35 HYPERURICEMIA IN CYCLOSPORINE (CsA) TREATED RENAL ALLOGRAFT RECIPIENTS: EVIDENCE FOR UNDEREXCRETION OF URATE.

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The hyperuricemia associated with CsA therapy was studied in 400 renal transplant recipients receiving either prednisone-cyclosporine (PC, n=122) or prednisone-azathioprine (PA, n=176). When the serum creatinine was less than 2 mg%, hyperuricemia was more common in the PC group (65.8%) than in the PA group (23.3%, $p < .005$) and the mean serum urate was greater in the PC than the PA group (8.24 ± 2.43 mg% vs. 6.35 ± 1.61 mg%, $p \leq .005$). Serum urate was greater in the PC than in the PA group at all levels of renal function (p=.032). Seventy-nine percent of PC patients treated with diuretic had elevated serum urate compared to 48% in those not receiving diuretic ($p \leq 0.01$), the respective values in the PA group were 37% vs. 11% ($p \leq 0.005$). Twelve individuals (PC n=6, PA n=2, normal volunteers n=4) had extended studies of urate metabolism. PC patients had large urate pools (493-998 $mg/m^2/d$) and a normal rate of turnover (275-410 $mg/m^2/d$). The urate clearance in PC patients ranged between 2.00 and 3.96 ml/min while it was between 4.83 and 8.23 ml/min in the others. Enzyme activity of HGPRT and APRT were normal in all individuals. We conclude that hyperuricemia in CsA treated renal allograft recipients is due to decreased renal urate excretion related to the drug and exacerbated by concommitant diuretic use.

36 DEOXYNUCLEOTIDE METABOLISM IN CULTURED CELLS EXPOSED TO 3'-DEOXY-3'-AZIDOTHYMIDINE

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3'-azido-3'-deoxythymidine (AZT) affected deoxynucleotide metabolism in the human cell lines, HL-60, H-9, K-562, and RPMI-8402. The respective ED 50 values of AZT for growth were 670, 110, 100, and 20 µM. When cells were exposed to 200 µM AZT, dTTP and dGTP decreased and dCTP increased. The increase in dCTP is in contrast to previous reports [Furman, et al., Proc. Nat. Acad. Sci. USA (1986) 83, 8333-8337]. Recovery of the dNTP pools was generally seen at 8 to 24 hrs. Pools of dCTP were the last to recover. Changes in the dNTP pools induced by AZT are consistant with a decline in dTTP affecting the allosteric regulation of ribonucleotide reductase.

After 24 hrs in 200 µM AZT, AZT-5'-monophosphate reached 2800, 4700, 15700, and 12800 µM in the HL-60, H-9, K-562 and RPMI-8402 cells, respectively. The corresponding AZT-5'-triphosphate levels were 25, 15, 60, and 45 µM. Thymidine and deoxyuridine in the media increased after treatment of the cells with AZT. All cell lines treated with AZT secreted AZT-5'-monophosphate.

Radiolabeled thymidine was used to study thymidine anabolism in cells treated with 20 µM AZT for 4 hrs. Pools of dTMP increased 3, 2, 4, and 7 fold in the respective cell lines. These data support the idea that thymidylate kinase is inhibited by AZT-5'-monophosphate (Furman et al., ibid) and suggest that cells in culture are able to overcome the effects of AZT on deoxynucleotide metabolism by expansion of their dTMP pool.

37 RENAL HANDLING OF HYPOXANTHINE AND XANTHINE IN NORMAL SUBJECTS AND IN CASES OF IDIOPATHIC RENAL HYPOURICEMIA

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Pyrazinamide(PZA) was used to study the renal handling of hypoxanthine, xanthine and uric acid in two normal subjects and in four patients with idiopathic renal hypouricemia. In normal subjects the suppression rate by PZA in the excretion of uric acid, hypoxanthine or xanthine is different for each compound. In a patient with postsecretory reabsorption defect, the ratio of uric acid clearance to creatinine clearance was reduced from 45.8 to 0.6% by PZA, the ratio of hypoxanthine clearance to creatinine clearance from 31.9 to 16.6% by PZA, and the urinary excretion of xanthine from 10.0 to 3.0 ug/min. PZA suppressed all the excretion of the three compounds. This effect is similar to in normal subjects. In three patients with presecretory reabsorption defect, PZA showed almost no suppressive effect on the ratio of uric acid clearance to creatinine clearance, the ratio of hypoxanthine clearance to creatinine clearance and the urinary excretion of xanthine. This similar suppressive or unchanged effect of PZA in response to both normal and morbid conditions suggests that the renal handling of hypoxanthine and xanthine, especially xanthine, is similar to that of uric acid.

38 LESCH-NYHAN SYNDROME DUE TO A SINGLE NUCLEOTIDE CHANGE IN THE HYPOXANTHINE-GUANINE PHOSPHORIBOSYLTRANSFERASE GENE ($HPRT_{Yale}$).

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Complete deficiency of hypoxanthine-guanine phosphoribosyltransferase (HPRT) causes Lesch-Nyhan syndrome. We have identified the mutation in a cDNA clone encoding $HPRT_{Yale}$, isolated from a subject with Lesch-Nyhan syndrome. This mutant enzyme is characterized by normal mRNA and protein concentrations, no residual catalytic activity and cathodal migration in polyacrylamide gel electrophoresis. We have cloned and sequenced a full-length cDNA (1378bp) from cultured lymphoblasts derived from the subject $HPRT_{Yale}$. A single nucleotide change (G→C) has been identified in its coding region. This transversion predicts an amino acid substitution from glycine (GGC) to arginine (CGC) in codon 71, explaining the cathodal migration of $HPRT_{Yale}$. Inclusion of the bulky arginine side chain in place of glycine probably disrupts protein folding.

39 SELECTIVE TOXICITY OF PURINE NUCLEOSIDES TO HUMAN T-LEUKAEMIC CELLS.

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In vitro cytotoxicity of various purine nucleosides and purine enzyme inhibitors, alone or in combination, and of the alkylating agent mafosfamide incubated for 4/ 24h has been studied in 17 leukaemic cell lines of various phenotypes and normal bone marrow cells. The purine nucleosides/inhibitors included: 2'chlorodeoxyadenosine (CdAdo), 2'deoxyadenosine (dAdo), 3'deoxyadenosine (3'dAdo), adenosine, adenine arabinoside (ara-A), deoxyguanosine (dGdo), guanine arabinoside (Ara-G), 2-deoxycoformycin (dCF) and 8-aminoguanosine (8-AG). T-lymphoblastic cell lines were found to be the most sensitive to the toxic effects of the purine analogues. Marked and selective inhibition of T-cell growth was shown by the combinations dCF with either dAdo or ara-A, of 8-AG with dGdo and by CdAdo or Ara-G alone. These compounds even at high concentrations produced only partial inhibition of the growth of normal bone marrow cells in in vitro assays (CFU-GM and CFU-GEMM) except for CdAdo which inhibited the formation of CFU-GEMM colonies. dCF plus 3'dAdo was toxic to all the cell lines at the concentrations employed, as well as to CFU-GM and CFU-GEMM and so was mafosfamide. The high therapeutic index of some of the purine nucleosides after a relatively short exposure period makes them candidates for selective in vitro removal of residual neoplastic cells in autologous BMT for acute lymphoblastic leukaemia of T-cell origin.

40 INABILITY OF POLY-ADP-RIBOSYLATION INHIBITORS TO PROTECT PERIPHERAL BLOOD LYMPHOCYTES FROM THE TOXIC EFFECTS OF ADA INHIBITION.

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The effectiveness of two inhibitors of poly-ADP-ribosylation, nicotinamide and 3-aminobenzamide to rescue resting and PHA-stimulated lymphocytes damaged by the combination of deoxycoformycin (dCF) and deoxyadenosine (dAdo) has been evaluated. Incubation with dCF (10^{-3}M) and dAdo (10^{-4}M) for 18h inhibited protein and RNA synthesis in unstimulated lymphocytes and impaired the cells to respond to PHA stimulation or to give rise to T-cell colonies in methyl-cellulose. Viability studies showed predominantly dead cells at day 4 in both the unstimulated and PHA-stimulated lymphocytes, whether or not the drugs were removed at 18h. Cell viability at day 4 increased from 13.7% to 41.1% with 3mM nicotinamide and to 28.8% with 5mM 3-aminobenzamide. Although nicotinamide was able to sustain the levels of NAD and reduce the fall in cell ATP concentration, the inhibition by dCF plus dAdo of protein synthesis, RNA synthesis and ability of cells to form colonies in cellulose or to respond to PHA was not reversed. Use of physiological concentrations of dCF (10^{-6}M) and dAdo (10^{-6}M), though producing less pronounced effects on cell viability, protein and RNA synthesis still caused toxicity even in the presence of nicotinamide. We conclude that inhibition of ADP-ribosylation with nicotinamide or 3-aminobenzamide does not protect cells in vitro from dAdo toxicity with ADA inhibition and is thus not likely to give significant clinical benefit in ADA deficiency.