Short reports

Clostridial toxins in neonatal necrotising enterocolitis

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SUMMARY Clostridium difficile cytotoxic toxin was found in the faeces or gut content of five of 39 neonates with necrotising enterocolitis (NEC). Toxin concentrations were uniformly low and did not differ from those found in healthy neonates. C difficile is unlikely to be involved in the pathogenesis of NEC. Stools from 33 babies with NEC were also tested for C perfringens alpha toxin, with negative results.

The cause of neonatal necrotising enterocolitis (NEC) has not yet been established. Although intestinal ischaemia has been widely accepted as an explanation, some recent observations have pointed more to an infective aetiology. Clostridium difficile is known to colonise the neonatal intestine and is a major cause of pseudomembranous colitis in adults. Since C difficile cytotoxic toxin has been found in the faeces of asymptomatic neonates, its presence in the stools of babies with NEC cannot alone be regarded as evidence of a causal relation. Furthermore, differences in the frequency of faecal cytotoxic toxin between affected and asymptomatic neonates, may be a reflection of differing degrees of environmental colonisation with C difficile rather than evidence of a true pathogenic role. For these reasons our study was undertaken using a quantitative cytotoxic toxin assay to determine whether there was any difference in the concentrations of toxin in the stools of babies with NEC compared with healthy neonates. In addition, since surgical intervention is reserved for the most severe cases of NEC, a group of surgically treated babies was treated separately to see if the severity of the disease might be reflected in the toxin concentration.

Clostridium perfringens also colonises the neonatal gut and is a recognised cause of various forms of necrotising enteritis in man (Pigbel, Darmbrand) and the newborn of other species (lamb dysentery, piglet enteritis). The lethal and necrotising alpha toxin of C perfringens is produced by all five types of this bacterial species and is an enzyme (lecithinase) which splits phospholipid complexes. This reaction was used to devise an assay to detect the possible presence of free alpha toxin in stools and intestinal content derived from babies with NEC.

Patients and methods

Samples of faeces or intestinal content were obtained from neonates either at their hospital of origin at the onset of symptoms, or immediately upon their arrival after transfer to the Hospital for Sick Children, Great Ormond Street. Thirty-nine neonates with NEC from 19 different hospitals were studied.

Surgical neonates. Twenty-two neonates with severe NEC underwent surgery. At the time of operation intestinal content was expressed from resected bowel and then stored at −20°C.

Medical neonates. Seventeen babies with clinical or radiological evidence of NEC who did not undergo surgery were studied. Faecal specimens consisted of the first passed after arrival at the Hospital for Sick Children, Great Ormond Street, or, in the case of babies remaining in their own units, the first stool collected after the onset of symptoms of NEC. Babies who failed to pass stools within 24 hours of the diagnosis of NEC were not included in the study. Samples were stored at −20°C.

Asymptomatic neonates. Although the prevalence of faecal cytotoxic toxin in asymptomatic neonates varies in different hospitals, it was reasoned that the concentration of toxin found in the stools of healthy subjects would be unlikely to depend on environmental colonisation. For this reason samples were obtained from 25 neonates at three different hospitals (The Hospital for Sick Children, Great Ormond Street, Queen Charlotte’s Hospital, and Kings College Hospital, London). Samples were obtained only from neonates with no history of gastrointestinal...
nal symptoms. No formal attempt was made to match for factors such as gestational age, birth asphyxia, and umbilical catheterisation, but this group was drawn from a typical 'at risk' population ranging in gestational age from 29 to 40 weeks (mean 32 weeks). Samples were stored as above.

**Toxin assay.** Fluid stools or intestinal content were centrifuged without further dilution, but formed stools were weighed and then converted to a suspension after the addition of a recorded volume of physiological saline. The supernatant was passed through a membrane filter of pore size 0.45 µm (Millipore UK).

**Cytopathic toxin.** Serial dilutions of filtrate were added to monolayers of human embryonic lung fibroblasts and the assay was performed in the presence of either *Clostridium sordellii* antitoxin or an equal volume of physiological saline. The monolayers were examined by inverted microscope after incubation at 37°C for 24 and 48 hours. The titre was expressed as the highest dilution of the filtrate that retained a definite cytopathic effect on the monolayer.

**C Perfringens alpha toxin.** The lecithovitellin test as described by Batty was modified for small volumes of sample. On each occasion the assay was performed the sensitivity of the lecithovitellin was tested by the inclusion of a positive control filtrate known to contain alpha toxin (derived from a broth culture of an alpha toxin producing strain of *C perfringens* Type A NCTC 8237).

**Statistical method.** Fisher's exact test was used for statistical analysis.

**Results** (see Table)

<table>
<thead>
<tr>
<th>Group</th>
<th>No with toxin positive stools or intestinal content*</th>
<th>Toxic titre*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surgically treated (NEC) (n=22)</td>
<td>3</td>
<td>1:3 \times 10^2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1:3 \times 10^2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1:1 \times 10^2</td>
</tr>
<tr>
<td>Medically treated (NEC) (n=27)</td>
<td>2</td>
<td>1:5 \times 10^2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1:1 \times 10^2</td>
</tr>
<tr>
<td>Asymptomatic neonates (n=25)</td>
<td>3</td>
<td>1:4 \times 10^2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1:3 \times 10^2</td>
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<tr>
<td></td>
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<td>1:3 \times 10^3</td>
</tr>
</tbody>
</table>

*Groups not comparable—no statistical analysis.

Non-significant—Fisher's exact test.

Cytopathic toxin neutralised by *C sordellii* antiserum was found in the faeces of 8 of the 64 neonates studied (12.5%). The concentrations of toxin were uniformly low (less than 1 \times 10^2) and there were no statistically significant differences in toxin concentration between the groups. Filtrates derived from 33 neonates with NEC were also tested for *C perfringens* alpha toxin and were uniformly negative. Lecithinase activity was detected in one sample, but since this was not neutralised by the appropriate antitoxin, it could not be ascribed to the presence of toxin.

**Discussion**

Several authors have investigated the possibility that *C difficile* and its toxins might be implicated in the pathogenesis of neonatal NEC. In 1978 Chang and Areson reported their inability to show cytopathic toxin in the faeces of any of 18 babies with NEC or 32 asymptomatic neonates. Stoll et al have also reported uniformly negative toxin assays in both affected and unaffected neonates. Paradoxically, other authors have described cytopathic toxin as a relatively common finding in the faeces of healthy neonates and infants. These conflicting results almost certainly reflect differing degrees of environmental colonisation with *C difficile* in the authors' various units.

Cashore et al identified *C difficile* cytopathic toxin in the stools of some babies with NEC, but not in their control group. It was suggested that the organism might play a role in some cases of the disease. Two subsequent papers have shown that toxin may be found in the stools of babies with NEC and asymptomatic neonates being nursed on the same unit.

Our study was designed to examine the possibility that the concentrations of cytopathic toxin found in the stools of babies with NEC might be higher than those found in healthy neonates. This was not the case. When toxin was found in the stools of babies with severe NEC (those being treated surgically) it was present at the same concentration as when toxin was found in the stools of babies with mild NEC (being treated medically) or asymptomatic neonates. In no patient did the concentration of cytopathic toxin exceed 1 : 1 \times 10^3. In contrast the stools or intestinal content of infants with the particular form of enterocolitis associated with Hirschsprung's disease have been tested on the same assay system and found to contain high concentrations of toxin (up to 1 : 1 \times 10^6). Our results add further weight to the view that *C difficile* does not play an important role in the aetiology of NEC.

*C perfringens* has been shown to colonise the
human alimentary tract within the first few days of life. This organism has also been shown to be responsible for disease characterised by bloody diarrhoea, patchy intestinal necrosis, and pneumatisis intestinalis both in man and in the newborn of other species. Unfortunately, the primary lethal necrotising exotoxins of *C perfringens* may prove difficult to detect after their production in vivo. With the exception of the lecithinase and haemolysis tests for alpha toxin, there are no reliable in vitro tests for these primary lethal toxins. Furthermore, certain toxins may be readily destroyed by trypsin or fixed to tissue, thus hindering detection in faecal contents. Our failure to detect free alpha toxin in the stools of babies with NEC may genuinely reflect its lack of involvement in the disease process. It is also possible that toxin produced locally at a site of necrosis is rapidly broken down or fixed to tissue and is for these reasons undetectable in intestinal content.

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References

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## Increased vomiting induced by an antiemetic drug

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**Summary** A case of progressive vomiting in a boy aged 5 months is reported. Vomiting was secondary to an obstruction in the antrum of the stomach caused by a mass consisting of alginate.

Vomiting in infants may be caused by various stomach disorders. As the illness is often transient it is commonly treated symptomatically with further investigation reserved for persistent cases only. The following case history shows, however, that symptomatic treatment may lead to further problems.

**Case report**

A boy aged 5 months was referred with suspected gastro-oesophageal reflux as the cause of his persistent vomiting, which had been present from the very first days of life. Initially, his physical development had been normal, but from week 16 onwards his body weight had not increased and traces of blood were occasionally found in the vomitus. Symptomatic treatment with Nutriton, (a food thickening agent, dosage two to three measures/feed) had been unsuccessful, as were Motilium (domperidon) (6 mg/day), and Gaviscon (15 ml/day). Radioccontrast studies performed a few days before referral had failed to show either diaphragmatic hernia or gastro-oesophageal reflux. Gastric emptying was normal and no foreign matter was seen in the stomach.

Further investigation was requested including determination of oesophageal pH and endoscopic studies of the oesophagus and stomach. To our