Acute lymphoblastic leukaemia: trimethoprim resistant organisms during treatment

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SUMMARY A cross sectional study was carried out in children receiving treatment for acute lymphoblastic leukaemia to determine the prevalence of trimethoprim resistant organisms in their gut flora and to compare this with a control population. There was a significantly higher prevalence of trimethoprim resistant bacteria in the study group (61%) compared with controls (14%). A longitudinal study showed that emergence of these organisms was intermittent during treatment.

Children receiving chemotherapy for acute lymphoblastic leukaemia are at increased risk of infection by *Pneumocystis carinii*. A combination of trimethoprim and sulphamethoxazole, co-trimoxazole, has proved useful in preventing such infections¹ and is now included in the treatment protocol for acute lymphoblastic leukaemia. Co-trimoxazole has been used in an attempt to decrease numbers of enterobacteriaceae in faeces of adults and children receiving chemotherapy for leukaemia and to prevent bacterial infection.^{2–5} Recently, such regimens have been associated with an increased intestinal colonisation by trimethoprim resistant coliforms, and several patients have developed serious infection with these bacteria.^{6,7} A further complication is that resistance to both trimethoprim and sulphamethoxazole can be encoded on plasmids, and these plasmids may also code for resistance to other antimicrobials, including gentamicin, ampicillin, and chloramphenicol.⁸ Thus administration of co-trimoxazole may select for bacteria resistant to many antimicrobials. Although the emergence of bacteria resistant to trimethoprim in adults with leukaemia who are receiving long term co-trimoxazole is well documented,² there is little information available for children. We present the results of a year's prospective survey into the prevalence and incidence of trimethoprim resistant coliforms in children with acute lymphoblastic leukaemia receiving long term co-trimoxazole.

Patients and methods

All patients attending the regional oncology unit, Alder Hey Children's Hospital, for treatment of

acute lymphoblastic leukaemia were entered into the survey. Nasal swabs, throat swabs, and faeces were inoculated on to Direct Sensitivity Test agar (Oxoid Ltd, Basingstoke), incorporating 7% (vol/vol) lysed horse blood and trimethoprim (4 mg/l), and incubated aerobically at 37°C for 18 hours. Colonies growing on the selective plate were identified using standard techniques and their susceptibility to a series of antibiotics was determined using a controlled disc diffusion method.⁸ Minimum inhibitory concentrations were determined using an agar incorporation method, as described previously.⁹ The survey was designed in two parts.

Firstly, a cross sectional survey was carried out to determine the prevalence of trimethoprim resistant aerobes in the intestinal flora of all children receiving chemotherapy for acute lymphoblastic leukaemia. Specimens were collected over two weeks when children were attending as outpatients. This was compared with the prevalence of carriage of trimethoprim resistant bacteria in the faeces of 568 children who were not receiving co-trimoxazole.

Secondly, a longitudinal study was carried out on children presenting with acute lymphoblastic leukaemia for the first time. Specimens were obtained before induction and at the beginning of prophylaxis and thereafter at monthly intervals. Three patients received co-trimoxazole daily and the remaining patients thrice weekly, at dosages of trimethoprim 160 mg/m²/day and sulphamethoxazole 800 mg/m²/day.

Statistical analyses were performed using χ^2 test with Yates's correction for small numbers where applicable.

Results

In the prevalence study trimethoprim resistant bacteria were not isolated from the anterior nares or throats of any children, nor were trimethoprim resistant Gram positive bacteria isolated from any site. Of 41 children who received prophylaxis with co-trimoxazole, 25 (61%) were excreting trimethoprim resistant bacteria in their faeces. In contrast, significantly fewer ($p < 0.001$) of the control children (79 of 568, (14%)) were excreting trimethoprim resistant bacteria. All isolates obtained were Gram negative. A greater variety of trimethoprim resistant bacteria were obtained from the control group, but *Escherichia coli* was the predominant isolate in both groups (Table 1). All the resistant bacteria had minimal inhibitory concentrations of greater than 800 mg/l trimethoprim. Twenty four (96%) of the trimethoprim resistant bacteria specimens isolated from the study group were also resistant to sulphamethoxazole, whereas 65 (82%) of those obtained from the control group were similarly resistant. None of the isolates obtained from the study group was resistant to gentamicin or tobramycin, but 22 (88%) were resistant to streptomycin and spectinomycin. Twenty three (92%) of the trimethoprim resistant bacteria from the study group were also resistant to ampicillin and ticarcillin and 77 (97%) of those from the control group were similarly resistant.

Thirteen children were entered into the longitudinal study. Two of the children (15%) were already excreting trimethoprim resistant strains of *E. coli* before they received chemotherapy (Table 2). They both continued, intermittently, to excrete trimethoprim resistant *E. coli* of the same biotype as the original isolates. One child also excreted a trimethoprim resistant strain of *serratia* on a single occasion. Seven patients (54%) who were surveyed for a total of 22 months did not acquire trimethoprim resistant bacteria. Five of these patients were followed up for only one to two months. Of the four patients (36%) who acquired resistant bacteria, only one had acquired them by the second month, two by

Table 2 Longitudinal study: prevalence of trimethoprim resistant organisms

Case No	Months from beginning of study											
	0	1	2	3	4	5	6	7	8	9	10	11
1	C	C	—	—	—	—	—	—	—	—	C	—
2	—	—	C	C	C	—	—	C	C	—	—	—
3	—	—	—	—	—	—	—	—	—	—	—	—
4	C	—	—	C	S	C	C	—	—	—	—	—
5	—	—	—	—	C	—	—	—	—	—	—	—
6	—	—	—	C	—	C	—	—	—	—	—	—
7	—	—	—	P	C	C	—	—	—	—	—	—
8	—	—	—	—	—	—	—	—	—	—	—	—
9	—	—	—	—	—	—	—	—	—	—	—	—
10	—	—	—	—	—	—	—	—	—	—	—	—
11	—	—	—	—	—	—	—	—	—	—	—	—
12	—	—	—	—	—	—	—	—	—	—	—	—
13	—	—	—	—	—	—	—	—	—	—	—	—

C=*E. coli*; S=*Serratia*; P=*Proteus*.

the third month, and one by the fourth month. Once colonisation had occurred, it did not follow that the patient continued to be colonised; indeed, intermittent excretion of resistant bacteria was the rule. There was no difference in risk of acquisition of resistant bacteria between children receiving daily or thrice weekly co-trimoxazole.

Discussion

Sulphamethoxazole and trimethoprim inhibit two enzymes necessary for bacterial synthesis of folic acid. The combination, co-trimoxazole, was introduced in the hope that the chances of mutations in two genes would be low and thus the development of resistance slight. Indeed, in comparing long term treatment of recurrent urinary tract infections with co-trimoxazole or trimethoprim alone Pearson *et al* found a decreased incidence of recurrence from 41.4% to 6.3% with co-trimoxazole and only six infections were due to trimethoprim resistant bacteria.¹⁰ In another study patients given either co-trimoxazole or trimethoprim for four weeks were found to have a decreased number of faecal coliforms, but resistance to trimethoprim was encountered.⁷ This occurred mainly in patients given trimethoprim alone. The rapid emergence of increased resistance to trimethoprim in the community was shown in a more recent study in which co-trimoxazole, trimethoprim, or placebo was given to a healthy group of students for only two weeks.⁶ High levels of trimethoprim and sulphonamide resistance were encountered in the co-trimoxazole or trimethoprim groups. *E. coli* isolated was predominantly trimethoprim resistant.

Initial reports on the prophylactic use of co-trimoxazole in patients with leukaemia have shown a decrease in the number of infective episodes.³⁻⁵

Table 1 Trimethoprim resistant bacteria isolated from faeces in 41 patients and 568 controls

Bacteria isolated	Study group	Control group
<i>Escherichia coli</i>	22	51
<i>Proteus</i>	2	8
<i>Citrobacter</i>	1	1
<i>Klebsiella</i>	0	11
<i>Acinetobacter</i>	0	5
<i>Enterobacter</i>	0	2
<i>Serratia</i>	0	1

Subsequent reports have indicated that such treatment selects for trimethoprim resistant coliforms¹¹ and patients could become infected with such bacteria.

In children with leukaemia co-trimoxazole is given prophylactically in an attempt to prevent *Ps. carinii* infections. During the study no child became infected. There is no doubt, however, that the use of co-trimoxazole in our patients selected for trimethoprim resistant bacteria. Sixty one per cent of those who received prophylaxis with co-trimoxazole were found to be excreting trimethoprim resistant bacteria, mainly *E. coli*, whereas only 14% of children who did not receive prophylaxis with co-trimoxazole were excreting such bacteria. Co-trimoxazole also apparently coselected for resistance to sulphonamides as 96% of the trimethoprim resistant bacteria isolated from the children with leukaemia were resistant compared with 82% of the control population. Most isolates from both groups were resistant to ampicillin and ticarcillin, but there was no evidence of resistance to gentamicin or tobramycin. The fact that 88% of the trimethoprim resistant bacteria were also resistant to streptomycin and spectinomycin may provide an insight into the genetics of this resistance as genes for resistance to trimethoprim, streptomycin, and spectinomycin are located on a single transposon (Tn7).¹²

No patient became infected with trimethoprim resistant bacteria during treatment with co-trimoxazole. It is noteworthy that trimethoprim resistant Gram positive bacteria were not selected by this prophylactic regimen as 70% of episodes of bacteraemia in our patients with leukaemia are due to Gram positive bacteria, such as *Staphylococcus aureus* and *Streptococcus pneumoniae* (unpublished data). In the longitudinal study 15% of patients were already colonised by trimethoprim resistant coliforms before treatment, which is in close agreement with the prevalence of resistance in the control population. Colonisation did not occur until the second month of prophylaxis and in most patients was intermittent.

As it is necessary to include prophylaxis for pneumocystis infection it is fortunate that although co-trimoxazole selects for resistant coliforms in the gastrointestinal tract, such bacteria, to date, have rarely caused serious infection. It would also seem that co-trimoxazole has not yet selected for plasmids that encode resistance to many different antimicrobials and, in particular, to gentamicin or tobramycin. Regimens for treatment of acute lymphoblastic leukaemia, however, have become increasingly in-

tensive, with a high incidence of profound neutropenia and increased side effects from chemotherapy. This, in particular, gives increased potential for invasive infections of gut origin and remains so throughout continuing treatment.¹³ Vigilance is therefore necessary with regard to the increased prevalence of trimethoprim resistant bacteria in the gastrointestinal tract of children with leukaemia and the potential for selecting resistance to many commonly used antimicrobials.

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