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Disturbance of 6-mercaptopurine metabolism by cotrimoxazole in childhood lymphoblastic leukaemia

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Summary. *The effect of cotrimoxazole on the utilization of 6-mercaptopurine (6MP) was studied in a group of children receiving remission maintenance treatment for lymphoblastic leukaemia (ALL). This was done by measuring the level of an active metabolite of 6MP, 6-thioguanine nucleotide (6TGN), and comparing it both with the drug dose and with subsequent neutropenia in the presence or absence of concurrent cotrimoxazole.*

In children who were not taken cotrimoxazole, the concentration of 6TGN showed a significant positive correlation with the dose and a significant negative correlation with the absolute neutrophil count 2 weeks later. In those who were taking the antibiotic both these relationships were lost.

This suggests that cotrimoxazole can interfere with both the absorption and the cytotoxicity of 6MP and may, in turn, alter its antileukaemic effect.

Introduction

Long-term cotrimoxazole therapy has been successfully used to prevent *Pneumocystis carinii* pneumonitis developing in children receiving immunosuppressive drugs [4]. For this reason it is now widely prescribed as part of remission maintenance therapy in lymphoblastic leukaemia (ALL), but it appears to have been adopted without detailed study of what effect it may have on the primary disease, either directly or by interference with the metabolism of the cytotoxic drugs used. A recent report has suggested that cotrimoxazole may be synergistic with methotrexate in producing an enteropathy [8], but we have been unable to find any documentary evidence concerning possible interaction with the other mainstay of remission maintenance, 6-mercaptopurine (6MP). To investigate this possibility and as part of a broader study of 6MP metabolism in ALL, based on the assay of its active metabolite, 6-thioguanine nucleotide (6TGN) [3, 6, 7], we have compared children who were taking cotrimoxazole with those who were not.

Patients and methods

Red blood cell (RBC) 6TGN assays were performed in children with ALL who were being treated according to the MRC trial schedule, UKALL VIII. The duration of remission maintenance therapy varied from 4 weeks to 2 years.

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The dose of 6MP was adjusted weekly on a sliding scale of 100%, 75%, 50%, or 0% from a baseline dose of 75 mg/m^2, in response to neutropenia or thrombocytopenia at the time of prescription. The children also received a weekly dose of oral methotrexate, adjusted to parallel the 6MP from a baseline dose of 20 mg/m^2. Vincristine was given monthly (1.5 mg/m^2), as was a 5-day course of prednisone (40 mg/m^2). Children in the first 27 weeks of maintenance therapy received continuous cotrimoxazole (150 mg/m^2 per day), after which time it was withdrawn for the remaining 120 weeks.

Blood samples (0.5 ml) for 6TGN assay were obtained at the time of venepuncture for vincristine injections in all cases. 6TGN was assayed fluorimetrically following extraction from 100 µl packed RBCs (8×10^8 RBCs), by a technique described in detail elsewhere [6]. The absolute neutrophil count (ANC) was measured in the routine blood counts at the clinic visit 2 weeks later.

Children studied while receiving cotrimoxazole were compared with those who had ceased to take it. The latter group included, in some cases, the same children later in their course of treatment. The RBC 6TGN concentration was compared with the daily dose of 6MP (corrected for surface area) prescribed for the child during the week prior to assay, and with the ANC 2 weeks afterwards.

Statistical analysis was by Pearson's product moment correlation co-efficient and Student's *t*-test.

Table 1. Mean 6-MP dose, 6TGN concentration, and ANC in children with and without cotrimoxazole

		With cotrimoxazole (n = 35)	Without cotrimoxazole (n = 49)
6MP dose (mg/m^2)	Mean	44.86	47.10
	Range	0–82	0–83
	SD	25.94	28.70
6TGN concentration (pmol/8 × 10^8 RBCs)	Mean	234	203
	Range	36–802	0–665
	SD	169	138
ANC 2 weeks later (× 10^9/l)	Mean	1.45	1.83
	Range	0.07–7.0	0.2–6.64
	SD	1.25	1.50
Mean duration of 6MP medication (months)		6.2	16.0

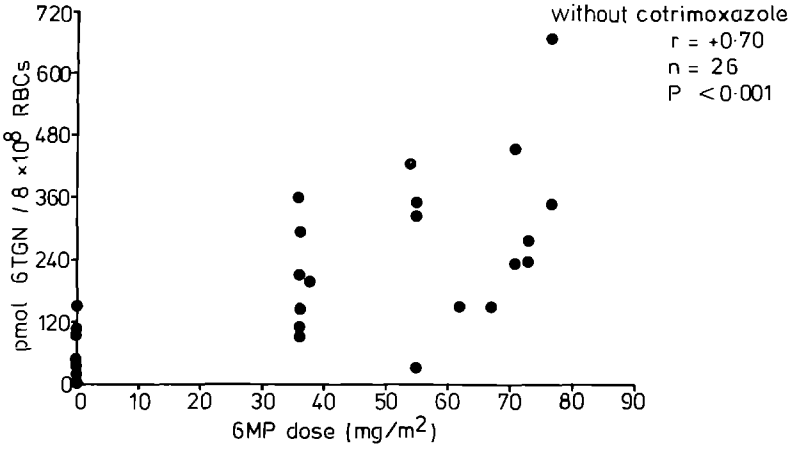

Fig. 1. Without cotrimoxazole. The relationship between 6MP dose and 6TGN concentration in eight children studied both with and without cotrimoxazole

Table 2. Effect of cotrimoxazole on the relationship between 6MP dose and 6TGN concentration and between 6TGN concentration and ANC

		With cotrimoxazole (n = 35)	Without cotrimoxazole (n = 49)
Correlation of dose and 6TGN	r	+ 0.2640	+ 0.4598
	P	NS	< 0.001
Correlation of 6TGN and ANC	r	− 0.1028	− 0.4808
	P	NS	< 0.001

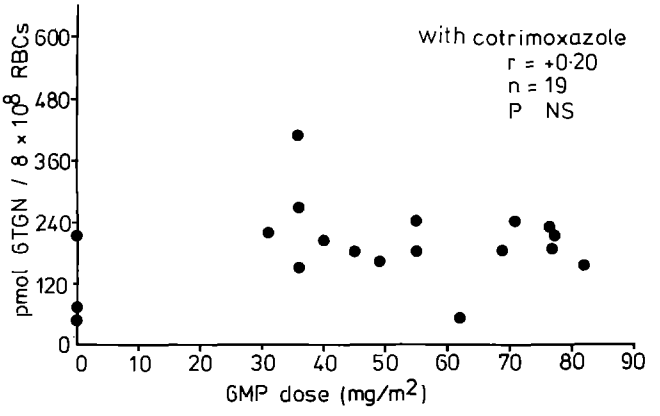

Fig. 2. With cotrimoxazole. The relationship between 6MP dose and 6TGN concentration in eight children studied both with and without cotrimoxazole

Results

Eighty-four red cell 6TGN assays were performed in 22 children, 11 girls, and 11 boys, with an age range of $2^1/_2$–16 years. Thirty-five measurements of 6TGN were obtained from children who were taking cotrimoxazole and 49 from children who were not. Eight children (3 girls and 5 boys) appear in both groups, as, according to protocol, they ceased to take cotrimoxazole after 27 weeks.

The results are shown in Tables 1 and 2. There was a significant positive correlation between dose and 6TGN concentration ($P < 0.001$) and a significant negative correla-

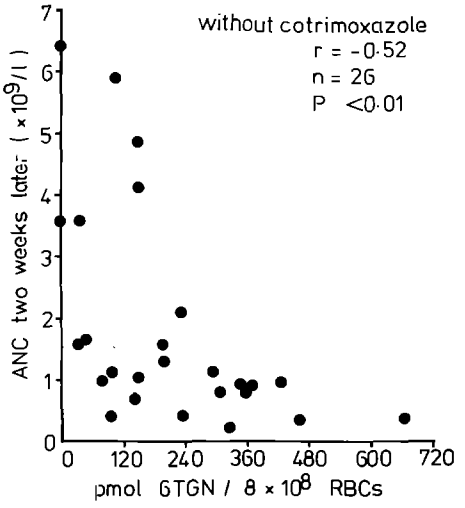

Fig. 3. Without cotrimoxazole. The relationship between 6TGN and ANC in eight children studied both with and without cotrimoxazole

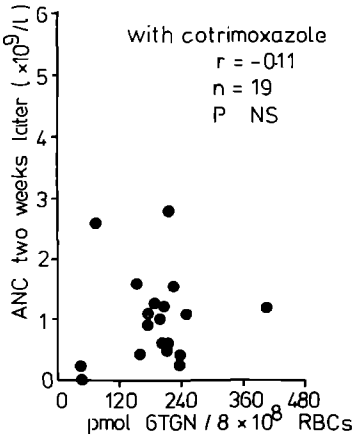

Fig. 4. With cotrimoxazole. The relationship between 6TGN concentration and ANC (ANC) in eight children studied both with and without cotrimoxazole

Table 3. Mean 6MP dose, 6TGN concentration, and ANC in the eight children both with and without cotrimoxazole

		With cotrimoxazole (n = 19)	Without cotrimoxazole (n = 26)
6MP dose (mg/m^2)	Mean	47.16	40.15
	Range	0–82	0–77
	SD	25.75	28.44
6TGN concentration (pmol/8 × 10^8 RBCs)	Mean	192	210
	Range	48–407	0–665
	SD	81	160
ANC 2 weeks later (× 10^9/l)	Mean	1.1	1.86
	Range	0.07–2.8	0.2–6.4
	SD	0.718	1.75

tion between 6TGN concentration and ANC 2 weeks later ($P < 0.001$) in children who were not receiving cotrimoxazole. In children who were taking cotrimoxazole there was no correlation either between dose and 6TGN concentration or between 6TGN concentration and ANC. These observations are strengthened when the same children are studied both with and without cotrimoxazole. The results recorded in the eight children appearing in both groups are presented in detail in Figs. 1–4 and Table 3.

The possibility that cotrimoxazole was interfering with the assay for 6TGN was explored by the addition of cotrimoxazole to the assay system in vitro. No such effect was found.

Discussion

These results suggest that cotrimoxazole may influence the utilization of 6MP at two levels. First, the loss of correlation between the 6MP dose and RBC 6TGN concentration suggests interference by cotrimoxazole either with absorption of 6MP or with its metabolism to 6TGN. Secondly, the loss of correlation between 6TGN level and ANC 2 weeks later suggests either an independent effect of cotrimoxazole on the neutrophil count or an influence on the myelotoxic effect of 6TGN. A third possibility, that cotrimoxazole interferes in vitro with the assay for 6TGN, was considered, but no such effect was found.

Because of the structure of the UKALL VIII protocol, the children taking cotrimoxazole are not exactly comparable with those who are not. The schedule requires that continuous cotrimoxazole is only given during the first 27 weeks of remission maintenance. It is possible that the duration of antileukaemic treatment rather than cotrimoxazole might be the variable interfering with 6MP utilization. However, when children receiving cotrimoxazole were divided into two groups according to the duration of prophylaxis (2 months and under, over 2 months) no increase in correlation of dose and 6TGN concentration, or of 6TGN concentration and myelosuppression, was found with the passage of time.

Cotrimoxazole appeared merely to produce loss of predictability of absorption and metabolism of 6MP and of its cytotoxic effect, rather than a definite trend towards potentiation or inhibition. There was no significant difference in the mean dose, mean 6TGN concentration, or mean ANC between those receiving cotrimoxazole and those not. Neither was there any significant difference in the ratio of dose to 6TGN concentration or of 6TGN concentration to neutrophil suppression between the two groups.

The loss of predictability of 6MP utilization is consistent with there being several theoretical levels of possible interaction with cotrimoxazole. Absorption may be affected because of an alteration of bowel flora by long-term cotrimoxazole. The suggested combined effect of methotrexate and cotrimoxazole in producing malabsorption during maintenance chemotherapy [8] could influence the predictability of 6MP absorption. Binding of 6MP to plasma protein has been described [9], although more information is required regarding the nature and significance of this. The possibility of competition between 6MP, or active 6MP metabolites, and cotrimoxazole for protein binding warrants consideration. Cotrimoxazole has been reported to exert a direct myelosuppressive effect [2] and there have been several reports of severe toxicity produced by cotrimoxazole in patients who already have early megaloblastic changes [1, 5, 10]. This might be expected to apply to children receiving antimetabolites.

It can be concluded that cotrimoxazole interferes with the pharmacokinetics of 6MP, but in what precise way and with what significance cannot be said. Neither can any inference be drawn on parallel interference with other drugs. It is important, however, to realize that interference by cotrimoxazole with the antileukaemic effect of the primary treatment may occur, and the mechanisms involved should be investigated further. Its widespread use has evolved in the belief that it is effective in preventing pneumonitis and does no harm. This may not be true and its optimal use should be better defined.

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