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Research report

Persistent cognitive deficits, induced by intrathecal methotrexate, are associated with elevated CSF concentrations of excitotoxic glutamate analogs and can be reversed by an NMDA antagonist

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ABSTRACT

For patients with acute lymphoblastic leukemia or non-Hodgkin lymphoma, intrathecal (IT) methotrexate (MTX) significantly reduces the risk of relapse within the central nervous system, but is associated with neurotoxic sequelae. We established a rat model of MTX-induced cognitive deficits to further investigate the underlying pathophysiology and to develop protective therapeutic interventions. IT MTX 0.5 mg/kg was administered to 10-week old male Long Evans rats. Cerebrospinal fluid (CSF) was collected for measurement of folate, homocysteine, and excitotoxic glutamate analogs. Recognition and spatial memory were tested in the novel object recognition (NOR) task and the object placement (OP) task, respectively.

Four doses of IT MTX in a two-week period induced cognitive deficits persisting at least three months after the final injection. CSF concentrations of the excitotoxic glutamate analogs homocysteic acid and homocysteine sulfinic acid were increased relative to baseline for the same three-month period. Dextromethorphan, a noncompetitive antagonist at the *N*-methyl-D-aspartate receptor, administered at a dose of 2 mg/kg intraperitoneally twice daily for a total of four doses, improved cognitive function among the MTX-treated rats, with no effect on control rats. Although this improvement was transient, each repeated treatment with dextromethorphan was followed by normalization of cognitive function.

In conclusion, IT MTX induces persistent alterations in glutaminergic tone that may contribute to persistent cognitive deficits. Treatment with a glutamate receptor antagonist such as dextromethorphan may ameliorate the negative cognitive outcomes observed among patients with leukemia or lymphoma treated with repeated doses of prophylactic IT MTX.

1. Introduction

For patients with acute lymphoblastic leukemia or non-Hodgkin lymphoma, intrathecal (IT) administration of methotrexate (MTX) significantly reduces the risk of relapse within the central nervous system and consequently increases disease-free survival. However, IT MTX is associated with neurotoxic sequelae. Severe neurotoxicity, including seizures, paralysis, and coma, has been described following IT MTX [1], but is relatively uncommon. More frequently, MTX induces subtle deficits in cognitive function. For example, survivors of therapy for childhood leukemia, who typically receive more than two dozen doses of IT MTX during 2–3 years of therapy, demonstrate an increased rate of deficits in working memory and executive function, leading to impaired school and occupational performance [2–5].

The multifactorial pathophysiology underlying MTX-induced cognitive dysfunction has been the subject of recent reviews [1,6,7]. One contributing factor may be an increase in excitotoxic amino acids within the central nervous system. MTX exerts its antineoplastic effect by depleting intracellular stores of reduced folate, a necessary cofactor for *de novo* purine and thymidine synthesis, thereby preventing DNA replication (Fig. 1). A secondary effect of MTX is a decrease in the availability of reduced folate for remethylation of homocysteine to methionine. As homocysteine increases intracellularly, metabolites of homocysteine appear, including other glutamate analogs (homocysteic acid and homocysteine sulfinic acid) as well as aspartate analogs formed

Abbreviations: aCSF, artificial cerebrospinal fluid; CA, cysteic acid; CSA, cysteine sulfinic acid; CSF, cerebrospinal fluid; EAA, excitotoxic amino acids; HCA, homocysteic acid; HCSA, homocysteine sulfinic acid; Hcy, homocysteine; iGluR, ionotropic glutamate receptor; IP, intraperitoneal; IT, intrathecal; IT1, single IT injection; IT4, four IT injections within two weeks; MTX, methotrexate; NMDA, *N*-methyl-D-aspartate; NOR, novel object recognition; OP, object placement.

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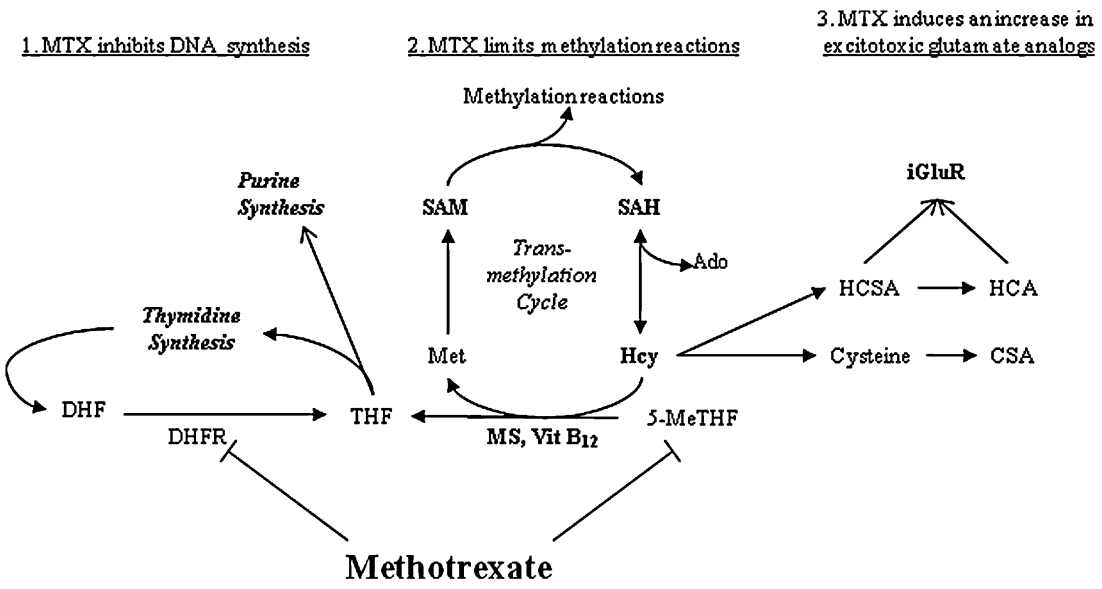

Fig. 1. Simplified schematic pathway of some folate-mediated reactions, highlighting the possible neurotoxic effects of methotrexate exposure. (1) Methotrexate (MTX) inhibits dihydrofolate reductase (DHFR), depleting intracellular pools of reduced folate (tetrahydrofolate; THF) available for purine and thymidine synthesis. (2) MTX exposure limits remethylation of homocysteine (Hcy) to methionine (met), leading to a reduction in methylation of myelin basic protein and neurotransmitter precursors. (3) Increased concentrations of Hcy are metabolized to the glutamate analogs, homocysteic acid (HCA) and homocysteine sulfinic acid (HCSA), which are excitotoxic agonists at ionotropic glutamate receptors (iGluR). Additional abbreviations: SAM: S-adenosylmethionine; SAH: S-adenosylhomocysteine; Ado: adenosine; CSA, cysteine sulfinic acid.

via the transsulfuration pathway (e.g. cysteic acid and cysteine sulfinic acid). Homocysteine and these related sulfur-containing excitotoxic amino acids are agonists at ionotropic glutamate receptors, including the *N*-methyl-D-aspartate (NMDA) receptor. It is well-established that abnormally high concentrations of homocysteine and other excitotoxic amino acids are associated with severe neurotoxicity, including seizures and neuronal death [8–11]. Dextromethorphan, an uncompetitive antagonist at the NMDA receptor has been used clinically to reverse severe neurotoxic sequelae of MTX therapy [12]. Using a rat model of MTX-induced cognitive dysfunction [13], the studies described here were undertaken to assess whether excitotoxic amino acids contribute to more subtle toxicity after IT MTX, including focal cognitive deficits.

2. Methods

2.1. Materials

Methotrexate (MTX), dextromethorphan hydrobromide and other chemicals were purchased from Sigma (Saint Louis, MO) unless otherwise stated. Methanol and water (HPLC grade) was purchased from Fisher Scientific (Pittsburgh, PA). For injection, MTX was diluted in artificial cerebrospinal fluid (aCSF; Na^+ 150 mM, K^+ 3 mM, Ca^{2+} 1.4 mM, Mg^{2+} 0.8 mM, P 1.0 mM, Cl^- 155 mM, in double distilled water). All injected solutions were sterilized by filtering through 0.22 μm syringe filters.

2.2. Animals

Eight-week-old male Long Evans rats were obtained from Charles River Laboratories (Wilmington MA). Rats were housed in groups of 2 or 3 with *ad lib* food (LabDiet 5001) and water with a 12-12 light/dark cycle. All studies were conducted following the 'Guide for the Care and Use of Laboratory Animals' and were approved by the Animal Institute Committee of the Albert Einstein College of Medicine. A total of 199 rats was used in these experiments.

2.3. Intrathecal injection and CSF collection

Intrathecal injection was carried out by transcutaneous cisternal magna puncture, as previously described [13]. Briefly, rats were anesthetized with inhaled 5% isoflurane/95% oxygen, and positioned in the lateral decubitus position. A 25-gauge butterfly needle was inserted into the cisterna magna. Correct positioning of the needle was verified by outflow of cerebrospinal fluid (CSF). MTX, 0.5 mg/kg, in 100 μL of aCSF was injected over 30–60 s. Control animals were injected with an equal volume of aCSF. IT injections were administered on one of two schedules (Table 1): a single IT injection (IT1) or four IT injections over a period of 10 days (IT4). Animals were treated in cohorts of approximately 20 rats (half injected with MTX and half with aCSF), and each treatment condition was replicated in at least three independent cohorts.

CSF was collected by gravity at multiple time points, as indicated in the results. Samples with gross contamination by blood were not analyzed (approximately 20% of all specimens). CSF samples were placed immediately in ice, then centrifuged briefly to remove any cellular elements. Supernatants were stored at −80 °C until further analysis.

2.4. Dextromethorphan

Dextromethorphan 2 mg/kg was administered intraperitoneally (IP) twice daily for a total of four doses to both MTX-treated and control rats, beginning after the 1-month behavioral testing. This dose and schedule were chosen to resemble the therapy given to the children described by Drachtman et al., to reverse severe MTX-induced neurotoxicity [12].

2.5. Cognitive testing

Recognition and spatial memory were tested in the novel object recognition (NOR) task and the object placement (OP) task respectively, and were carried out as

Table 1
Experimental schema.

Time (after last IT injection)	Treatment, testing, and specimen collection	
Baseline	CSF collection; IT1: MTX or aCSF IT × 1 dose	CSF collection; IT4: MTX or aCSF IT × 4 doses within 2 weeks
1 week	Behavioral testing	
4 weeks (1 month)	Behavioral testing; CSF collection	Behavioral testing; CSF collection
5 weeks		DM twice daily IP × 4 doses; Behavioral testing
8 weeks (2 months)		Behavioral testing; CSF collection
9 weeks		DM twice daily IP × 4 doses; behavioral testing

Abbreviations: CSF, cerebrospinal fluid; MTX, methotrexate 0.5 mg/kg; aCSF, artificial cerebrospinal fluid; DM, dextromethorphan 2 mg/kg; IT, intrathecal; IP, intraperitoneal.