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5'-METHYLTHIOADENOSINE (MTA) PHOSPHORYLASE FROM *LEISHMANIA DONOVANI*. George W. Koszalka and Thomas A. Krenitsky. Burroughs Wellcome Co., Experimental Therapy Department, Research Triangle Park, NC, USA

A nucleoside phosphorylase specific for 6-amino purine nucleosides has been purified from extracts of promastigotes of the pathogenic protozoan *Leishmania donovani*. Unlike the three nucleoside hydrolases previously isolated from this organism (Koszalka and Krenitsky, *JBC*, 254, 8185-8193, 1979), this enzyme was capable of nucleoside synthesis from pentose-1-phosphate and adenine or selected analogues. The purified enzyme had a pI value of 6.2, a molecular weight of 86,000 daltons, and a pH optimum for adenosine phosphorolysis between 5.6 and 6.5. The energy of activation for the synthetic direction with adenine and Rib-1-P was 8600 cal/mole, while that for the phosphorolytic direction was 11,300 cal/mole.

Substrates for the phosphorolytic direction in decreasing order of substrate efficiency (Rel  $V_{max}/K_m$ ) were: 5'-methylthioadenosine (26), 2'-deoxyadenosine (12.5), 5'-deoxyadenosine (11.8) and adenosine (0.7). The  $K_m$  values ranged from 1-46  $\mu$ M. Adenine arabinoside, inosine, 2'-deoxyinosine, guanosine, and 2'-deoxyguanosine were neither substrates nor potent inhibitors. Adenine and various 2-substituted adenine analogues were substrates with Rib-1-P, whereas 4-amino-pyrazolo(3,4-d)pyrimidine and the 3-deaza-, 7-deaza-, and 8-aza-adenine analogues were all non-substrate competitive inhibitors. This enzyme catalyzed nucleoside synthesis from adenine and Rib-1-P, 2'-dRib-1-P or 5'-methylthio-Rib-1-P. A marked difference between this protozoal MTA phosphorylase and the mammalian counterpart is its ability to synthesize and cleave 2'-deoxyadenosine.

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HEMATOLOGIC TOXICITY PRODUCED BY PARENTERAL 6-THIOGUANINE IN MAN IS DOSE AND SCHEDULE DEPENDENT AND HIGHLY VARIABLE IN SEVERITY. John S. Kovach, Theresa C. Hu, and Joseph Rubin, Mayo Clinic and Foundation, Department of Oncology, Rochester, MN, USA

Although plasma concentrations of 6-thioguanine after oral administration in man have been shown to be highly variable, 6-TG has been used almost exclusively orally in therapy of human cancers. We carried out the first Phase I trial of 6-TG given intravenously daily for 5 days in 40 patients with advanced solid tumors and normal bone marrow function. 6-TG was given by rapid IV infusion in increments ranging from a daily dose of 15 to 65 mg/m<sup>2</sup>. Dose limiting leukopenia associated with thrombocytopenia at times occurred at daily doses of 55-65 mg/m<sup>2</sup>. Several patients at these doses had no toxicity whatsoever and tolerated multiple courses of the drug without evidence of cumulative toxicity. Plasma and red cell concentrations of parent compound were similar in all individuals. No metabolites were measurable by hplc. Plasma elimination best fit a one-compartment model. The terminal half-life ( $t_{1/2}$ ) was 20 minutes on day 1 and on day 5. We (unpublished data) and Konits et al (*Cancer Chemo Pharmacol* 8:199, 1982) previously demonstrated that high doses of 6-TG (700 mg/m<sup>2</sup>) given IV on a single day do not consistently produce hematologic toxicity, although  $t_{1/2}$  is more than 10 times greater than after the doses used in this study. Preliminary statistical analyses suggest an effect of dose, prior treatment, and possibly sex on the extent of hematologic toxicity. Supported by NCI contract CM27548.

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A LYMPHOHEMATOPOIETIC STEM CELL MODEL FOR ADENOSINE DEAMINASE (ADA) DEFICIENCY. Joanne Kurtzberg, Michael L. Greenberg, Sara Chaffee, and Michael S. Hershey. Duke University Medical Center, Departments of Medicine and Pediatrics, Durham, NC, USA.

In two reported instances treatment with the ADA inhibitor deoxycoformycin (dCF) has resulted in abrupt conversion from a T lymphoblastoid to a promyelocytic phenotype in patients presumed to have acute stem cell leukemia. We have established the DU.528 cell line from leukemic cells of one such patient, in whom we had documented biochemical consequences of ADA inhibition (dATP and S-adenosylhomocysteine accumulation) during dCF treatment (Hershey et al, *PNAS* 81:253, 1984). In culture and as a xenograft in nude mice, DU.528 displays the ability to undergo differentiation into lymphoid, myeloid, erythroid and megakaryocytic lineages, properties of a pluripotent stem cell. In various experiments dCF + 2-20  $\mu$ M Ado and dAdo induce 20-80% of T lymphoblastoid DU.528 cells to acquire myeloid surface antigens and functional characteristics, and 20-60% to synthesize hemoglobin. Treatment of nude mice bearing DU.528 xenografts with dCF induces T-lymphoblastoid to promyelocytic conversion, similar to the transformation observed *in vivo*. We hypothesize that the pluripotent lymphohematopoietic stem cell may be a target for effects of ADA substrates in genetic ADA deficiency, and that interference with lymphoid differentiation rather than lymphocyte viability may be responsible for the selective absence of lymphoid lineages in ADA deficiency. The DU.528 cell line provides a unique model for examining the biochemical basis for effects of purines on lymphohematopoietic differentiation.

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RENAL HANDLING OF URIC ACID UNDER METHYLCLOTHIAZIDE ALONE OR ASSOCIATED WITH TRIAMTERENE

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The influence of 2 diuretics (D) on renal handling of uric acid (UA) was evaluated. 8 normal male volunteers (age : 32.5  $\pm$  4.6, BW : 68  $\pm$  8 kg, H : 175  $\pm$  6 cm) received in random order placebo (P), methyclothiazide (MCT, 2 mg) alone or with triamterene (75 mg, MCT + T) for 4 days. Urine (u) was collected every 12 hours and blood (b) was drawn at days 1 and 5, for measurement of : b UA (micromol/l), excreted (u UA) and filtered (f UA) UA (micromol/min) and fractional clearance (f Cl UA). D increased (p < .05) b UA and f UA (40.78 for P, 56.92 for MCT, 50.06 for MCT + T) ; u UA varied not significantly (ns) and nycthemeral variation in u UA is maintained. In P, u UA = 1.88 + 0.04 f UA, r = .60, p < .001, the same correlation is observed with MCT + T, but u UA is too low for f UA in MCT. In P, f Cl UA (.099 + .027) is negatively correlated with b UA (312  $\pm$  48) = -.007 + 30.27/b UA, r = .69, p < .001 ; in MCT + T f Cl UA is ns decreased (.084 + .017) but normally correlated with b UA (366 + 61) ; in MCT f Cl UA is further decreased (.074 + .021, p < .01 vs P) despite similar increases in b UA (394  $\pm$  55), hematocrit, proteins, renin activity, aldosteronemia and higher cumulative sodium excretion in MCT + T than in MCT.

Conclusion : There is no tendency for MCT + T to lessen MCT induced changes in renal handling of UA, together with higher natriuretic activity.

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6-THIOPURINE RIBOSIDE ANALOGS: THEIR TOXICITY AND METABOLISM IN *LEISHMANIA DONOVANI* AND MAMMALIAN CELLS. Stephen W. LaFon, Naomi K. Cohn,

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The growth of *Leishmania donovani*, mouse L cells and Detroit 98 cells were differentially inhibited by 6-thiopurine riboside analogs. The analogs studied were 4-thiopurinol riboside, 7-thioformycin, 6-thio-9-deazapurine riboside, 6-methylthio-9-deazapurine riboside and 6-thio-8-azapurine riboside as well as 6-thiopurine riboside itself. These nucleosides were selectively metabolized by the three cell lines to their respective 5'-monophosphates (5'-MP) attaining cellular concentrations ranging from 6 to 103 pmol/10<sup>6</sup> cells for the parasite and 0 to 2700 pmol/10<sup>6</sup> cells for the mammalian cells. There were no 5'-di- or 5'-triphosphate metabolites formed from these nucleosides. Only 6-thiopurine riboside itself was susceptible to cleavage of its glycosyl bond during the incubations. The sulfhydryl moieties of the thiopurine riboside analogs also were resistant to oxidation, methylation and cleavage during the incubations. In general, the toxicity of these compounds to *L. donovani*, L cells and Detroit 98 cells correlates well with the levels of 5'-MP formed from the respective nucleoside.

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STUDIES OF PURINE BIOSYNTHESIS IN CHINESE HAMSTER CELL MUTANTS DEFICIENT IN ADENYLOSUCCINASE. Paul K. Laikind, Harry E.

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Purine biosynthesis and growth rate studies were carried out on a mutant of Chinese Hamster Ovary Cells (CHO-Ade I) deficient in the adenylylase biosynthetic enzyme Adenylosuccinase (ASase). The ratio of the rate of adenylylase to guanylate synthesis are altered in CHO-Ade I (3:88) compared to the nearly equal distribution of these nucleotides (49:51) in the wild type parent line CHO-K1. The decrease in the ratio of adenylylase to guanylates results from an apparent stimulation in the rate of guanylate synthesis in addition to the expected reduced rate of adenylylase synthesis. In comparison to the wild type CHO cells the ASase deficient cells show an increased accumulation of hypoxanthine-containing compounds in both the cell and media (excreted) fractions. CHO-Ade I cells display a marked elevation in the activity of the branch point enzyme adenylosuccinate synthetase suggesting a direct regulation of this enzyme by either adenylosuccinate or the ultimate product adenylylates. Similar to patients with a deficiency of this enzyme SAICA riboside and succinyladenosine are excreted into the medium by CHO-Ade I cells.