Enteropathogenic *Esch. coli* gastroenteritis in premature infants and children treated with fosfomycin

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Baquero, F., Cañedo, T., Rodriguez, A., and Jaso, E. (1975). *Archives of Disease in Childhood*, 50, 367. **Enteropathogenic *Esch. coli* gastroenteritis in premature infants and children treated with fosfomycin.** Forty-two infants, some premature, with enteropathogenic *Esch. coli* (EPEC) gastroenteritis were treated with an oral suspension of fosfomycin in a dose of 100 and 200 mg/kg per day. After the treatment there were 11 secondary clinical infections (6 reinfections and 5 relapses) which received a second treatment with fosfomycin. In total, 53 treatments were made with fosfomycin and in 92% of the cases there was both clinical and bacteriological cure. 93% of the EPEC strains were sensitive to fosfomycin *in vitro*, their minimum inhibitory concentrations being <64 µg/ml. The concentration of fosfomycin in blood and faeces was assayed by a diffusion plate microbiological method in a group of these children, showing that this antibiotic is partly absorbed and the rest eliminated in the faeces, where its concentration was found to be very high. Tolerance of the product was good, and there were neither toxic nor side effects.

Fosfomycin is a new wide spectrum antibiotic described by Hendlin *et al.* (1969) and Stapley *et al.* (1970). It is very active *in vitro* against *Esch. coli* (Zimmerman *et al.*, 1970; Kestle and Kirby, 1970; Martin *et al.*, 1970; Moreno-Lopez *et al.*, 1971) and has practically no toxicity (Miller *et al.*, 1970). The absorption of an oral dose in man is approximately 30 to 40% (Rodriguez and Mata, 1968-1969; Foltz, Wallick, and Rosenblum, 1970). The absence of toxicity and the high activity against *Esch. coli* made us try this new antibiotic on a group of 39 premature babies and infants with gastroenteritis and 3 carriers of enteropathogenic *Esch. coli* (EPEC).

Material and methods

Patients. Table I shows the age and sex of the 42 children studied. 90% were less than 3 months old and 40% were premature. 39 of them had diarrhoea with two previous stool cultures positive for EPEC. It is important to emphasize that 76% of the children were infected with EPEC in hospital. The study was made during a hospital epidemic caused by the 0126:K71B16 serotype; for this serotype the proportion of children

infected in hospital was 94%. In 36 children there were other disorders as listed in Table II.

Table III shows the treatments used before fosfomycin. In 32 children (79%) an antibiotic had been used and in 19 of these the treatment lasted more than a week without obtaining any clinical or bacteriological improvement.

Treatment and dosage. The fosfomycin calcium salt was used as a suspension containing 50 mg/ml of active product. It was given orally as the only antibiotic, in doses of 100 or 200 mg/kg per day divided into 3 or 4 units for an average of 7 days.

Bacteriological study. The selection and diagnosis of all the cases were made by stool culture and immunofluorescence. The following culture media were used: MacConkey agar, Selenite broth, SS agar, MRS agar, Mycobiotic agar, and Mannitol-Salt agar. Colony counts were made on fresh specimens of faeces, which were homogenized and then diluted 1/10 to 1/10 000; 0.05 ml of each dilution were plated out on Cysteine-Lactose-Electrolyte-Deficient agar (Oxoid). The direct immunofluorescence examination was made on the 1/10 dilution of the faeces using polyvalent antisera conjugated to fluorescein isothiocyanate, contrast stained by rhodamine.

EPEC serotyping was done with antisera using the

TABLE I
Characteristics of the patients studied

Sex	Age (d)					Age (m)		Total
	0-10	11-20	21-30	31-60	61-90	3-12	12	
Males	2(1)	5(2)	6(4)	4	3	2		22(7)
Females	1(1)	3(2)	7(6)	5(1)	2	1	1	20(10)
Total								42(17)

Clinical states	Primary cases	Secondary cases
Serious	18	—
Mild	21	11
Carriers	3	—

Numbers in parentheses indicate prematures.

TABLE II
Concomitant states

Digestive apparatus	
Intestinal malrotation 2; postoperative omphalocele 1; oesophageal atresia 1; anal atresia 1; megacolon 1; malabsorption dystrophy 2	
Circulatory-respiratory apparatus	5
Pre/postoperative cardiopathy with/without hepatomegaly 3; pulmonary agenesis 1; transposition of main arteries 1	
Infections	13
Bronchopneumonia 3; otitis 5; pharyngeal carriers of <i>Pseudomonas</i> 2; bacteriuria by EPEC and <i>Enterobacter</i> 1; hepatitis 1; sepsis by <i>Esch. coli</i> 1;	
Nervous system	1
Cerebral postanoxic paralysis 1	
Others	9
Mongolism 1; hypocalcaemia 1; rickets 3; anti-D isoimmunization 1; transfusion reaction 1; hydrocele 2	

The antibiotic sensitivity of the strains to fosfomycin, other antibiotic and chemotherapeutic agents, was examined by the disc-plate test. The minimum inhibitory concentrations (MIC) of each strain to fosfomycin was determined by the agar dilution method using techniques recommended by WHO (Ericsson and Sherris, 1971). An inhibition zone of 10 mm round a 30 µg disc was considered to be the minimum reflecting a therapeutic response to the antibiotic. The criteria recommended by the suppliers (Pasteur Institute) were followed for the other antibiotics. Bacteriological examination of the faeces was repeated every 3 days during the course of treatment, 3 days after ending it, and afterwards between the first and fourth weeks.

Fosfomycin determinations. Determinations of fosfomycin in blood and faeces were made using a plate-diffusion microbiological method with MB-1159 *Erwinia atroseptica* as the test organism (H. Wallick and W. Maiese, personal communication, 1969). Concentrations for the standard curve were 0.5, 0.75, 1.0, 1.5, and 2.0 µg/ml, and dilutions were made in human serum or pH 8.0 Tris buffer (0.05 mol/l) depending upon the assay. Serum samples were diluted in human serum and stool samples in buffer to approximately 1.0 µg/ml. At the working dilutions no stool bacterial interference was seen in the determinations.

Evaluation criteria and secondary infections. We call mild cases those which do not require intravenous fluids and serious cases those needing intravenous drips and frequently having electrolyte disturbances. The criteria of cure were based on the following parameters: the remission of diarrhoea, recovery of lost weight, and elimination of EPEC from faeces. When EPEC were eliminated without clinical response, there was said to be a bacteriological cure. A failure was recorded when there was neither a clinical nor a bacteriological cure. Reinfections were considered to be those cases in which a different serotype or antibiotic of EPEC was

TABLE III
Previous antibiotic treatments in 42 patients

	No. (%)
No previous antibiotic treatment	3 (7.1)
Antibiotic treatment unrelated to gastroenteritis	6 (14.3)
Previous treatment for gastroenteritis	32 (78.6)
Nature of previous treatment	
Colistin alone	12
Colistin and nifuroxazide	11
Nifuroxazide and nalidixic acid	5
Sulphonamides-neomycin	4

method of the Pasteur Institute (Le Minor, 1972). Polyvalent OB and specific OB/O antisera were used; for the latter, the minimum agglutinating values were 1:200 for the O titre and 1:1600 for the B titre.

TABLE IV
Distribution by serotypes of the 57 strains of EPEC isolated

Serotypes	Primary cases		Secondary cases	Total (%)
	1 Type only isolated	2 Types isolated	1 Type only isolated	
018:K21B75	—	—	2	2 (3)
055:K59B5	1	—	1	2 (3)
0111:K58B4	12	—	2	14 (25)
0119:K69B14	4	—	1	5 (9)
0125:K70B15	2	4	—	6 (11)
0126:K71B16	19	4	5	28 (49)
Total strains	38	8	11	57 (100)
	38 (90.5%)	4 (9.5%)	11	
Total cases	42		11	53

found. When the EPEC in the faeces had the same serotype and antibiotype, then it was considered to be relapse.

Results

Microbiological study. All the stool cultures of treated children grew EPEC. In 4 cases there was a mixed infection with two different serotypes. The 57 EPEC strains found were distributed (Table IV) throughout 6 different serotypes, the most frequent being 0126:K71B16 (49%) and 0111:K58B4 (25%).

The quantitative stool cultures and the direct immunofluorescence gave congruent results. In the primary infections the average participation colony count of EPEC in relation to the total count of aerobic-bacterial flora was 76%, including 14 cases in which EPEC in pure culture was isolated. The EPEC count varied between 10^8 and 10^{10} colonies/g faeces. In the secondary infections, however, the participation of EPEC in relation to the total aerobic-bacterial flora was smaller (41% average), the counts also being lower than in the primary infections. In 66% of the stool cultures made before treatment, in addition to EPEC, there were small numbers of *Enterobacter*, *Streptococcus*

faecalis, and nonserotyped *Esch. coli* (Table V).

The sensitivity of the 57 EPEC strains found at the beginning of the treatment to fosfomycin and other antibiotics and chemotherapy agents is summarized in Table VI. Gentamicin and fosfomycin are the most active agents. In the Fig. it can be seen that all the fosfomycin-sensitive

FIG.—Cumulative percentages of the values of minimal inhibitory concentrations of strains of EPEC isolated, and correlation with the diameter of the zone of inhibition.

TABLE V
Accompanying intestinal flora

Organism	Before treatment (%)	3-4th day of treatment (%)	3 days after treatment (%)
<i>Esch. coli</i> nonserotyped	11.3	52.1	67.3
<i>Enterobacter cloacae</i>	52.8	66.7	71.0
<i>Streptococcus faecalis</i>	34.0	31.3	23.2

Note: The percentages in this table correspond to the cases from whose stools the indicated organisms were isolated.

TABLE VI

Susceptibility of 57 strains of EPEC isolated from 42 primary cases and 11 secondary cases

Antibiotic or chemotherapeutic	Concentration of disc (µg)	% of susceptible strains
Gentamicin	30	100
Fosfomicin	30	93.0
Nitrofurantoin	300	88.1
Colistin	10	69.7*
Nalidixic acid	30	57.1
Cephalothin	30	52.4
Tetracycline	30	21.4
Novobiocin	30	14.3
Erythromycin	15	9.5
Neomycin	30	44.0
Streptomycin	30	2.4
Kanamycin	30	2.4
Carbenicillin	100	0
Ampicillin	25	0
Chloramphenicol	30	0
Sulphonamides	50	0

*Ordinarily the percentage susceptibility to colistin of the EPEC strains is greater than this in the clinic.

strains had an MIC of 64 µg/ml or less, with an inhibition zone diameter bigger than 10 mm; only the 4 strains resistant to 30 µg fosfomicin disc exceeded this value. These were strains of *Esch. coli* type 0111:K58B4 and in spite of their comparative *in vitro* resistance, they were eliminated from the faeces with the same ease as the sensitive strains, probably because the concentrations of active fosfomicin in the intestinal

tract were much greater than the MIC values of the strains. In all cases, primary and secondary, oral administration of fosfomicin was followed by the elimination of EPEC from the faeces within 3 days of treatment. Secondary infections appeared 2 weeks after stopping treatment and then only in children who for other reasons had stayed in hospital, EPEC being found again in the stool cultures of 11 children.

According to our criteria, out of the 11 secondary infections we considered 6 to have reinfections (14%) and 5 relapses (12%). All cases developed mild diarrhoea.

Bacteriological studies showed (Table V) that the action of fosfomicin was specific against the causal micro-organism (EPEC) without appreciably altering the rest of the bacterial flora. It is difficult to explain why nonpathogenic *Esch. coli* were selected during the fosfomicin treatment. The selection of *E. cloacae* could be based on the frequent resistance of this species to fosfomicin.

Fosfomicin blood and faecal concentrations. Table VIIA shows the results of blood levels in two groups of children treated with doses of fosfomicin of 100 and 200 mg/kg per day. Though after the first dose the group of children treated with the smaller dose had an average blood level lower than the second group treated with twice the dose, after a few days of treatment the first group, who were all premature babies, showed levels much higher than

TABLE VII

(A) *Blood levels after oral administration of fosfomicin*

Treatment and dosage	Average blood levels (µg/ml)			
	After the 1st dose (h)			During treatment on 3rd to 7th d: 1 h
	1	2	6	
11 patients (8 prematures) 100 mg/kg per day in doses of 25 mg/kg SD	2.8 (0.6)	2.9 (0.7)	2.7 (0.9)	14.2 (2.3)
12 patients 200 mg/kg per day in doses of 50 mg/kg SD	4.6 (1.6)	4.3 (0.5)	3.4 (0.4)	7.8 (0.9)

(B) *Fosfomicin concentration in faeces*

Treatment and dosage	Average of 11 cases on 3-4th d of treatment
11 patients 200 mg/kg per day in doses of 50 mg/kg	3.7 µg/g, faeces just eliminated (maximum value 8.55 µg/g; minimum value 1.33 µg/g)

SD, standard deviation.

TABLE VIII
Summary of results

	Primary cases (and %) (18 serious, 21 mild, and 3 carriers)	Secondary cases (and %) (all mild; 6 reinfect- ions and 5 relapses)	Total (%)
Patients treated	42 (100)	11 (100)	53 (100)
Bacteriological cures	42 (100)	11 (100)	53 (100)
Clinical cures	38*(90.4)	11 (100)	49 (92.4)

*The 4 remaining cases were given successful corrective dietetic treatment for symptoms of malabsorption.

those of the second group, no doubt due to the accumulation of fosfomycin due to the physiological renal insufficiency in premature children.

In another group of 11 children, treated with 200 mg/kg per day given in 4 doses (Table VIIB) the concentration of fosfomycin in faeces was determined. It was not intended to study quantitatively elimination through the rectal route, but to determine the active antibiotic concentration.

Clinical results. Table VIII summarizes the results of fosfomycin treatment of 42 patients who received a total of 53 treatments. Though EPEC was always eliminated quickly, in 4 cases diarrhoea only ceased after adequate dietary treatment. The 11 secondary infections all responded well to a second course of fosfomycin, with no appreciable development of EPEC resistance to it: in one case EPEC MIC increased from 8 to 64 µg/ml. Clinical progress was excellent in all patients, except for the 4 mentioned above, and the general condition improved rapidly, even in the seriously ill patients. The stools became normal in nature and frequency after the third day of treatment. Excluding the 3 carriers, the average number of stools fell from 7 per day before treatment to 3 per day after. Weight, which was decreasing or static before treatment, increased gradually as the children improved and diarrhoea stopped. The average increase was 7.4% at the end of treatment, and 13.4% for the 17 premature children.

Fosfomycin was shown to be more active *in vivo* than *in vitro*, since the 4 EPEC initially resistant were also eliminated, owing no doubt to the high fosfomycin concentrations obtained *in situ*, far exceeding the MIC of these micro-organisms. The results did not appear to differ according to the dosage used (100 or 200 mg/kg per day).

Tolerance and toxicity. There were no side effects, superinfections, or candidiasis, and no evidence of toxicity from the blood analysis data. Transaminase levels did not change with fosfomycin

treatment, since the average SGOT values before, during, and after treatment were 46, 47, and 49 Reitman-Frankel units and SGPT 31, 36, and 30 Reitman-Frankel units. In no case did we find any increase in the values of transaminase, urea, creatinine, and alkaline phosphatase.

Discussion

The treatment of intestinal bacterial infections with antibiotics was questioned by Dixon (1965) and Aserkoff and Bennett (1969), who noted a longer period of excretion of the pathogenic flora in the treated patients than in nontreated ones. Technical progress in fluid replacement and in the general care of the child with diarrhoea have considerably improved the prognosis of these patients, but we believe that antibiotic therapy can improve it further, especially in the acute phase of the disease. In the intestinal invasive growth phase of EPEC, antibiotics can probably reduce the pathogenic EPEC population and so the total amount of enterotoxin produced, decreasing the pathophysiological effects of this substance. Also, as the pathogenic EPEC population is reduced in the baby, antibiotic therapy could help prevent acute epidemic dissemination, which is very important in children's hospitals.

It is possible that the wide spectrum antibiotics, especially high doses, cause suppression of the normal antagonist flora permitting a possible superinfection, relapse, or reinfection. The possibility of affecting the normal antagonist flora is probably more important in the subacute or chronic phase of the disease corresponding to the spontaneous resettlement of this flora. This could explain the negative effect of the prolonged antibiotherapy in the proportion of carriers of enteropathogenic organisms after the acute bacterial diarrhoea. Haltalin, Nelson, and Kusmiesz (1973) have shown the necessity of using absorbable antibiotics in the treatment of shigellosis, and the same may be true for some EPEC gastroenteritis.

Because fosfomycin is absorbed it provides therapeutic serum levels and a high intraluminal con-

centration in active form. With the dose used, it eliminates EPEC rapidly from the stool without appreciably altering the normal enteric flora. The EPEC strains found were sensitive to fosfomycin and no appreciable EPEC resistance developed in the 53 treatments. Hendlin *et al.* (1970) showed that the development of resistance to fosfomycin *in vitro* is of a chromosomal nature and that the resistant strains are defective metabolic mutants that lose the original virulence of the pathogenic strains: they cannot transfer *in vitro* their bacterial resistance to fosfomycin, indicating that this is not of a plasmidic nature. This antibiotic was well accepted and tolerated by children and no toxicity was detected. Fosfomycin can be considered to be a valuable antibiotic for the treatment and epidemiological control of EPEC infections in hospitalized children.

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