ELECTROPHEROTYPES OF ROTAVIRAL RNA FROM CASES OF INFANTILE DIARRHEA IN URUGUAY

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Received May 13, 2002; accepted June 14, 2002

Summary. – Electropherotypes of human rotavirus isolates from infants with acute diarrhea belonging to two populations with different clinical features were determined. Thirteen electropherotypes were identified in total 69 isolates; 46 (66.6%) isolates had long RNA migration patterns and 23 (33.3%) isolates had short migration pattern. One of the long-pattern electropherotypes (47.82% of the total electropherotypes) was predominant. It was detected in both populations almost throughout the whole period of the study, while other electropherotypes were found only occasionally. The co-circulation of long and short electropherotypes was not frequent.

Key words: rotavirus; RNA electropherotypes; infantile diarrhea

Introduction

Rotaviruses are the most important cause of acute diarrhea in infants and young children, both in developing and developed countries worldwide (Kapikian and Chanock, 1996). Rotaviruses contain a double-stranded RNA genome consisting of 11 segments. Polyacrylamide gel electrophoresis (PAGE) of the genome allows identification of different patterns which are constant and characteristic for a particular rotavirus isolate (Clarke *et al.*, 1981; Estes *et al.*, 1984). The technique divides electropherotypes into short and long RNA patterns, based on large differences in the mobility of segments 10 and 11 of the genome (Espejo *et al.*, 1977, 1979; Kalica *et al.*, 1981). Both, long and short eletropherotypes may also exhibit different mobility patterns of other segments.

Electropherotyping has been applied extensively to molecular epidemiological studies of rotavirus diarrhea worldwide in a large variety of geographical and environmental backgrounds (Buitenwerf *et al.*, 1983; Follett *et al.*, 1984; Rodger *et al.*, 1981; Svensson *et al.*, 1986; Tam *et al.*, 1986; Steele and Alexander, 1987; Hortal *et al.*, 1986; Tietzová *et al.*, 1995; Superti *et al.*, 1995; da Silva *et al.*, 2000). Co-circulation of different electropherotypes, predominance of some strain at a specific time period, and a sequential pattern of appearance of a given electropherotype each year (Estes *et al.*, 1984) are common features arising from these studies.

An extensive diversity in electropherotypes observed among the group A human rotaviruses has been considered the result of two mechanisms: accumulation of point mutations and genetic reassortment between co-circulating strains. Recently a study carried out over six years by Watanabe *et al.* (2001) showed that at least a part of electrophoretic diversity observed among rotavirus strains is due to genetic reassortment between strains concurrently circulating in the human population.

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Abbreviations: CASMU = Centro de Asistencia del Sindicato Médico del Uruguay; CSIC = Comisión Sectorial de Investigación Científica; ELISA = enzyme-linked immunsorbent assay; PAGE = polyacrylamide gel electrophoresis; PBS = phosphate-buffered saline; SDS = sodium dodecyl sulphate

Studies on enteric pathogens in children with acute diarrhea in Montevideo, Uruguay, have identified rotavirus in 14.0–19.5% of the children admitted to the Public Children's Hospital "Pereira Rossell" in Montevideo, Uruguay (Ferrari *et al.*, 1985; Torres *et al.*, 2001; Hortal *et al.*, 1986). The relative incidence was apparently higher in children from middle-income social groups according to a recent study carried out by Ramírez *et al.* (2001) at a prepaid institution (CASMU) where rotavirus was associated with an acute diarrhea in 45% of the cases. In Uruguay with a typical temperate climate, the rotavirus seasonal variation appears all the year round, with a higher percentage occurring in winter in the population attending public hospitals (Hortal *et al.*, 1986) and in autumn in children attending the CASMU Clinic (Ramírez *et al.*, 2001). The only investigation of electropherotypes of circulating rotaviruses, performed in Uruguay between May 1982 and April 1984, showed the occurrence of different patterns only in the long electropherotype, during all the whole period studied (Hortal *et al.*, 1986).

The aim of this study was to investigate the occurrence of different rotavirus electropherotypes causing infantile diarrhea in children admitted at the public hospitals "Pereira Rossell" (1990–1993) and CASMU (1996–2001) in Montevideo, Uruguay. As it has been reported previously (Ramírez *et al.*, 2001), clinical features of these children with acute diarrhea were different. It was therefore interesting to study features of the rotaviruses circulating in the population admitted at these hospitals.

Materials and Methods

Stool specimens were collected at the Public Children's Hospital "Pereira Rossell" from hospitalized children with acute diarrhea during 1990–1993, and at the CASMU Clinic from children as ambulatory or hospitalized patients treated for diarrhea during 1996–2000. All the children under study were less than two years old. Sixty-nine samples previously positive by a commercial ELISA kit detecting rotavirus group A antigens were selected for characterization by polyacrylamide gel electrophoresis (PAGE). Stool specimens were frozen and stored at -20°C until processing.

RNA extraction and PAGE. A 20% of stool suspension in phosphate-buffered saline pH 7.1 (PBS) was used for RNA extraction with sodium dodecyl sulphate (SDS) and phenol-chloroform (1:1) Finally, RNA was ethanol-precipitated and kept at -20°C. The precipitated RNA was pelleted by centrifugation at 10,000 x g for 15 mins and dissolved in 30 µl of the sample buffer (2% SDS, 10% glycerol, 5% 2-mercaptoethanol, 0.005% bromophenol blue and 0.062 mol/l Tris). PAGE was performed according to Laemmli (1970) in a 7.5% separating gel and a 3% concentrating gel. The gels were stained with silver nitrate or ethidium bromide.

Results and Discussion

Sixty-nine stool specimens previously characterized by ELISA were analyzed by PAGE for electrophoretic profile. Although we could not determine the incidence of individual rotavirus electropherotypes in a given year because the number of specimens analyzed in individual years was very limited and different (1 to 19), the results of the electropherotyping allowed the following conclusions. Both the "long and "short" major classes of rotavirus electropherotypes were observed among 69 rotavirus isolates. Among them, 46 (66.6%) belonged to the long pattern and 23 (33.3%) to the short one. These results confirm previous results of studies from different countries where the prevalence of long pattern over the short pattern has been observed (Houly *et al.*, 1986; Broor *et al.*, 1993; Tietzová *et al.*, 1995; Ojeh *et al.*, 1995; Pereira *et al.*, 1983). A diversity in both long and short patterns has been found, distinguishing 13 electropherotypes: LA, LB, LC, LD, LE, LF, LG (long pattern) and SA, SB, SC, SD, SE, SF (short pattern), as shown in Fig. 1 (only predominant RNA profiles are shown). Electropherotype LA was by far the most frequently detected electropherotype, occurring in 33 of 69 (47.82%) isolates studied.

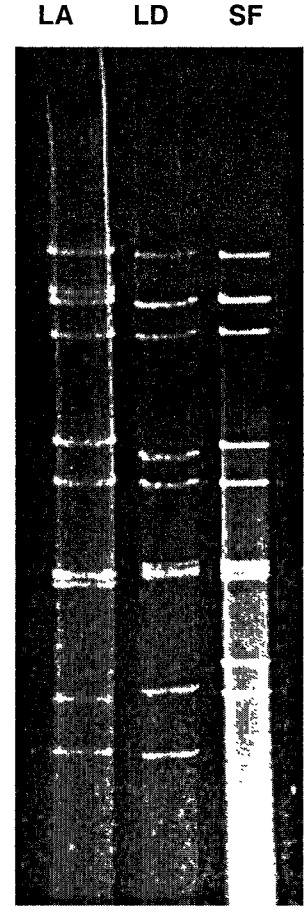

Fig. 1

Predominant rotavirus electropherotypes

LA, LD = long pattern SF = short pattern.

The circulation of a single predominant RNA electropherotype with minor co-circulation variants – a typical feature in rotavirus epidemiology – (Estes *et al.*, 1984; Steele *et al.*, 1993), was also observed in our study during some years. Throughout our study the most frequent LA electropherotype circulated for several years according to the results obtained in similar studies (Albert *et al.*, 1982; Rodger *et al.*, 1981). However, some published data indicate that most of the electro-

Table 1. Rotavirus electropherotypes in 1990–1993 and 1996–2001

Year	No of isolates	Electropherotypes (%)												
		Short						Long						
		SA	SB	SC	SD	SE	SF	LA	LB	LC	LD	LE	LF	LG
1990	1	100	–	–	–	–	–	–	–	–	–	–	–	–
1991	10	20	10	–	–	–	–	10	–	–	50	10	–	–
1992	9	–	–	–	–	–	–	55.6	22.2	11.1	–	–	11.1	–
1993	1	–	–	–	–	–	–	100	–	–	–	–	–	–
1996	7	–	–	–	–	–	–	100	–	–	–	–	–	–
1997	19	–	–	5.26	31.58	10.53	52.63	–	–	–	–	–	–	–
1998	12	–	–	–	–	–	–	91.67	–	–	–	–	8.33	–
1999	6	–	–	–	–	–	–	100	–	–	–	–	–	–
2000	2	–	–	–	–	–	–	100	–	–	–	–	–	–
2001	2	–	–	–	–	–	–	–	–	–	–	–	–	100

pherotypes occurred for short periods of time, in which certain RNA electropherotype emerged and disappeared within a few months (Steele and Alexander, 1987).

In this study with a limited number of specimens and in a study by Hortal *et al.* reported earlier (1986) with a considerable number of specimens analyzed, the co-circulation of major short and long electropherotypes was observed rarely or not at all.

No marked differences in the electropherotype features or in circulating patterns could be found in relation to the two groups of population studied, based on clinical features (Ramírez *et al.*, 2001). Unfortunately, it was not possible to study the specimens from both clinics at the same time. Furthermore, some electropherotypes as LA and LF were detected in both populations.

Although the genotyping of rotaviruses using RT-PCR or sequencing is progressively substituting serotyping methods, the analysis of migration of the genomic RNA fragments from rotavirus, evidenced by electropherotypes continues to be a powerful tool for molecular epidemiological studies, indicating the existence of genetic diversity among co-circulating rotaviruses, with the appearance of new strains, and shifts in the prevalence of some of them. These shifts suggest that rotavirus strains may evolve rapidly by genetic reassortment, as has been shown with rotaviruses of different animal origin or of the same host species under cell culture conditions (Garbarg-Chenon *et al.*, 1984; Ward *et al.*, 1988), or as was evidenced by the characterization of unusual rotavirus isolates from epidemiological studies (Iizuka *et al.*, 1994; Kaga *et al.*, 1994; Matsuno *et al.*, 1988). Recently, a direct evidence of reassortment has been reported (Watanabe *et al.*, 2001) by comparing the electropherotypes of the stool rotavirus specimens obtained from children over a period of six years. Although nucleotide sequencing is needed to accurately establish the relationship between reassortants and their parent strains, the comparison by electropherotypes is a prerequisite for the selection of possible candidates for reassortant. Thus, we think that electropherotypes are still very important. Further combination with nucleotide sequencing may support the hypothesis that frequent genetic reassortment among co-circulating strains, together with an accumulation of point mutation, operates as the principal mechanism generating the genetic variability of human rotaviruses in nature.

Acknowledgements. The work of J.A. was supported in part by a grant from Comisión Sectorial de Investigación Científica (CSIC), Universidad de la República. A.D. had the benefit of a fellowship from UNESCO/PEDECIBA. The authors thank Ms. A.M. Navarro for her technical assistance.

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