samples were available within the five day period after introduction of antibiotic treatment. Quantitative studies of enterobacteriaceae showed that there was a rapid fall in the mean $\log_{10}$ colony forming units (cfu)/g dry weight associated with administration of vancomycin and aztreonam and no fall in numbers in the group receiving vancomycin and gentamicin (figure). In the group receiving vancomycin and aztreonam, inhibitory concentrations of aztreonam were detected in faecal samples. Inhibitory concentrations of gentamicin were not detected in the group receiving vancomycin and gentamicin.

The proportion of neonates in each group for whom samples were available at each time were similar. The proportion of neonates at 14, 21, and 28 days from whom enterobacteriaceae were isolated was significantly higher in the group which received vancomycin and gentamicin than in the group which received vancomycin and aztreonam (table 2). There were no



Quantitative changes in enterobacteriaceae in faecal flora with intravenous vancomycin and aztreonam or gentamicin; ○=vancomycin and aztreonam ●=vancomycin and gentamicin. The arrow indicates the start of intravenous antibiotics and vertical bars the SD.

Table 1 Clinical features of neonates receiving vancomycin and aztreonam or vancomycin and gentamicin

	Vancomycin and aztreonam (n=40)	*Vancomycin and gentamicin (n=41)*
Median gestational age in weeks (range)	27 (24–32)	28 (24–32)
Median birth weight (g)	1040	910
<1000 g (%)	47·5	53·7
Umbilical artery catheter (%)	72·5	63·4
Intraventricular haemorrhage diagnosed by ultrasound (%)	40·0	29·3
Pneumothorax (%)	12·5	7·3
Treatment for patent ductus (%)	22·5	36·6
Outborn (%)	37·5	43·9
Bacteraemia in first week (%)	5·0	2·4
IPPV >24 hours (%)	92·5	80·5
Apgar score <5, at 5 minutes or IPPV more than 4 minutes from birth (%)	60·0	70·7
Hypoglycaemia (%)*	7·5	7·3

IPPV, intermittent positive pressure ventilation.
*Glucose concentration <1·2 mmol/l.

Table 2 Percentage with enterobacteriaceae isolated at each postnatal age

Postnatal age (weeks)	*Group*	*No of samples*	*% With enterobacteriaceae*
1	VA	31	19·4
	VG	35	34·3
2	VA	31	25·8
	VG	35	60·0
3	VA	30	30·0
	VG	29	75·9
4	VA	18	50·0
	VG	23	87·0

VA refers to neonates who received vancomycin and aztreonam and VG to neonates who received vancomycin and gentamicin. The differences in percentage with enterobacteriaceae at 2, 3, and 4 weeks of postnatal age: χ^2 at 2 weeks=7·8 ($p<0·01$), at 3 weeks=12·4, and at 4 weeks=6·7.

Table 3 Outcome of neonates treated with vancomycin and aztreonam and vancomycin and gentamicin

	Group	
	Vancomycin and aztreonam (n=40)	*Vancomycin and gentamicin (n=41)*
No who died	6	4
No with necrotising enterocolitis		
Definite	0	6
Possible	14	13
No with chronic lung disease	9	5
Median hospital stay (days)	40	43

differences in the proportions of samples from the two groups from which anaerobes, enterococci, yeasts, or staphylococci were isolated at each postnatal age.

The proportion of neonates who died before discharge from hospital or developed chronic lung disease and the median duration of hospital stay were similar in the two groups (see table 3). A larger proportion of neonates in the group which received vancomycin and gentamicin (six of 41) developed definite necrotising enterocolitis compared with the group which received vancomycin and aztreonam (six of 41 *v* 0 of 40; p 0·028; Fisher's exact test). To be sure that this difference was not attributable to differences between the two groups other than antibiotic treatment, the effect that each of the clinical characteristics had on outcome was examined in a logistic regression model. The only variable to have a significant effect on the risk of necrotising enterocolitis independently of antibiotic group was the presence of an umbilical artery catheter; an umbilical artery catheter was associated with a reduced risk of necrotising enterocolitis.

All of those that developed definite necrotising enterocolitis had received vancomycin and gentamicin preceding the onset of clinical signs of necrotising enterocolitis. A similar proportion of neonates in the two groups developed possible necrotising enterocolitis. Faecal samples were available from the 48 hour period preceding the onset of five of the six episodes of definite necrotising enterocolitis. Enterobacteriaceae were present preceding all of these five episodes.

Discussion

Intravenously administered antibiotics may have a profound influence on the pattern of development of the gastrointestinal flora of premature neonates.[10] In this study we have shown a reduction in bowel colonisation with enterobacteriaceae associated with the intravenous administration of aztreonam but not gentamicin. The majority of premature neonates in intensive care units receive antibiotics. The clinical signs of bacterial infection are nonspecific, so that many receive antibiotics in the absence of laboratory or radiological evidence of infection. The pattern of antibiotic usage varies considerably between neonatal units,[11] yet there have been few studies comparing the impact of different regimens on the development of the neonatal faecal flora.

In this study we have presented evidence suggesting that intravenously administered aztreonam and vancomycin by comparison with gentamicin and vancomycin reduces faecal colonisation with enterobacteriaceae. Although both aztreonam and gentamicin are excreted in the bile, the peak serum concentrations of aztreonam are considerably higher than those of gentamicin and this may explain the differences in faecal antimicrobial activity. The antimicrobial activity of biliary excreted gentamicin may also have been reduced by anaerobic conditions, low intraluminal pH, and the presence of divalent cations such as calcium ions. No attempt was made to separate the two groups of neonates within the intensive care unit, so that cross colonisation was not prevented. Prevention of cross colonisation might have led to greater differences in the patterns of bacterial colonisation between the two groups.

If enterobacteriaceae are involved in the pathological process leading to necrotising enterocolitis then its difference in incidence between the two groups could be explained by modification of the bowel flora before or soon after the onset of that pathological process. However, other explanations cannot be excluded such as direct effects of antibiotics on the bowel. Past attempts to prevent necrotising enterocolitis through modification of the gastrointestinal flora by orally administered aminoglycosides have not produced conclusive results,[12–14] although the small number of neonates included and the rapid selection of aminoglycoside resistant enterobacteriaceae after the use of oral aminoglycosides[12] may have contributed to the inconclusive nature of these studies.

This study certainly does not refute the hypothesis that colonisation of the bowel of premature neonates with enterobacteriaceae contributes to the pathogenesis of neonatal necrotising enterocolitis. It suggests that the specific antibiotics used for the intravenous treatment of neonates with suspected infection may influence the risk of the subsequent development of necrotising enterocolitis.

1 Speer ME, Taber LH, Yow MD, Rudolph AJ, Urteaga J, Waller S. Fulminant neonatal sepsis and necrotizing enterocolitis associated with a 'nonenteropathogenic' strain of Escherichia coli. *J Pediatr* 1976;**89**:91–5.
2 Stanley MD, Null DM, deLemos RA. Relationship between intestinal colonization with specific bacteria and the development of necrotizing enterocolitis. *Pediatr Res* 1977; **11**:543.
3 Westra-Meijer CMM, Degener JE, Dzoljic-Danilovic G, Michel MF, Mettau JW. Quantitative study of the aerobic and anaerobic faecal flora in neonatal necrotising enterocolitis. *Arch Dis Child* 1983;**58**:523–8.
4 Modi N, Damjanovic V, Cooke RWI. Outbreak of cephalosporin resistant Enterobacter cloacae in a neonatal intensive care unit. *Arch Dis Child* 1987;**62**:148–51.
5 Carbonaro CA, Clark DA, Elseviers D. A bacterial pathogenicity determinant associated with necrotizing enterocolitis. *Microb Pathog* 1988;**5**:427–36.
6 Hoy C, Millar MR, Mackay P, Godwin PGR, Langdale V, Levene M. Quantitative changes in faecal bacteriology preceding necrotising enterocolitis in premature neonates. *Arch Dis Child* 1990;**65**:1057–9.
7 LeFrock JL, Smith BR, Chandrasekar P, *et al.* Efficacy and safety of aztreonam in the treatment of serious Gram-negative infections. *Arch Intern Med* 1987;**147**:325–8.
8 Umana MA, Odio CM, Castro E, Salas JL, McCracken GH Jnr. Evaluation of aztreonam and ampicillin vs amikacin and ampicillin for the treatment of neonatal bacterial infections. *Pediatr Infect Dis J* 1990;**9**:175–80.
9 Lennette EM, Balows A Hausler WJ, Shadomy HJ, eds. *Manual of clinical microbiology*. 4th Ed. Washington: American Society for Microbiology, 1985.

10 Bennet R, Erikson M, Nord CE, Zetterstrom R. Faecal bacterial microflora of newborn infants during intensive care management and treatment with five antibiotic regimes. *Pediatr Infect Dis J* 1986;5:533–9.
11 Hammerschlag MR, Kleins JO, Herschel M, Chen FCJ, Fermin R. Patterns of use of antibiotics in two newborn nurseries. *N Engl J Med* 1977;**296**:1268–9.
12 Boyle R, Nelson JS, Stonestreet BS, Peter G, Oh W. Alterations in stool flora resulting from oral kanamycin prophylaxis of necrotising enterocolitis. *J Pediatr* 1978;**93**: 857–61.
13 Egan EA, Mantilla G, Nelson RM, Eitzman DV. A prospective controlled trial of oral kanamycin in the prevention of neonatal necrotizing enterocolitis. *J Pediatr* 1976;**89**: 467–70.
14 Grylack LJ, Scanlon JW. Oral gentamicin therapy in the prevention of neonatal necrotising enterocolitis. *Am J Dis Child* 1978;**132**:1192–4.