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THE RELATIONSHIP BETWEEN DIHYDROOROTIC ACID DEHYDROGENASE AND IN VITRO AND IN VIVO CYTOSTATIC EFFECTS OF BREQUINAR (DUP-785; NSC 368390).

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DUP-785 (Brequinar sodium) is a novel potent inhibitor of the pyrimidine de novo enzyme dihydroorotic acid dehydrogenase (DHO-DH). We studied the relationship between DHO-DH activity and in vitro growth-inhibitory effects and in vivo antitumor effects of DUP-785. Seven cell lines from different histological origin were continuously exposed to DUP-785 for 48 hr. The human squamous carcinoma cell line 14C was most sensitive (IC50 0.19 μM), three other cell lines had a comparable IC50 of about 0.4 μM, while rat hepatoma H35 and murine leukemia L1210 had a IC50 of 2.6 and 5.9 μM, resp. DHO-DH activity varied between 15 and 20 nmol/hr.10^6 cells in the cell lines tested. Inhibition by 1.3 μM DUP-785 was 80-90% in the most sensitive lines, compared to 50% in H35 and 30% in L1210. The sensitivity of two murine colon tumors was investigated in vivo by injection of 50 mg/kg at day 0, 4, 8 and 12. The doubling time of Colon 26 for untreated and treated tumors was 2.7 and 2.8 days, resp, while for Colon 38 these values were 5.1 and 8.3 days, resp. DHO-DH activity was 230 and 167 nmol/hr per mg protein, resp. In both tumors 50 mg DUP-785/kg decreased DHO-DH activity to below 10% after 4 hr, which was partly recovered after 1 day. After 1 day the concentration of uridine nucleotides in Colon 26 decreased by 30% and in Colon 38 by 50%, followed by an increase of 130 and 180%, resp, after 4 days. In conclusion; in vitro the extent of inhibition of DUP-785 was related to the growth-inhibition, but in vivo this relation was less evident.

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FLUOROPYRIMIDINE METABOLISM IN HUMAN HEAD AND NECK CANCER XENOGRAFTS AND MURINE COLON TUMORS.

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Human head and neck xenografts (HNX) tumor lines represent an unique model to study the action of anticancer drugs. 1/4 HNX lines resistant to 5-fluorouracil (5FU) was sensitive to its analog 5'deoxy-5-fluorouridine (5'dFUR). To explain these differences we studied metabolism of 5FU and 5'dFUR in 4 HNX lines (DU, KE, E, G) and for comparison also in two murine colon carcinoma lines (Colon 26 and 38). Initial conversion of 5'dFUR to 5FU catalyzed by pyrimidine nucleoside phosphorylase (PNP), was highest in Colon 26, 15-20 times lower in DU, KE and Colon 38 and intermediate in both other tumors. The Km for 5'dFUR in all tumors was 1-2 mM. PNP also catalyzes further anabolism of 5FU to fluorouridine (FUR) or 2'-deoxyfluorouridine (FUdR); the same pattern of activity was found as with 5'dFUR as substrate. In all HNX tumors 5FU conversion to FUdR was 5-10 fold than of 5FU to FUR; in the colon tumors this was 3 fold. The conversion of 5FU to the active nucleotides FUMP and FdUMP (via FUR and FUdR, resp) was measured by addition of ATP to the PNP assay; FUMP synthesis showed the following pattern: Colon 26 >> DU > G > E > KE >> Colon 38, and FdUMP synthesis: Colon 26 > DU = KE > E > G = Colon 38. For the direct conversion of 5FU to FUMP the following pattern was observed: Colon 26 $\geq$ Colon 38 > KE > E = DU = G. Colon 26, 38 and KE were sensitive to 5'dFUR. Conclusion: the anabolism of 5'dFUR to 5FU and subsequently to nucleotides (via 5FU-> FUMP) may be related to the differential sensitivity of the tumors.

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IN VITRO AND IN VIVO INHIBITION OF THYMIDYLATE SYNTHASE OF HUMAN COLON CANCER BY 5-FLUOROURACIL.

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Thymidylate synthase (TS) is a key enzyme in the synthesis of dTTP and a target for 5-fluorouracil (5FU), used for the treatment of colorectal cancer. The metabolite FdUMP inhibits TS by formation of a ternary complex with TS and 5,10-methylene tetrahydrofolate (CH_2THF). Its extent of formation and stability determine the effect of 5FU. To establish a relation with antitumor activity of 5FU we measured in vitro TS activity and binding of FdUMP to TS in biopsies (immediately frozen in liquid nitrogen) of primary colon tumors and healthy mucosa from 7 patients. At optimal CH_2THF concentration and 10 μM dUMP TS activity was 3-6 fold higher than at 1 μM, both in tumors and mucosa. In mucosa TS activity at 10 μM dUMP was 44-107 pmol/hr per mg protein; in tumors from the same patient TS activity was always higher, but varied considerably between 52 and 3000, which is possibly related to tumor heterogeneity. 10 nM FdUMP inhibited TS activity 70-90%. The number of FdUMP binding sites at optimal CH_2THF concentrations was 0.1-0.3 pmol FdUMP/mg protein in tumors and <0.1 in mucosa, but always 2-3 fold higher in the tumor. In vivo FdUMP binding was determined in tumor biopsies of patients obtained 1.5-3 hr after treatment with 500 mg 5FU/m^2. FdUMP binding to the ternary complex was still complete; all binding sites were occupied by FdUMP. Currently the FdUMP binding in samples obtained at later time-points is being measured. In conclusion; TS activity was higher in tumors; inhibition by FdUMP was not only observed in vitro but also in vivo.

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SENSITIVITY TO PURINE ANTAGONISTS IN CHILDHOOD LEUKEMIA ASSESSED BY THE AUTOMATED MTT-ASSAY.

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We showed that in childhood common acute lymphoblastic leukemia (c-ALL), 5'nucleotidase (5'NT) positive cases have a poorer prognosis than 5'NT negative cases. This might be due to the breakdown of the cytotoxic nucleotides of 6-mercaptopurine (6-MP) by high cytoplasmic 5'NT activities. Alternatively, ecto 5'NT can provide purine requirements of the purine salvage pathway and therefore rescue cells from a blockage of purine de novo synthesis by 6-MP and/or methotrexate. To test these hypotheses we need to determine the relation between these enzymes and drug sensitivity. We have adapted the MTT assay, which has only been reported on in studies dealing with established cell lines, to assess the chemosensitivity of cells obtained directly from patients. The assay is based on the reduction of MTT to formazan by living but not by dead cells. Formazan production is quantitated automatically with a microplate spectrophotometer. Incubation of ALL cells with 6-MP (4-125 µg/ml) and 6-thioguanine (1.6-50 µg/ml) for 2-4 days resulted in dose-response curves covering the range from 0% to 100% cell survival. Comparison of the MTT assay with a dye exclusion assay in 10 patients with ALL demonstrated an identical success rate and comparable dose-response curves for both assays. Because automated quantitation of the chemosensitivity of leukemic cell samples involving about 80 drug concentrations takes only a few minutes with the MTT assay, this assay is a rapid, efficient, and objective method of measuring sensitivity to purine antagonists in ALL patients.

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THE INOSINIC BRANCH POINT AND ITS HORMONAL REGULATION. EVALUATION THROUGH A MATHEMATICAL MODEL.

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We present a simplified model of purine de novo synthesis consisting in: (1) the administration of [^{14}C]formate, (2) the determination in the nucleotides, of both concentrations and specific activity, (3) the calculation of the apparent rate constants (k_1-k_2-k_3) for overall reactions involved in IMP, AMP and GMP formation, through a system of differential equations. We have evaluated the channeling of IMP into either GMP or AMP ("the inosinic branch point") in vivo. The extraction of acid-soluble nucleotides, hydrolysis, purification of purine bases, determination of quantity and specific radioactivity after [^{14}C]formate administration, was carried out according to Pizzichini et al., 1985 (1). In the liver, kidney and other organs, of normal, adrenalectomized, castrated rats, we found (1) a different pattern for each type of tissue, (2) a preferential channeling of IMP into AMP under normal conditions, with remarkable variations in the individual situations. We were able to conclude that this critical metabolic point is under hormonal control.

(1) Pizzichini M., Di Stefano A., Marinello E. (1985) It.J. Bioch. 34(5), 305-312.

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PURINE DE NOVO SYNTHESIS AND INOSINIC BRANCH POINT IN VIVO IN DIFFERENT TISSUES. A BIOMATHEMATICAL MODEL.

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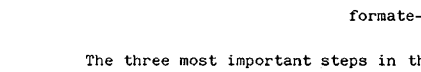
We have elaborated a biomathematical model of purine de novo synthesis in vivo, which is visualized as a series of monomolecular reactions, leading from formate to IMP, from IMP either to AMP or to GMP:



The three most important steps in this sequence are evaluated in terms of apparent rate constants (k_1-k_2-k_3) through a system of differential equations, which includes the concentration and the specific radioactivity of the nucleotides after [^{14}C]formate administration. Thus, we were able to follow (1) the rate of purine de novo synthesis in vivo in different rat organs (liver, kidney, spleen, heart), levator ani muscle, gastrocnemius and extensor digitorum longus, (2) the channeling of IMP into GMP or AMP (the "inosinic branch point"). Formation of IMP was most rapid in spleen, very fast in kidney, liver, heart, and lower in the muscles. The conversion of IMP into AMP was preferential in all tissues, an unexpectedly high rate of reaction IMP --> GMP was evident in the heart.