The Relative Contribution of Drug Concentration and Duration of Exposure to Mouse Bone Marrow Toxicity during Continuous Methotrexate Infusion

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SUMMARY

The effects of exposure of bone marrow to specific methotrexate (MTX) concentrations were studied by constant infusion of the drug into C57BL mice. The residual marrow nucleated cell count was determined in 168 mice at specific intervals. *In vitro* culture of colony-forming cells (CFU-C) was also performed in 69 of these mice. Duration of exposure varied from 12 to 72 hr. Plateau plasma MTX concentrations were studied in the range from $10^{-6}$ to $10^{-5}$ M. The total number of nucleated cells per femur fell to a nadir of 30% of control for all drug concentrations studied. The nadir was reached earliest with the highest drug concentrations. The percentage of CFU-C per $7.5 \times 10^4$ nucleated cells plated increased after 48-hr infusions compared to the percentage after 24-hr infusions. This increase was seen at all plasma concentrations studied. The total number of CFU-C per femur at plasma MTX concentrations above $10^{-6}$ M decreased in the first 24 hr to 40% of control, but then the number significantly increased to 66% of control between 24 and 48 hr. In contrast, no change was observed in CFU-C per femur between 24 and 48 hr during constant infusion at plasma concentrations below $10^{-6}$ M. Wright’s-stained smears showed no change in the differential count of marrow specimens at 24 and 48 hr that might account for the increased percentage of CFU-C at 48 hr. The increase in CFU-C per femur during high-dose infusions is probably the result of recruitment of CFU-C. The increased percentage of CFU-C suggests recruitment at the lower concentrations as well, but selective elimination of non-CFU-C cells cannot be excluded. Marrow [6-3H]deoxycytidine incorporation studies in vivo during exposure to $10^{-7}$ M MTX showed that the phenomenon of recruitment observed in *vitro* was initiated during the absence of DNA synthesis in vivo.

INTRODUCTION

MTX, a folic acid analog with potent antineoplastic activity, is used in a variety of clinical schedules and combination therapy regimens in man (1, 8, 11). Although the pharmacokinetics of MTX has been investigated in detail in both man (20) and animals (3), the relationship of drug concentration and duration of tissue exposure to cell kill is still poorly understood. Previous studies in which single-bolus doses of MTX (3) were used to study toxicity to normal tissues, particularly bone marrow (4, 6, 23) and intestinal mucosa (13), have shown that plasma MTX concentrations above $1 \times 10^{-6}$ M (9) are associated with inhibition of DNA synthesis in bone marrow, whereas the threshold for inhibition of intestinal mucosal DNA synthesis appears to be somewhat lower, approximately $5 \times 10^{-6}$ M. Constant infusion of MTX to achieve a plasma level of $2 \times 10^{-6}$ M revealed an initial suppression of [3H]dUdR incorporation into DNA of marrow, followed by a spontaneous recovery of [3H]dUdR incorporation despite continued exposure to the drug (25). Using the spleen colony assay for marrow colony-forming units (CFU-S), Bruce et al. demonstrated (5, 6) an initial rapid decrease of CFU-S after multiple doses of MTX with no further decline after 12-hr exposure. In none of these studies was it possible to quantify the relationship between drug concentrations, duration of exposure, and marrow cell kill.

More recently, using constant-infusion devices in mice, we obtained evidence for the following sequence of changes in bone marrow colony-forming cells during continuous exposure to $10^{-5}$ M MTX; (a) rapid depression of deoxycytidine incorporation into DNA and depletion of nucleated cells in the marrow, reaching a maximum within 24 hr; (b) recruitment of new myeloid CFU-C despite continued drug infusion and progressive decrease in nucleated cells; and (c) rapid resumption of DNA synthesis in surviving cells upon removal of the drug (15, 16).

These findings prompted further investigation of the effects of drug level and duration of exposure on marrow cytotoxicity and clonogenic capacity over a wide range of plasma MTX concentrations (from $10^{-6}$ to $2 \times 10^{-6}$ M) maintained for periods up to 72 hr. The results indicate that the degree of bone marrow toxicity is a function of both drug concentration and duration of exposure during the initial period of rapid cell kill. Eventually, there is a common plateau of residual nucleated cells for all drug concentrations tested. In addition, the time course of adaptive response in marrow is also a function of concentration and duration of exposure.