Changes of Cerebral Biopterin and Biogenic Amine Metabolism in Leukemic Children Receiving 5 g/m² Intravenous Methotrexate

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ABSTRACT

Acute or subacute neurologic disorders can be observed in patients receiving high-dose methotrexate therapy for lymphoblastic leukemia or malignant tumor. Impairment of biopterin metabolism leading to decreased availability of monoamine neurotransmitters has been suggested to explain methotrexate neurotoxicity. To investigate such a mechanism, we have measured prospectively by HPLC the concentrations of total biopterin, homovanillic acid, and 5-hydroxyindolacetic acid in cerebrospinal fluid of 57 children with acute lymphoblastic leukemia. A sequential analysis of cerebrospinal fluid was performed for each patient: cerebrospinal fluid samples were obtained before therapy and after each of the four high-dose methotrexate infusions during the CNS prophylaxis phase. A significant increase of total biopterin concentrations in cerebrospinal fluid was observed after high-dose methotrexate therapy compared with the pretreatment values. No cumulative effect was noted. In contrast, no significant

variation of the homovanillic acid and 5-hydroxyindolacetic acid levels was observed in cerebrospinal fluid. However, individual analysis revealed a transient decrease of homovanillic acid and 5-hydroxyindolacetic acid concentrations in cerebrospinal fluid of six children. The increase of total biopterin mimicking that observed in inherited dihydropteridine reductase deficiencies suggests that methotrexate inhibits the regenerating system of biopterin in the brain of patients undergoing high-dose methotrexate therapy. (*Pediatr Res* 37: 151–154, 1995)

Abbreviations

HVA, homovanillic acid
5HIAA, 5-hydroxyindolacetic acid
MTX, methotrexate
DHPR, dihydropteridine reductase

Children with leukemia or malignant tumor receiving intrathecal or i.v. MTX may develop acute or subacute encephalopathy (1, 2), suggesting a direct effect of the drug on the brain metabolism. The hypothesis of impaired neurotransmitter synthesis has been proposed to explain the occurrence of these abnormalities (3). The biogenic amine neurotransmitters are produced in the brain at a rate-limiting hydroxylation from precursor amino acids. Tetrahydrobiopterin is the essential cofactor of this hydroxylation step. DHPR maintains adequate levels of tetrahydrobiopterin, and the inherited deficiency of DHPR leads to impaired neurotransmitter amine synthesis and severe neurologic disease (4). It has been suggested that MTX,

which *in vitro* inhibits DHPR (5), could lead to a deficiency of tetrahydrobiopterin and therefore of biogenic amine neurotransmitters (3). Such a hypothesis is important to test because of potential substitutive therapy with neurotransmitter precursors and tetrahydrobiopterin as reported in inherited DHPR deficiencies (6). Previous studies did not completely support the hypothesis of impaired neurotransmitter synthesis, but these works concerned leukemic children receiving intrathecal MTX therapy (7, 8). Pharmacokinetic studies have demonstrated that distribution of MTX in cerebrospinal fluid compartments varies considerably after administration by lumbar puncture (9). By contrast, continuous i.v. infusion of high-dose MTX can provide a stable drug concentration in the CNS at a level that is suspected to inhibit DHPR in the brain (10). To evaluate the potential repercussion of MTX therapy on the brain metabolism of monoamine neurotransmitters, we prospectively measured the levels of total biopterin and metabo-

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lites of biogenic amines (5-HIAA, HVA) in cerebrospinal fluid of 57 leukemic children receiving i.v. 5 g/m² MTX.

METHODS

Patients. Fifty-seven children (23 girls and 34 boys) with acute lymphoblastic leukemia from the pediatric hematology departments of Lille, Nantes, and Poitiers University Hospitals were treated between August 1989 and January 1993 according to the Children's Leukemia Cooperative Group protocol (11). The mean age of the patients was 6 y (range, 1 to 16 y; median, 5 y). After induction and consolidation phases, all children received daily 6-mercaptopurine and four i.v. high-dose MTX courses (5 g/m² every 2 wk). Intrathecal administration of MTX was performed at the end of each MTX infusion after cerebrospinal fluid collection. All the patients were in complete remission and none of them had CNS involvement at the time of the MTX therapy. High-dose MTX was given as a 24-h i.v. infusion with alkaline hydration. Folinic acid rescue (15 mg/m² given orally every 6 h) was initiated 12 h after the end of MTX infusion. None of the patients had clinical evidence of neurologic dysfunction during the study. After randomization, 19 high-risk children received i.v. cytosine arabinoside (1 g/m²) administered 12 and 24 h after the start of each MTX infusion. All patients were observed sequentially before receiving any therapy and after the four MTX courses. Permission to include each child in the study was obtained by informed consent from the parents. The study was approved by the ethics committee of the Catholic University Hospitals of Lille.

Cerebrospinal fluid analysis. Cerebrospinal fluid samples were obtained by routine lumbar puncture at initial diagnosis of hemopathy before any treatment and immediately at the end of each MTX infusion just before intrathecal therapy. After collection for standard cytochemical analysis, a 1-mL cerebrospinal fluid sample was collected directly into a plastic tube and stored at -70°C until analysis. Cerebrospinal fluid levels of total biopterin, 5HIAA, and HVA were determined in a reference center by HPLC as previously described (12, 13).

Statistics. Statistical analysis of the data was performed with SAS software (SAS Institute, Inc., Cary, NC). Normality of the values distribution was assessed using the Shapiro-Wilk test. Repeated measures analysis of variance was performed to test intrapatient differences between the five values of total biopterin, 5HIAA, and HVA (before and after the four MTX courses). Student-Newman-Keuls test was used if a significant difference was seen. *t* test was used to compare patients receiving cytosine arabinoside with those not receiving it. Significance was evaluated at the *p* < 0.05 level.

RESULTS

For all patients, total biopterin, 5HIAA, and HVA concentrations were available before any treatment. Two hundred eighty-five cerebrospinal fluid samples from 57 patients were analyzed. Large interpatient variations of total biopterin, 5HIAA, and HVA levels were observed (Table 1). A normal distribution was observed only for biopterin values. Statistical analysis was performed after logarithmic transformation of 5HIAA and HVA values.

A significant increase of biopterin concentrations after the MTX therapy was observed compared with the values before MTX therapy (analysis of variance, *p* < 0.001). The biopterin levels after the four MTX courses were not statistically different. For 5HIAA values, a significant rise was observed only for the second cycle (*p* = 0.027) compared with pretreatment values. No difference was seen for HVA levels. Children receiving or not receiving cytosine arabinoside did not exhibit different biopterin, 5HIAA, and HVA concentrations in cerebrospinal fluid. Six of the children had transient falls of 5HIAA and HVA levels after i.v. high-dose MTX (Table 2). None of them experienced concomitant acute or subacute neurologic abnormality. Patients 2 and 3 had received concomitant high-dose cytosine arabinoside.

DISCUSSION

Systemic administration of high-dose MTX as been shown to produce acute or subacute encephalopathy (1). Patients with these disorders usually present with seizures or focal neurologic signs such as hemiparesis. The mechanism of this MTX-induced neurotoxicity remains unknown. A transient defect in biogenic amine neurotransmitter synthesis has been suggested (3). This hypothesis is supported by studies demonstrating that intraventricular administration of MTX in monkeys induces seizures and a reduction of neurotransmitter catabolite levels in cerebrospinal fluid (14). Tetrahydrobiopterin is the required cofactor of hydroxylases for biogenic amine synthesis from the amino acid precursors tyrosine and tryptophan (Fig. 1). DHPR is essential for maintaining efficient tetrahydrobiopterin levels. This regenerating system of the cofactor of hydroxylases is inhibited *in vitro* by high concentrations of MTX (5). Inhibition of DHPR in the brain by MTX leading to tetrahydrobiopterin deficiency and therefore to impairment of biogenic amine synthesis might explain the occurrence of neurologic signs observed in a few patients after MTX therapy (3).

Hyperphenylalaninemia and increased levels of total biopterin were reported in serum of patients receiving systemic

Table 1. Mean ($\pm$ SD) of total biopterin (BIO), 5HIAA, and HVA concentrations (nmol/L) in cerebrospinal fluid before treatment and after the four high-dose MTX courses

	Before treatment	Course 1	Course 2	Course 3	Course 4
BIO	<i>n</i> = 57 24.7 $\pm$ 9.9	<i>n</i> = 56 30.7 $\pm$ 11.5	<i>n</i> = 55 33.4 $\pm$ 10.1	<i>n</i> = 55 32.4 $\pm$ 11.1	<i>n</i> = 57 31.4 $\pm$ 10.9
5HIAA	<i>n</i> = 57 132.5 $\pm$ 53	<i>n</i> = 55 148.3 $\pm$ 58.6	<i>n</i> = 57 163.1 $\pm$ 66	<i>n</i> = 57 152.2 $\pm$ 63	<i>n</i> = 56 161.4 $\pm$ 110
HVA	<i>n</i> = 57 365.4 $\pm$ 161.7	<i>n</i> = 55 396.4 $\pm$ 172	<i>n</i> = 57 396.5 $\pm$ 132	<i>n</i> = 57 423.8 $\pm$ 169	<i>n</i> = 56 393.6 $\pm$ 147

Table 2. Selective cases of transient fall of 5HIAA and HVA levels (nmol/L) in cerebrospinal fluid after MTX therapy

	Case 1: M 5 y.o.			Case 2: F 2 y.o.			Case 3: M 13 y.o.			Case 4: F 3 y.o.			Case 5: M 5 y.o.			Case 6: F 7 y.o.		
	BIO	5HIAA	HVA	BIO	5HIAA	HVA	BIO	5HIAA	HVA	BIO	5HIAA	HVA	BIO	5HIAA	HVA	BIO	5HIAA	HVA
Before therapy	24.3	165	591	28.2	167	287	18.5	102	205	21.7	151	503	23.1	120	261	33.5	141	467
Course 1	23.6	110	355	28.7	99	163	24.9	62	87	39.5	186	551	26.9	47	97	32.1	174	709
Course 2	31.3	114	401	33.6	178	482	21.1	72	157	37.1	158	457	41.1	136	434	38.3	166	316
Course 3	27.2	81	295	36.6	131	464	28.2	66	93	41.5	252	675	35.8	115	663	31.1	103	201
Course 4	39	81	339	41.4	166	493	25.2	73	95	41.5	81	269	28.6	104	376	41.6	58	163

M, male; F, female; BIO, total bipterin; y.o., years old.

Figure 1. Interaction of tetrahydrobiopterin (THB) in the initial step of biogenic amine synthesis. THB is a necessary cofactor for hydroxylation of tyrosine (TYR), phenylalanine (PHE), and tryptophan (TRP). MTX inhibits *in vitro* DHPR, the enzyme required for the regeneration of THB from dihydrobiopterin (DHB). 5HT, 5 hydroxytryptophan.

high-dose MTX (15, 16). In addition, serum phenylalanine levels normalized or fell significantly after tetrahydrobiopterin administration in patients with hyperphenylalaninemia induced by MTX therapy (17). Hyperphenylalaninemia and high total bipterin concentrations are also observed in serum of children with inherited DHPR deficiency (18). These reports strongly suggest impairment of DHPR activity in peripheral compartments after MTX therapy.

Pinkerton *et al.* (7) reported a rise of bipterin levels in serum but not in cerebrospinal fluid of children with acute lymphoblastic leukemia undergoing induction (including intrathecal MTX) or maintenance (including oral or intramuscular MTX) chemotherapy. This observation can be explained by the poor diffusion in the CNS of low-dose MTX administered by venous or intrathecal route. However, these authors reported increased bipterin concentrations in cerebrospinal fluid of children who had completed 2 y of maintenance therapy, suggesting a cumulative effect of repeated MTX administrations. We chose to conduct our prospective study in children undergoing systemic high-dose MTX because pharmacokinetic studies demonstrated that i.v. 5 g/m² MTX can provide drug levels in CNS that can potentially inhibit cerebral DHPR (19). The present study shows a significant rise of bipterin levels in cerebrospinal fluid after 5 g/m² MTX infusion. Such biochemical findings are similar to those observed in inherited DHPR deficiency (18). In these patients, an increased level of total bipterin reflects the accumulation of dihydrobiopterin with a concomitant decrease in tetrahydrobiopterin. Because of high instability of reduced bipterins, measurement of tetrahydrobiopterin in cerebrospinal fluid was not performed in our

multicentric study. However, we previously reported a slight but significant decrease of tetrahydrobiopterin in a selected group of children receiving i.v. high-dose MTX therapy (16). In contrast, Hyland *et al.* (20) reported the absence of decreased tetrahydrobiopterin concentrations in a few patients receiving intrathecal MTX. No cumulative effect of the successive courses of MTX on the total bipterin level in cerebrospinal fluid was noted in our study. However, our patients were observed over a period of a only few months.

No significant variation of cerebrospinal fluid neurotransmitter metabolite levels was observed in this study except a modest but significant increase in HVA levels after the second course of high-dose MTX. The absence of reduced concentrations of monoamine catabolites can be explained by an insufficient decrease of tetrahydrobiopterin to induce alteration of monoamine metabolism. Individual analysis revealed a transient decrease of these metabolites in six patients, but none of them experienced neurologic abnormalities. However, such metabolic changes constitute a propensity for the occurrence of neurologic abnormalities. We previously reported the occurrence of acute neurologic dysfunction in a child with decreased 5HIAA and HVA concentrations in cerebrospinal fluid after high-dose i.v. MTX (3 g/m²) infusion (21). Individual susceptibility can explain the occurrence of such neurologic perturbations in a context of low 5HIAA and HVA levels. In contrast, Silverstein *et al.* (8) reported a gradual rise of 5HIAA and HVA levels in leukemic children after 1 mo of treatment with weekly intrathecal MTX, but it was impossible to determine whether these variations are caused by delayed egress from cerebrospinal or enhanced turnover. Although cytosine arabinoside has no reported effect on biogenic amine metabolism, these authors related an unexpected decrease of 5HIAA and HVA levels in five of six patients who had received intrathecal cytosine arabinoside alone. Interestingly, high-dose cytosine arabinoside systemic infusions, which provide high drug concentrations in the CNS, had no significant influence on these metabolite concentrations in our study. Individual analysis revealed that among our six patients with a transient decrease of 5HIAA and HVA levels only two had received cytosine arabinoside in association with high-dose MTX.

The present work demonstrated an effect of high-dose MTX infusions on bipterin metabolism in the CNS but the absence of stigmas of significant impairment of monoamine synthesis. However, individual analysis showed a decrease in cerebrospinal monoamine catabolite levels in a few children but without neurologic dysfunction. The data in this prospective study are compatible with the hypothesis of an inhibition of the regen-

erating system of bipterin after systemic MTX therapy. We previously reported a successful substitutive treatment with neurotransmitter precursors and tetrahydrobiopterin in a child with a subacute encephalopathy related to a defect of tetrahydrobiopterin synthesis after MTX therapy (22). Because of potential replacement therapy, additional investigations are needed in children with acute neurologic dysfunction after high-dose MTX therapy.

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