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Small intestinal transit time affects methotrexate absorption in children with acute lymphoblastic leukemia

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Summary. *Serum methotrexate concentrations have been measured in 28 children with acute lymphoblastic leukaemia (ALL) following PO administration under standard conditions. Small-intestinal transit time, measured by the time taken for lactulose to pass from mouth to caecum, has been related with methotrexate absorption parameters.*

Small intestinal transit times ranged from 30 to 240 min. Children with longer transit times had later times of peak methotrexate concentration and tended to have a more erratic methotrexate absorption profile with two peaks. There appears to be an optimal transit time between 90 and 105 min for methotrexate absorption, with both faster and slower small-intestinal transit times producing lower peak concentrations as a fraction of the dose.

Introduction

The efficacy of methotrexate in maintenance regimens for children with acute lymphoblastic leukaemia (ALL) is well established [35]. Various studies have recently shown variability in the absorption and unpredictable serum levels of methotrexate after PO administration [2, 9, 13, 20, 30]. One contributory factor is the concurrent administration of food [31]. However, even when it is administered to fasting patients there is still a marked difference in both the peak levels and the timing of the peak concentrations [2, 30]. Gross enterocyte function, as measured by the conventional 1-h blood xylose level, does not appear to be contributory [32].

Gastric emptying and intestinal motility have been shown to affect absorption of some drugs in adults [5, 11, 12, 16, 18, 33]. We have therefore related mouth-to-caecum transit time, as measured by estimation of breath hydrogen following a dose of lactulose PO, with various parameters of methotrexate absorption in a group of children with ALL.

Patients and methods

In all, 28 children (14 male, 14 female) with ALL in remission were studied. Their ages ranged from 2.8 to 13.5 years (mean 7.2 years), and the duration of treatment varied from 3 to 35 months (mean 15.7 months). They were undergoing treatment according to one of four regimens; UKALL VI (5 children), a modified UKALL VI regimen (2), UKALL VIII (10), and a modified ALGB 6801 regimen (11). In the last two regimens methotrexate was given once a week, whilst it was given on 5 consecutive days each month in the first two.

The absorption studies were performed under standardised conditions apart from the dose of methotrexate. All studies were performed in the early morning. The children were fasted overnight and did not eat or drink for 1 h after methotrexate administration, but subsequently ate or drank normally. During the studies the children were encouraged to play and be involved in relatively normal activities.

No other drugs were given concurrently and the children had received only 6-mercaptopurine during the previous 6 days. At least 14 days had elapsed since the last dose of vincristine and 9 days since the last dose of prednisone.

All children received methotrexate as 2.5-mg tablets (Lederle). The dosage of methotrexate varied from 2.3 mg/m^2 to 20.4 mg/m^2, this being the child's usual dose of methotrexate.

Capillary blood samples were obtained at regular intervals for 48 h after tablet ingestion using a simple skin puncture device (Monolet). The samples were centrifuged and sera stored at −20° C until analysis.

Methotrexate concentrations were assayed with a radioimmunoassay using an I^{125} radiolabel, which was sensitive to methotrexate concentrations of 450 pg/ml (10^{-9} *M*) [1]. The antiserum used was specific for substances containing the 2–4 diaminopteridine structure. The major methotrexate metabolite 7-hydroxymethotrexate cross-reacted by 0.23% and 4-amino 4-deoxy N10 methylpteroic acid by 81%.

Concurrently with the methotrexate absorption study each child's small-intestinal transit time was measured by determining the time taken for lactulose to pass from mouth to caecum, as indicated by a rise in breath hydrogen concentration of more than 10 ppm above baseline following a dose of 5 g/m^2 lactulose PO [3–5]. Breath samples were obtained at 15-min intervals for up to 5 h. In older children collection of end-expiratory air was by a slight modification of the method described by Pearman et al. [27]. Younger children did not tolerate this technique and a modification of a simple Wignan's blow-out device [15] was used. The hydrogen content of the breath was detected by means of an electrochemical technique [3].

For each methotrexate absorption curve the parameters analysed were: the peak (maximum) concentration of methotrexate, the time of the peak, and the area under the serum concentration time curve up to 48 h after PO methotrexate administration (AUCo). The peak concentration and the AUCo were both also expressed as a fraction of the dose

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administered. The study was passed by the ethical committee of Newcastle Area Health Authority (T).

Statistical evaluation involved Kendall's rank correlation for relating parameters with the non-normally distributed small-intestinal transit time. Comparison between groups with different small-intestinal transit times involved the Kruskal-Wallis non-parametric analysis of variance, or the Mann-Whitney U-test if only two groups were involved.

Results

Variability in methotrexate absorption

With the dosage range 2.11 mg/m^2–20.27 mg/m^2 the peak concentration varied from 0.143 μ*M* (65 ng/ml) to 2.103 μ*M* (955 ng/ml). When expressed as the peak concentration as a fraction of the dose, there was a greater than eight-fold variation, with a range 0.0095–0.081 and mean of 0.033 (Fig. 1). The time of the maximum methotrexate concentration varied from 0.5 to 3 h, with a mean of 1.2 h (Fig. 1). In five children there were two peaks in their methotrexate absorption profile (Fig. 2).

The area under the plasma methotrexate concentration/time curve varied from 1.088 μ*M*/h to 7.095 μ*M*/h. There was a greater than five-fold variation in AUC as a fraction of the dose, the range being 0.042–0.239 with a mean of 0.110 (Fig. 1).

Variability in the small-intestinal transit time

The small-intestinal transit time varied from 30 to 240 min in a non-normal distribution, with a median of 75 min (Fig. 3). There was no difference in the small-intestinal transit time between males (median 67.5 min) and females (median 75 min) ($P = 0.37$). There was a trend for increasing small intestinal transit to be associated with increasing age ($\tau = 0.25$, $P = 0.04$), but there was no rank correlation between either dose of methotrexate ($\tau = 0.14$) or duration of therapy ($\tau = 0.09$) and the small-intestinal transit time.

Relationship between time of peak methotrexate concentration and small-intestinal transit time

There was no significant rank correlation between the time of the maximum methotrexate concentration and the small intestinal transit time in the 28 children overall ($\tau = 0.27$, $P = 0.08$). If, however, in those children who had two peaks in their methotrexate absorption profile the time of the later peak was considered, there was a rank correlation between small-intestinal transit time and the time of the peak methotrexate concentration ($\tau = 0.61$, $P = 0.005$) (Fig. 4).

Those children who had two peaks in their absorption profile had a significantly longer small-intestinal transit time (median 150 min) than the remainder median 60 min ($P = 0.0004$). There was no difference in their age, dose of methotrexate, duration of treatment, or male-to-female ratio.

Relationship between small-intestinal transit time and parameters of methotrexate absorption

The peak concentrations as a fraction of the dose in groups of children with different transit times are shown in Fig. 5. There is a significant difference among these groups (Kruskall-Wal-

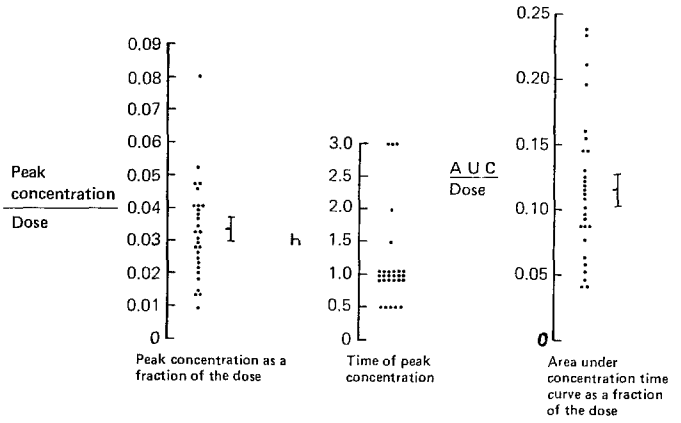

Fig. 1. The peak concentration as a fraction of the dose, area under the serum concentration time curve as a fraction of the dose, and the time of the peak concentration in the 28 children studied. (means ± SEM)

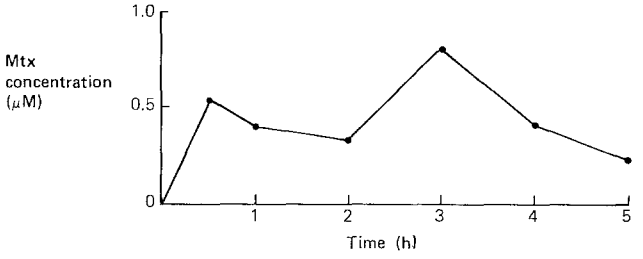

Fig. 2. Methotrexate absorption profile with two peak concentrations

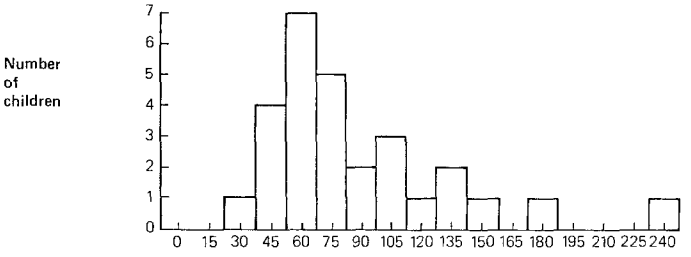

Fig. 3. Distribution of the small-intestinal transit time in the 28 children

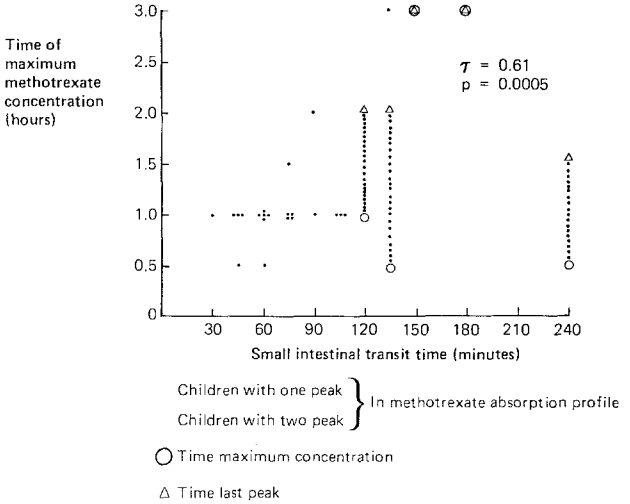

Fig. 4. Relationship of the time of the peak methotrexate concentration and the small-intestinal transit time

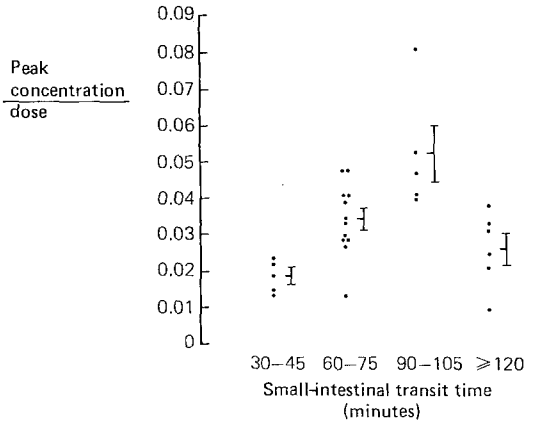

Fig. 5. Peak concentration as a fraction of the dose in groups of children according to their small-intestinal transit time. (means ± SEM)

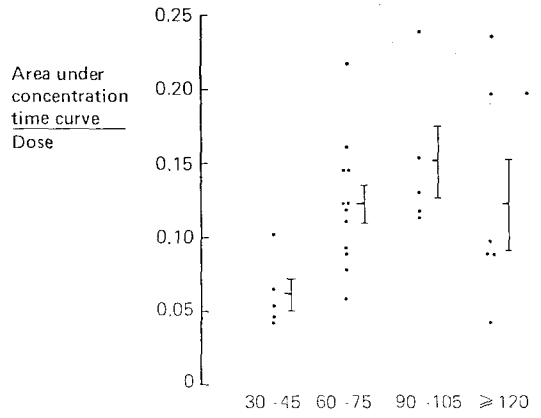

Fig. 6. Area under the serum concentration time curve as a fraction of the dose in groups of children according to their small-intestinal transit time (means ± SEM)

lis $H = 16.82$, $P = 0.0008$). Between individual groups the difference was significant when 30–45 min was compared with 90–105 min ($P < 0.01$) and when ≥ 120 min was compared with 90–105 min ($P < 0.05$).

Similarly, there was a significant difference among groups with different small-intestinal transit times for the area under the curve as a fraction of the dose ($H = 9.16$, $P = 0.027$). The only comparison to reveal a significant difference between individual groups was that of 30–45 min and 90–105 min ($P = 0.019$).

When the children with a small-intestinal transit time of 90 or 105 min were compared with the other children, both the peak concentration and the area under the curve as a fraction of the dose were significantly greater ($P = 0.0018$ and $P = 0.038$).

Discussion

These results confirm previous reports of considerable inter-individual variability in methotrexate absorption in children with ALL [2, 9, 13, 20, 30]. This variability included the three aspects, reported here, of peak concentration and AUC, both as a fraction of the dose, and the time of the peak methotrexate concentration. Thus serum methotrexate levels are unpredictable in children following identical doses PO.

Previously, little attention has been paid to the reasons for the interindividual variability in the absorption of methotrexate, and those studies that have considered this aspect have focused on differences in intestinal function. Freeman-Narrod suggested that abnormal enterocyte function as determined by the D-xylose absorption test may be a factor [14], although this was not confirmed by Pinkerton [32].

Variation in gastrointestinal motility has not previously been investigated as a cause of variability in methotrexate absorption. Gastric emptying has been related to the absorption of other drugs [25]. With paracetamol a good correlation has been shown between the rate of gastric emptying and the rate of drug absorption [16], and pharmacological modification of gastric emptying with metochlorpramide or propantheline has produced changes in peak concentration and rate of absorption [24]. However, few reports have related the mouth-to-caecum transit time (including both gastric and intestinal components) to drug absorption and its interindividual variability.

Measurement of the small intestinal transit time by the breath hydrogen technique is a simple non-invasive assessment of intestinal transit [4]. It is especially applicable to the age range of the children in this study, including those between 2 and 5 years. The technique measures the time taken for the inert sugar lactulose to pass from the mouth to the caecum and therefore does not discriminate between gastric and intestinal components of transit. Measurement of gastric emptying would allow more detailed analysis of gastrointestinal motility in those children with slow transit times, but methods to study this are invasive, employ a radioisotope, or require the child to be still for a long period of time [34]. The finding of a fast transit time, however, implies that both gastric and intestinal components are rapid.

This hydrogen breath technique has been shown to allow accurate determination of mouth-to-caecum transit time; there is a good correlation between results obtained by this method and those obtained by the appearance of PEG in terminal illeal aspirates when both lactulose and PEG are simultaneously ingested [4]. However, the small-intestinal transit time in one individual is probably not constant, i.e., there is also intraindividual variability [21, 29].

The dose of lactulose used was the minimum dose to produce an easily detected rise in breath hydrogen. Higher doses were avoided as these have been shown to shorten the small-intestinal transit time [4, 21). Also, the solution of lactulose was isotonic to prevent an osmotic influence on gastric emptying [25].

The object of the investigation was to study methotrexate absorption in conditions as similar as possible to those in which the children usually took the methotrexate tablets. For this reason the children were encouraged to play and be active and not to lie down, as this retards gastric emptying and delays absorption [26]. Similarly, capillary rather than venous blood sampling was used as this is less painful and gastric stasis can be caused by pain or trauma [25]. Another variable altering gastric motility, the volume of fluid ingested, was controlled [25].

In all children a rise in breath hydrogen occurred following lactulose ingestion, allowing the small-intestinal transit time to be determined. The median small-intestinal transit time obtained when 5 g/m^2 lactulose was used was in agreement with previously reported values when 10 g lactulose was given to adults [4, 21]. However, the range of values in adults

(28–118 min) was not as wide as that reported here (30–240 min) [4].

The radioimmunoassay employed in this study cross-reacts with 4-amino 4-deoxy N10 methylpteroic acid by 81%. This metabolite has been inconsistently found in the serum and urine of patients at later times following high-dose methotrexate administration [6, 10, 19]. No study has yet shown its presence after low-dose PO administration, although we are investigating this at present. Even if this metabolite was present at later time intervals it should not significantly affect early absorption parameters.

Analysis of the relationship between the time of the peak methotrexate concentration and small-intestinal transit was confused by the presence of two peak concentrations in five children. If the latter peak was considered in these children or if these children were excluded from the analysis there was a significant rank correlation between these two variables. Those children with two peaks had an erratic absorption profile, and this has been described with other drugs when there is delayed gastric empyting [11].

Five of the six patients with a small-intestinal transit time greater than 105 min had two peaks in their absorption profile. These facts may suggest that the long small-intestintal transit time in these children might be due to delayed gastric empyting.

There appeared to be an optimal small-intestinal transit time of 90–105 min for methotrexate absorption, resulting in higher peak concentrations and AUC. Both shorter and longer small-intestinal transit times resulted in lower methotrexate absorption parameters. For children with a rapid transit time of 30–45 min the lower peak concentrations and AUC may be explained by an insufficient time for dissolved methotrexate to be exposed to the aborptive sites. As in the rat, methotrexate has been shown to be absorbed by an active transport system, probably shared with pteroyl glutamic acid (folic acid), limited to the jejunum [7, 8, 17, 36].

Rapid intestinal transit has been directly related to malabsorption of other substances, including drugs. The absorption of cholesterol, which is normally only partially absorbed, is reduced with increased transit times [33], as is glucose in patients who have rapid transit after gastrectomy [5]. In addition, xylose, which is probably absorbed by both passive and active means, has been shown to be poorly absorbed when transported rapidly through the gastrointestinal tract [12]. Even the absorption of carbohydrate, protein, and fat is reduced when the transit time is decreased by various means [18]. Further, indirect, supportive evidence for this concept is derived from pharmacological modification of digoxin absorption [23]. Increasing gastrointestinal motility with metoclorpramide decreases the absorption of digoxin tablets, while reducing the motility with propantheline increases the absorption. No effect of propantheline was seen with liquid digoxin [23].

Rapid transit may also cause insufficient time for tablet dissolution, which has been shown to be a rate-limiting factor in the absorption of some drugs [22].

The effect of rapid intestinal transit is probably maximally seen with drugs which have slow tablet dissolution, are absorbed from restricted areas of the gastrointestinal tract, and are normally submaximally absorbed. This latter effect may be of relevance for methotrexate, as the impairment of intestinal function, which has been described in children with ALL [28], may reduce the efficacy of methotrexate absorption, so that transit time is a critical factor.

Children with transit times slower than 105 min had lower peak concentrations, but not smaller AUCs, than children with transit times of 90–105 min. An explanation for this may be delayed gastric emptying, producing slow release of the drug from the stomach with a late, low, peak concentration, whilst the effect on AUC is not as pronounced. These findings are in agreement with those of other studies, in which gastric emptying was directly determined, and late reduced peak levels with usually unchanged AUCs have been described when there is slow gastric emptying [11, 16, 24, 25].

Knowledge of the reasons for variability in methotrexate absorption may be useful when the prognostic significance of this variability is determined. Studies investigating this aspect are currently being performed in the MRC UKALL VIII trial. If a particular profile of methotrexate absorption is found to be prognostically superior, then pharmacological alteration of intestinal transit, e.g., by metoclorpramide or loperamide, and thus of the methotrexate absorption profile may be of value.

The finding of a significant correlation between intestinal transit time and the bioavailability of a drug may have important implications not only for methotrexate but for many other drugs which were previously thought to be rapidly and reliably absorbed from the gastrointestinal tract.

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