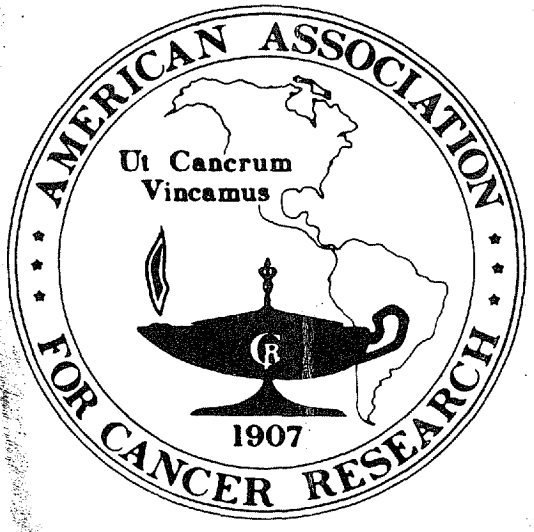
EIGHTY-SECOND

annual meeting of the
American Association
for Cancer Research

PROCEEDINGS

May 15-18, 1991
Houston Texas



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1920

Folate Analogues: Nonpolyglutamylatable inhibitors of thymidylate synthase (TS) and GAR-formyltransferase (GARFTase). Nair, M.G., Abraham, A., Kisliuk, R.L., McGuire, J.J., Galivan J. Univ. of South Alabama, Mobile, AL 36688, Tufts University, Boston, MA 02111, Roswell Park Cancer Institute, Buffalo, NY 14263, and Wadsworth Center for Laboratories and Research, Albany, NY 12201.

We have previously shown that replacement of the L-glutamate moiety of classical 4-amino antifolates such as methotrexate and 10-deazaaminopterins with a 4-methylene glutamate results in a) potent inhibition of dihydrofolate reductase, b) high level of antifolate activity during continuous exposure to tumor cells, c) enhanced ability to inhibit folinic acid transport to tumor cells relative to methotrexate and d) complete loss of substrate activity to folylpolyglutamate synthetase. These studies have now been extended to other antifolates, by replacing the glutamate moiety of TS inhibitors PDDF and DMPDDF and the GARFTase inhibitor DDATHF, with 4-methylene glutamate. The new compounds are non-polyglutamylatable and effective inhibitors of folinic acid transport to H35 hepatoma cells. However, the 4-methyleneglutamate derivatives are much weaker antifolates than their predecessors as judged by their ability to inhibit the growth of H35 hepatoma and CCRF-CEM human leukemia cell growth. The results clearly indicate that polyglutamylation is a major determinant of non-DHFR type antifolate cytotoxicity.

1921

Reversal of the toxicity but not the antitumor activity of Lometrexol by folic acid.
Grindey, G.B., Alati, T., and Shih, C. Lilly Research Labs. Indianapolis, Ind. 46285.

Lometrexol, 5,10-dideazatetrahydrofolic acid (DDATHF) has broad spectrum activity and good therapeutic index in murine and human xenograft carcinomas. However, severe toxicity was observed during initial Phase I trials (R.L. Nelson, pers. comm.). DDATHF (12.5-50 mg/kg/day X 5) is non-toxic and achieves >95% inhibition at all dose levels using C3H mammary carcinoma. In C3H mice placed on a folate-free diet for two weeks, DDATHF toxicity is increased 100-fold. Antitumor activity is markedly reduced with only 21% and 88% inhibition of tumor growth observed at maximally tolerated doses of 0.25 and 0.5 mg/kg, respectively. High doses of folic acid in the drinking water completely reverse both toxicity and activity. In contrast, low doses of folic acid (0.003%) prevent this dietary-induced toxicity and >90% inhibition of tumor growth is achieved from 6.25-50 mg/kg of DDATHF. The tight-binding of DDATHF to the folic acid transport protein may be involved in these observations. The activity and toxicity of related analogs under these conditions is under evaluation. These results support the use of low doses of oral folic acid to reduce the toxicity of DDATHF in clinical trials.

1922

Trimetrexate resistance in human colon cancer cells is associated with acute induction of dihydrofolate reductase. JL Grem, DM Boarman, P Daychild, CJ Allegra. Medicine Branch, NCI, Bethesda, MD.

The ID_{50} for TMTX (24 h exposure (exp.)) in a cloning assay was 0.44 μM in SNU-C4 (C4) cells, while HCT-116 (116) and NCI-H630 (630) cells were 37- and 200-fold less sensitive. We studied several potential resistance mechanisms. Uptake and retention of [^{14}C]TMTX were similar in all 3 lines. Differences in dTTP, ATP and GTP depletion with TMTX did not account for the variable sensitivity, nor did differences in baseline DHFR properties including catalytic act., binding capacity and binding affinity. Thymidylate synthase act. was 45% and 73% lower in 116 and 630 cells. Exp. to 1 μM TMTX for 24 h resulted in a signif. increase (1.8- to 2.2-fold) in total DHFR content in the 2 resistant lines (in binding assays and Western blot analysis). The increase in DHFR content during TMTX was evident by 6-12 h and was prevented by concomitant exp. to the protein synthesis inhibitor cycloheximide. The relevance of this phenomemon was suggested by rapid recovery of free, biologically active DHFR and [6-^{3}H]dUrd incorporation after TMTX removal in the resistant cells.

1923

An altered K_t for the reduced folate transport system confers resistance to Lometrexol ((6R)DDATHF). O.Russello, B.A.Moroson, A.R.Cashmore, A.D.Cross, G.P.Beardsley. Dept. Pediatrics, Yale Univ.,New Haven, CT 06510.
A highly DDATHF resistant human lymphoblastic leukemia cell line (CCRF-CEM/CR 14) was obtained by further exposure of a previously developed resistant cell line (CR15), to 1 mM (6R)DDATHF. The CR15 line has defective polyglutamylation, but normal levels of GAR TFase and normal reduced folate transport. The derived CR14 line can be mantained in 0.1 mM (6R)DDATHF, 5 orders of magnitude above the ED 50 for the parent cell line. The CR14 line showed the same polyglutamylation defect seen in CR15. Kinetic parameters for GAR TFase from CR14 were not different from those from the parent cell line. Study of the transport system revealed an increased K_t, but no differences in V_{max} or number of binding sites. The same alterations in affinity were found for (6S)DDATHF, methotrexate and leucovorin.(CA 50721,IST and AIRC, Italy.)

1924

High-level methotrexate resistance in a human breast cancer cell line secondary to a novel membrane translocation defect.
Pinard M-F and Jolivet J. Institut du Cancer de Montréal, 1560 Sherbrooke east, Montréal H2L 4M1.

A 1,000-fold methotrexate (MTX) resistant ZR-75-1 human breast cancer cell line with deficient polyglutamylation (J Biol Chem 259: 10793, 1984) was found to be unable to accumulate MTX during short exposures to high drug concentrations. MTX initial uptake was only mildly abnormal with a 2.3-fold increased K_t in the resistant compared to the wild type cells and no V_{max} alterations. At MTX concentrations ≥10μM, initial uptake kinetics became identical and the cytotoxicity curves superimposable in both sensitive and resistant cells. The latter still could not accumulate or transstimulate MTX however, suggesting abnormal translocation of the reduced folate/MTX membrane carrier across the cell membrane and likely explaining the almost absent polyglutamate formation. Impaired MTX translocation can thus be responsible for high levels of drug resistance.

1925

In vivo antitumor activity and metabolism of a series of open chain folate based GAR transformylase inhibitors.
Mullin, R.J., Bigham, E.C., Duch, D.S., Perone, R., Keith, B.R., Smith, G.K., and Waters, K.A. Wellcome Research Laboratories 3030 Cornwallis Rd., R.T.P., NC 27709

The activity of 5-deazaacylotetrahydrofolate (5-DACTHF) an inhibitor of purine de novo biosynthesis and potential antitumor agent has been reported. This study compares the properties of 5-DACTHF and a series of analogs, 2′-F, 3′-F, 10-S and 10-CH_2. All analogs have similar IC_{50} values for inhibition of MCF-7 cell growth, GAR transformylase, and methotrexate uptake by Molt-4 cells, a measure of cellular uptake potential. Only 5-DACTHF, 2′-F, and 3′-F demonstrate significant inhibition of colon 38 adenocarcinoma growth in vivo. This correlates with the Km of these compounds for folylpolyglutamate synthetase. In support of this correlation, 24 hours after dosing, only those compounds with antitumor activity were detectable in tumor tissue, and they were present nearly exclusively as the polyglutamated species despite similar blood levels for all compounds. These results indicate that, following uptake, polyglutamation represents a critical step in the in vivo anti tumor activity of these compounds.