13 David M.O. Becroft, Dianne R. Webster, H.Anne Simmonds, Lynnette D. Fairbanks, J.Douglas Wilson and Leo I.Phillips. Princess Mary Hospital For Children, Auckland, New Zealand

HEREDITARY OROTIC ACIDURIA : FURTHER BIOCHEMISTRY

We report the results of new biochemical studies in D.G., the longest surviving patient with hereditary orotic aciduria. He is now 23 years old and in good health and regular employment. In 1984 on a normal diet he excreted 0.72mmol orotic acid/mmol creatinine. His plasma orotic acid was 20µmol/l and his renal clearance of orotic acid 295ml/min (creatinine clearance 93ml/min), confirming net renal secretion of orotic acid. His plasma uridine was 50µmol/l. His erythrocyte nucleotides are within the normal range except for raised levels of UDPG eg 84 and 113 µmol/l (normal 36±8SD). Incorporation of 1mmol/l adenine into nucleotides by intact erythrocytes is normal, indicating PP-ribose-P levels are not increased. Studies of immune function have been normal and include normal intracytoplasmic Ig levels in resting and PWM-stimulated lymphocytes. Several times in recent years he has stopped taking his normal dose of 3g uridine/day, which has resulted in large increases in urine orotic acid levels and in megaloblastic anaemia. These results indicate the continued requirement for de novo pyrimidine synthesis in adulthood.

14 FUNCTIONAL ANALYSIS OF A REMARKABLE EUKARYOTIC PROMOTER, THE HUMAN ADENOSINE DEAMINASE PROMOTER. Theo M. Berkvens, Dinko Valerio, Geert Weeda, Marja G.C. Duyvesteijn, Ben M.M. Dekker, Hans v.Ormondt, P. Meera Khan and Alex v.d.Eb. Sylvius Laboratories, State University of Leiden (NL).

We have cloned and characterized the human gene for adenosine deaminase (ADA)(ref.1). One surprising feature of this gene is the lack of the characteristic eukaryotic promoter elements (i.e. a TATA and a CAAT box) in the region (0-135) upstream of the cap site (+1). Nevertheless, this upstream region, which is extremely GC-rich (82%), was shown to have promoter activity in a transient expression assay (ref.1). It was hypothesized that the remarkable symmetrically disposed GC-rich motifs found in this promoter region play an important role in the regulation of the ADA gene. This was deduced from their striking homology with elements found in other genes (e.g. those for DHFR and PGK), especially with those elements in the SV-40 early promoter which have been proven to bind specific transcription factors (SP1) intimately (ref.2).

To investigate the ADA promoter in more detail, we linked the promoter region to the chloramphenicol acetyl transferase (CAT) gene and determined its activity in cell lines derived from various tissues and representing different stages of differentiation. By in vitro mutagenesis of the ADA promoter and by competition experiments involving cotransfection with specific SV-40 early promoter sequences we are attempting to resolve the role of the GC-rich motifs in the functioning of this remarkable promoter.

Ref.1: D.Valerio et al., The EMBO J.vol.4(2) 1985, in press.
2: D.Gidoni et al., Nature 312: 409-413 (1984).

15 MECHANISM OF CYTOTOXICITY OF 2-CHLORO AND 2-BROMO-DEOXYADENOSINE FOR A HUMAN LYMPHOBLASTIC CELL LINE, CCRF-CEM. Raymond L. Blakley, Min-chi Huang and Rainer Koob. St. Jude Children's Research Hospital, Division of Biochemical and Clinical Pharmacology, Memphis, TN 38101 U.S.A.

Cytotoxicity as measured by inhibition of growth and DNA synthesis (IC_{50}) and by decrease in clonogenicity (EC_{50}) has been compared with that of dAdo. IC_{50} for growth and for DNA synthesis is similar in all 3 cases. IC_{50} for 2-CldAdo (0.045 µM) is lower than for 2-BrdAdo (0.068 µM) and dAdo (0.9 µM). For 2-CldAdo and 2-BrdAdo, EC_{50} is somewhat higher than IC_{50} and much greater in the case of dAdo. About 20% of cells are resistant to concentrations of 2-BrdAdo $>> EC_{50}$ but viability is decreased to approximately 2% by 18 hr exposure to 2-CldAdo at 20 x EC_{50} or to dAdo at 3 x EC_{50}. Treatment with 2-CldAdo or dAdo sufficient to cause large losses in viability result in accumulation of cells at the G_1/S border. The nucleosides are converted over 24 hr to increasing levels of the triphosphates. The latter inhibit reduction of CDP and of ADP by L1210 ribonucleotide reductase with IC_{50} in the order: 2-BrdATP < 2-CldATP < dATP << ara-ATP. In intact CCRF-CEM cells dAdo specifically decreases dGTP with other dNTPs increasing, whereas 2-CldAdo and 2-BrdAdo decrease dCTP and dATP with a transitory decrease in dGTP but dGTP and dTTP are elevated above controls by 24 hr. Data indicate that inhibition of ribonucleotide reductase possibly contributes to inhibition of DNA synthesis but is probably not the lesion critical for cell death. Supported by American Lebanese Syrian Associated Charities.

16 HORMONE-INDUCED ACCELERATION OF PHOSPHORIBOSYL-PYROPHOSPHATE (PRPP) AND OF PURINE SYNTHESIS IN MOUSE LIVER IN VIVO. Pnina Boer, Sara Brosh and Oded Sperling, Tel-Aviv Univ School of Medicine, Beilinson Med Center, Dept of Clin Biochemistry, Petah-Tikva, Israel.

The effect of i.p. administration of several hormones on PRPP concentration and on the rate of de novo purine synthesis in the liver was studied in mice. PRPP was assayed enzymatically in livers removed 15 min following the administration of the hormones. The rate of de novo purine synthesis in the liver in vivo was gauged by the incorporation of precursor (^{14}C)formate into total liver purines. (^{14}C)formate was administered intraveneously 15 min following the hormones and incorporation into liver purines allowd for 4 min.Glucagon (2mg/kg), L-epinephrine (1mg/kg), db cAMP (40mg/kg), angiotensin II (65ug/kg) oxytocin (25U/kg)and vasopressin (25U/kg), were found to increase PRPP concentration by 2 to 6 folds and to accelerate the rate of purine synthesis by 2 to 3 folds.

All the above hormones are known to exert glycogenolytic effect in the liver, through either cAMP-dependent or calcium-dependent mechanisms. The increased synthesis of PRPP, which causes the acceleration of purine synthesis, could reflect a metabolic effect of the hormones, such as the glycogenolytic-induced increased formation of substrate Rib-5-P,or activation of PRPP synthetase by an as yet unidentified mechanism.

17 PURIFICATION AND PROPERTIES OF HUMAN DEOXYCYTIDINE KINASE. Christina Bohman and Staffan Eriksson, Karolinska Institutet, Medical Nobel Institute, Department of Biochemistry I, Stockholm, Sweden. Deoxycytidine kinase is the principal deoxynucleoside salvage enzyme in humans. It plays a central role in regulation of DNA precursor metabolism in lymphocytes. The enzyme is involved in the pathogenicity of certain inherited immunodeficiency diseases, associated with high deoxynucleoside levels. We have purified the enzyme from human T-leukemic cells in culture (CEM-cells) and from human leukemic spleen (chronical lymphatic leukemia of B-cell type). A similar specific activity was found in crude extracts from both CEM-cells and leukemic spleen. Therefore the latter constitutes a good source for purification of the enzyme. From 1000 mg spleen approximately 10 µg purified enzyme was obtained, and thus the cell content of the enzyme is very low. The purification involves ammonium sulfate precipitation, DEAE-Sephadex chromatography and affinity chromatography on dTTP-Sepharose. The preparation is now highly purified and on SDS-gel electrophoresis 3-4 bands are apparent. In the purified fractions, deoxyguanosine also served as a substrate for the enzyme but with a 50-fold higher K_m value than deoxycytidine.

18 SYNERGY OF METHOTREXATE AND 6-MERCAPTOPURINE ON CELL GROWTH AND CLONOGENICITY OF CULTURED HUMAN T-LYMPHOBLASTS.

Jos P.M. Bökkerink, Ronney A. De Abreu, Jan P.R.M. van Laarhoven, Marinka A.H. Bakker, Tilly W. Hulscher, Egbert D.A.M. Schretlen and Chris H.M.M. de Bruyn.
Centre for Pediatric Oncology SE Netherlands, Dept. of Pediatrics, University Hospital St. Radboud, 6500 HB Nijmegen, The Netherlands.

Cell growth of Molt-4 cells was hardly influenced by a 24 hrs incubation with 6-mercaptopurine (6MP) concentrations under 5 µM or by a 24 hrs incubation with 0.002 µM methotrexate (MTX). After a 24 hrs incubation with 0.02 µM MTX the cell growth was inhibited partially and with 0.2 µM MTX the cell growth was inhibited completely.
In experiments, where the cells were incubated for 24 hrs with 2 µM 6MP after 24 hrs preincubation with 0.02 µM MTX, a greater inhibition of cell growth was obtained than in experiments with 0.02 µM MTX alone.
In experiments, where 2 µM 6MP and 0.02 µM MTX were added simultaneously to the cells or 6MP was added 24 hrs prior to MTX inhibition, no synergy was observed.
The results of the experiments on soft-agar clonogenicity of the Molt-4 cells, were found comparible to those of cell growth.