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## A Simplistic Individualization Method for 6-Mercaptopurine in Acute Lymphoblastic Leukemia Children

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**Abstract:** The aim of this study was set up a simplistic but low-cost method for 6-mercaptopurine (6-MP) individualization chemotherapy for Acute Lymphoblastic Leukemia (ALL) children in developing China. The blood samples of ten ALL children with 6-MP chemotherapy were collected in the morning on 7th day and 14th day of the onset of therapy respectively and 6-thioguanine nucleotides (6-TGN) levels in Red Blood Cell (RBC) were measured by RP-HPLC (reverse phase-high performance liquid chromatography). Meanwhile on 14th day and 21st day the blood samples of ten ALL children were collected in the morning respectively and determined White Blood Cell (WBC) counts to monitor myelotoxicity. The ideal target level range of 6-TGN was set up between 275~750 pmol/8×10<sup>8</sup> RBC. If the level of 6-TGN in the sample of 7th day was more than 1000 pmol/8×10<sup>8</sup> RBC, the 6-MP dose starting since 8th day was adjusted to 50% of the original dose. If the level of 6-TGN in the sample of 7th day was more than 750 pmol/8×10<sup>8</sup> RBC, the 6-MP dose starting since 8th day was adjusted to 60% of the original dose. The results showed that the 6-TGN levels in ten ALL children ranged from 264~866 pmol/8×10<sup>8</sup> RBC in the samples of 7th day. Among them only two children had high levels of 6-TGN more than 750 pmol/8×10<sup>8</sup> RBC who showed after-effect myelotoxicity with low WBC on 14th day. After the adjustment of 6-MP dose since 8th day, ten ALL children had 6-TGN levels ranging from 270~450 pmol/8×10<sup>8</sup> RBC and no one had a high level of 6-TGN on 14th day. On 21st day ten ALL children had normal WBC counts and showed no myelotoxicity. We concluded that the 6-MP dose adjustment by 6-TGN levels is a simplistic but low-cost individualized method for 6-MP chemotherapy in ALL children in developing China.

**Key words:** 6-mercaptopurine (6-MP), 6-thioguanine nucleotides (6-TGN), individualization chemotherapy, acute lymphoblastic leukemia

### INTRODUCTION

6-Mercaptopurine (6-MP) is one of the major drugs used in maintenance therapy for Acute Lymphoblastic Leukemia (ALL). As an inactive prodrug, 6-MP requires absorption, cellular uptake and intracellular anabolism to nucleotides for cytotoxic activity. These nucleotides are ultimately incorporated into DNA and RNA, resulting in cell death. 6-MP may be anabolized to nonmethylated nucleotides or may undergo methylation by the enzyme thiopurine methyltransferase (TPMT) to S-methylated nucleotides (Bostrom and Erdmann, 1993). 6-thioguanine nucleotides (6-TGN) are the major cytotoxic metabolites of 6-MP. Due to different enzymatic activity of TPMT that deactivate the 6-MP, ALL children taking same dose of 6-MP cannot reach the same levels of 6-TGN in blood. Some children with low enzymatic activity of TPMT have a high level of 6-TGN and cannot endure the severe myelotoxicity (Aarbakke *et al.*, 1997). The best

individualization for 6-MP therapy is to conduct gene type analysis which costs expensively for individual child in developing country. Since China is a developing country and has a limited medical care resource, this study was aimed to build a simple but low-cost individualization method for 6-MP therapy through the determination of 6-TGN level in Red Blood Cells (RBC) by HPLC.

### MATERIALS AND METHODS

**Patients:** Ten ALL remission children, aged from 3-8 years old, 5 female and 5 male, who received the maintain 6-MP + methotrexate (MTX) chemotherapy for at least 2 months in Zhujiang Hospital in 2004 were enrolled in this study. The first 7 days the start dose of 6-MP was 75 mg m<sup>-2</sup> day. The dose on 8th day was adjusted by the 6-TGN levels in RBC sample of 7th day in maintain treatment (Rostami-Hodjegan *et al.*, 1995). The target level range of

6-TGN was 275~750 pmol/8×10<sup>8</sup> RBC. If the level of 6-TGN in the blood sample was more than 1000 pmol/8×10<sup>8</sup> RBC, the dose was adjusted to 50% of the original dose. If the level of 6-TGN in the blood sample was more than 750 pmol/8×10<sup>8</sup> RBC, the dose was adjusted to 60% of the original dose. On 14th day the 6-TGN was assayed once more. On 14th day and 21st day the WBC was counted as a myelo-toxicity index.

**Sample process and HPLC assay:** Two milliliter of blood from cubitus vein was collected in heparin anti-coagulation tube. RBC were separated by centrifugation at 1140 rpm, 4°C for 10 min and washed with Hanks solution 2 times first at 1140 rpm, 4°C for 10 min and then at 2280 rpm, 4°C for 10 min. The cells were counted and the concentration was adjusted to 4×10<sup>6</sup> μL<sup>-1</sup>. Eight hundred microliter of 3.75 mmol L<sup>-1</sup> dithiothreitol (DTT) and 500 μL of 1.5 mol L<sup>-1</sup> sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) were added to 200 μL RBC sample. The sample was kept at 100°C for 1 h. Then the sample was cooled down and adjusted its pH about 11.6 with 3.4 mol L<sup>-1</sup> sodium hydroxide (NaOH) solution. The alkaline sample was added 8 mL of Phenyl Mercury Acetate (PMA) adduct (43.8 mg PMA + 1.5 g 1-pentanol+100 mL methylbenzene) and vortexed for 10 min and centrifuged at 3270 rpm, 4°C for 5 min. Six milliliter methylbenzene layer was blew to dry with nitrogen. The residue was dissolve in 200 μL of 0.1 mol L<sup>-1</sup> hydrochloric acid (HCl) and 20 μL was injected to measure by HPLC according the reference (Ma *et al.*, 2000) with slight modification. Briefly, the instrument was LC-6A HPLC System (Shimadzu, Japan), with C18 column 5 μm (4 mm×250 cm), methanol-water (5:95) as mobile phase, 0.9 mL min<sup>-1</sup> of flow rate and wavelength at 300 nm, column temperature at 25°C, with sensitivity of 1.0 AUFS.

## RESULTS AND DISCUSSION

On 7th day and 14th day, respectively, the blood samples of ten ALL children were collected and measured by RP-HPLC. The 6-TGN levels of ten samples ranged from 264~866 pmol/8×10<sup>8</sup> RBC on 7th day. Among

them two children had high levels of 6-TGN more than 750 pmol/8×10<sup>8</sup> RBC. Due to after-effect of 6-MP myelotoxicity for about 2 weeks, the two children showed with low WBC count on 14th day. After the adjustment of dose, ten children had 6-TGN levels ranging from 270~450 pmol/8×10<sup>8</sup> RBC and no one had a high level of 6-TGN on 14th day. And after another 7 days, that was on 21st day, ten children had normal WBC counts and showed no myelotoxicity with (Table 1).

Individualized chemotherapy for childhood acute lymphoblastic leukemia is a trend in clinic compared with the conventional chemotherapy. Patients who received individualized doses had significantly fewer courses of treatment with systemic exposures below the target range than did patients who received conventional doses. Among the patients with B-lineage leukemia, those who received individualized therapy had a significantly better outcome than those given conventional therapy; the mean rates of continuous complete remission at five years were 76 and 66%, respectively (Evans *et al.*, 1998). By measurement of the 6-TGN level we adjust the dose of 6-MP in order to avoid the myelo-toxicity. We enact the 6-TGN level range of 275~750 pmol/8×10<sup>8</sup> RBC. If the level of 6-TGN in the sample was more than 750 pmol/8×10<sup>8</sup> RBC, the dose was reduced to 50~60% of the original dose. The low WBC levels in two children with myelotoxicity were restored to normal until on 21st day. The results showed that the it might take 2 weeks to restore WBC levels to normal after the dose was cut-down, which suggested that the change of 6-TGN levels is ahead of change of WBC.

The concentration of 6-MP and its metabolites in target cells reflect the efficacy of chemotherapy. However, the number of tumor cells in remission ALL children was decreased significantly and it disabled the determination of 6-MP in tumor cells. Since the metabolites of 6-MP was accumulated in RBC and steady state levels which corresponding to lymphocyte could be reached after a long time of chemotherapy, the levels of 6-TGN in RBC could replace the levels of 6-TGN in tumor cells (Rostami-Hodjegan *et al.*, 1995; Schmiegelow and Bruunshuus, 1990). 6-TGN was present in all the red cell

Table 1: 6-TGN levels in RBC and WBC count at different time in maintain treatment

Patients	Original 6-MP dose (mg m <sup>-2</sup> day <sup>-1</sup> )	6-TGN on 7th day (pmol/8×10 <sup>8</sup> RBC)	WBC on 14th day (×10 <sup>9</sup> L <sup>-1</sup> )	Adjust 6-MP dose (mg m <sup>-2</sup> day <sup>-1</sup> )	6-TGN on 14th day (pmol/8×10 <sup>8</sup> RBC)	WBC on 21st day (×10 <sup>9</sup> L <sup>-1</sup> )
1	75	785	1.6	45	368	3.7
2	75	866	1.5	45	389	3.6
3	75	421	2.9	75	418	3.3
4	75	264	3.9	75	270	4.0
5	75	309	3.8	75	310	3.8
6	75	278	3.8	75	290	4.0
7	75	345	3.7	75	350	3.9
8	75	312	3.9	75	333	3.9
9	75	432	3.6	75	450	3.5
10	75	286	4.1	75	297	4.4

fractions after 3 days 6-MP administration. With respect to the monitoring of therapy, the concentration of 6-TGN after 7 to 10 days of 6-MP could be used to predict eventual steady-state concentrations using a simple model (Rostami-Hodjegan *et al.*, 1995). In the study of 12 boys and 19 girls 2-16 years of age on oral 6-MP and MTX maintenance therapy for non-B-cell ALL, Schmiegelow and Bruunshuus (1990) found that (a) during maintenance therapy, 6-TGN accumulate in the erythrocytes (E-6-TGN); (b) for patients receiving an unchanged dose of 6-MP, no significant correlation could be demonstrated between the mean E-6-TGN and the dose of 6-MP ( $r = -0.11$ ,  $p = 0.28$ ) (31 patients); (c) among 21 patients receiving 50-75 mg m<sup>-2</sup> 6-MP, a variation of up to 3 orders of magnitude in mean E-6-TGN could be demonstrated, with the interindividual Coefficient of Variation (CV) in mean E-6-TGN for these patients being 0.31; (d) the median intraindividual CV in E-6-TGN at an unchanged dose of 6-MP was 0.11 (range, 0.04-0.18) and (e) the degree of myelodepression as measured by the mean white cell count was related to mE-6-TGN ( $r = -0.55$ ,  $p = 0.0006$ ). These results indicate that E-6-TGN could be a useful parameter for monitoring 6-MP maintenance chemotherapy, although this needs to be explored in prospective studies.

Although three kinds of 6-MP intracellular metabolites in RBC (6-TGN, 6-thioinosine monophosphate, 6-methyl-mercaptopurine) could be measured simultaneously by RP-HPLC (Ma *et al.*, 2000), we only determined the 6-TGN levels in RBC. In the genomic era, the most effective individualization for chemotherapy is to conduct gene type analysis for every cancer patient. 6-MP individualization chemotherapy for ALL children is no exception. However, gene type analysis costs expensively for individual child in developing country and most grass-root hospital lack necessary analysis professional and facility. Our work

supplies a simplistic but low-cost individualization for 6-MP chemotherapy. Our clinical practice showed that it's feasible method to individualize 6-MP just by determination of 6-TGN in blood sample and adjusting 6-MP dose correspondingly with 6-TGN levels.

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