

23

COORDINATE REGULATION OF ADENOSINE DEAMINASE, PURINE NUCLEOSIDE PHOSPHORYLASE AND TERMINAL DEOXYNUCLEOTIDYL TRANSFERASE mRNA LEVELS BY PHORBOL ESTERS IN HUMAN THYMOCYTES. Amos Cohen and Hector Martinez-Valdez, The Hospital for Sick Children, Division of Immunology/Rheumatology, Research Institute, Toronto, Ontario

Canada.

Phorbol esters caused reversible decrease in the levels of terminal deoxynucleotidyl transferase (TdT) and adenosine deaminase (ADA) mRNAs and an increase in the level of purine nucleoside phosphorylase (PNP) mRNA. The structural specificity of the phorbol esters affecting ADA, TdT and PNP mRNA levels correlates with their effectiveness in the activation of protein kinase (PKC). The effect of phorbol esters on TdT and ADA mRNA levels is attributed to an apparent decrease in stability of their mRNAs. The changes observed in ADA, TdT and PNP mRNA levels closely simulate the behaviour of these enzyme activities during T-cell differentiation *in vivo*. Moreover, similar changes in ADA, TdT and PNP activities were observed in immature pre-B and T-lymphoid cell lines expressing TdT activity.

The presence of high ADA and low PNP activities in immature lymphocytes may have a role in maintaining balanced intracellular dGTP/dATP pools which are required for TdT activity (Cohen, A. et al. J. Biol. Chem. 258:12334). Expression of TdT activity is a requirement for the diversification of immunoglobulin (Ig) and T-cell antigen receptor (TcR) genes. Thus, upon completion of Ig and TcR gene rearrangement PKC activation may mediate the programmed changes in these enzyme levels to those observed in mature lymphocytes.

24

FUNGALBIONICS-A NEW CONCEPT OF THE ETIOLOGY OF GOUT AND HYPERURICEMIA. A.V. Costantini, Univ of Calif School of Med San Francisco Ca U.S.A./Multicenter Fungalbionic Research Project. Address all mail to European Office Am Muehlbach 13, 7800 Freiburg, West Germany

Fungalbionics implicates fungi and fungal metabolites as the cause of gout/hyperuricemia. The mechanisms are not the usual patterns of invasive-type mycoses nor of mycotoxicoses, but rather incorporate occult features of both mechanisms. Gout and/or hyperuricemia can be induced in fowl by the mycotoxins oosporein, ochratoxin and by oosporein-producing fungi. Gouty tophi have been induced in primates by aflatoxin. Fungi produce preformed uric acid, preformed urate crystals, lipoproteins, glycosaminoglycans and glutamates, excess of which are found in gout. Gouty tophi are granulomatous and possess all of the features of delayed hypersensitivity. Giant cells in avian and human gouty tophi contain asteroid bodies which are fungal in origin. Fungal-like spherules have been found in avian gouty lesions; cultural and immunological electron microscopy studies are in progress. The findings in acute gout are those of an acute infection. All drugs used in treating gout/hyperuricemia are antifungal. Griseofulvin, an antifungal antibiotic, is as effective as colchicine in gout. Both are antitubulins and arrest fungal cell division. Probenecid, allopurinol, corticosteroids, NSAIDs, possess antifungal activity. The fungalbionic concept gives a unitarian explanation of gout, hyperuricemia and related diseases and findings.

25

THE ACTIVITY OF AA-193, A NEW URICOSURIC AGENT, IN ANIMALS. Takashi Dan, Hiroshi Koga, Etsuro Onuma, Haruko Tanaka, Haruhiko Sato and Bunya Aoki. Chugai Pharmaceutical Co., Ltd., Exploratory Research Laboratories, Gotemba-shi, Shizuoka 412, Japan.

A new uricosuric agent, 5-chloro-7,8-dihydro-3-phenylfuro[2,3-g]-1,2-benzisoxazole-7-carboxylic acid (AA-193) was compared with other uricosurics in the rat, mouse and cebus monkey, because the effects of those drugs were known to be highly species-dependent.

Probenecid and tienilic acid which in the human kidney block the reabsorption of filtered and secreted urate, increased the urate excretion in rats. But benzbromarone, an inhibitor of the latter reabsorption in man, did not have the uricosuric activity. Thus, the post-filtered reabsorption of urate is probably dominant in rats. We found that in rats AA-193 is the most potent uricosuric yet reported. In mice, probenecid not only had so-called paradoxical actions but stimulated the urinary urate wasting after administration of pyrazinamide. Aspirin also responded paradoxically. These data suggested that the renal transport system of urate in the mouse is similar to that in man. AA-193 as well as benzbromarone enhanced the urate excretion dose-dependently in normal condition, but the effects were different in pyrazinamide-suppression tests in mice. In cebus monkeys, the uricosuric and hypouricemic effects of AA-193 were more potent than those of probenecid and similar to those of tienilic acid, but less than those of benzbromarone. Benzbromarone had a considerable role in post-secreted reabsorption in the monkey.

Conclusion: AA-193 is a new class of uricosuric agent that controls the renal reabsorption of filtrated urate particularly.

26

CAN RISK SCORES FOR VASCULAR DISEASE IN GOUT PATIENTS BE IMPROVED ?

Lynda G. Darlington and James T. Scott
Epsom District Hospital Kennedy Institute of
Rheumatology Unit Rheumatology
Epsom, Surrey. U.K. London W6 U.K.

Gout patients are known to have risk factors which predispose to vascular disease, including hyperlipidaemia, hypertension, obesity and Type A personalities in association with hyperuricaemia and a high alcohol intake.

In this study a scoring system was devised in which risk factors for vascular disease were scored using scales based on the relative importance of these factors in the pathogenesis of vascular disease.

For each of 36 patients with primary gout a risk score was determined and each patient was advised and treated according to his or her own risk factors.

Patients were seen every three months, at which times their risk scores were recalculated and further advice given to see whether vascular risk could be reduced.

Risk scores ranged from 2 - 15, with a mean value on entering the study of 8.4. 9 patients reduced their risk score by 3 or more at one or more stage in follow up while 4 increased their score by 3 or more. Using paired t tests to compare risk scores for the group after various lengths of follow up there were significant falls in risk score at months 3 and 9 ($p < 0.05$ and < 0.002).

These results should be seen in the context of a small study but suggest that intensive counselling may beneficially affect risk scores for vascular disease in gout patients.

27

STRUCTURAL CONSEQUENCES OF POINT MUTATIONS IN NINE HUMAN HPRT VARIANTS. Beverly L. Davidson, Thomas D. Palella, Shin Fujimori, William N. Kelley. University of Michigan, Department of Internal Medicine, Ann Arbor, Michigan, USA.

Complete deficiency of hypoxanthine guanine phosphoribosyltransferase causes the Lesch-Nyhan Syndrome (LNS). Partial deficiency of HPRT causes severe, precocious gout. The molecular basis for HPRT deficiency has been determined previously in this laboratory by amino acid sequence analysis in three subjects and by cloning of mutant cDNA sequences in six subjects. In each case a single point mutation resulting in a single amino acid substitution was identified. The structural consequences of these mutations were examined using secondary structure prediction techniques. The mutations in HPRT-London and HPRT-Yale abolish putative β -turns while the mutation in HPRT-Midland predicts an additional β -turn. A sequence predicted to be in random coil structure in HPRT-Munich and HPRT-Ashville is replaced by β -sheet structure and a region of putative β -sheet in HPRT-Ann Arbor is replaced by α -helix. The predicted structural consequences of the mutations in HPRT-Flint, HPRT-Kinston, and HPRT-Toronto were unremarkable using these techniques.

28

CEREBROSPINAL FLUID CYCLIC NUCLEOTIDE ALTERATIONS IN THE LESCH-NYHAN SYNDROME. N. Lawrence Edwards, Dept of Medicine, Univ of Florida and VAMC, Gainesville, Florida and Michael V. Johnston, and Faye S. Silverstein Dept of Neurology and Pediatrics, Univ of Michigan, Ann Arbor, Michigan, USA.

Cyclic nucleotides in the central nervous system may have "first" messenger roles as well as the classic "second" messenger effects. Such primary actions may include regulation of neurotransmitter synthesis, control of certain cellular movements and effects on both tropic and developmental processes. Low cyclic AMP (cAMP) levels are reported in spinal fluids (CSF) from patients with certain basal ganglion diseases, i.e. Parkinson's and advanced Huntington's disease. Cyclic GMP (cGMP) in the CSF is felt to reflect central cholinergic activity. In an effort to find a suitable biochemical marker of disease activity in the Lesch-Nyhan syndrome (L-NS) we made serial measurements of cAMP and cGMP on deproteinized CSF extracts using a radioimmunoassay. Three boys with L-NS at ages 29, 68, and 128 months had 5 CSF determinations over the subsequent 40 months period. Seventeen age-matched patients undergoing leukemia workup and showing no evidence of CNS disease were used as controls. The control group had values of 1.4 ± 0.6 pmol/ml for cAMP and 0.1 ± 0.05 for cGMP. The L-NS subjects had cAMP level of 4.3 ± 2.1 and cGMP of 1.1 ± 0.7 . The correlation (Pearson's) between cAMP and cGMP levels in the L-NS subjects was $r = 0.83$ and in the controls only 0.26. These findings raise the possibility of an as yet undescribed CSF metabolite with caffeine-like activity in the L-NS spinal fluid that may block cyclic nucleotide phosphodiesterase activity and lead to CSF cyclic nucleotide elevations.