Antimicrobial susceptibility

Strains of *H. influenzae* type b isolated from spinal fluid in both cases were resistant to ampicillin and chloramphenicol by disc diffusion test. Minimum inhibitory concentrations and minimum bactericidal concentrations were determined in duplicate by standard tube dilution methods using heminmenadione broth culture and inoculum of 10^5 colony forming units. The results are shown in the Table.

Discussion

In 1981, 14% of the strains of *H. influenzae* b found in Mexico were resistant to ampicillin, but no resistance to chloramphenicol had been found.⁴ Chloramphenicol was therefore the drug of choice for severe infections caused by this micro-organism. Clinicians and microbiologists need to be warned of the recent emergence of strains resistant to both ampicillin and chloramphenicol throughout the world to recognise such cases promptly and be able to provide appropriate treatment.

Resistance of *H. influenzae* b to ampicillin and chloramphenicol is due to plasmid R responsible for β -lactamase and acetyltransferase, respectively.¹ Such resistance strains may therefore increase. As other authors suggest,^{5,6} we believe that third

generation cephalosporins such as cefotaxime or latamoxef (moxalactam) should be considered the drugs of choice in the treatment of such patients.

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Suspected rotavirus encephalitis

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SUMMARY A 9 month old boy with suspected rotavirus encephalitis developed infantile spasms and delayed psychomotor development. Anti-rotavirus antibodies in cerebrospinal fluid, blood, and faeces were studied.

Rotavirus is an important agent of acute viral gastroenteritis in children.¹ There are few reports of central nervous system involvement associated with rotavirus infection. Salmi *et al* described two patients, one of whom developed fatal Reye's syndrome and the other encephalitis with slow recovery.² Afebrile convulsions in patients with rotavirus gastroenteritis have been noted especially in Japan.^{3,4} It is not clear whether the aetiology of central nervous system involvement is the direct

invasion of the virus into the central nervous system. The present paper describes a case of acute encephalitis accompanying rotavirus gastroenteritis. During the course of disease, the patient suffered infantile spasms and delayed psychomotor development as sequelae. Rotavirus antigens and antibody titres were examined in sera, cerebrospinal fluid (CSF), and stool specimens.

Materials and methods

Complement fixation test against human rotavirus, Odelia strain, serotype 4, was determined by micro-complement fixation method. Enzyme linked immunosorbent assay (ELISA) for detecting anti-rotavirus IgG, IgA, and IgM antibodies was carried out by methods described previously.⁵ Sera were diluted 100-fold and CSF 10-fold with buffer.

Positive standard sera diluted 10-, 100-, 1000-, and 10 000-fold were always used simultaneously. Stool specimens were diluted 10-fold with dilution buffer containing 10% fetal calf serum.

Polyacrylamide gel electrophoresis of genome ribonucleic acid from stool samples was carried out by methods described elsewhere.⁶ Negatively stained rotavirus with 3% uranyl acetate was examined under electron microscope. Latex agglutination test using anti-Odelia strain rotavirus antibody was performed. Concentrations of immunoglobulins G, A, and M were determined by nephelometry.

Case report

The patient was a 9 month old Japanese boy, the only child of healthy parents. He had had normal development until watery diarrhoea and vomiting occurred on 9 February 1985. Diarrhoea continued for five days. He had tonic and clonic convulsions on 11 February. Body temperature was 37.1°C. He was admitted to hospital and treated intravenously with nitrazepam and phenytoin. Next morning he was transferred to Teikyo University Hospital because of recurrent intractable convulsions with disturbance of consciousness.

Physical examination on admission showed body temperature of 38.3°C; pulse rate of 189 beats per minute; blood pressure of 138/66 mmHg; no response to stimulation of pain; poor light reflex; bulging anterior fontanelle; decreased deep tendon reflexes, abdominal wall superficial reflex, and cremaster muscle reflex; and no evidence of Kernig's or Babinski's signs.

Laboratory data on admission were as follows: haemoglobin 11.6 g/dl, white blood cell count $29.6 \times 10^9/l$, C reactive protein 4(+), aspartate aminotransferase activity 267 IU, alanine aminotransferase activity 393 IU, lactate dehydrogenase activity 1730 IU, total protein 66 g/l, sodium 133 mmol/l, potassium 4.9 mmol/l, chloride 96 mmol/l, glucose 10 mmol/l (181 mg/100 ml), and ammonia 118 µg/100 ml. Lumbar puncture showed protein 11 mg/100 ml, glucose 5 mmol/l (91 mg/100ml), and initial pressure 120 mmH₂O. Serum IgG was 834 mg/100ml (normal 750±300), IgA 189 mg/100ml (normal 40±25), and IgM 123 mg/100ml (normal 66±34). A chest x ray film showed consolidation in the right upper field. Electroencephalography showed diffusely slow waves, 2-3 Hz. Brain computed tomography revealed brain oedema.

Nitrazepam and phenytoin were used again but were not effective. Convulsions continued for three hours until intravenous injection of phenobarbital. Loss of consciousness without convulsion continued for nine days. After that, recurrent respiratory

infection and convulsion of less than five minutes occurred occasionally with fever (38-39°C) during admission to hospital. Vitamin B₆ (30 mg/kg) was added to phenobarbital (4 mg/kg) orally.

Temporarily improved electroencephalographic findings showed suppression burst pattern. Infantile spasms started in June. Adrenocorticotrophic hormone therapy was effective in decreasing convulsions. Delayed development was pronounced.

Viral examination. Rotavirus was detected from stool specimens for three days after admission by latex agglutination, electron microscopy, and polyacrylamide gel electrophoretic analysis of genome ribonucleic acid. Rotavirus antigen was not detected from CSF specimens by latex agglutination. No viruses were recovered from throat swab and CSF specimens. Antibodies to influenza, rubella, measles, mumps, cytomegalovirus, adenovirus,

Figure Anti-rotavirus antibodies in sera and stools. Samples were diluted as described in Materials and methods.

Table Anti-rotavirus antibodies and total immunoglobulins in cerebrospinal fluid

	Rotavirus specific*			Non-specific (mg/100ml)		
	IgG	IgA	IgM	IgG	IgA	IgM
Feb 15	22	18	23	ND	ND	ND
Mar 1	257	34	64	1.00	0.26	0.1
May 20	939	36	13	2.07	0.26	0.09
Jul 25	806	40	12	4.01	0.42	<0.01
Oct 2	1160	55	13	2.34	<0.01	<0.01

*ELISA value, optic density $\times 1000$. Cerebrospinal fluids were diluted 10-fold by buffer. ND=not done.

herpes virus, and enterovirus were not increased in paired sera and CSF specimens on 12 February and 22 March.

Complement fixation test in sera showed rotavirus infection, while the complement fixation titre did not increase in CSF. Specific anti-rotavirus IgG, IgA, and IgM antibodies were noted in sera. Anti-rotavirus IgA antibody was also found in stools (Figure).

Anti-rotavirus IgG, IgA, and IgM antibodies were determined in CSF by ELISA (Table). Specific IgG concentration was calculated as follows. (1) Total IgG in serum and in CSF were examined each time. (2) Standard curve of anti-rotavirus IgG was drawn using 10-, 100-, 1000-, and 10 000-fold diluted standard serum. Optic density at 100- and 1000-fold diluted standard serum was determined to be 100 and 10 units, respectively. Each optic density in serum or CSF was converted into units. (3) Specific IgG concentration = anti-rotavirus IgG (unit) in CSF/total IgG in CSF: anti-rotavirus IgG (unit) in serum/total IgG in serum. Specific IgG concentration was 3.0 on 20 May and 1.4 on 25 July.

Discussion

Rotavirus is suspected to be a possible agent of Reye's syndrome and encephalitis,¹ although rotavirus antigens have not yet been detected in the central nervous system.

The electropherotype of the genome ribonucleic acid in our patient belonged to subtype F, a common type that has been reported previously.⁶

We do not know of any reports describing anti-rotavirus antibody in CSF specimens found by ELISA.

The anti-rotavirus IgG antibody and total IgG in CSF gradually increased during the course of the disease, and total IgG decreased thereafter. Specific IgG concentration in CSF is sufficient evidence to

conclude a diagnosis of rotavirus encephalitis in this patient because the value of more than one indicates production of antibodies in the central nervous system.

Rotavirus causes exanthema in 4% of patients, a possible sign of viraemia.¹ This may support the case for invasion of rotavirus into the central nervous system through blood vessels.

We have attempted in vitro to inoculate rotavirus into central nervous system cells, MGC cells, and astrocytoma cell line.⁴ Rotavirus was found in the nerve cells by fluorescent antibody method, but cytopathic effects could not be detected. Even after the third passage, virus antigens were noted in the cells, providing evidence that rotavirus can persist in nerve cells.

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