205 ACYCLOVIR'S ANTIHERPETIC ACTIVITY IS POTENTIATED BY INHIBITION OF RIBONUCLEOTIDE REDUCTASE

Thomas Spector, Devron R. Averett, Donald J. Nelson, Catherine U. Lambe, Robert W. Morrison, Jr., Marty H. St Clair and Phillip A. Furman

Experimental Therapy, Organic Chemistry and Virology Departments. Wellcome Research Laboratories, Research Triangle Park, NC 27709

Compound A723U, a 2-acetylpyridine thiosemicarbazone, was found to inactivate herpes simplex virus (HSV) ribonucleotide reductase. Inactivation occurred only while the enzyme was catalyzing the reduction of substrate and at a maximum rate of 17 per hr. A723U inhibited virus replication (ED_{50} = 3-5 μM) at concentrations that were not toxic to the confluent host cells. It also exhibited mutually potentiated anti-HSV activity with acyclovir (ACV). Sub-inhibitory concentrations of either compound significantly decreased the ED_{50} of the other compound. As expected, A723U caused the dGTP pool to decrease. However, it also caused the level of ACV-triphosphate to markedly increase. The consequence is a facilitation of the binding of ACV-triphosphate to its target enzyme, HSV DNA polymerase.

206 URATE OXIDASE ENCAPSULATED IN ERYTHROCYTES

U. Sprandel, Medical Polyclinic, University of Munich, FRG

Erythrocytes have been proposed as cellular carriers for enzyme replacement therapy. Urate oxidase was encapsulated in erythrocytes using different methods. Average encapsulation was found to be 7 % of the added enzyme activity using a dialysis technique and 1.75 % using a direct hemolysis procedure. Binding of enzyme to the membrane could be excluded. Optimum enzyme activity of urate oxidase encapsulated in erythrocytes was found to be at pH 9.5 and 42° C. Suspension of urate oxidase loaded erythrocytes in uric acid containing medium was followed by an initial decrease of uric acid which could be related to the available space inside the cells. After 40 minutes steady-state levels were reached and the further decrease of uric acid showed a direct relation to the added amount of entrapped enzyme. Within 24 hours there was a loss of 8 %, within 7 days of 15 % of the enzyme activity, which could be related to hemolysis. This system was not studied in vivo, since it was not possible to elevate uric acid in a laboratory animal. However, these experiments demonstrate, that it would be possible to decrease elevated uric acid levels by urate oxidase loaded erythrocytes in human.

207 MURINE LYMPHOCYTES AND LYMPHOCYTE LINES SECRETE ADENOSINE DEAMINASE(ADA).

Phyllis R. Strauss, Biology Department, Northeastern University, Boston MA USA

ADA is found in the sera of all mammals except the horse and the concentration of serum adenosine (Ado)is correspondingly low (<1 uM). In this study we examine the possible origin of extracellular ADA. Cells from freshly isolated mouse spleen were incubated in phosphate buffered saline containing 5 mM glucose and 0.1% bovine serum albumin(BSA) for up to 2 hrs; S49 thymoma cells were incubated in serum-free Dulbecco's modified Eagle's medium containing 0.1% BSA for up to 24 hrs. Cells were rapidly separated from incubation medium by centrifugation through a layer of silicone oil. Intracellular and extracellular ADA was measured by determining the rate of conversion of ^{3}H Ado(1 uM,6 uCi/ml) to inosine+hypoxanthine. To account for extracellular ADA due to cell lysis, intracellular and extracellular lactate dehydrogenase was also measured. On several occasions S49 cells which had been incubated for 20-23 hrs were washed, resuspended and incubated for further time intervals. The appearance of ADA activity in the extracellular medium was time and cell concentration dependent and could not be accounted for by dead or leaky cells. Between 7-10% of the initial intracellular activity was secreted during an 8 hr period. We conclude that lymphocytes secrete ADA. Supported by NSF PCM 77-25434 and ONR 14-82-K0283.

208 AN EXPLANATION FOR THE HETEROGENEITY IN B LYMPHOCYTE ECTO-5'-NUCLEOTIDASE (ECTO-5'-NT) ACTIVITY IN PATIENTS WITH HYPOGAMMAGLOBULINEMIA.

Linda F. Thompson, Richard D. O'Connor, Julie M. Ruedi, and John F. Bastian. Scripps Clinic and Research Foundation, Department of Immunology, La Jolla, CA USA.

Ecto-5'-NT activity was measured in human B cells at different stages of development and compared with the B cells' capacity to synthesis Ig in vitro. Ecto-5'-NT activity was higher in adult B cells from peripheral blood, spleen, or lymph node than in B cells from fetal spleen or cord blood (27.9±12, 29.2 and 33.8 vs 5.02 and 5.59±2.8 nmol/hr/10^6 cells, respectively). B cell ecto-5'-NT activity increases shortly after birth and is reflected by gradual increases in total lymphocyte ecto-5'-NT activity from 7.0±2.1 in cord blood, to 14.0±4.2 nmol/hr/10^6 cells in normal infants at 6 mo. B cells from cord blood or 6 mo. old infants could synthesize IgM, but not IgG, in response to Epstein Barr virus (EBV). Adult peripheral B cells separated into mature pokeweed mitogen (PWM)-responsive and less mature PWM-nonresponsive fractions by mouse RBC rosetting had equivalent total ecto-5'-NT activity. Thus, in normal human B cell development, the ability to synthesize IgG in vitro in response to EBV or PWM is acquired subsequent to the acquisition of ecto-5'-NT activity. Therefore, patients with hypogammaglobulinemia might have either normal or low B cell ecto-5'-NT activity depending upon the location of their block in B cell differentiation. Of 11 patients studied, 4 had normal B cell ecto-5'-NT activity while 7 had reduced activity (15.4-58.2 and 2.8-13.2 nmol/hr/10^6 cells, respectively).

209 ECTO-5'-NUCLEOTIDASE (ECTO-5'-NT) CAN USE IMP TO PROVIDE THE TOTAL PURINE REQUIREMENTS OF MITOGEN-STIMULATED HUMAN T CELLS AND HUMAN B LYMPHOBLASTS.

Linda F. Thompson. Scripps Clinic and Research Foundation, Department of Immunology, La Jolla, CA USA.

The ability of mitogen-stimulated human T cells or human B lymphoblasts to derive their total purine requirements from inosine 5'-monophosphate (IMP), inosine (HxR), or hypoxanthine (Hx) was compared. Con A-stimulated T cells were treated with aminopterin (Ampt) to inhibit purine synthesis de novo; thymidine (TdR) was added as a source of pyrimidines. Under these conditions, 30 μM IMP, HxR, and Hx showed comparable abilities to support ^{3}H-TdR incorporation into DNA or ^{3}H-leucine incorporation into protein at rates equal to those of untreated control cultures. Similar results were found when azaserine was used to inhibit purine synthesis de novo. In parallel experiments, Ampt inhibited the growth of WI-L2 B lymphoblasts by >95% and this growth inhibition could be overcome by 30 μM IMP, HxR, or Hx (plus TdR). However, with cell line #1254, a derivative of WI-L2 lacking detectable ecto-5'-NT activity, only HxR and Hx (plus TdR), but not IMP (plus TdR), were able to restore the growth inhibition due to Ampt. Thus, the catalytic activity of ecto-5'-NT is sufficient to meet the total purine requirements of mitogen-stimulated human T cells or rapidly-dividing human B lymphoblasts, suggesting that this enzyme could play a role in purine salvage when purine synthesis de novo is limited and/or an extracellular source of purine nucleotides is available. Such conditions might exist in developing thymus, spleen and bone marrow where massive cell death occurs.

210 A COMPARATIVE STUDY OF ALLOPURINOL AND PENTOSTAM IN THE TREATMENT OF VISCERAL LEISHMANIASIS:

Mohsen Tobaigy, Johannes Mejer, Birgit Peitersen, Hohiuddin Ansari, Paul Poll. Departments of Medicine, Pediatric & Histopathology, King Fahd Central Hospital, Gizan, Saudi Arabia.

Allopurinol has been shown to inhibit the in vitro growth of leishmaniasis Donovani (1). Recently allopurinol has also been reported effective in the treatment of visceral leishmaniasis with a response rate of 14 out of 16 treated cases (2). The purpose of the present study is to compare the clinical effect of allopurinol versus pentostam at fixed dosages (30 ml/kg and 10 mg/kg daily for 30 days respectively) in the treatment of visceral leishmaniasis. 16 patients were included. 7 patients received allopurinol and 9 pentostam treatment. The clinical effect was monitored using a scoring system. 5 parameters were included each gave 1 point for improvement and 0 point for no improvement. In an average the patients in the allopurinol group scored 1 1/7 point and the pentostam group 3 7/9 point within the first month of treatment. The difference is significant ($p < 0.01$ Wilcoxon test).

Conclusion: Although allopurinol improve the clinical condition of the patients to some extent pentostam treatment was superior in that respect.

(1) J.J.Marr: Purine Metabolism in Man - IV, Plenum Press, New York, Editor: C.H.M.M. Bruym, H.A.Simmonds & M.M.Muller 1984, p. 231-237.

(2) T.K.Jha: Transaction of the Royal Society of Tropical Medicine and Hygiene. Vol. 77, 204-207, 1983.