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COMMENTARY

HIGH DOSE METHOTREXATE THERAPY: INSECURE RATIONALE?*

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Methotrexate (MTX, 4-amino-10-methylpteryolglutamic acid) an anti-folate first used in the late 1940s for treatment of acute lymphoblastic leukemia in children [1], is now one of the most commonly used and thoroughly studied anti-neoplastic agents. During 35 years of clinical use, individual doses of MTX have increased from 0.5 mg to >33 g/m^2, a 66,000-fold increase. The rationale for the empiric use of high dose MTX (HDMTX) was established during the late 1960s [2], with Goldin *et al.* [13] having established the laboratory basis for leucovorin rescue a decade earlier. The apparent clinical success of HDMTX in the treatment of osteogenic sarcoma has furthered the use of regimens using HDMTX [4]. Concurrent clinical trials, however, of HDMTX versus low or moderate doses of drug either in single agent or multi-drug regimens were not done. Recently, results of clinical trials have suggested that HDMTX may not be significantly better than more conventional doses of MTX in the treatment of head and neck tumors, breast cancer and lymphomas [5–7]. Furthermore, it was not until 1984, in this journal, that tumor MTX concentrations following HDMTX were reported [8]. Subsequently, we have shown that MTX polyglutamates in tumor biopsy samples following low dose MTX (approximately 50 mg total dose in three or four divided doses) were 40% of the concentrations reported after high dose therapy (10 g/m^2). Thus, with a several hundred-fold increase in the dose of MTX, the tumor MTX polyglutamates increased by only a factor of two [9].

The absence of clinical trials proving the superiority of HDMTX over conventional MTX combined with laboratory data showing that the time of exposure to MTX may be a more important correlate of cell kill than the maximum concentration achieved in the extracellular milieu [10] has led several groups to an investigation of the time/dose relationship of MTX action and the regulation of folate and MTX accumulation *in vivo*. These issues will be discussed in this review of the rationale for the use of HDMTX therapy, as summarized at an NCI-sponsored symposium for anti-folate compounds.‖

Elevated concentrations of MTX in the plasma could facilitate passive entry of MTX into tumor cells, potentially circumventing drug resistance secondary to defective transport. This theory and many of those to follow are based on work done with cell lines grown and selected *in vitro*. Tissue culture medium often has 100–1000 times the folate concentration found in plasma, and this folate is present as folic acid, not the physiologic form, 5-methyltetrahydrofolate. The discrepancies in the folate concentration and form could influence the study of MTX/folate homeostatis *in vitro*. Specifically, work from several laboratories has shown that the MTX transport mutants, selected *in vitro*, cannot efficiently transport the natural serum folate, 5-methyltetrahydrofolate [11], and will not grow well *in vivo*. Accordingly, their use as a model-system for MTX accumulation may not be warranted.

An increased intracellular MTX content could potentially overcome drug resistance secondary to an increased or altered dihydrofolate reductase, the presumed key intracellular target. Despite the well documented, massive overproduction of the presumed primary target enzyme, dihydrofolate reductase (DHFR) *in vitro* (reviewed in Ref. 12), a primary cell isolate from a patient who has failed therapy with MTX, containing extraordinarily high, or even minimally elevated DHFR activity has yet to be well documented. Studies of the DHFR content of primary tumors and normal tissue obtained at biopsy and autopsy have shown that human tissue contains little DHFR activity compared to human and rodent cell lines *in vitro* and tissues obtained from laboratory animals [13]. Our laboratory and Goldie with his associates have found that leukemic blast cells (ALL and AML), as well as normal bone marrow cells, contain less than 10% of the DHFR activity of human leukemia cells *in vitro* [13, 14]. Whether this is due to tumor heterogeniety, cell cycle specificity of the enzyme, or another as yet undefined factor is not clear, but it suggests that cells *in vivo* could be inhibited by much lower concentrations of MTX than cells growing *in vitro*. It should also be

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noted that, when the amount of DHFR found in brain tumor biopsy samples [13] is compared to the amount of MTX found in tumor biopsy samples [9] obtained after children had taken low dose (20–50 mg) MTX, the MTX is >50 times the DHFR content (pmol/g wet weight). In addition, authors describing DHFR gene amplification in cells obtained from patients resistant to MTX have not reported elevated levels of DHFR enzyme activity [15–17].

The increase in intracellular MTX could potentiate the formation of MTX polyglutamate derivatives, thereby increasing the intracellular half-life of the drug and prolonging the inhibition of DHFR. MTX polyglutamate formation has been associated with an increase in cell kill and with the inhibition of the thymidylate synthase, *in vitro* [18, 19]. The possibility, however, that MTX polyglutamates might be associated with increased toxicity must not be overlooked. Children with ALL take 20–50 mg MTX/m^2 weekly for 2–3 years. The accumulation of MTX as a polyglutamate could be at the expense of the folates in normal tissue. Note that a marked folate deficiency has already been reported in human red blood cells and liver [20, 21]. Laboratory animals, treated with repetitive "low" dose MTX for 20–50 weeks, have also been found to have a striking folate deficiency in brain tissue [22, 23]. Thus, an increase in the cytotoxicity of MTX secondary to polyglutamate synthesis may not be associated with an increase in the therapeutic index.

It is also possible that, as a result of feedback regulation, too great a concentration of MTX monoglutamate may inhibit MTX$(Glu)_n$ synthesis. This is based on studies of MTX and folate homeostasis *in vivo* and *in vitro*, demonstrating a reciprocal relationship between folate (MTX) concentrations and polyglutamate formation [24–29]. There was no more MTX(GLU_n) in rat hepatic tissue after a 2-hr infusion of MTX at 10^{-4} M than following an infusion at 10^{-6} M [30], despite a transient but significant increase in the hepatic MTX concentration during the infusion at 10^{-4} M MTX. Thus, at least in these situations, transport was not limiting to the intracellular metabolism of MTX and, if MTX(Glu_n) are necessary for cytotoxicity, HDMTX could theoretically limit its own efficacy.

HDMTX therapy will result in an increase in the time of exposure to a minimally required cytotoxic concentration of MTX. This is based upon results of studies which allowed the suggestion that the time of exposure to MTX is more important than the peak extracellular concentration both *in vivo* and *in vitro* [10, 31]. The initial clinical trial of aminopterin used only 0.5 to 1.0 mg/day [1]. The early studies of Djerassi *et al.* [31] showed that 18- to 24-hr infusions of 50–100 mg of MTX were effective in treating children with acute lymphoblastic leukemia, but too toxic. Recent studies from our laboratory and that of Kolhouse and associates have demonstrated a saturable, specific receptor-mediated transport for MTX via the folate transport pathway in rapidly growing cells *in vitro* [32, 33]. In these studies, maximum receptor-mediated transport of MTX occurred at an extracellular concentration of only 50–100 nM. This suggests that the use of repetitive low dose MTX to maintain the plasma at only 100–200 nM MTX may increase the therapeutic index by creating a situation where only a susceptible cell population will accumulate drug through specific biologically active pathways (in this case, active, receptor-mediated folate transport).

MTX resistance may be seen in mitotically active cells containing low levels of thymidylate synthetase. These populations may be more sensitive to greater concentrations of MTX. Should this be true (i.e. low thymidylate synthetase), one could also suggest that the "salvage pathway" is more important than the *de novo* synthesis of thymidine monophosphate. Therefore, one would not expect the cells to be more sensitive to MTX which primarily inhibits *de novo* synthesis of DNA precursors, simply because more drug is present. In other words, if the cells are not reliant on a particular biochemical pathway, inhibiting that pathway may not increase cell kill.

Treating to a maximally tolerated dose (MTD) has been the generally accepted dogma in cancer chemotherapy. This empiric approach was mandated in the past by the absence of specific disease (tumor) oriented drugs, and this approach did contribute to the current cure rates for diseases such as leukemia and lymphoma. The concept that "more is better" may still be applicable to the use of cycle-dependent, non-phase specific drugs such as alkylating and interchelating agents for which a significant dose response can be generated. However, as we learn more about the mechanism of action of anti-metabolites, it may be that more drug per unit time is not necessarily better. Treating to an MTD may actually eliminate the possibility of increasing the therapeutic index since treating to normal tissue tolerance offers little margin for error. Time, as shown for MTX, may be a more critical variable than peak concentration, especially if tumor cells are more efficient at accumulating the drug than non-malignant host tissue.

In summary, our current understanding of MTX pharmacodynamics; its relationship to and effects on folate metabolism; the few clinical trials showing no significant improvement in cure rate with high versus low dose MTX; the lack of documentation that high dose MTX significantly increases the tumor MTX concentration; and the increased expense, hospital time and potential morbidity and mortality associated with high dose therapy suggest that the scheduling of MTX in the treatment of patients with cancer needs to be evaluated more completely.

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