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Methotrexate Accumulates to Similar Levels in Animals Transplanted with Normal *versus* Drug-resistant Transgenic Marrow[1]

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ABSTRACT

Gene transfer and expression of methotrexate (MTX)-resistant variants of dihydrofolate reductase (DHFR) in normal hematopoietic cells is a potential strategy to permit administration of larger doses of MTX by alleviating drug toxicity in normal cells and tissues that are drug sensitive. We have previously demonstrated that transplantation of marrow from transgenic mice expressing drug-resistant DHFRs conferred upon normal recipient animals resistance to MTX at levels that are usually toxic for hematopoietic and gastrointestinal (GI) tissues. One explanation for the observed protection from GI toxicity by drug-resistant marrow is that MTX could be cleared more rapidly in animals maintaining a more healthy hematopoietic system. To evaluate this possibility, we carried out MTX pharmacokinetic studies in mice that received transplanted transgenic marrow expressing either of two different DHFR variants, administering increasing doses of MTX up to 4 mg/kg/day. Animals received i.p. injection precisely every 24 h. Every 4 days, three animals from each group were sacrificed, and their plasma and intestines were assayed for MTX. Animals transplanted with transgenic Arg-22 DHFR drug-resistant marrow maintained hematocrit levels that were about 4-fold higher at 3 weeks after transplant than those of untreated animals or animals that received normal marrow cells. Animals that received normal marrow did not survive beyond 25 days and did not accumulate higher levels of MTX than animals that received a transgenic marrow transplant. Untreated animals exhibited a higher rate of survival (36 days) but again did not accumulate higher levels of MTX than the transgenic marrow recipients. When the experiment was repeated using transgenic Tyr-22 DHFR marrow, the levels of MTX in the plasma or GI tissues did not differ significantly between groups. Intestinal concentrations of MTX in both experiments were about 4–5-fold higher than those in the plasma. These results indicate that protection from MTX toxicity conferred by expression of drug-resistant DHFR activity in the marrow is not the result of a higher rate of MTX clearance from the circulation in comparison with control animals but a true resistance of hematopoietic and GI tissues to MTX. The maintenance of antifolate levels in animals protected from MTX toxicity implies that this procedure should not compromise the antitumor efficacy of MTX.

INTRODUCTION

DHFR[3] (EC 1.5.1.3) catalyzes the NADPH-dependent conversion of 7,8-dihydrofolate to 5,6,7,8-tetrahydrofolate. The enzyme is necessary for maintaining intracellular pools of tetrahydrofolate and its derivatives, which are essential cofactors for biosynthetic reactions requiring one-carbon unit transfer (1). MTX is a competitive inhibitor of DHFR and depletes reduced folate pools, resulting in decreased DNA and RNA synthesis and hence inhibition of cell growth. MTX has been widely used as an antiproliferative agent in the treatment of a variety of tumors (2, 3). Major limitations with MTX treatment are bone marrow toxicity, GI toxicity, and the emergence of drug-resistant tumor cells (4, 5). The introduction of MTX-resistant DHFR variants into normal hematopoietic cells is a potential therapeutic strategy for permitting administration of larger doses of MTX (6). Several variants of DHFR have been characterized and used as mediators of drug resistance in cultured mammalian cells and in animals (7–10).

To study the effect of MTX-resistant DHFR expression in whole animals, we have established several inbred lines of FVB/N transgenic mice expressing MTX-resistant DHFR activity (11, 12). We found that marrow transplanted from these transgenic animals into normal recipients conferred resistance to MTX at levels that cause both hematopoietic and GI toxicity (12, 13). Several investigators have transduced murine bone marrow cells with drug-resistant DHFR variants and transplanted these transduced cells into mice, rendering recipient animals more resistant to antifolates (14–17). The mechanism by which drug-resistant DHFR transgenic marrow is able to protect animals from systemic MTX toxicity is not currently understood. One possibility is that transgenic hematopoietic cells may penetrate tissues such as GI tissues, thus contributing to normal GI structure and function. However, it is also possible that animals transplanted with drug-resistant marrow may eliminate MTX more efficiently, thus protecting the animal from MTX toxicity (18).

To address the latter possibility, we carried out the present pharmacokinetic study to determine the levels of MTX in the plasma and intestine of animals transplanted with either drug-resistant transgenic marrow or normal marrow. As described below, we found that normal animals did not accumulate MTX to higher levels than animals transplanted with drug-resistant DHFR transgenic marrow. These results imply a cellular/molecular basis for the chemoprotection observed in animals transplanted with drug-resistant marrow and have significant implications for the clinical application of MTX resistance gene transfer in human cancer therapy trials.

MATERIALS AND METHODS

Animals and Bone Marrow Transplantation. FVB/N mice were obtained from the NIH Animal Supply Facility (Frederick, MD). Transgenic animals used in this study were established in the FVB/N strain as described previously (11, 12). Animals were provided food and water *ad libitum*. BMT was conducted as described previously (13). Briefly, marrow was flushed from the long bones of the hind limbs of donor mice into DMEM without additives. Marrow cells were washed twice by centrifugation at $1000 \times g$ in a Beckman (Fullerton, CA) TJ-6 for 15 min at room temperature. A single-cell suspension was prepared in DMEM by passage through a syringe fitted with a 27-gauge hypodermic needle. Eight-week-old recipient mice were irradiated at 800 rads using a cesium 137 source 1 day before transplant. Bone marrow cells were injected via the tail vein in 0.5-ml samples.

A total of two experiments were carried out. In the first experiment, animals were irradiated at 800 rads and then transplanted with 1×10^7 normal bone marrow cells or 1×10^7 Arg-22 DHFR transgenic [line 04, (11)] marrow cells (experiment 1). In the second experiment, animals were irradiated at the same dose and then transplanted with either 1×10^7 normal bone marrow cells or 1×10^7 Tyr-22 DHFR transgenic [line 11, (12)] marrow cells (experiment 2). Normal, unirradiated, untreated animals were included in both experiments as a control group. MTX was administered daily to all animals at increasing doses up to 4 mg/kg/day.

MTX Preparation. MTX stock solution of 55 mM was prepared in 10 mM Tris-HCl (pH 7.5) and diluted in PBS [137 mM NaCl, 3 mM KCl, and 10 mM

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[3] The abbreviations used are: DHFR, dihydrofolate reductase; BMT, bone marrow transplant; MTX, methotrexate; GI, gastrointestinal; H_2F, 7,8-dihydrofolate.

sodium/potassium phosphate (pH 7.4)]. The UV absorption spectrum of the drug used for injection was in close quantitative agreement with previous reports (19). MTX stock solution and dilutions were stored at −20°C until use.

Pharmacokinetic Study. Pharmacokinetic studies were carried out using female animals with a mean body weight of 24 grams. Animals were weighed and given MTX daily at a dose of 1 mg/kg/day on days 1 through 4 (after BMT), 2 mg/kg/day on days 5 through 8, and 4 mg/kg/day for the duration of the experiment. i.p. injections using a 27-gauge hypodermic needle were carried out precisely 24 h after the previous injection. On days 12, 16, 21, 26, and 31, injections were withheld from three mice in each experimental group that were sacrificed instead. Blood was taken from the abdominal aorta of the sacrificed animals using sodium citrate as an anticoagulant. Plasma was separated by centrifugation for 10 min at 14,000 rpm in an Eppendorf centrifuge 5415C (Hamburg, Germany) and kept frozen at −20°C until assayed. The entire intestine was removed, placed in ice-cold buffer consisting of 0.5 M Tris-HCl (pH 7.5), sectioned longitudinally, cleaned, and then soaked in fresh ice-cold buffer for 5 min. The tissue was blotted to remove excess liquid, placed in preweighed sample vials, and frozen at −20°C until assayed. Intestinal samples were processed as described previously with modifications (20, 21). Briefly, samples were supplemented with buffer (at a 3:10 ratio) consisting of 0.05 M Tris-HCl (pH 7.5) and homogenized using a postmounted homogenizer (Power-Gen), boiled for 5 min, vortexed, and centrifuged at 1,500 × *g* for 10 min in a Beckman TJ-6. The supernatant was cleared once more by centrifugation at 14,000 rpm for 10 min in an Eppendorf centrifuge. The tissue samples were measured against a standard curve constructed similarly using normal tissue.

Histopathological Analysis. Liver, ileum, sternum, and femur were harvested from the sacrificed animals, fixed in 10% phosphate-buffered formalin (bone samples were then decalcified in 10% formic acid), embedded in paraffin, sectioned, mounted, and stained with H&E. Analysis of tissue samples was undertaken without prior knowledge of animal identity.

MTX Macroassay for Plasma. Plasma MTX concentrations in experiment 1 were determined using a spectrophotometric assay for DHFR inhibition based on the procedure described by Falk *et al.* (22). The DHFR enzyme assay was carried out in a Beckman DU 7400 spectrophotometer equipped with a circulating water bath maintained at 37°C. Aliquots of 0.385 ml of standards, blanks, or unknowns were added to 1-ml quartz cuvettes, followed by the addition of 0.305 ml of enzyme reaction mixture. The reaction mixture consisted of 10 ml of 0.5 M Tris-HCl (pH 7.5), 5 ml of 1.5 M KCl, 0.5 ml of 5 mg/ml NADPH, and 0.15 ml of 0.4 unit/ml DHFR. The mixture was inverted to mix and allowed to incubate in the prewarmed cuvette holder for 5 min. An aliquot of 0.06 ml of H_2F (4.2 mg/ml) was added to start the reaction, and the change in absorbance at 340 nm was recorded for 6 min. MTX concentrations in test samples were determined by comparison to a MTX standard curve in the linear range.

MTX Microassay for Plasma. Levels of MTX in plasma obtained for experiment 2 were determined with a DHFR enzyme inhibition assay as modified from Widemann *et al.* (23). Briefly, the assay was carried out in a Bio-Tek (Winooski, VT) FL600 microplate reader with the temperature maintained at 37°C. Aliquots of 0.02 ml of standards, blank plasma, or unknowns were pipetted into discrete wells of Costar 96-well cluster plates (Cambridge, MA). A 0.055-ml aliquot of enzyme mixture [consisting of 6 ml of 0.05 M Tris-HCl (pH 7.5), 0.4 ml of 5 mg/ml NADPH, and 0.5 ml of 0.2 unit/ml DHFR] was added to the wells. The plate was incubated for 5 min in the microplate reader, whereupon 0.125 ml of H_2F (4.2 mg/ml) was added, and the plate was shaken for 1 min and read for 20 min at 340 nm. The linear portion of each reaction was used for subsequent analysis.

MTX Microassay for Intestine. MTX concentrations in the intestine were assayed as described for the plasma microassay with the following variations. Aliquots of 0.125 ml of standards or unknowns were added into discrete wells of Costar 96-well cluster plates. A 0.1-ml aliquot of enzyme mixture [consisting of 10 ml of 0.05 M Tris-HCl (pH 7.5), 5 ml of 1.5 M KCl, 1 ml of 5 mg/ml NADPH, and 0.3 ml of 0.4 unit/ml DHFR] was added to the wells. The plate was incubated for 5 min in the microplate reader at 37°C, 0.025 ml of H_2F (4.2 mg/ml) was added, and the plate was shaken for 1 min and read for 20 min at 340 nm.

Statistical Analysis. Statistical comparison of MTX concentrations between groups was carried out using ANOVA (StatView v.4.2; SAS Institute Inc., Cary, NC). Probability values of less than 0.05 were considered to be significant.

Reagents. MTX (amethopterin), dihydrofolic acid (H_2F), DHFR from bovine liver (macroassay), and NADPH were purchased from the Sigma Chemical Co. (St. Louis, MO); DHFR from *Lactobacillus caseii* (microassay) was obtained from Biopure Corp. (Cambridge, MA); and blank mouse plasma was obtained from Harlan Bioproducts (Madison, WI). All other chemicals were reagent grade or better.

RESULTS

Transplantation with Arg-22 or Tyr-22 DHFR Transgenic Marrow Protects Animals from MTX Toxicity. In previous studies, transgenic animals expressing an Arg-22 variant DHFR were found to be resistant to MTX (11), as were normal FVB/N animals transplanted with Arg-22 transgenic bone marrow cells (13). The Tyr-22 variant of DHFR has been shown to have a higher level of catalytic activity than Arg-22 (10). To determine whether chemoprotection is associated with decreased MTX levels in animals transplanted with transgenic marrow, we determined MTX levels in plasma and GI tissue extracts at regular intervals after transplantation.

Experiments 1 and 2 were carried out using Arg-22 and Tyr-22 DHFR transgenic marrow, respectively. Three mice from each group were sacrificed on days 12, 16, 21, 26 (day 27 for the Tyr-22 marrow), and 31 (day 30 for Tyr-22 marrow) to assay plasma and GI tissues for MTX. Hematocrit levels were assessed weekly as an indicator of the overall response to MTX (Fig. 1, *A* and *B*). Mice transplanted with Arg-22 DHFR transgenic marrow exhibited hematocrit levels 2–3-fold higher than those in animals transplanted with normal marrow on day 14 and 4-fold higher on day 21. Animals transplanted with Tyr-22 DHFR transgenic marrow exhibited hematocrit levels that were 2-fold higher than those in animals transplanted with normal marrow on day 14 and 2-fold higher than those in animals that received no BMT on day 23. In experiment 1, all of the animals transplanted with normal marrow were moribund by day 26, whereas in experiment 2, all of the animals transplanted with normal marrow were moribund by day 17. In both experiments, animals that did not receive a transplant exhibited reduced hematocrit levels by day 21 that declined progressively as the experiment proceeded. Animals transplanted with transgenic DHFR Arg-22 marrow had mean hematocrit values of 33 on days 7 and 14, which increased to 40 by day 21, whereas animals that received transgenic DHFR Tyr-22 marrow had a mean hematocrit of 43 on day 23, which declined to 32 on day 30. In both experiments, animals transplanted with transgenic marrow thus demonstrated a high degree of resistance to MTX. Statistical analysis of hematocrits in both experiments using ANOVA showed that there was a significant difference between the hematocrits of animals transplanted with transgenic marrow and those of animals transplanted with normal marrow or untreated animals. The hematocrits from both experiments followed a similar pattern.

Accumulation and Persistence of MTX in Plasma. The pharmacokinetics of MTX accumulation in plasma from experiment 1 (with Arg-22 DHFR transgenic marrow) and experiment 2 (with Tyr-22 DHFR transgenic marrow) are shown in Fig. 2, *A* and *B*. As observed in previous studies (13), all animals transplanted with normal marrow were moribund by day 26 and exhibited GI and hematopoietic toxicity, as evidenced histologically by severe GI atrophy and marrow aplasia (data not shown; see Ref. 13). Unirradiated, untreated control animals survived longer. Animals transplanted with Arg-22 DHFR transgenic marrow (experiment 1) accumulated the highest levels of MTX among the three experimental groups, reaching a level of nearly 40 nM on day 21. Statistical analysis of the plasma MTX levels in experiment 1 using one-way ANOVA indicated that there was no significant difference between animals transplanted with normal marrow and untreated animals. However, MTX levels were significantly

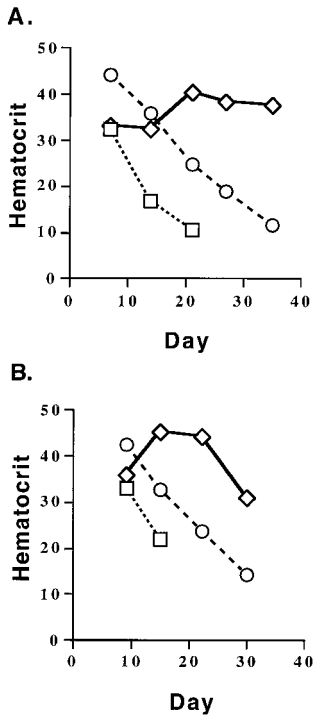

Fig. 1. Mean hematocrit levels as a measure of time. *A*, two sets of mice were irradiated and transplanted (as described in "Materials and Methods") with Arg-22 DHFR transgenic marrow cells or with normal FVB/N marrow cells. A group of unirradiated animals that did not receive transplants was included as a control. Each group of mice was given MTX at increasing doses up to 4 mg/kg/day. SDs of the mean hematocrit values were less than 15%, except for the value at day 14 for the group that received normal marrow (SD = 20%). *B*, mean hematocrit levels in animals transplanted with Tyr-22 DHFR transgenic marrow. Two sets of mice were irradiated and transplanted with (as described in "Materials and Methods") Tyr-22 DHFR transgenic or normal FVB/N marrow cells. A group of unirradiated, untreated mice was also included as a control. SDs of the mean hematocrit values were less than 15%. □, animals transplanted with normal marrow; ◇, animals transplanted with DHFR transgenic marrow; ○, normal animals.

higher in animals transplanted with transgenic marrow than in either animals receiving normal marrow or untreated animals. This shows that far from clearing MTX more efficiently, animals transplanted with Arg-22 DHFR transgenic marrow tolerated higher plasma concentrations of the drug than either of the other two groups. In experiment 2, there were no significant differences in plasma MTX between the three groups. A replot of plasma MTX *versus* hematocrit (data not shown) indicated that although the hematocrits of animals transplanted with transgenic marrow were high, the plasma MTX concentrations of all of the different groups overlapped, further substantiating that animals transplanted with DHFR transgenic marrow did not clear MTX at a faster rate than the other two groups.

MTX Concentrations in the Intestine. Intestinal levels of MTX (Fig. 3, *A* and *B*) were much higher than those found in plasma. Accumulation of high MTX levels in the intestine has been reported previously and has been attributed to the presence of MTX in biliary secretions into the intestinal lumen (24). Intestinal MTX levels were similar in both experiments and did not differ significantly between groups or days of drug administration. The assessed intestinal MTX levels in both experiments thus indicate that animals transplanted with DHFR transgenic marrow do not clear MTX at a faster rate than the other two groups from this major site of MTX toxicity.

DISCUSSION

Previous studies have shown that transplantation of marrow that has been genetically engineered to express drug-resistant DHFR confers upon recipient animals resistance to MTX at levels that cause systemic toxicity, particularly bone marrow toxicity and GI toxicity (12–16). However, the mechanism by which drug-resistant marrow may protect nonhematopoietic tissues is not understood. One possibility is that the more healthy hematopoietic system maintained in animals transplanted with drug-resistant marrow may in some way bring about more efficient elimination of the drug from sensitive tissues, thus protecting the animals from MTX toxicity. However, we found that MTX levels were not higher in the plasma or intestine of normal mice or mice transplanted with normal marrow in comparison with animals that received DHFR transgenic marrow. These results demonstrate that the chemoprotection afforded by drug-resistant DHFR expression in hematopoietic cells (12, 13) is not based on increased elimination of MTX in comparison with control animals.

Previous studies (25) reporting the plasma levels of MTX as a function of time after a single i.p. injection in mice indicated a distribution half-life of 30 min and an elimination half-life of approximately 12 h. Thus, in the course of daily injections, plasma MTX

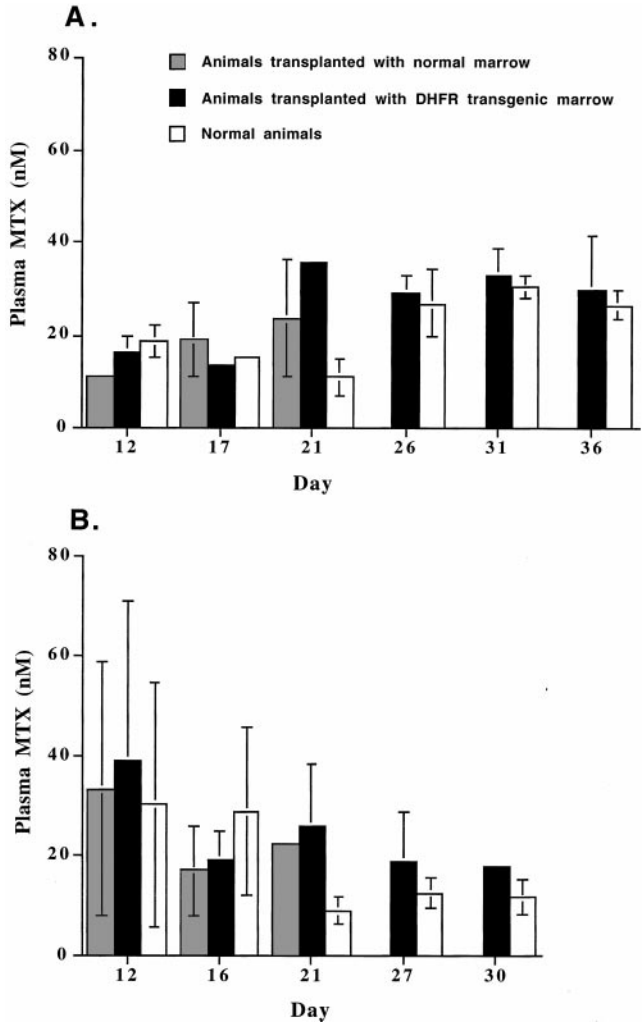

Fig. 2. MTX levels in plasma. MTX was administered precisely every 24 h. Values shown are the mean of three individual mice/group, except as indicated below. *A*, accumulation of MTX in mouse plasma in animals transplanted with Arg-22 DHFR transgenic marrow, normal marrow, or in untreated animals. For the values obtained from animals transplanted with normal marrow (day 12), $n = 1$; for the values obtained from animals transplanted with transgenic marrow (days 17 and 21), $n = 2$; and for the values obtained from animals that received no transplant (day 17), $n = 2$. *B*, accumulation of MTX in mouse plasma of animals transplanted with Tyr-22 DHFR transgenic marrow, normal marrow, or in untreated animals. For the values obtained from animals transplanted with normal marrow (day 21), $n = 1$; for the values obtained from animals transplanted with transgenic marrow (day 30), $n = 2$; and for the values obtained from animals that received no transplant (day 30), $n = 6$.

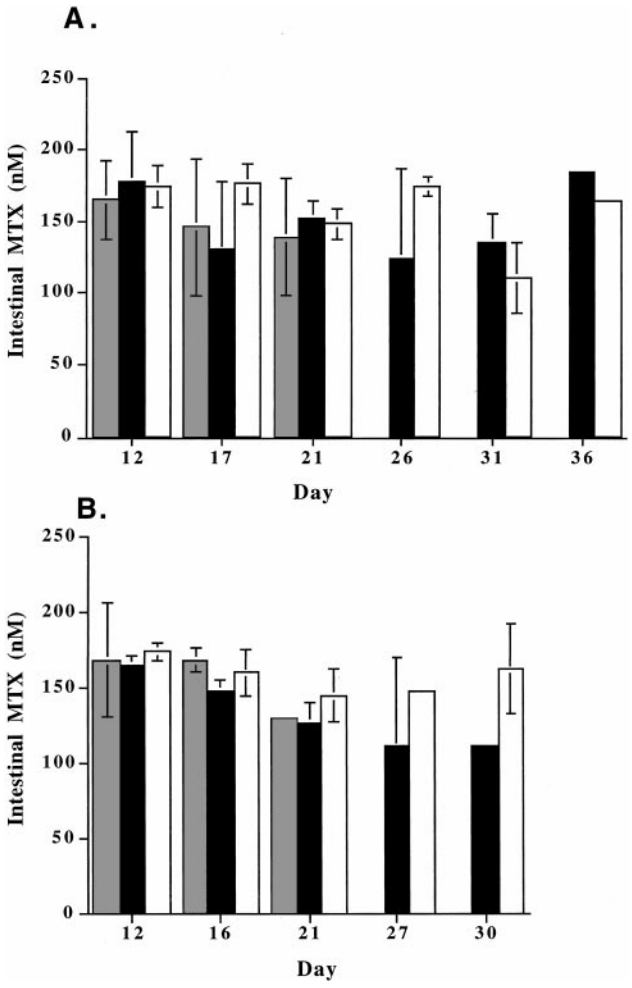

Fig. 3. Concentration of MTX in intestine after administration of 4 mg/kg/day. MTX concentrations were about 5–10 times higher than those seen in the plasma. *A*, MTX levels in animals transplanted with Arg-22 DHFR transgenic marrow, normal marrow, or in untreated animals. For the values obtained from animals transplanted with transgenic marrow (day 36) and animals that received no transplant (day 36), $n = 2$. *B*, MTX levels in animals transplanted with Tyr-22 DHFR transgenic marrow, normal marrow, or in untreated animals. For the values obtained from animals that received normal marrow transplant (day 21), $n = 1$; for the values obtained from animals transplanted with transgenic marrow (day 30), $n = 1$; and for the values obtained from animals that received no transplant (days 27 and 30), $n = 2$ and 6, respectively. ▨, animals transplanted with normal marrow; ■, animals transplanted with DHFR transgenic marrow; □, normal animals.

levels should reach steady state within 3–4 days at a given dose. Under these conditions, we observed no significant difference in MTX levels between animals transplanted with normal marrow *versus* transgenic marrow, although the mean hematocrit of animals that received DHFR transgenic marrow was 2–3-fold higher than that of animals that received normal marrow or that of untreated animals. Interestingly, although the hematocrits of this DHFR transgenic marrow transplant group were high, the plasma MTX concentrations overlapped those of the other two groups.

Intestinal levels of MTX from both experiments conducted in this study were similar and were approximately 4–10 times higher than those in the plasma. Previous studies of intestine (4) have shown that in mice injected with 5 mg/kg MTX, drug concentrations reached steady state at about 16 h, which is consistent with the results seen here. There was no significant difference between normal and transgenic marrow recipients, again indicating that the level of MTX in animals receiving transgenic marrow was not lower than that of the normal marrow transplant group. Although we observed some variability in the plasma MTX levels, the consistency of the GI levels underscores the relative lack of pharmacokinetic difference among the three experimental groups. This is especially striking, considering the significant intestinal toxicity experienced in the irradiated normal marrow group. This further supports the lack of a pharmacokinetic basis for protection from GI toxicity by Arg-22 or Tyr-22 DHFR expression in hematopoietic cells.

MTX is an effective chemotherapeutic agent in the treatment of a variety of solid tumors and leukemias (2, 3). The pharmacology and toxicology of MTX and related analogues have been under intensive study in both animals and humans (4, 26–30). Plasma clearance and distribution rates in tissues have been established and can be correlated with the action of the drug in tumor and normal tissue. Previous studies have shown that the limiting toxicity of MTX is associated with the biochemical effects of the drug at sites of proliferating normal tissue, primarily in the small intestine (4, 5, 27). The clinical usefulness of MTX is also limited by the development of drug resistance in tumor cells (31, 32). This development of drug resistance is not accompanied by resistance in normal cells, thereby increasing the toxicity of MTX to normal cells as the dose of MTX is escalated to treat the drug-resistant tumor (33).

If normal tissues could be rendered less sensitive to MTX, then this would allow administration of higher MTX doses and an increased likelihood of antitumor efficacy. One potential way to bring this about is by transfer of a drug resistance gene into hematopoietic cells. There are several drug resistance genes that have been investigated in chemoprotection studies, including the DHFR gene (7, 15, 34), the multiple drug resistance gene 1 (*MDR1*; Refs. 35–37), and the O^6-methylguanine methyltransferase gene (38, 39). DHFR has attributes that make it a particularly attractive mediator of drug resistance, including the availability of several mutants of mammalian DHFRs that confer high-level resistance to antifolates (17, 34, 40, 41). These mutant forms of DHFR consist mostly of substitutions at amino acids 22 and 31, which contribute to the active site of the enzyme (7, 10). Although numerous drug-resistant DHFR mutants have been studied, the most well characterized mutant is the Leu-22 to Arg substitution (7) that has been used extensively as a dominant selectable marker in mammalian systems by virtue of its high level of resistance to MTX (42, 43). However, this highly MTX-resistant mutant is severely impaired catalytically (10, 44). A Leu-22 to Tyr substitution generated by saturation mutagenesis in this laboratory (10) resulted in a murine DHFR that was 10-fold more catalytically active than the Arg-22 mutant and, at the same time, nearly as resistant to MTX as Arg-22.[4] Subsequent studies by Lewis *et al.* (45) and Ercikan-Abali *et al.* (46) on the human Tyr-22 DHFR mutant confirmed the high catalytic activity and drug-resistant character of the variant enzyme. We have generated several lines of transgenic mice expressing MTX-resistant DHFR activity and detailed the extent of MTX resistance conferred by expression of the murine Arg-22 and Tyr-22 DHFR mutants *in vivo* (12, 13). Marrow transplanted from these transgenic animals into normal recipients protected the recipients from both hematopoietic and GI toxicity.

MTX clearance is largely attributed to the kidney, but there have also been studies showing that MTX may be associated with hematopoietic cells that could participate in the MTX clearance process (47). The time points picked in the experiments reported here were chosen because they represent the initial, intermediate, and late stages of hematopoietic deterioration in normal marrow transplant recipients undergoing MTX administration. The results are of considerable significance in that if animals receiving drug-resistant marrow were in fact clearing the drug more effectively than the control animals, then

[4] P. A. Peiper, D. A. Evenson, A. Rosowsky, R. S. McIvor, and C. R. Wagner. Resistance of murine dihydrofolate reductase to antifolates: Effects of substituting phenylalanine-31 by serine and leutine-22 by tyrosine, manuscript in preparation.

this improved clearance would also protect tumor tissues from the drug as well as GI tissues. However, the results obtained from this study clearly indicate that the chemoprotection from MTX toxicity conferred by expression of drug-resistant DHFR activity in the marrow is not due to an increased rate of MTX clearance from the circulation but rather to a true resistance of hematopoietic and GI tissues to accumulated levels of MTX. We conclude that the chemoprotective effect afforded by drug-resistant marrow transplanted into recipient animals is based at the cellular and/or molecular level in normal sensitive tissues, rather than at the level of circulating MTX. Experiments aimed at elucidating the mechanism of this chemoprotection are currently being undertaken using the DHFR transgenic model system for MTX resistance described in this study.

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