Importance of 6-mercaptopurine dose in lymphoblastic leukaemia

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Abstract
To explore the possibility that higher total dosage of 'maintenance' treatment may have contributed to the recent improvement in outlook of children in the United Kingdom with lymphoblastic leukaemia, details of the amount of 6-mercaptopurine prescribed during the first two years of treatment were studied in an unselected cohort of children diagnosed between 1973 and 1987. Eighty-five patients were studied, 30 diagnosed before and 55 after 1980. The group diagnosed after 1980 showed an 18% improvement in relapse free survival at five years. Their median total dose of 6-mercaptopurine had increased by 22%, whereas according to the protocol it should have risen by an average of only 9%. After 1980 boys were prescribed significantly more 6-mercaptopurine than girls, and had fewer dose reductions because of myelosuppression.

These findings support the clinical impression that after 1980 an important therapeutic difference resulting from the new United Kingdom acute lymphoblastic leukaemia protocols was an increase in the amount of 6-mercaptopurine that children actually received as a result of changes in prescribing guidelines rather than dose. They also provide further evidence that boys tolerate 6-mercaptopurine better than girls, which may be related to the still unexplained difference in prognosis between the sexes.

During the last decade there has been a striking improvement in long term survival for children with lymphoblastic leukaemia in the United Kingdom. Before 1980 treatment protocols were somewhat vague about what modifications in dose were permitted during the 'maintenance' phase of treatment, with the result that many clinicians veered away from doses that caused myelosuppression to minimise morbidity. With the implementation of the United Kingdom acute lymphoblastic leukaemia (UKALL) VIII protocol in 1980, which required a much greater degree of compliance by doctors to a strict regimen of permitted modifications in doses, two notable differences gradually became apparent. Firstly, there was an increased incidence of morbidity and mortality as a result of the treatment and secondly, despite this, there was a 20% improvement in long term survival.

The two antileukaemic drugs that were most affected by the changes in prescribing practice were 6-mercaptopurine and methotrexate, both of which were used during the 'maintenance' phase of treatment. Of these 6-mercaptopurine was likely to be the most important as the dose of methotrexate was relatively small.

It is important to understand that the main differences between protocols before and after 1980 are related to adjustments in dose rather than 'target' protocol doses. Our hypothesis was, therefore, that children treated for acute lymphoblastic leukaemia since 1980 had been exposed more 6-mercaptopurine by having the dose reduced less frequently and that this could be an important factor in their better prognosis. We also suspected that, based on preliminary work on the pharmacokinetics of 6-mercaptopurine, there may be a difference between the sexes in tolerance of 6-mercaptopurine, and it was to explore these two suppositions that the present study was undertaken.

Patients and methods

PATIENTS
Children with acute lymphoblastic leukaemia who were attending the Children's Hospital, Sheffield and who had completed two years continuing chemotherapy in their first remission were studied. The patients were divided into two groups, those diagnosed before 1980 (December 1973 to February 1980) and those diagnosed after 1980 (March 1980 to April 1990). In the group diagnosed before 1980 consecutive cases who received chemotherapy according to the Medical Research Council trial protocols UKALL III (arms 'ordinary' A, B, C, and D and 'ordinary modified' A and E) and UKALL V (arms 'continuous' and 'gaps') were studied. In the group diagnosed after 1980 children receiving chemotherapy according to protocols UKALL VIII (arms A and B) and UKALL X (arm A) were studied. Only arm A of UKALL X was used because the effect on the outcome of treatment of the additional blocks of intensification treatment in arms B, C, and D of the trial was not known. Only children with no mediastinal mass and diagnostic white cell counts of less than 20x10^9/l were eligible for UKALL III and V, so when comparisons were made between patients diagnosed before and after 1980, children with T-cell acute lymphoblastic leukaemia and white cell counts of more than 20x10^9/l at diagnosis were excluded from the group diagnosed after 1980. When the group diagnosed after 1980 was studied on its own, children with all immunological types of acute lymphoblastic leukaemia (except B-cell) and all diagnostic white cell counts were included.
Children were studied from the time that they started receiving 6-mercaptopurine during the phase of treatment directed towards central nervous system prophylaxis and throughout eight subsequent cycles of 'maintenance' chemotherapy. All the protocols are described in detail elsewhere, and the differences in 6-mercaptopurine regimens among them are shown in the table. All contained an induction phase of four to five weeks when they received weekly vincristine intravenously (1·5 mg/m²), daily oral prednisolone (40 mg/m²), and asparaginase (crisantisapase) intramuscularly, the doses and times varying depending on the protocol. UKALL VIII A and X also included two intravenous doses of daunorubicin (45 mg/m²). Induction was followed by a central nervous system prophylaxis phase of two to three weeks during which cranial radiotherapy was given (24 Gy in UKALL III and V, and 18 Gy in UKALL VIII and X). This was accompanied by weekly intrathecal methotrexate, and 6-mercaptopurine was started during this phase. 6-Mercaptopurine was given as a single daily oral dose, the dose being dependent on the protocol. Treatment was continued either continuously (UKALL III 'ordinary' and modified A, VIII, X and V 'continuous') or with a one week gap every three (UKALL III modified E) or four weeks (UKALL V 'gaps'), together with weekly methotrexate orally (20 mg/m²) and monthly vincristine intravenously (1·5 mg/m²) with five days of oral prednisolone (40 mg/m²). 'Maintenance' treatment was given for two to three years depending on the protocol.

In all the protocols the dose of 6-mercaptopurine was reduced if myelosuppression occurred. In UKALL III and V the aim was to keep the total white cell count at 2-3·10⁹/l, but instructions were not specific and modification of the dose was largely at the clinicians' discretion.

In UKALL VIII and X much stricter criteria for modifying the doses of 6-mercaptopurine and methotrexate were introduced; the drugs were reduced to 50% or 0% of the target dose if the neutrophil count fell to less than 1 or less than 0·5·10⁹/l. They were restarted when the counts rose above the threshold and built up to the target dose again over a period of three weeks.

In the latter part of UKALL VIII and throughout UKALL X oral co-trimoxazole was given during 'maintenance' chemotherapy. Children on UKALL VIII received the drug daily for the first 27 weeks, and those on UKALL X took it three times weekly throughout treatment.

PREScribing of 6-MERCAPTOpurINE
The actual amounts of 6-mercaptopurine prescribed for each child during central nervous system prophylaxis and the first 24 months of 'maintenance' treatment were extracted from the records. They were converted into mg/m² for each three month period and then added together. For those diagnosed after 1980 the longest uninterrupted period at the target dose and the number of times that the dose was reduced because of myelosuppression were also extracted.

This was not done for the group diagnosed before 1980 because few children were ever given the target dose and because there was no consistency in the degree of myelosuppression that led to a reduction in dose, so the results could not be compared.

STATISTICAL ANALYSIS
The Mann-Whitney U test was used to compare medians. Duration of remission between the groups was compared by the log rank test. When considering control of the disease by treatment, deaths during remission and second malignancies were not counted as 'events' but were censored at the time of their occurrence.

Results
In the group diagnosed before 1980 there were 30 eligible children (19 boys and 11 girls). Nineteen children were treated according to UKALL V (11 boys and eight girls), and 11 according to UKALL III (eight boys and three girls). The ages at diagnosis ranged from 2 to 11 years (median 5·5), and the diagnostic white cell counts ranged from 0·3·16·3·10⁹/l (median 4·2). All had 'non-T-cell' acute lymphoblastic leukaemia. At the end of April 1990 length of...
follow up ranged from 27 to 193 months (median 147).

In the group diagnosed after 1980 there were 55 children (30 boys and 25 girls). Forty-four (24 boys and 20 girls) were consecutively enrolled in UKALL VIII (arms A and B) and 11 (six boys and five girls) were consecutively enrolled in UKALL XA. The ages at diagnosis ranged from 0-3-13 years (median 4) and the white cell counts at diagnosis ranged from 0-8—670 x 10^9/l (median 9.0). The immunological disease types were distributed as follows: 65% were common acute lymphoblastic leukaemia, 16% were pre-B, 5% were null, 5% were T-cell acute lymphoblastic leukaemia, and 9% were unclassifiable. At the end of April 1990 the length of follow up ranged from 23 to 121 months (median 76). When this group was matched with the group diagnosed before 1980, 15 children who had T-cell acute lymphoblastic leukaemia or white cell counts of more than 20 x 10^9/l at diagnosis were excluded to make the groups comparable.

PRESCRIBING OF 6-MERCaptopurINE
Group diagnosed before 1980
Before 1980 modifications of the dose of 6-mercaptopurine if myelosuppression occurred were left to the clinicians’ discretion, with the aim of keeping the white cell count between 2 and 3 x 10^9/l. Prescribing of 6-mercaptopurine under these relaxed guidelines was compared with prescribing under the stricter regimens after 1980. Children diagnosed before 1980 received considerably lower cumulative doses of 6-mercaptopurine (range 22 106 to 49 563 mg/m^2, median 31 103) than comparable patients treated after that date (range 22 820 to 50 777 mg/m^2; median 40 499) (fig 1). The median difference between the two groups was 7743 mg/m^2 (95% confidence interval (CI) 4141 to 10 964 mg/m^2, p<0.0001).

This increase in the amount of 6-mercaptopurine that was prescribed cannot be accounted for solely by differences between protocols before and after 1980. The percentage increase in the median prescribed dose after 1980 was 22%, but the average increase in the ’target’ cumulative dose of 6-mercaptopurine specified in the protocols after 1980 was only 9%. The largest difference was in UKALL III ‘ordinary modified’ E in which the potential dose of 6-mercaptopurine was 34% less than the potential dose after 1980, but only three children in the group diagnosed before 1980 received chemotherapy according to this protocol.

When boys and girls were analysed separately the increase in the amount of 6-mercaptopurine prescribed after 1980 was more pronounced for boys than for girls. Before 1980 boys’ doses ranged from 23 365 to 49 563 mg/m^2 (median 31 019) and after 1980 ‘standard risk’ boys’ doses ranged from 29 746 to 50 777 mg/m^2 (median 41 711). The median difference was 9025 mg/m^2 (95% CI 4264 to 13 302, p<0.004).

For girls doses prescribed before 1980 ranged from 22 106 to 39 843 mg/m^2 (median 31 187) and after 1980 (‘standard risk’) doses ranged from 22 106 to 47 175 mg/m^2 (median 37 560). The median difference was 6394 mg/m^2 (95% CI 540 to 11 419 mg/m^2, p<0.04). Among those diagnosed before 1980 there was no significant difference in the amount of 6-mercaptopurine prescribed to boys compared with girls.

Group diagnosed after 1980
In the group diagnosed after 1980 (‘all risk’) the median cumulative dose of 6-mercaptopurine for boys was 42 099 mg/m^2 (range 29 746–50 777) whereas for girls it was 38 151 mg/m^2 (range 22 820–47 175) (fig 2). The median difference between the prescribed dose for boys compared with that for girls was 3647 mg/m^2 (95% CI 222 to 7468 mg/m^2, p<0.03).

The greater tolerance to 6-mercaptopurine shown by boys after 1980 was also reflected in the duration of the longest uninterrupted period they spent at the target dose of 75 mg/m^2. Their median time was 19 weeks (range 5–82) whereas for girls it was 13 weeks (range 2–45) (p<0.05) (fig 3). Girls also had more reductions in doses because of myelosuppression that were specified in the protocols (range 5–23, median 14.0) than boys (range 2–20, median 10.5) (p<0.03).

Non-compliance could potentially have affected these results, but children in this study group were also having red blood cell 6-thioguanine nucleotides (an active metabolite of 6-mercaptopurine) measured regularly as part

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**Figure 1** Cumulative dose of 6-mercaptopurine (mg/m^2) in study groups before (n=30) and after (n=40) 1980. Horizontal lines indicate median values (before 1980, 31 103 mg/m^2, after 1980, 40 499 mg/m^2).

**Figure 2** Cumulative dose of 6-mercaptopurine (mg/m^2) in boys (n=30) and girls (n=25) diagnosed after 1980. Horizontal lines indicate median values (boys 42 099 mg/m^2, girls 38 151 mg/m^2). Closed symbols indicate patients who subsequently relapsed.
of another study and non-compliance was not
detected by this technique.

OUTCOME OF TREATMENT
Outcome of treatment among the children
studied reflected national trends in the United
Kingdom. At the time of analysis there had
been nine relapses (two girls and seven boys in
the group diagnosed before 1980 compared with
four in the group diagnosed after 1980 ('standard
risk') who were all boys. Log rank analysis indi-
cated that there was an 18% improvement after
1980 in those still in first remission at five years
($\chi^2=3.9, p<0.05; 95\% \text{CI } 0.08 \text{ to } 36.76\%)$.

In the 'all risk' group diagnosed after 1980,
outcome of treatment was also examined in rela-
tion to the amount of 6-mercaptopurine that
had been given. There were seven relapses in
the whole group (all boys). There was an esti-
mated 15% increase in numbers remaining in
remission at five years for those who received
less than the group median cumulative dose of
6-mercaptopurine (one relapse) compared with
those receiving more than the group median
dose (six relapses) ($\chi^2=3.69, p<0.07; 95\% \text{CI }$
$3.6 \text{ to } 33.2\%$) (fig 4). Five of the seven
relapses occurred in the upper quartile for 6-
mercaptopurine dosage.

There were no differences between the
groups above and below the median dose of
6-mercaptopurine in diagnostic white cell count
or type of leukaemia. There was an apparent
excess of boys in the group above the median
(18 boys and nine girls) and particularly in the
upper quartile (11 boys and three girls) but this
was not significant.

Discussion
This single centre study with homogeneity of
prescribing practice both before and after 1980
provides objective data to support the sugges-
tion made by the Medical Research Council
Working Party that more rigid prescribing cri-
teria for drugs used in 'maintenance' treatment
have contributed to the improved outlook for
children with acute lymphoblastic leukaemia
diagnosed after 1980. In our patients the
amount of 6-mercaptopurine that was prescrib-
ed undoubtedly increased after 1980, and
much more than could be explained by changes
in target doses specified in protocols. This was
associated with a significant improvement in
relapse free survival.

Because of the parallel adjustment of doses of
6-mercaptopurine and methotrexate during
'maintenance' chemotherapy it also follows that
cumulative doses of methotrexate must have
increased after 1980 as well. We believe that this
is less likely to have had such a large effect on
outcome, as the doses of methotrexate that are
used are small.

Other studies have also shown an association
between the amounts of 6-mercaptopurine and
methotrexate prescribed during 'maintenance'
chemotherapy and subsequent relapse of
leukaemia. In studies in which children were
not treated with doses large enough to cause
toxicity,67 either because of the dose given in
the protocol,6 or because physicians did not
comply,7 the children who received less treat-
ment did worse. In contrast, Silberman et al
found that a small group of children with acute
lymphoblastic leukaemia who received less than
half their potential dose of 6-mercaptopurine
because of pronounced toxicity related to treat-
ment did better than similar patients who had
less toxic reactions.

Our group diagnosed after 1980 show the
same paradoxical phenomenon. Within this
group most children were being treated with
doses large enough to cause toxicity and were
occasionally experiencing myelosuppression.
Those who had more toxic reactions, and who
were therefore being prescribed less 6-
mercaptopurine seemed less likely to relapse,
whereas those who tolerated uninterrupted
target doses were more likely to relapse. This
observation was not significant, but taken in
the context of other studies points to a suggestive
trend.

As more children are being prescribed as
much 6-mercaptopurine as they can tolerate,
individual differences in metabolism of the drug
are becoming apparent. In the group diagnosed
after 1980 the cumulative dose of 6-mercaptop-
urine that was tolerated by different patients
ranged from 43% to 97% of the potential dose
using the same prescribing guidelines. This
almost certainly reflects underlying differences
in the metabolism of 6-mercaptopurine among
patients.
As well as differences among patients the group diagnosed after 1980 also showed a significant difference in the tolerance of 6-mercaptopurine between the sexes, the boys being prescribed more 6-mercaptopurine and spending longer uninterrupted periods at the target dose specified in the protocol than girls using the same prescribing guidelines. This has been noted before over shorter times and presumably reflects a sex difference in the metabolism of 6-mercaptopurine. This difference in tolerance between the sexes was not noted in the group diagnosed before 1980. The reason may be that with the earlier ‘gentle’ approach to treatment, doses of 6-mercaptopurine were not influenced as much by tolerance in either sex. In our study group boys had a worse outcome of treatment than girls, and it was striking that the boys most tolerant of 6-mercaptopurine subsequently relapsed more often.

The worse prognosis for boys with acute lymphoblastic leukaemia remains unexplained but perhaps sex differences in the metabolism of 6-mercaptopurine may in large part be responsible. It may not be a coincidence that hypoxanthine-guanine phosphoribosyltransferase (the main enzyme responsible for converting the inactive native drug to its cytotoxic metabolites) is sex linked, and we are currently exploring this possibility.

In the meantime the findings of this single centre study clearly indicate that one of the chief differences between treatment protocols before and after 1980 is the amount of 6-mercaptopurine that was prescribed during ‘maintenance’ treatment as a result of changed criteria for reducing doses rather than a difference in recommended maximum doses in the protocols. Improvement in outlook for children in the United Kingdom with acute lymphoblastic leukaemia may in large part be because of this. The future intention to titrate the dose of 6-mercaptopurine to myelotoxicity in all children may prevent children who are more tolerant of the drug being inadvertently undertreated. It may, by the same mechanism, eliminate the difference in prognosis between the sexes.

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