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Influence of Orally Administered Antibiotics on Faecal pH and Volatile Fatty Acid Concentrations of Infants and Young Children

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Faecal specimens were collected from infants and children with common infections who presented to the Paediatrics Outpatient Department of the National Children's Hospital, San Jose, Costa Rica or to the Paediatrics Clinic, Texas Tech University Health Sciences Center, Lubbock, Texas. Specimens were obtained before administration of various antibiotics, 7 d after therapy was begun and 28 d after completion of treatment. The antibiotics prescribed were either amoxicillin, ampicillin, cephalexin, choramphenicol, dicloxocillin, erythromycin, penicillin or trimethroprim/sulphamethoxazole. Faecal concentrations of total and individual volatile fatty acids (VFA) and faecal pH levels were determined and compared before, during and after treatment with the various antibiotics. Specimens, collected during the same time periods from a group of healthy infants and children who were not taking antibiotics, were evaluated to determine if normal fluctuations occurred between samplings. There were no significant differences in the Texas subjects in total VFA concentrations and pH levels between the patients receiving antibiotics and healthy participants who did not. With the exception of erythromycin administered to Costa Rican patients, therapeutic doses of the antibiotics did not significantly alter total VFA concentrations or pH levels of the faeces. Variations in concentrations of individual VFAs as a consequence of treatment were demonstrated only in Costa Rican patients receiving either erythromycin or cephalexin; however, the patterns of change were similar to those observed in untreated control participants from Texas and were therefore not attibuted to antibiotic administration. These results are in accordance with our previous studies which show that absorbable antibiotics, administered to mice in therapeutic doses, do not significantly alter total VFA concentrations or pH levels of caecal contents and do not increase the susceptibility of the animals to oral challenge with enteric pathogens.[10] We speculate that the use of absorbable antibiotics does not compromise natural resistance against infections of the intestinal tract of humans.

KEY WORDS—Antibiotics; Faeces; Children.

INTRODUCTION

Acute diarrhoea is an important childhood illness because of its high prevalence and the high associated mortality, especially in developing countries.[16] Several reports indicate that the risk of diarrhoea caused by enteric pathogens increases when humans and animals are treated with antibiotics.[5,6,7,18,19] There is evidence from animal studies that the administration of antibiotics increases susceptibility to colonisation with a variety of enteric pathogens including *Vibrio cholerae*, *Salmonella enteritidis*, *Pseudomonas aeruginosa*, and *Shigella flexneri*.[1,2,8,11,17] The increased susceptibility is thought to result from disruption of the indigenous intestinal flora, producing conditions that favour multiplication of enteric pathogens.

The mechanisms by which the indigenous intestinal flora provide protection against colonisation by enteric pathogens are not completely understood, but there is compelling evidence that short chain volatile fatty acids (VFA) generated by flora components as metabolic end-products play a critical role.[3,4,14] Studies in our laboratory have demonstrated that the VFA, in concentrations found in

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caecal contents of conventional mice, inhibit the multiplication of *P. aeruginosa, Salmonella typhimurium, S. flexneri* and enterotoxigenic *Escherichia coli in vitro*.[9,13,17] The organisms multiplied however in the presence of VFA in concentrations observed in the contents of streptomycin treated mice.[10] Streptomycin, when administered to mice in high doses, significantly lowers caecal VFA concentrations and increases intraluminal pH levels and reduces colonisation resistance to challenge with enteric pathogens.[10,17] However, orogastric administration of amoxicillin, erythromycin, chloramphenicol and trimethoprim/sulphamethoxazole to juvenile mice in doses identical on a weight basis to those given to infants and children in the present study, neither significantly lowered caecal VFA concentrations, nor increased caecal pH levels.[10] When these mice were challenged, susceptibility to colonisation with enteric pathogens apparently was not increased.

The purpose of this study was to determine if oral administration of antibiotics to infants and children to treat a variety of infections influenced faecal pH levels and VFA concentrations in the same manner.

MATERIALS AND METHODS

Study population

Infants and children under 5 y of age who presented to the Paediatric Outpatient Department of the National Children's Hospital, the Social Security Trust, San Jose, Costa Rica, or to the Paediatric Clinic, Texas Tech University Health Sciences Center, Lubbock, Texas, with common infections (otitis media, streptococcal pharyngitis, impetigo and sinusitis) were enrolled in this study. Patients who had been treated with antibiotics within a month preceding the visit or whose main complaint was suggestive of an acute enteric infection were excluded.

Design of study

After the patients with the acute minor infections were evaluated, antibiotics were prescribed. The choice of antibiotics depended on availability, the specific illness and the prescribing preference of the participating physicians. The different antibiotic preparations studied reflected a variety of classes including some widely prescribed in the US and Costa Rica and some available over the counter, without a prescription, in developing countries. Amoxicillin at 40 mg/kg/day, trimethoprim/sulphamethoxazole (TMS) at 10 mg/kg/day of the trimethoprim component, erythromycin ethylsuccinate 40 mg/kg/day or penicillin V potassium at 25 mg/kg/day were administered at the Texas Tech University Clinic. Ampicillin trihydrate at 70 mg/kg/day, cephalexin at 40 mg/kg/day, dicloxacillin at 25 mg/kg/day, erythromycin stearate at 40 mg/kg/day, chloramphenicol palmitate at 50 mg/kg/day or trimethoprim/sulphamethoxazole (TMS) at 10 mg/kg/day of the trimethoprim component were administered in Costa Rica.

Faecal specimens were collected from the patients before antibiotic administration, 7 d after therapy was begun and 28 d after completion of treatment. Hospital specimens were collected by a technician; post-antibiotic specimens were collected either by the mother, an attendant or by a technician. Specimens were placed in screw capped containers that were tightly closed and refrigerated until they were collected. Although patients from Texas or Costa Rica served as their own controls, specimens from healthy children who were not treated with antibiotics collected over a similar time period were included, in order to measure normal variations of faecal VFA concentrations and pH levels and to determine if these factors differ in healthy and infected infants and children.

Measurement of pH and VFA concentration in faeces

Immediately after arrival at the laboratory, both in Lubbock and San Jose, the pH values of faecal specimens were determined potentiometrically, and the specimens then frozen at −70°C. Faecal specimens from Costa Rica were shipped to Lubbock by air express in sealed containers packed with dry ice. All specimens were thawed and 1 g of each homogenised with 1 ml of distilled water. The homogenates were acidified with 0·1 ml/g of 50 per cent H_2SO_4 and 1 ml of anhydrous ethyl ether added to extract short-chain VFA. The ether-faecal content homogenate was mixed in a culture tube by inverting the tube 20 times and venting occasionally to release the pressure. The mixture was centrifuged at 2500 rpm using an IEC clinical centrifuge (International Equipment Co., Needham Heights, MA) for 10 min to break the emulsion and the tubes placed in a −70°C freezer until the aqueous phase froze. The ether extract was then injected onto the column of a gas chromatograph containing a thermal conductivity detector (series 550; Gow-Mac Instruments Co., Bridgewater, N.J.). The chromograph was equipped with two stainless steel

columns (182·9 by 0·32 cm) packed with 10 per cent SP-1000 plus 1 per cent H_3PO_4 on Chromosorb WAW (100/120 mesh). The flow rate of helium, the carrier gas, was 120 ml/min. The injection port temperature was 175°C, the column 145°C and detector 155°C. Peaks of VFA were identified and quantified by reference to different concentrations of VFA standards. VFA concentrations were reported as micro-equivalents per gram wet weight of faeces.

Statistical analysis

Statistical evaluations of the significance of differences in pH levels and VFA concentrations were performed using the Fisher's least significant difference and the Duncan Neuman-Keul tests.

RESULTS

A total of 117 of the 126 initial participants completed the study, 48 in Texas and 69 in Costa Rica. In Lubbock 11 participants served as healthy, untreated comparison patients, 13 patients received amoxicillin, 14 received erythromycin ethylsuccinate, seven received TMS and three received penicillin. In Costa Rica, 14 patients received ampicillin trihydrate, 11 received dicloxocillin, 13 received cephalexin, 14 received TMS, 11 received erythromycin stearate and six received chloramphenicol palmitate. Three faecal samples were obtained from all of the enrolled participants before, during and after treatment. Of the nine patients who did not complete the study, some were lost to follow up while others, who required a change in antibiotic during therapy due to either treatment failure or adverse reactions to an antibiotic therapy, were excluded from analysis.

Total faecal VFA concentrations of healthy, untreated comparison patients in Texas and corresponding patients with infections were not significantly different, nor were there differences in total VFA concentrations between patients from Texas and patients from Costa Rica. Although there were fluctuations in the concentrations of total VFA of specimens from both Texas and Costa Rica collected before, during or after treatment, these did not follow consistent patterns and were not significantly different from the concentrations measured in specimens from the healthy participants. Among the Texas patients, there were no statistically significant changes in total faecal VFA concentrations attributable to antibiotic use (Table 1). A significant decrease in total faecal VFA was observed, on the other hand, when Costa Rican patients were given erythromyin. Among the untreated participants in Texas, the specimens obtained during the second sampling period contained significantly greater total VFA than specimens obtained during the third sampling period indicating fluctuations of VFA do occur over time, which may reflect diet or other factors.

When individual VFA were considered, administration of erythromycin to Costa Rican patients caused significant decreases in faecal acetic, propionic, isobutyric, butyric, isovaleric, and valeric acid concentrations (Table 2). Cephalexin administration in Costa Rica also caused a significant decrease in faecal butyric acid concentration and increase in isobutyric acid concentration. However, specimens obtained from the healthy participants in Texas exhibited similar significant variations in concentrations of propionic and valeric acids from one collection period to another.

There were no significant differences between the pH levels of specimens obtained from Texan or Costa Rican patients receiving antibiotics and from untreated healthy participants. Additionally, there were no significant differences in pH among specimens from patients before, during, or after treatment with any of the antibiotics included in the study (Table 3).

DISCUSSION

With the exception of erythromycin stearate, administered to Costa Rican patients, none of the antibiotics that were studied significantly altered total faecal VFA concentrations. We are unable to explain why erythromycin stearate produced an effect while erythromycin ethylsuccinate, that has the same antibacterial activity and bioavailability and is pharmacokinetically identical, did not. It is likely, however, that all variations in VFA concentrations that we observed were natural fluctuations and were not induced by antibiotic administration. Changes in individual VFA that occurred after erythromycin stearate administration were not significantly different from changes occurring over time in healthy untreated participants from Texas.

The results of our studies are similar to those obtained by Hoverstad *et al.*[12] who administered oral antibiotics to healthy adult volunteers and measured faecal VFA concentrations. Hoverstad and co-workers found that commonly used anti-

Table 1. Total VFA concentrations of faeces of children who received oral antibiotics and of untreated controls

Antibiotics	Total VFA		
	First Specimen	Second Specimen	Third Specimen
Untreated, Texas n=11	51·35±14·24*	92·60±24·67§	31·39± 3·26
Treated, Texas	Before	During	After
Amoxicillin n=13	89·21±11·98	109·11±22·84	78·61±14·02
Erythromycin n=14	70·36±11·64	69·65±24·36	97·12±34·35
Penicillin n=3	104·85±30·20	85·98±19·61	111·78± 4·54
TMS n=7	61·20±12·61	55·58±10·79	69·51±12·81
Treated, Costa Rica	Before	During	After
Ampicillin n=14	47·31± 7·13	51·93±10·54	49·26± 5·42
Cephalexin n=13	62·83±10·77	54·67±11·83	52·90± 8·85
Chloramphenicol n=6	35·98±13·66	20·33± 4·43	50·15±10·09
Dicloxacillin n=11	68·20±16·62	83·59±15·04	44·23± 9·21
Erythromycin n=11	72·00±11·93	36·68± 5·88†	53·16± 7·45
TMS n=14	56·05± 5·62	53·55± 8·11	65·62±10·42

*Mean microequivalents acid per gram of faeces ± standard error of the mean.
†Significantly lower value than either before of after treatment ($p<0·05$).
§Significantly greater value than third specimen ($p<0·05$).

biotics that are absorbed from the intestine (erythromycin, co-trimoxazol, doxycycline, nalidixic acid and ofloxacin) had little effect on VFA concentrations whereas non-absorbable antibiotics such as bacitracin and vancomycin significantly reduced concentrations of these acids. The antibiotics that we investigated are normally absorbed from the intestine.

The findings in this study are in accord with our previous studies[10] which showed that orogastric administration of absorbable antibiotics to mice did not significantly alter caecal VFA concentrations or, with the exception of chloramphenicol, influence caecal pH levels. Colonisation resistance against *S. typhimurium*, *S. flexneri* or enterotoxigenic *E. coli* was not affected by administration of these antibiotics to mice, while a non-absorbable antibiotic, streptomycin, given to mice diminished caecal VFA concentrations and reduced colonisation resistance to the above organisms.

Examination of eight anatomical areas of the human gastrointestinal tract by Moore and Holdeman[15] revealed that the faecal flora is representative of the flora of the ascending, transverse

Table 2. Effects of selected oral antibiotics on faecal VFA concentrations

		Acetic	Propionic	Isobutyric	Butyric	Isovaleric	Valeric
Untreated	First	28·05 ± 7·16*	10·69 ± 14·12	2·95 ± 1·51	7·14 ± 2·68	1·25 ± 0·43	1·39 ± 0·30
Texas	Second	39·56 ± 13·40	28·78 ± 9·91†	4·47 ± 2·05	8·26 ± 1·83	8·19 ± 4·88	3·44 ± 0·88†
n = 11	Third	17·38 ± 1·94	6·98 ± 0·70	0·76 ± 0·24	4·32 ± 0·84	1·18 ± 0·46	9·77 ± 0·20
Erythromycin	Before	35·22 ± 3·95	14·99 ± 3·79	1·60 ± 0·55	15·53 ± 5·40	3·03 ± 0·91	1·53 ± 0·81
Costa Rica	During	24·09 ± 3·32§	6·91 ± 1·24§	0·42 ± 0·24§	6·84 ± 2·25§	0·41 ± 0·28§	0§
n = 11	After	29·24 ± 3·30	15·59 ± 3·15	0·45 ± 0·32	6·43 ± 1·42	0·99 ± 0·44	0·27 ± 0·19
Cephalexin	Before	36·22 ± 5·45	15·64 ± 6·28	0	9·40 ± 1·81	0·92 ± 0·20	0·63 ± 0·29
Costa Rica	During	33·64 ± 6·58	14·25 ± 5·73	9·93 ± 0·09†	5·39 ± 1·22§	0·87 ± 0·21	0·43 ± 0·23
n = 13	After	28·00 ± 5·02	14·43 ± 3·21	0·39 ± 0·23	7·13 ± 0·95	2·24 ± 0·54	0·66 ± 0·34

*Mean microequivalents per gram of faeces ± standard error of the mean.
†Significantly greater value than before treatment ($p < 0·05$).
§Significantly lower value than before treatment ($p < 0·05$).

Table 3. pH values of faeces of children who received oral antibiotics and of untreated controls

Antibiotics	pH		
	First Specimen	Second Specimen	Third Specimen
Untreated, Texas n=11	6·56±0·37*	7·20±0·29	7·03±0·24
Treated, Texas	**Before**	**During**	**After**
Amoxicillin n=13	6·93±0·28	6·78±0·25	6·94±0·28
Erythromycin n=14	6·81±0·27	7·08±0·29	7·49±0·23
Penicillin n=3	6·78±0·11	7·05±0·38	6·86±0·23
TMS n=7	6·90±0·40	6·59±0·39	6·97±0·33
Treated, Costa Rica	**Before**	**During**	**After**
Ampicillin n=14	6·23±0·31	6·10±0·32	6·00±0·34
Cephalexin n=13	6·37±0·31	6·18±0·35	6·95±0·15
Chloramphenicol n=6	5·56±0·45	6·07±0·28	6·45±0·43
Dicloxacillin n=11	6·10±0·34	6·63±0·23	6·67±0·44
Erythromycin n=11	5·91±0·33	6·22±0·18	6·46±0·35
TMS n=14	6·35±0·29	6·05±0·28	5·84±0·27

*Mean pH value of faeces ± standard error of the mean.

and descending colon and rectum. These investigators state that the feces should contain the bacterial populations or bacterial activities present in the colon. It is not unreasonable to assume, therefore, that the VFA concentrations in the human colon are similar to the concentrations present in the faeces and that these acids at physiological pH levels interfere with the colonisation of the human intestinal tract by enteric pathogens.

Our study indicates that commonly administered non-absorbable antibiotics have little effect on human intestinal VFA concentrations or pH levels. We speculate, therefore, that their use does not compromise natural resistance against enteric pathogens mediated by VFA and hydrogen-ions in the human colon.

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