43 ON THE MECHANISM OF DEOXYRIBONUCLEOSIDE TOXICITY IN HUMAN T-LYMPHOBLASTOID CELLS; REVERSAL OF GROWTH INHIBITION BY ADDITION OF CYTIDINE. Yvonne Dahbo and Staffan Eriksson, Karolinska Institutet, Medical Nobel Institute, Department of Biochemistry I, Stockholm, Sweden. High levels of deoxyadenosine and deoxyguanosine in patients with inherited deficiency of either adenosine deaminase or purine nucleoside phosphorylase, respectively, are considered to be responsible for the associated immunological disorder. The mechanism involves phosphorylation to the corresponding deoxyribonucleoside triphosphates which subsequently inhibit the CDP reducing activity of ribonucleotide reductase. Addition of deoxycytidine protects cells from the cytotoxic effects of deoxyadenosine and deoxyguanosine by competition for phosphorylation and by replenishing dCTP, the apparent limiting DNA precursor. Addition of cytidine, but not uridine, led to a reversal of deoxyguanosine and thymidine growth inhibition, comparable to that obtained with deoxycytidine. Analysis of the intracellular nucleotide pools showed that increased levels of cytidine ribonucleotide were sufficient to overcome the inhibitory effects of dGTP and dTTP on CDP reduction, thereby circumventing a depletion of the dCTP pool. A partial reversal of deoxyadenosine toxicity was also obtained with addition of cytidine. The present results may encourage the use of cytidine in combination with deoxycytidine as a pharmacological regime in treatment of immunodeficiency disease associated with increased deoxyribonucleotide levels.

44 INCREASED LEVEL OF RIBONUCLEOTIDE REDUCTASE IN DEOXYADENOSINE RESISTANT ADENOSINE DEAMINASE DEFICIENT HUMAN HISTIOCYTIC LYMPHOMA CELLS. Yvonne Dahbo, Dennis A. Carson+ and Staffan Eriksson, Karolinska Institutet, Medical Nobel Institute, Department of Biochemistry I, Stockholm, Sweden, +Scripps Clinic and Research Foundation, Department of Basic and Clinical Research, La Jolla, California. The DHL-9 wild type cell line is a human histiocytic lymphoma naturally devoided of deoxyadenosine deaminase activity. From this cell line a mutant, DHL-9,dAR-2, resistant to high concentrations of deoxyadenosine has been selected. To be cytotoxic deoxyadenosine has to be converted to the corresponding deoxyribonucleoside triphosphate, which is an inhibitor of ribonucleotide reductase. In this study we tested if the high resistance of DHL-9,dAR-2 to deoxyadenosine was due to either a higher level of ribonucleotide reductase or to an alteration of the structure of the nucleotide binding subunit, M1 of ribonucleotide reductase. The mutant was found to have a two to five times higher ribonucleotide reductase activity than the wild type in crude cell extracts. The mutant activity appeared to be as sensitive to dATP inhibition as the wild type enzyme. By SDS-gel electrophoresis followed by immunoblotting it was found that the M1-subunit level was 2-3-fold higher in the dAR-2 extracts than in the wild type. This increased protein M1-level may well explain the 10-20-fold higher resistance to deoxyadenosine of the dAR-2 cell line, but the reason for increased M1-production remains to be clarified.

45 ISOLATION OF ADENOSINE DEAMINASE (ADA) cDNA SEQUENCES, CONTAINING THE ACTIVE SITE, BY COMPLEMENTATION IN ESCHERICHIA COLI. Mary Jo Danton and Mary Sue Coleman. Univ. of Kentucky, Lexington, KY 40536. The inherited deficiency of the purine salvage enzyme adenosine deaminase is associated with severe B and T-cell dysfunction. In order to investigate the ADA molecule, we have obtained cDNA clones encoding ADA gene sequences. Clones were isolated by metabolic complementation of E. coli SØ200, which has a deletion of the ADA gene. The human T lymphoblast cell line, Molt 4, was the source of mRNA from which cDNA was synthesized. The cDNA sequences were inserted into the Pst 1 site of pBR322 and the recombinant plasmids were used to transform E. coli SØ200. Bacterial colonies that phenotypically expressed adenosine deaminase were isolated by metabolic screening. We obtained cDNA clones that varied in size from 200 to 1000 bp. None of the ADA clones obtained were able to grow on selective media containing the ADA inhibitor deoxycoformycin. This implies that even the shortest clones encode the active site of the enzyme. Restriction mapping and comparison to published sequences showed that all the short clones lie in the central region of the gene. Supported by grant CA26391.

46 STUDY TO DETERMINE RELATIVE HYPERURICAEMIC EFFECTS OF THE DIURETIC DRUGS BUMETANIDE AND FRUSEMIDE.

by
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The aim of this study was to see whether bumetanide had less hyperuricaemic effect that frusemide, using doses of equal diuretic potency.

Thirty adult patients who required diuretic therapy had plasma and urinary urate estimations and plasma sodium and potassium measurements performed on a low purine diet.

They were then given either bumetanide or frusemide in doses of equal diuretic potency and urate, sodium and potassium levels were measured over the months that followed.

Both drugs caused hyperuricaemia and there was no significant difference between bumetanide and frusemide in their hyperuricaemic effects ($p > 0.05$).

47 STUDY OF THE EFFECT OF HYPOURICAEMIC THERAPY ON SERUM LIPID LEVELS IN GOUT PATIENTS

by

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25 patients with primary gout had fasting serum uric acid and lipid levels determined before hypouricaemic therapy.

These uric acid and lipid levels were repeated after biochemical equilibrium had occurred on allopurinol or probenecid and in the absence of significant changes in weight.

No significant changes occurred in cholesterol, triglyceride or high density lipoprotein cholesterol levels (paired 't' tests - $p > 0.05$).

There were no significant correlations of serum lipid levels with each other, with uric acid values or with weight.

These findings suggest that hypouricaemic therapy in gout patients has no effect on serum lipid levels.

48 A MOLECULAR SURVEY OF HPRT DEFICIENCY. Beverly J. Davidson, Thomas D. Palella, James M. Wilson, Peter E. Daddona, Timothy O'Toole, and William N. Kelley, University of Michigan Medical Center, Department of Internal Medicine and Biological Chemistry, Ann Arbor, Michigan, USA.

Virtually complete deficiency of hypoxanthine phosphoribosyltransferase (HPRT) results in the Lesch-Nyhan syndrome. Partial deficiency of HPRT results in gout. We have previously sequenced the normal enzyme and documented the amino acid substitution in four mutant forms of the enzyme. We have undertaken a molecular genetic survey of cell lines derived from HPRT-deficient patients in order to define specific mutations for which protein sequencing is impractical or impossible. Lymphoblasts derived from 17 patients deficient in HPRT (9 with the Lesch-Nyhan syndrome; 8 with partial HPRT deficiency) were studied. Northern blot analysis of mRNA derived from these cell lines was used to determine the molecular size and relative concentrations of message. Three cell lines had no detectable HPRT mRNA. In the remaining cell lines, all but one exhibited normal molecular size (1.6 Kb); the exception had a molecular weight of 1.8 Kb. Relative concentrations of mRNA ranged from slightly diminished in 3, normal in 11 and increased in 3. To more completely define the molecular abnormality, we have generated a cDNA library derived from mRNA obtained from one patient's cultured B-lymphoblasts. From this library, a mutant HPRT cDNA was cloned. This cDNA is 1200 base pairs in length and has restriction maps with Hae III, Hinc II and Hind III identical to normal HPRT-cDNA. This approach provides a direct method for defining the mutation in all $mRNA^+$ $HPRT^-$ subjects. From this survey we conclude that analysis of mutant HPRT at the DNA and mRNA level provides a powerful tool to define specific mutations.