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Original Article

The effect of high-dose methotrexate on the improvement of symptoms and renal function of pediatric patients with acute lymphoblastic leukemia

Huan Zhou[1], Chong Wen[2]

[1]*Department of Neonatology, The Central Hospital of Wuhan, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei, China;* [2]*Department of Traditional Chinese Medicine, The Central Hospital of Wuhan, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei, China*

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Abstract: Objective: To explore the clinical effect and influence of high-dose methotrexate (HD-MTX) on renal function in pediatric patients with acute lymphoblastic leukemia (ALL). Methods: The clinical data of 115 pediatric patients with ALL were retrospectively analyzed, and patients were divided according to the risk of pediatric leukemia, into a high-risk group (n = 59) and a standard-risk group (n = 56). We examined the expression of 7-day creatinine clearance (Ccr) and cystatin C (Cys) before and after treatment, and the 5-year survival of the two groups of patients. Results: Both groups had significantly decreased Ccr and Cys levels after treatment as compared to the levels before treatment, and the difference was statistically significant (P = 0.000). The expression of Ccr in the standard-risk group after treatment was higher than that in the high-risk group, and the expression of Cys in the standard-risk group after treatment was significantly lower than that in the high-risk group, and the differences were significant (P = 0.000). There was no significant difference in the incidence of side effects between the high-risk and standard-risk groups (P > 0.05). The survival rates of the two groups were high, and there was no significant difference (P = 0.389). Conclusion: The clinical efficacy of HD-MTX for the treatment of pediatric ALL was significant, and the survival rate was improved, but toxic side effects were frequent, and it is necessary to alleviate these effects expeditiously.

Keywords: Methotrexate, acute lymphoblastic leukemia, treatment, renal function

Introduction

As one of the most common malignant tumors in children, leukemia has a high incidence in young children. The American Cancer Society report showed that the disease incidence and the number of deaths are increasing annually [1, 2]. Acute lymphoblastic leukemia (ALL) is predominant in pediatric patients, and its incidence in pediatric leukemia is 75% or higher [3]. ALL is a heterogeneous hematologic malignancy, and a large number of primitive or immature lymphocytes are found in the bone marrow slides of ALL patients [4]. A large number of proliferative abnormal lymphocytes massively invade each organ of the body, which results in the inhibition of normal hematopoiesis and is deleterious to healthy growth and development [5]. Therefore, finding an effective regimen is an urgent issue for clinicians.

Several new treatment techniques for ALL have been developed recently, including targeted gene-directed therapy and immunotherapy [6, 7]. However, the principal treatment for ALL is still chemotherapy, within which high-dose methotrexate (HD-MTX) is the most important component [8]. Some scholars suggest that the intrathecal injection of a combination of three drugs (MTX, glucoside, and dexamethasone) and HD-MTX can replace chemotherapy in patients diagnosed with primary ALL [9]. Clinically, the safe dosage of HD-MTX is 1-5 g/m^2, but the application of MTX will induce some side effects and adverse reactions [10]. Therefore, calcium folinate is always used for rescue 36 h after administering HD-MTX, and sufficient hydration, alkaline treatment, and dynamic renal function detection are provided. However, the effect of different dosages of MTX on side effects and prognosis is controversial [11, 12].

Table 1. Clinical data of the two groups

Group	Marked risk group (n = 56)	High risk group (n = 59)	X^2	*P* value
Sex			0.443	0.506
Man	30 (50.85)	25 (44.64)		
Woman	29 (49.15)	31 (55.36)		
Age			0.360	0.549
> 12 year	39 (66.10)	34 (60.71)		
≤ 12 year	20 (33.90)	22 (39.29)		
Stature			0.975	0.324
> 140 cm	40 (67.80)	33 (58.93)		
≤ 140 cm	19 (32.20)	23 (41.07)		
Weight			1.393	0.238
> 50 kg	20 (44.44)	25 (55.56)		
≤ 50 kg	39 (55.71)	31 (44.29)		
Educational status			0.351	0.554
< Primary school	47 (79.66)	47 (83.93)		
≥ Primary school	12 (20.34)	9 (16.07)		
Family situation			2.230	0.135
Single parent	5 (8.47)	10 (17.86)		
Parents	54 (91.53)	46 (82.14)		
Nation			1.723	0.189
Han nationality	55 (93.22)	55 (98.21)		
Minority ethnic	4 (6.78)	1 (1.79)		

This study aims to clarify the feasibility, adverse effects, and effect on prognosis of HD-MTX in children with ALL, and to provide better references for clinicians.

Methods and materials

This study retrospectively analyzed the clinical data of 115 children with ALL admitted from Wuhan Central Hospital of Huazhong University of Science and Technology. According to the risk of leukemia, the children were divided into a high-risk group (n = 59) and a standard-risk group (n = 56). The study was approved by the medical ethics committee of our hospital, and all the family members of the children were informed and signed the informed consent. This study has been approved by the Ethics Committee of Wuhan Central Hospital of Huazhong University of Science and Technology. All study participants had given their written informed consent before participating in the study.

Inclusion and exclusion criteria

The inclusion criteria were as follows: younger than 18 years without congenital heart disease or liver and kidney dysfunction; complete clinical treatment record without other malignant tumors; and the family members were cooperative to the treatment and follow-up.

The exclusion criteria were as follows: patients with chronic renal insufficiency, recent infections, or serious dysfunction of important organ functions and immune dysfunction; kinship between the two groups of children.

Principal drugs

Vincristine was purchased from Guangdong Lingnan Pharmaceutical Co., Ltd (approval No.: H20065857), asparaginase from Beijing Shuanglu Pharmaceutical Co., Ltd. (approval No.: H20057369), daunorubicin from Hisun Pfizer Pharmaceutical Co., Ltd. (Approval No.: H33020925), prednisone from Guangdong Huanan Pharmaceutical Group Co., Ltd. (Approval No.: H44020682), cyclophosphamide from Jiangsu Heng Rui Pharmaceutical Co., Ltd (Approval No.: H10950290), cytarabine from Hisun Pfizer Pharmaceutical Co., Ltd. (Approval No.: H20054695), MTX from Guangdong Lingnan Pharmaceutical Co., Ltd (Approval No.: H20074246), dexamethasone from Cisen Pharmaceutical Co., Ltd (Approval No.: H37021969), calcium folinate from Jiangsu Heng Rui Pharmaceutical Ltd. (Approval No.: H20080718), calcium folinate from Guangdong Lingnan Pharmaceutical Co., Ltd (Approval No.: H20040396), sodium bicarbonate tablets from Tianjin Lisheng Pharmaceutical Co., Ltd. (Approval No.: H12020220), sodium bicarbonate from Hainan Pharmaceutical Factory Pharmaceutical Factory Two (Approval No.: H41023005), and 6-mercaptopurine from Zhejiang Zhebei Pharmaceutical Co., Ltd. (Approval No.: H33020001).

Treatment grouping and methods

Patients in the high-risk group were treated with VDLP (vincristine, daunorubicin, L-asp, and prednisolone) induction therapyand the early intensive CAM (cyclophosphamide, cytarabine, and 6-mercaptopurine (6-MT)) regimen, followed by 5.0 g/m^2 MTX to prevent central ner-

Table 2. Clinical efficacy of high-dose methotrexate in the two groups

Group	Recure	Effective	Of no avail	X^2	*P* value
Marked risk group (n = 56)	42 (75.00)	8 (14.29)	6 (10.71)	0.218	0.641
High risk group (n = 59)	33 (55.93)	18 (30.51)	8 (13.56)		

Table 3. High-dose methotrexate concentration in the blood at 48 hours

Group	> 1 μmol·L^{-1}	0.1~1 μmol·L^{-1}	< 0.1 μmol·L^{-1}
Marked risk group (n = 56)	11 (19.64)	30 (53.57)	15 (26.79)
High risk group (n = 59)	17 (28.81)	29 (49.15)	13 (22.03)
X^2	1.312	0.225	0.352
P	0.252	0.636	0.553

Table 4. Expression of 7-day creatinine clearance (Ccr) and cystatin C (Cys) in both groups before and after treatment

Group	Pretherapy	Post-treatment
Ccr (mL/min)		
Marked risk group (n = 56)	90.25±16.50	83.52±15.60*,**
High risk group (n = 59)	91.33±15.93	76.88±13.84*
Cys (mg/L)		
Marked risk group (n = 56)	1.12±0.15	1.62±0.30*,**
High risk group (n = 59)	1.09±0.11	1.93±0.42*

*There was a significant difference between the expression before and after treatment (P < 0.05). **There was a significant difference between the standard-risk group and the high-risk group after treatment (P < 0.05).

vous system leukemia. Patients in the standard-risk group were treated with VDLP induction chemotherapy and CAM intensive chemotherapy and afterwards administered 3.0 g/m^2 MTX for preventative treatment. MTX was administered by the following method: 1/10 (≤ 500 mg) of the total amount was quickly dripped intravenously within 30 min and the remaining amount was uniformly dripped within 24 h. Thirty to 120 min after the quick administration, the intrathecal injection of the three-drug combination was administered. Then, 24 h after the injection, calcium folinate (15 mg/m^2) was applied for rescue once every 6 h. At the first administration, calcium folinate was injected intravenously, and afterwards it was administered orally 6-8 times. If the serum MTX concentration at 48 h was > 1 μmol/L, calcium folinate was increased to reduce the blood concentration to ≤ 0.1 μmol/L, and if the blood concentration of MTX was ≤ 0.1 μmol/L, calcium folinate was discontinued. During the 3 days before and after HD-MTX treatment, patients were administered $NaHCO_3$ 1.0 g, 3 times per day. Five percent $NaHCO_3$ (5 mL/kg) was dripped intravenously on the day of treatment, and the blood pH of the patients remained ≥ 7.0. Vital organs, such as liver, kidney, and gastrointestinal tract, were simultaneously hydrated for protection on the treatment day and on the third day after treatment. Furthermore, 6-MT (50 mg/m^2) was simultaneously administered with HD-MTX for a total of 7 days. Blood MTX concentration was measured by a rapid blood concentration meter (Abbott TDxFLx fluorescence polarization immunoassay analyzer), Ccr and Cys were tested using Hitachi 7600 automatic biochemical analyzer (Hitachi, Japan).

Endpoints

The primary endpoint was symptom improvement. Symptoms were graded as the following stages: recovery; effectiveness, and ineffectiveness were respectively considered when the symptoms and signs disappeared completely, basically disappeared, and did not disappear. The total effective rate = (number of patients at recovery stage + number of patients at effective stage)/total case number × 100%. The blood concentration of MTX at 48 h was detected using a fluorescence polarization immuno-analyzer. The expression of creatinine clearance (Ccr) and cystatin C (Cys) was observed 7 days before and after treatment, and the 5-year survival rate was observed. Secondary endpoints included gastrointestinal reactions, liver function damage, renal damage, skin allergies.

Statistical analysis

This study used SPSS20.0 software package (Shanghai Becca) to carry out the statistical analysis of the collected data, and GraphPad Prism 7 (Shanghai Becca) was used to create graphical representations, in which the count data were expressed as rate (%) and analyzed

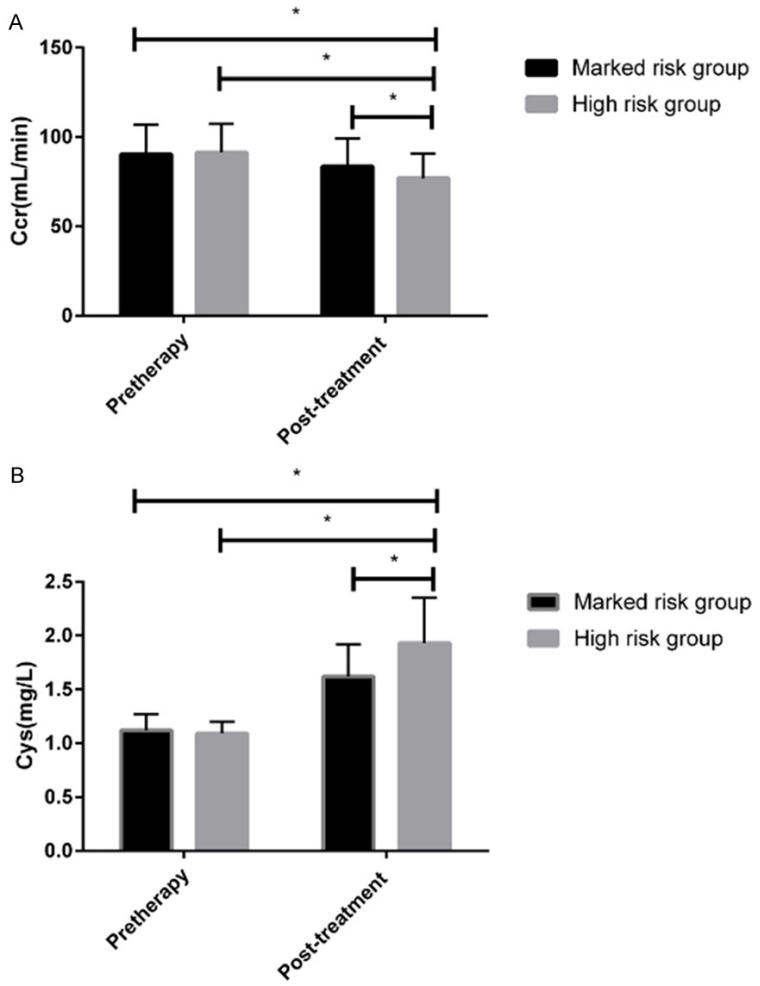

Figure 1. The expressions of Ccr and Cys in the two groups before and after treatment. A. The expression of Ccr in the standard-risk group after treatment was higher than that in the high-risk group. B. The Cys expression in the standard-risk group was lower than that in the high-risk group, and the difference was statistically significant (P < 0.05).

by chi square test. Data are expressed as mean ± standard deviation (mean ± SD), intra-group comparisons were performed using a paired t-test, and the inter-group comparisons were performed using an independent sample t-test. P < 0.05 was considered statistical significance.

Results

Patient characteristics

We compared the clinical data of the two groups and found no statistical difference in sex, age, height, weight, education, family status, or ethnic group between the high-risk group and the standard-risk group (P > 0.05), which indicated that the two groups were comparable (**Table 1**).

Clinical efficacy of HD-MTX

The patients' conditions were significantly improved after treatment in both groups, and the total effective rate of treatment in the two groups was not statistically different (P > 0.05) (**Table 2**).

Blood MTX concentration at 48 h

We used the fluorescence polarization immuno-analyzer to detect the blood concentration of MTX at 48 h in the two groups of patients and found that the proportion of patients with a blood drug concentration > 1 μmol/L in the high-risk group was not significantly different from that in the standard-risk group (P > 0.05). The proportion of patients reaching safe blood concentration in the standard-risk group was also not significantly different from that in the high-risk group (P > 0.05; **Table 3**).

Expression of Ccr and Cys before and after treatment

We examined the expressions of Ccr and Cys in the two groups before and after treatment and found that there was no statistical difference in the expressions of Ccr and Cys before treatment between the two groups (P > 0.05). Both groups showed significantly decreased expression after treatment compared to those before treatment (P < 0.05). The expression of Ccr in the standard-risk group after treatment was higher than that in the high-risk group, and the Cys expression in the standard-risk group was lower than that in the high-risk group, and the difference was statistically significant (P < 0.05 for both; **Table 4**, **Figure 1A** and **1B**).

Toxicity and side effects

We compared the total incidence of toxicities and side effects in the two groups and found

Table 5. Toxicity and adverse reactions

Group	Digestive tract reaction	Liver function damage	Renal function impairment	Skin sensibility	Total incidence of adverse reactions	X^2	*P* value
Marked risk group (n = 56)	15 (26.79)	4 (7.14)	5 (8.93)	2 (3.57)	26 (46.43)	0.065	0.779
High risk group (n = 59)	12 (20.34)	6 (10.17)	5 (8.47)	3 (5.08)	26 (44.07)		

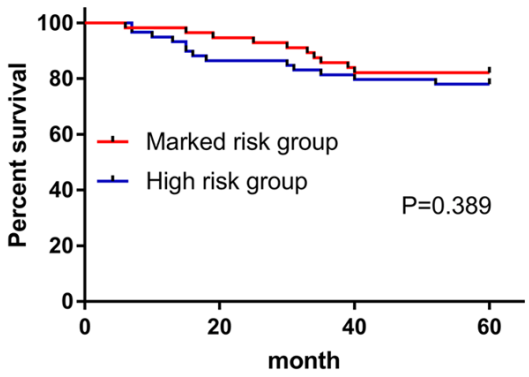

Figure 2. The survival rate at 5 years after treatment in both groups.

that the total incidence of side effects in the high-risk group was not significantly different from that in the standard-risk group (P > 0.05; **Table 5**).

Survival

We calculated the survival rate at 5 years after treatment. The 5-year survival rate in the high-risk group was not different from that in the standard-risk group (P = 0.389 **Figure 2**).

Discussion

In clinical practice, the principal ALL treatment is induction chemotherapy using the VDLP regimen. However, some studies have found that chemotherapy not only increases the incidence of adverse reactions, but also reduces treatment compliance and inhibits patient recovery [13].

Clinical studies have shown that most chemotherapy drugs have difficulty in passing through the blood-brain barrier and blood-seminiferous tubule barrier after entering the body, which leads to difficulty in entering the testicular and central nervous systems, which can lead to relapse [14]. Studies have shown that MTX suppresses the asylum leukemic cells by penetrating the blood-brain barrier and blood-seminiferous tubule barrier after reaching a certain concentration [15]. At present, HD-MTX, which reduces resistance to chemotherapy drugs, is usually administered in combination with calcium folinate for the treatment of ALL, so as to alleviate the symptoms of the disease and achieve a therapeutic effect [16]. Although the therapeutic effect of the regimen is satisfactory, patients generally experience increased sever adverse effects. A previous study showed that the timely detection of blood drug concentration in children and the administration of calcium folinate at appropriate time points are useful to reduce the occurrence of adverse reactions [17].

Therefore, in this study, the pediatric patients were divided into two groups according to the severity of the disease, and two dosages, 3 g/m^2 and 5 g/m^2 were administered to the standard-risk group and the high-risk group, respectively. The efficacy, incidence of adverse reactions, and MTX blood concentration at 48 h were determined, and patients were followed-up to determine the 5-year survival rate. We evaluated the efficacy in the two groups of patients and found that the total effective rate in both the high-risk group and the standard-risk group was more than 85% after treatment with different concentrations, and there was no difference between the two groups, which was consistent with the results of Arend et al. [18], and implies that MTX has a good therapeutic effect in the treatment of ALL. We measured the blood concentrations at different time points and found that there was no difference in the 48-h blood concentration between the two groups. The underlying reason may be the MTX was converted to glutamate through hepatocyte metabolism after absorption, and the remainder was metabolized by intestinal bacteria, which reduces accumulation in the body.

We also examined the expression of Ccr and Cys before and after the treatment in the two groups of patients. As a metabolic product of creatinine, Ccr is a sensitive indicator of glomerular filtration dysfunction, whereas Cys is

an ideal endogenous marker to reflect the change in glomerular filtration rate. We found that Ccr after treatment in both groups was decreased significantly compared to that before treatment, whereas Cys was significantly increased compared to that before treatment. Moreover, Ccr after treatment in the standard-risk group was significantly higher than that in the high-risk group, and Cys after treatment in the standard-risk group was significantly lower than that in the high-risk group. The study by Csordas et al. [19] found that a high dose (5 g/m^2) significantly affected nephrotoxicity in children with ALL, which indicated that a high concentration (5 g/m^2) of MTX aggravates renal injury. Nephrotoxicity was significantly different in patients treated with a low concentration (3 g/m^2) of MTX. At the end of the study, we compared the side effects and survival of the two groups after treatment and found no statistical difference in the incidence of side effects in the two groups. Moreover, there was no difference in the 5-year survival rate between the two groups, and the survival rate was high. We speculate that this may be associated with the individual differences of the children. Therefore, this study and the previous literature proved that HD-MTX had a good efficacy in the treatment of pediatric ALL. However, the treatment of ALL by HD-MTX will induce renal impairment in pediatric patients, so it is necessary to choose proper doses according to the severity of the disease and the disease typing.

In conclusion, HD-MTX has significant clinical efficacy in children with ALL and is effective to improve the survival rate of the children, but in the process of treatment, toxicity and side effects easily occur and need to be properly alleviated.

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Disclosure of conflict of interest

None.

Address correspondence to: Chong Wen, Department of Traditional Chinese Medicine, The Central Hospital of Wuhan, Tongji Medical College, Huazhong University of Science and Technology, No. 26, Shengli Street, Jiangan District, Wuhan 430014, Hubei, China. Tel: +86 027 82211628; E-mail: chongwen5434664@163.com

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