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SHORT COMMUNICATION

Enhancement by caffeine of the growth inhibitory effects of antimetabolites in lymphoma L5178Y cells*

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Caffeine has manifold effects on cultured mammalian cells (review: Ref. 1). At relatively high concentrations it can by itself inhibit growth and induce mutation and chromosome aberrations [2, 3]. In addition, however, many studies have shown that concentrations of caffeine that inhibit neither growth nor incorporation of [^{3}H]thymidine into DNA potentiate the lethal effects of ultraviolet irradiation and of a variety of alkylating agents [4–13]. Although the precise mechanism or mechanisms by which caffeine exerts these effects are not known, the process of post-replicative repair of DNA is believed to be involved.

This paper reports observations of the enhancement by caffeine of the growth inhibitory effects of methotrexate, 6-methylmercaptopurine ribonucleoside, mycophenolic acid, hydroxyurea and 2′-deoxyadenosine in cultured murine lymphoma L5178Y cells. Methotrexate is an inhibitor of dihydrofolate reductase (EC 1.5.1.3), methylmercaptopurine ribonucleoside of amidophosphoribosyltransferase (EC 2.4.2.14), mycophenolic acid of inosinate dehydrogenase (EC 1.2.1.14), and hydroxyurea of ribonucleoside diphosphate reductase (EC 1.17.4.1), whereas enzymatic sites of action of deoxyadenosine have not been precisely defined. For the purposes of this paper, we are applying the term "antimetabolite" to hydroxyurea and deoxyadenosine as well as to the other drugs, as they all affect nucleotide metabolism. We are not aware that the enhanced cytotoxicity of combinations of caffeine and antimetabolites has previously been reported.

The lymphoma L5178Y cells used in this study were grown in stationary suspension culture in Fischer's medium supplemented with 10% horse serum (Grand Island Biological Co.). Stock cultures were maintained in logarithmic phase by dilution at least every 48 hr to a density of *ca.* 0.2×10^5 cells/ml. In the experiments reported here, cells were used at densities between 0.14 and 2.5×10^5 cells/ml, under which conditions the average generation time was 11 hr. Cell numbers were measured using a model Z_F Coulter Counter. When combinations of drugs were used, all were added simultaneously.

Deoxyadenosine, hydroxyurea, and methylmercaptopurine ribonucleoside were obtained from Sigma Chemical Co., methotrexate from Lederle Laboratories, mycophenolic acid from Dr. T. J. Franklin, and 2′-deoxycoformycin from Dr. G. A. LePage.

The results are shown in Fig. 1. In all of these experiments, cells were exposed to drugs for between 1 and 6 hr, after which the drugs were removed (i.e. the cells were collected by centrifugation and suspended in fresh medium). Growth was then measured during 38–54 hr after removal of drugs. Under these conditions, "re-growth" may depend on the extent of cell kill during the period of exposure to drug, to disturbances in the cell cycle, or to continued effects of intracellular drug that may still be present. The times of exposure to drug and the concentrations of the drugs that were used were chosen, on the basis of prior experience, to induce small to moderate degrees of growth inhibition in the absence of caffeine. These results (Fig. 1, panels A and D) and other studies have indicated that 2 mM caffeine by itself was not growth inhibitory under these conditions.

The interaction of caffeine and methotrexate (Fig. 1A) was studied both when cells were exposed to drugs for 1 hr and when a 6-hr treatment time was used. In both cases, the addition of caffeine led to an appreciably greater degree of apparent cell kill.

In studies with deoxyadenosine (Fig. 1B), an inhibitor of adenosine deaminase (EC 3.5.4.4), 2′-deoxycoformycin, was added to prevent detoxification of this compound [14, 15]. At the concentration used, the deoxycoformycin had no growth inhibitory effect of its own. Again, caffeine enhanced the growth inhibition produced by deoxyadenosine, although the growth rates of treated cells were less than control cells. In another experiment (data not shown), conditions were obtained in which there was no increase in cell number over 30 hr in the presence of deoxyadenosine. In this case, the combination of caffeine plus deoxyadenosine led to a progressive decrease in cell numbers. In one experiment, it was shown by the soft-agar cloning technique [16] that caffeine actually did increase cell killing by deoxyadenosine.

Caffeine enhanced the growth inhibitory effect of 100 μM hydroxyurea to a large extent (Fig. 1C), and this enhancement was also observed (data not shown) when higher concentrations (320 and 3200 μM) of hydroxyurea were used; these concentrations, of course, were themselves inhibitory. This enhancement of growth inhibition by combinations of hydroxyurea and caffeine was also observed when murine leukemia L1210 and human lymphoblast 6410 cells were used.

Finally, enhancement by caffeine of the growth inhibitory effects of methylmercaptopurine ribonucleoside (Fig. 1D) and of mycophenolic acid (Fig. 1E) was also observed in lymphoma L5178Y cells. In both of these cases, treated and control cells grew at approximately the same rates after recovery for 20 hr in the absence of drug.

The mechanism or mechanisms by which caffeine enhances the growth inhibitory effects of the antimetabolites are not known. Methotrexate (Refs. 17 and 18, and L. W. Brox, personal communication), 6-methylmercaptopurine ribonucleoside (R. W. Wood and J. F. Henderson, unpublished results), mycophenolic acid (J. K. Lowe and J. F. Henderson, unpublished results), hydroxyurea [19–21] and deoxyadenosine (L. W. Brox, personal communication) all either decrease intracellular concentrations of the four deoxyribonucleoside triphosphate substrates of DNA synthesis, or cause alterations in the relative concentrations of these metabolites, or both. If caffeine partially interferes with a process that requires deoxyribonucleoside triphosphates (such as DNA repair) then reduction or unbalance in their concentrations might further retard this process. This matter obviously requires further investigation.

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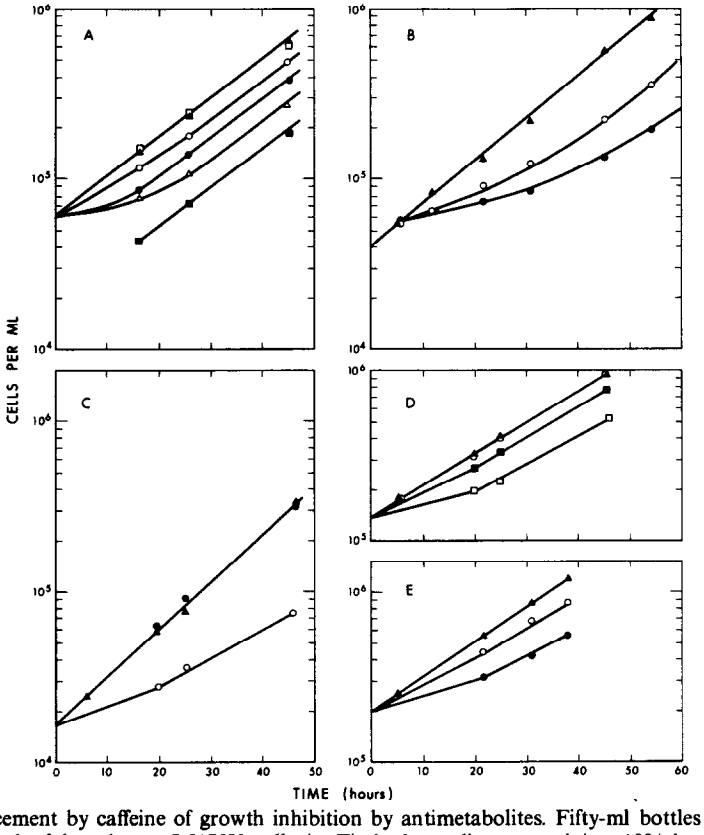

Fig. 1. Enhancement by caffeine of growth inhibition by antimetabolites. Fifty-ml bottles were inoculated with 10 ml of lymphoma L5178Y cells in Fischer's medium containing 10% horse serum at cell densities of between 0.2 and 1.2 × 10^5 cells/ml. Drugs were added and the cells were incubated for varying periods of time, after which the cells were collected by centrifugation at 800 *g* and resuspended in 20 ml of fresh warmed growth medium in the absence of drugs. The cultures were incubated at 37° and cell densities were measured for the next 38–54 hr. Results presented are averages of duplicate analyses, and are representative of those obtained in at least two experiments. (A) Methotrexate. Cells were incubated for 6 hr with no additions (▲), for 6 hr with 2 mM caffeine (□), for 1 hr with 1 μM methotrexate (○), for 1 hr with 1 μM methotrexate plus 2 mM caffeine (●), for 6 hr with 1 μM methotrexate (△), and for 6 hr with 1 μM methotrexate plus 2 mM caffeine (■). (B) Deoxyadenosine. All cultures contained 0.3 μg/ml of deoxycoformycin during the period of exposure to drug. Cells were incubated for 5 hr with no other additions (▲), for 5 hr with 50 μM deoxyadenosine (○), or with 50 μM deoxyadenosine plus 2 mM caffeine (●). (C) Hydroxyurea. Cells were incubated for 3 hr with no additions (▲), for 3 hr with 100 μM hydroxyurea (●), or for 3 hr with 100 μM hydroxyurea plus 2 mM caffeine (○). (D) Methylmercaptopurine ribonucleoside. Cells were incubated for 3 hr with no additions (▲), for 3 hr with 2 mM caffeine (○), for 3 hr with 5 μM methylmercaptopurine ribonucleoside (■), or for 3 hr with 5 μM methylmercaptopurine ribonucleoside plus 2 mM caffeine (□). (E) Mycophenolic acid. Cells were incubated for 2 hr with no additions (▲), for 2 hr with 1 μM mycophenolic acid (○), or for 2 hr with 1 μM mycophenolic acid plus 2 mM caffeine (●).

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