Short Communication

Intravenous Infusion of High Dose Methotrexate, Metabolism of Pteridines and Consequences for the Tetrahydrobiopterin Dependent Hydroxylases

J.-L. Dhondt, F. Millot, J.-M. Hayte, G. Forzy, F. Mazingue*, W. Pfeleiderer** and J.-P. Farriaux*

*Laboratoire de Biochimie, Faculté Libre de Médecine, 56 rue du port, 59046 Lille, France*** Service de Génétique et Maladies Héritaires du Métabolisme, CHU, Lille, France**** Fakultät für Chemie, Universität Konstanz, Postfach 5560, W-7750 Konstanz, Fed. Rep. of Germany*

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Introduction

Methotrexate (MTX) is one of the most common cytostatics used in patients with acute lymphoblastic leukemia for control of CNS relapse. However, high doses of the drug (HD-MTX) are required to obtain potentially cytotoxic CSF levels. As a corollary, neurotoxicity is a well-recognized complication of the therapy, but the mechanism of the direct acute effect of the drug on the brain remains unclear. It has been suggested that MTX may reduce concentration of tetrahydrobiopterin (BH₄) (1) by inhibiting dihydropteridine reductase (DHPR, EC 1.6.99.7) which maintains biopterin in a reduced state necessary for its cofactor activity in the synthesis of monoamine neurotransmitters from aminoacid precursors. However, data which have been previously reported (1, 2) did not completely support that suggestion, but in these works, the studies were concerning patients receiving maintenance therapy and variable doses of MTX.

Because of potential therapeutic prevention or cure of dysfunction of tetrahydrobiopterin dependent metabolisms, we have designed a study aimed to explore the relationships between MTX and pteridine metabolism, in an homogeneous group of patients given a constant HD-MTX dose (5 g/m²).

Material and Methods

Patients

Biopterin metabolism was studied in 68 patients (32 F/36 M), aged 6 months to 17 years, with acute lymphoblastic leukemia or non-Hodgkin's lym-

phoma. MTX (5 g/m²) was administered by continuous intravenous infusion over 24 hours. Usually, 4 cycles were performed, one each over 2 weeks; a total of 233 cycles were included. At the time of MTX treatment, patients did not presented signs of hepatic or renal dysfunction or evidence of CNS involvement. Permission to include each child in the study was obtained by informed consent from the parents. The study had the approval of the local ethics committee.

Samples

Blood samples (5 ml) were collected in EDTA or heparinized tubes, essentially for monitoring MTX levels. Samples were taken before (T0), 24 (T24), 48 (T48) and 72 (T72) hours after the beginning of perfusion. After centrifugation, a 1 ml aliquot was kept at -20 °C for phenylalanine and pteridine measurements.

At T24, lumbar puncture was performed for intrathecal administration of MTX, a 1 ml aliquot of CSF was immediately frozen and stored at -20 °C. Thus the following observations resulted exclusively from the intravenous administration of MTX. CSF samples (n = 99) obtained before the beginning of MTX cycles for surveillance of meningeal relapse have served as controls.

Assay

Unconjugated pteridines were measured according to a high performance liquid chromatographic (HPLC) procedure previously described (3, 4). After iodine

oxidation and ion-exchange clean-up, pteridines were separated on a SCX column (Whatman, USA) and detected by their fluorescence. Neopterin and biopterin concentrations presently reported represent the sum of oxidized and reduced forms. In addition, percentage of BH4 has been estimated by comparing results after acidic and alkaline oxidation in CSF samples collected on 1 mg/ml ascorbic acid.

MTX and its derivatives, phenylalanine, 5-hydroxyindoleacetic acid (HIAA) and homovanillic acid (HVA) were measured as previously described (4, 5).

Results and Discussion

MTX and peripheral metabolism of biopterin

Serum biopterin concentration was significantly higher at the end of the MTX infusion than before. When the sequence of cycles was considered, the highest net increase of serum biopterin was observed during the first cycle (Fig. 1) because of T0 values, before the first MTX cycle, not significant different from pretreatment values, whereas T0 values in subsequent cycles were higher.

Serum phenylalanine concentration was found elevated at the end of MTX infusion ($212 \pm 127 \mu\text{mol/l}$) when compared to T0 values ($98 \pm 42 \mu\text{mol/l}$, $p < 0.001$), with a 4-fold increase of the phenylalanine to tyrosine ratio. In contrast to the large inter-individual variations, maximal serum phenylalanine concentrations were of the same magnitude in each individual, suggesting individual predispositions.

Figure 1. Serum neopterin and biopterin concentrations before (T0) and at the end of the MTX infusion (T24) according to cycle number

* Student's test

** ns, not significant at $p = 0.01$

These results confirmed those previously reported (6, 7) showing a defective conversion of phenylalanine to tyrosine presumably due to a reduction of the hepatic tetrahydrobiopterin pool. The increase of serum biopterin and the positive correlation between phenylalanine and biopterin levels ($r = 0.787$) would suggest that MTX induces a release of biopterin out of the hepatic cells, which can be secondary to an inhibition of DHPR.

MTX and biopterin metabolism in central nervous system

CSF samples obtained at T24 showed a significant increase of biopterin ($30.3 \pm 11.9 \text{ nmol/l}$) when compared to values before MTX treatment ($24.0 \pm 9.3 \text{ nmol/l}$, $p < 0.001$).

A slight decrease of biopterin present as tetrahydrobiopterin (%BH4) in CSF of patients on MTX was observed [64.8 ± 15.2 ($n = 51$) vs $73.0 \pm 14.4\%$ before MTX ($n = 32$), $p < 0.01$]. A significant negative correlation was found between %BH4 and CSF concentrations of 2,4-diamino-6-hydroxymethyl-7-hydroxy-pteridine ($r = 0.499$) and 2,4-diamino-6-methyl-7-hydroxy-pteridine ($r = 0.476$), unconjugated pteridines presumed to result from a cleavage of methotrexate or 7-hydroxy-methotrexate at the C9-N10 bond (4). This observation gives a new insight

Figure 2. 7-hydroxy-2,4-diamino-pteridines and %BH4 in CSF of a patient during HD-MTX therapy.

in the possible mechanism of CNS bipterin perturbation. Especially, 2,4-diamino-pteridines have been reported as potent inhibitor of pteridine-dependent enzymes (8, 9). The present results suggest that they may be potent inhibitor of DHPR (Fig. 2) and their passage through the blood brain barrier (CSF/serum ratio = 8 to 11%) was found more important than that of MTX (3%).

The consequence on tetrahydrobiopterin dependent tyrosine- and tryptophan-hydroxylase was explored by measuring CSF catabolites (HIAA and HVA) of the respective monoamines. Since important varia-

tions of HIAA and HVA were observed with age, distributions of values before and during MTX cycles were compared (Fig. 3). Scatter of values and regression with age did not show significant differences for both metabolites, a finding also confirmed by paired-t test comparison of averaged individual values before and during MTX cycles. The absence of significant reduction of monoamine metabolites can be explained by a reduction of BH₄ not sufficient to impair monoamine metabolism; however, HIAA and HVA only reflect final catabolism of released monoamines and may be not informative on transient and rapid changes of monoamine synthesis.

Figure 3. Distribution of CSF HIAA and HVA concentrations with age in patients before and during MTX cycles. dashed lines represent 95% confidence intervals for slope of the regression line and 95% confidence bands of the distribution left: before MTX therapy right: at T24 of MTX infusion; the grey area represents distribution of values before MTX.

Conclusion

Biogenic amine neurotransmitters are produced in the brain by rate-limiting hydroxylation of precursor aminoacids, a step that is dependent on adequate levels of tetrahydrobiopterin cofactor. Following in vitro experiments showing that MTX can inhibit DHPR (10), it was tempting to hypothesize that MTX may, in vivo, reduce the concentration of the active form of the cofactor and therefore lower the synthesis of monoamines, and may contribute to act in the pathogenesis of neurological pathology noted in some patients on HD-MTX therapy.

The present results confirmed a direct effect of MTX on serum biopterin and phenylalanine levels with maximal concentrations observed at the end of the MTX infusion, which can be due to an inhibition of peripheral DHPR activity.

In contrast, the poor penetration of MTX and/or its metabolites into CSF can explain the absence of significant alteration of monoamine metabolism. Nevertheless, it remains possible that a suppressed amine synthesis can occur when altered blood brain barrier is responsible for higher MTX and derivatives accumulation or after intrathecal MTX. The latter possibility could not have been presently explored since CSF samples were collected before intrathecal MTX administration.

The presence of 7-hydroxy-diamino-pteridines in significant concentration (up to 5 $\mu\text{mol/L}$ in serum, 0.5 $\mu\text{mol/L}$ in CSF) and their slow clearance from the body rise the question of possible interferences of these compounds on folate- or pteridine-dependent enzymes. If such effects are confirmed, inclusion of a "second rescue" (tetrahydrobiopterin or monoamine

precursors) would be recommended in at risk patients, supporting the earlier proposal by Cotton (11).

Since the incidence of neurological symptoms following HD-MTX is low, at-risk factors may exist. Efforts to characterize such factors are in progress in order to be able to detect individual susceptibility for such condition.

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