Viruses excreted in neonatal stools

A. D. STEELE, L. S. STEINHARDT, J. J. ALEXANDER

Summary

Faecal specimens from 122 infants in the Neonatal Unit at Ga-Rankuwa Hospital were examined by electron microscopy for the presence of virus. In total 40% of the neonates were excreting virus. Rotavirus was the commonest, found in 33,5% of the infants, with small round viruses (SRVs) found in 12,3% and adenovirus seen in 1 baby. RNA analysis of the rotavirus genome revealed a similar electrophoretic band in all specimens. Most of the babies infected with rotavirus were less than 7 days old, and very little excretion occurred after 21 days of age. Only 8% of the babies excreting virus had any clinical signs of infection. Rotavirus infection appears to be endemic and SRV infection common in the Neonatal Unit at Ga-Rankuwa Hospital.


There have been many reports describing the presence of different viruses in the faeces of infants and young children.1-3 Rotavirus has been well documented as the commonest cause of acute infantile gastro-enteritis,4,5 while other viruses, such as adenovirus6 and astrovirus,7 have also been associated with outbreaks of gastro-enteritis. The role of a heterogeneous group of "small round viruses" (SRVs), which are often found in the stool, is still being explored.8 However, the presence of virus in the stool is not always associated with diarrhoea. This is especially true in the neonate population, in which virus excretion by asymptomatic infants has often been reported.9,10 As most of the human enteric viruses, including the SRVs, cannot easily be isolated - if at all - in tissue culture, an electron microscopic study was conducted at Ga-Rankuwa Hospital in order to investigate: (i) which agents are excreted in the faeces of neonates; (ii) the relative prevalence of such infections; (iii) the age distribution of virus excretors; and (iv) the clinical manifestations, if any, of infection.

Material and methods

Study population. Between May and August 1984 stool specimens were collected once a week on a random basis from infants admitted to the Neonatal Unit at Ga-Rankuwa Hospital (these include both term and pre-term neonates, mostly from the labour ward at Ga-Rankuwa Hospital but also from the surrounding clinics, home deliveries and peripheral hospitals). In total 150 faecal specimens from 122 neonates were examined by electron microscopy. The age range of the infants at the time of sampling was 1 - 53 days, with a mean of 12.8 days. Specimens were processed on the day of collection.

Electron microscopy. The clarified faecal extracts were ultracentrifuged at 30000 g for 1 hour to pellet the virus. The pellets were resuspended in microscopy diluent containing 0,1% bacitracin and mixed with an equal volume of 3% potassium phosphotungstate. A drop of the mixture was placed on a carbon-formvar-coated copper grid. Excess fluid was blotted off and the grid allowed to air-dry before examination in a Joel 1200 electron microscope.
Electrophoresis. The crude faecal extracts were used after an initial low-speed spin to sediment any macroscopic debris. The virus was disrupted with sodium dodecyl sulphate (SDS), and then deproteinised with a phenol-chloroform mixture. The RNA was precipitated overnight in absolute ethanol and the RNA pellet was dried before being resuspended in sample buffer. The gel electrophoresis was carried out in a 10% polyacrylamide gel for 18 hours at 100 V using the discontinuous buffer system of Laemmli. The gels were silver-stained for visualisation of the RNA bands.

Results
Rotaviruses, adenoviruses and SRVs were observed in the faeces of the neonates studied; prevalences of these infections are presented in Table I. Rotavirus was the most commonly observed agent, occurring in 27% of the neonates and also together with an SRV infection in 6.6% (Fig. 1). The rotavirus RNA migration patterns found by electrophoresis were all similar (Fig. 2), indicating that a single strain of rotavirus was present in the ward.

<table>
<thead>
<tr>
<th>Table I: Viruses Detected in the Faeces of 122 Neonates</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of Excretors</td>
</tr>
<tr>
<td>------------------</td>
</tr>
<tr>
<td>Rotavirus</td>
</tr>
<tr>
<td>SRVs</td>
</tr>
<tr>
<td>Rotavirus and SRVs</td>
</tr>
<tr>
<td>Adenovirus</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

The age range of the infants excreting virus was 1 - 42 days, with a mean of 8.6 days. Rotavirus was excreted very early in life, occurring in 43.3% of cases in the 1st week of life (Table II). A significantly higher proportion of infants less than 7 days old than of older ones were excreting rotavirus ($P = 0.01$). SRVs were also seen more commonly in infants less than 1 week old, and very little virus excretion was found after 21 days of age. In contrast...
TABLE II. AGE DISTRIBUTION OF THE EXCRETORS

<table>
<thead>
<tr>
<th>Age (d)</th>
<th>No. tested</th>
<th>Rotavirus-positive</th>
<th>SRV-positive</th>
<th>Adenovirus-positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 7</td>
<td>60</td>
<td>26</td>
<td>43,3±</td>
<td>10</td>
</tr>
<tr>
<td>8-21</td>
<td>59</td>
<td>12</td>
<td>20,3±</td>
<td>5</td>
</tr>
<tr>
<td>22-35</td>
<td>23</td>
<td>2</td>
<td>8,7±</td>
<td>—</td>
</tr>
<tr>
<td>&gt; 35</td>
<td>8</td>
<td>1</td>
<td>12,5±</td>
<td>—</td>
</tr>
</tbody>
</table>
*Statistically significant difference between age of < 7 days and older (P = 0.01).

adenovirus, which was only seen in 1 case, occurred at an older age — 29 days.

The 49 neonates who excreted virus were clinically assessed. Only 4 of these excretors (8,1%) had any clinical signs of illness; 3 had mild diarrhoea, while 1 had necrotising enterocolitis. All 4 of these symptomatic infections were associated with rotavirus excretion, although 1 of the neonates with mild diarrhoea also excreted SRVs.

Discussion

Rotavirus was found to be endemic in the Neonatal Unit at Ga-Rankuwa Hospital. The occurrence of this virus in the unit over a long period of time (our own unpublished data) and the natural self-limiting nature of the infection indicate that the virus is spread from baby to baby within the ward. The presence of a single strain of rotavirus in different maternity units world-wide has been reported several times.10,12

The occurrence of a single RNA electrophoretotype in this study demonstrates that a single strain of rotavirus is endemic in the ward.

Polyacrylamide gel electrophoresis of the 11 genome segments of rotavirus yields an easily recognisable pattern which is characteristic for each viral isolate. Although considerable variation in the RNA profiles exists among different strains of rotavirus, a standard distribution of the 11 segments into four size classes occurs among the group A rotaviruses. RNA segment bands 1 - 4 are denoted as class I, bands 5 and 6 as class II, bands 7 - 9 as class III, and bands 10 and 11 as class IV.13 The RNA profiles exhibited by the rotaviruses isolated in this study all conform to the basic group A rotavirus pattern.

The age distribution of the rotavirus excretors was of interest, since the neonates were being infected very early in life. This too indicates that the infection is endemic in the unit. The infants infected with rotavirus were mostly asymptomatic or clinical manifestations were mild, only 4 of 41 rotavirus excretors exhibiting any symptoms at all.

Many reports have been made of a heterogeneous group of SRVs excreted in human faeces which cannot be cultured and thus have not been characterised. These particles are at present usually identified by their appearance under the electron microscope (Fig. 2). They are normally not associated with symptoms and may be ubiquitous in faecal extracts, although they cannot be totally excluded from a role in causing diarrhoea.14

Adenoviruses have been demonstrated in infantile gastroenteritis,15,16 but have also been identified in stools from children who do not have enteritis.17 In this study adenovirus was seen in the stool from only 1 patient, aged 29 days, and would seem to have been introduced from outside the wards. No clinical symptoms were associated with excretion of this virus.

Rotaviruses were demonstrated to be endemic in the Neonatal Unit at Ga-Rankuwa Hospital and SRVs were also found to occur in substantial numbers of stools. Excretion of these viruses was not associated with symptoms of gastro-enteritis in the majority of cases, and so it appears that infection by these viruses in neonates is not a major problem. In fact, in terms of a prospective study done in Melbourne,18 an infection with rotavirus early in life might very well protect the infant from severe life-threatening illness at a slightly older age.

We wish to thank Professor I. T. Hay and Dr. J. B. Ellis of the Department of Paediatrics, MEDUNSA, and the sisters of the Neonatal Unit at Ga-Rankuwa Hospital for their assistance in the collection of specimens for this study. The study was supported in part by a grant from the South African Medical Research Council.

REFERENCES