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2421

Biological properties of (6S)-, (6R)-, and (6R,S)-leucovorin. McGuire, J.J., Russell, C.A., & Heitzman, K.J. Grace Cancer Drug Center, Roswell Park Cancer Institute, Buffalo, NY 14263

(6R,S)-leucovorin (LV) is used to selectively "rescue" the toxic effects of methotrexate (MTX) and to biochemically modulate the anti-tumor effects of fluoropyrimidines (FPs). (6S)-LV is biologically active, while (6R)-LV is assumed to be inert. We compared the biological properties of pure preparations of these isomers, with particular emphasis on their ability to form poly(γ-glutamyl) metabolites. (6S)-LV was ≥4000-fold more able than (6R)-LV to protect CCRF-CEM cells from the growth inhibitory effects of MTX; (6S)-LV modulated the growth inhibitory activity of FPs, while at an equivalent levels (6R)-LV did not. (6S)- and (6R,S)-LV were substrates for human and rat folylpolyglutamate synthetase (FPGS); (6R)-LV was essentially inactive both as a substrate and inhibitor of these FPGS. Lack of conversion to polyglutamates may thus contribute to the diminished biological activity of (6R)-LV. After nonenzymatic conversion of LV preparations to their respective 10-formyltetrahydrofolate forms, all forms were FPGS substrates; the V/K_m of the natural isomer was only 2-fold higher than that of the unnatural isomer. Stereospecificity of FPGS at C-6 of reduced folates is thus profoundly dependent on the type of 1-carbon substituent. Supported by CA43500 and CA16056.

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Improved clinical tolerance of lometrexol with oral folic acid. Young, C.W., Currie, V.E., Muindi, J.F., Saltz, L.B., Pisters, K.M.W., Esposito, A.J., and Dyke, R.W.*, Memorial Sloan-Kettering Cancer Center, New York, NY 10021, and *Eli Lilly and Co., Indianapolis, Ind. 46285

Lometrexol (LTX), an inhibitor of GAR transformylase that binds tightly to cellular folate binding protein (FBP), produced severe cumulative toxicity in its initial clinical trial (Proc. ASCO 9:76, 1990). In mice folate depletion increases the toxicity of LTX 100-fold; the therapeutic index is restored by oral folic acid supplementation (Proc AACR 32:324, 1991). Nineteen of 53 patients in our i.v. dose-finding trial received LTX in combination with oral folic acid. Dose-limiting toxicities were thrombocytopenia and anemia; mucositis and diarrhea were also observed. Without folic acid, the MTD of LTX was 2.7 mg/m2 twice weekly x 4 doses, every 28 to 35 days; cumulative toxicity was observed. With oral folic acid at 1 mg/day continuously, the recommended Phase II dose is 4 to 5 mg/m2 twice weekly x 4 doses at 28 day intervals; cumulative toxicity is absent. A patient with oropharyngeal cancer has had a Complete Response of 18 months duration; lesser antitumor effects were seen in 8 additional patients. Concomitant oral folic acid enhances tolerance to the toxic effects of lometrexol without eliminating therapeutic response to the drug. This effect could be mediated by a decrease in enterohepatic reabsorption of LTX following its biliary secretion.

2423

Development of a fluorescence HPLC method for analysis of lometrexol in biologic samples. Muindi, J.F., Currie, V.E., Shih, C.* and Young, C.W. Memorial Sloan-Kettering Cancer Center, New York, NY 10021, and *Lilly Research Laboratories, Indianapolis, Ind. 46285

Lometrexol, 5,10-dideazatetrahydrofolate (LTX), an inhibitor of GAR transformylase that binds tightly to folate binding proteins (FBP), is undergoing clinical trial because of its broad activity against transplanted tumors in mice. We are developing a sensitive and specific HPLC method for quantitation of LTX and possible metabolites in biologic fluids. Oxidized 10-deaza-pteridines are intensely fluorescent but N-10 pteridines and reduced 10-deaza-pteridines have negligible fluorescence. LTX in plasma or urine is freed from FBP by incubation in ammonium formate pH 3, then extracted by perchlorate precipitation. The protein-free extract is oxidized by exposure to manganese dioxide at 90°C for 10 minutes then chromatographed on a C18 reverse phase column using fluorescence detection with excitation at 330nm and emission at 425nm. In human serum and urine the assay is linear between 0 and 1000 nM; the lower limit of quantitation is 5 nM. The within day and between day coefficients of variation have been < 10% and < 15% respectively. Study of patients treated on Days 1, 4, 8, and 11 has confirmed drug accumulation and slowed clearance rates between Day 1 and Day 11.

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ICI D1694 resistant cell lines. A.L. Jackman, L.R. Kelland, M. Brown, W. Gibson, R. Kimbell, W. Aherne+ and I.R. Judson. The Institute of Cancer Research, Sutton, U.K. +Div.Biomedical Res. University of Surrey, Guildford, U.K.

Three cell lines, the mouse L1210 leukemia and the human CH1 and 41M ovarian lines (IC_{50} = 10, 25 and 13nM), were made resistant (step-wise increments 10-5000nM) to the thymidylate synthase (TS) inhibitor, ICI D1694. TS activity was raised significantly in the CH1:R line (3.5-fold). An ELISA method confirmed a raised level of the enzyme protein (~9-fold). After exposure to 0.1μM ^{3}H ICI D1694 (glu_1) for 24hrs, CH1:R cells accumulated normal levels of ^{3}H (~3μM) but whereas in the parental cells the polyglutamate derivatives, glu_{3-5}, predominated (80%) in the CH1:R cells glu_{2-3} were the major fractions (70%). The Ki values for glu_1 and glu_3 were unchanged for TS extracted from this line (82nM and 1.2nM respectively). Cross-resistance to FdUrd (25-fold) suggests that the small elevation in TS level may be a major mechanism of ICI D1694 resistance in this line and the change in polyglutamate profile may augment this (glu_2 is ~3-fold less active and glu_4 and glu_5 ~2-fold more active than glu_3 against TS). In the 41M:R cells decreased uptake via the reduced-folate carrier is the mechanism of resistance to ICI D1694 and although polyglutamation is reduced as a result of decreased uptake the pattern of polyglutamates is not significantly changed (predominantly glu_{3-5}). The L1210:R line has a small decrease in the initial velocity for uptake of ICI D1694 but its major mechanism of resistance is its failure to polyglutamate ICI D1694. Exposure of parental and L1210:R cells for 48hrs to 1μM ICI D1694 resulted in ~0.35μM glu1 in both cell lines but only the parental cells formed polyglutamates (~11μM). Cross-resistance studies with antifolates of defined mode of action confirm the proposed mechanisms of resistance for these cell lines.

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Polyglutamation of the thymidylate synthase (TS) inhibitor, ICI D1694. A.L. Jackman, W. Gibson, T.C. Stephens* and F.T. Boyle*. Inst. Cancer Res. Sutton, UK. *ICI Pharmaceuticals, Alderley Park, Macclesfield, Cheshire, UK.

ICI D1694 is a quinazoline antifolate that acts via inhibition of TS and is in clinical study. The potent activity of the drug against L1210 cells (IC_{50}=8nM) is due to the good cellular uptake via the reduced-folate carrier (RFC) and rapid intracellular polyglutamation (Jackman et al. Cancer Res. 51:5579-5586, 1991). Polyglutamation of ICI D1694 has been measured in the human cell lines, W1L2 lymphoblastoid (IC_{50}=6nM) and MCF-7 breast (IC_{50}=0.7nM). Cells were exposed to 100nM ^{3}H ICI D1694 (glu_1) and all the cellular ^{3}H was found as glu_{1-6}. After 30mins ~1μM ^{3}H drug equivalents were found intracellularly with 1% and 40% glu_1 respectively (glu_5 and glu_2 being the respective major metabolites). At 4hrs <10% of the cellular ^{3}H was glu_1 in both cell lines (W1L2 = glu_2~3%, glu_3~18%, glu_4~22%, glu_5~52%, glu_6~4%) (MCF-7 = glu_2~9%, glu_3~40%, glu_4~20%, glu_5~22%). By 24 hrs there was ~10μM ^{3}H intracellularly and glu_5 predominated in both cell lines (W1L2 ~60% and MCF-7 ~55%). W1L2 cells incubated for 4 hrs followed by 24hrs in drug-free medium did not lose a significant ammount of ^{3}H. Thus polyglutamation serves as a mechanism for drug accumulation and retention and in view of the potent TS inhibitory activity of the polyglutamates (glu_1 Ki=60nM; glu_4= 1nM), we conclude that ICI D1694 acts by metabolism to polyglutamates. Supporting this is the poorer growth inhibition of two analogues (7-CH_3 and 2-NH_2) of ICI D1694 (~150- and 50-fold respectively) despite being 6 and 1.5-fold better TS inhibitors. Studies indicate that the 7-CH_3 compound cannot be polyglutamated and that the 2-NH_2 compound is polyglutamated less readily due to poor cellular uptake, a result of its failure to use the RFC.

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Phase I trial of ICI D1694: a novel thymidylate synthase inhibitor. Clarke S.[1], Ward J.[1], Planting A.[2], Spiers J.[3], Smith R.[3], Verweij J.[2] and Judson I.[1] 1. Inst of Cancer Res, Sutton, Surrey, UK. 2. Rotterdam Cancer Inst, Rotterdam, Holland. 3. ICI Pharmaceuticals, Macclesfield, Cheshire, UK.

ICI D1694 is a water soluble quinazoline based thymidylate synthase (TS) inhibitor. Its predecessor, CB3717 showed anti-tumour activity, but further development was halted because of liver and kidney toxicities due to poor water solubility especially at acid pH. The phase I study of ICI D1694 began on 27/2/91 at the Royal Marsden Hospital (RMH) using a constant I.v infusion over 15 min, q 3/52. Patients have been treated at doses of 0.1, 0.2, 0.4, 0.6, 1.0, 1.6, 2.6 and 3.5mg/m^2. 27 pts (RMH 23, Rotterdam 4; 19M, 8F; mean age 57; ECOG PS 0:11, 1:13, 2:3) have received 57 courses (median 2, range 1-6). Tumour types include colon 9, sarcoma 4, head and neck 4, mesothelioma 3, breast 2, ovary 1, glioma 1, bile duct 1, carcinoid 1 and stomach 1. Mucositis has been seen in 4 pts (WHO grade 1:2, 2:2), at doses 0.4, 0.6, 1.0 and 1.6mg/m^2. One patient had a fall in EDTA clearance of 25% after one dose of 0.4mg/m^2 which persisted after stopping treatment at a time of generalised disease progression. No subsequent pt has had a fall in renal function. One pt developed grade 2 anaemia and leucopenia 1wk after a 2nd dose at 2.6mg/m^2. 2 pts (at 1.6 and 2.6mg/m^2) have developed asymptomatic abnormalities of liver function which require further investigation, both have progressive disease. Pharmacokinetic studies show a linear dose/auc relationship and clearance conforms to a 3 compartment model. No responses have yet been observed in 24 evaluable pts. The MTD has not yet been reached.

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