ENHANCEMENT OF METHOTREXATE CYTOTOXICITY BY URACIL ANALOGUES THAT INHIBIT DEOXYURIDINE TRIPHOSPHATE NUCLEOTIDOHYDROLASE (dUTPase) ACTIVITY

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INTRODUCTION

Impairment of DNA synthesis by lack of thymine nucleotides was long considered the critical cytotoxic event in cells exposed to methotrexate (MTX) (1). Recent work has shown, however, that the thymineless state is associated with increases in intracellular dUMP and dUTP with resulting uracil misincorporation into DNA (2-4). Subsequent unrepaired excisions of uracils by uracil-DNA-glycosylase (5,6) lead to DNA fragmentation (7-11).

We have found that these effects are more easily produced in some cell types, e.g., human lymphoblasts 8866, than in others, e.g., phytohemagglutinin (PHA)-stimulated lymphocytes (12). This accords with the known fact that cell lines differ in their sensitivity to MTX cytotoxicity (9). If uracil misincorporation into DNA is a critical event in the death of cells in these circumstances, it seemed likely that a cell's susceptibility to this state might be influenced by its levels of deoxyuridine triphosphate nucleotidohydrolase (dUTPase) (EC 3.6.1.23), which tends to exclude dUTP from DNA synthesis. In recent work we found that the levels of these enzymes do show differences in a variety of diverse cell types (12,13). We also have evidence correlating sensitivity to MTX cytotoxicity with dUTPase levels in some cell lines (14).

This paper presents evidence that certain uracil derivatives (here termed analogues), which inhibit dUTPase activity in intact cells or sonicates, may enhance MTX cytotoxicity as judged by (a) cell counts or cloning efficiency; (b) MTX-induced increases in [dUTP]/[dTTP] ratios; (c) uracil misincorporation into DNA; and (d) DNA strand breakage as assayed by the alkaline filter elution method (15) and end-labeling of new 3'-OH termini (16). Other methods used are discussed elsewhere (13,14).

RESULTS

Assays of dUTPase and Uracil-DNA-Glycosylase Levels in Various Cell Lines

An extensive survey showed wide variations in dUTPase and uracil-DNA-glycosylase levels in different cell lines (Fig.1). Since dUTPase levels...
vary in the course of the cell cycle, we regard these assay results as average levels of enzyme activity in actively growing nonsynchronized cultures. The glycosylase results with open points represent cell lines in which glycosylase was also assayed in the presence of 15 mM uracil. The results in right graph confirm that free uracil is a powerful inhibitor of glycosylase (5,6).

Seven cell lines that differ widely in their dUTPase levels were selected. These were studied further in an effort to determine whether higher dUTPase levels could be correlated with lower MTX sensitivity and vice versa.

Uracil Misincorporation into DNA: Apparent Influence of dUTPase Levels

Measurements were made of actual rates of misincorporation of uracil from [5-3H]dUrd into DNA under standard conditions. In all cell types studied, observed rates of uracil misincorporation into DNA (in pmoles per 100 pmoles DNA nucleotide) in control cells exposed to no MTX were <0.1, both in cultures lacking uracil in the medium and in ones to which 15 mM uracil had been added 24 hr before harvest. Rates in cells exposed to 10 μM MTX for 5 hr in the absence and presence of added uracil were as shown in Table 1. The data confirm that added uracil enhances recovery of misincorporated uracil in DNA by inhibiting its excision by glycosylase. They also suggest (but do not prove) an inverse correlation between MTX-induced uracil misincorporation and dUTPase level in all cell lines tested.

Additional Evidence Associating dUTPase Level with MTX Cytotoxicity

Correlation of Cell Count Effects of MTX with dUTPase Levels. Next, experiments were performed in which MTX cytotoxicity, as judged by effects of MTX on cell counts or cloning efficiency in standardized cultures, was compared with the average dUTPase level of each cell line. The results in Fig. 2 show an apparent inverse correlation between MTX cytotoxicity and dUTPase levels in all cell lines tested but two (PC-4 cells and 3T3-R500 cells), which demonstrated MTX resistance that appears unrelated to dUTPase level. The latter is known to be MTX-resistant (17).