1518

THE REGULATION OF FIBRINOGEN PRODUCTION INVOLVES AT LEAST ONE OTHER HEPATOCYTE GENE. D.M. Fowlkes (1), P.K. Lund (2), M. Blake (1), and J. Snouwaert (1). The University of North Carolina, Department of Pathology (1) and Physiology (2) and Program in Molecular Biology and Biotechnology, Chapel Hill, NC, USA.

It is currently thought that glucocorticosteriods have a direct effect on the transcription of the alpha, beta and gamma fibrinogen genes. However, our studies indicate that while corticosteriods play a role in fibrinogen production, this role is not due to transcriptional activation via glucocorticosteriod receptors. In initial experiments, we compared the levels of fibrinogen mRNA in hepatocytes isolated from hypophysectomized rats to those from control animals. The levels of mRNA in hypophysectomized rats, which produce little ACTH or corticosteriods, were significantly higher than the levels in control animals. Albumin mRNA levels were unaffected by hypophysectomy. These results are in opposition to those which we had anticipated. Based on previously published data, we had thought that physiologic deprivation of corticosteriods would lead to decreased levels of fibrinogen. We propose that these results are related to the negative feedback that corticosteroids have on Hepatocyte Stimulating Factor (HSF) production through a tightly controlled feedback circuit. To investigate the role of corticosteriods in fibrinogen gene regulation, we have conducted experiments with primary hepatocytes in culture and rat FAZA cells (continuous hepatoma cell line). There is a 4 to 5 fold increase in fibrinogen production when these cells are treated with HSF but no change when these cells are treated with dexamethasone alone. However, there is a marked additional increase in the production of fibrinogen with the combination of dexamethasone and HSF. Data gathered through kinetic analysis of this synergistic interaction suggest that the maximum response to HSF requires another gene product whose production is responsive to dexamethasone. Detailed analysis of the rate of transcription of the gamma fibrinogen gene, its processing and mRNA turnover suggests a specific role for this gene product in regulating fibrinogen synthesis. Characterization of this gene product will lead to greater understanding of the regulation of the Acute Phase Reactants.

1519

IDENTIFICATION OF VITAMIN K-DEPENDENT CARBOXYLASE ACTIVITY AND EVIDENCE FOR PROTHROMBIN SYNTHESIS IN ALVEOLAR TYPE II CELLS. R. Wallin and S. R. Rannels. Dept. of Physiology, The Milton S. Hershey Med. Ctr., The Pennsylvania State University, Hershey, Pa. 17033, U.S.A.

Prothrombin precursors have been shown to be present in microsomes from lung alveolar type II cells. Immunoblotting revealed two microsomal precursors of apparent mol. wt. 68 and 65 kDa. The 65 kDa protein appears to be the substrate for the vitamin K-dependent carboxylase. Fluorography of [^{14}C]-labeled precursors of vitamin K-dependent proteins in lung microsomes shows that the lung has several precursors that are not found in the liver. Pulmonary macrophages appear to be devoid of vitamin K-dependent carboxylase activity. However, type II epithelial cells have significant activity and this activity was unaltered when these cells were maintained in primary culture for 3 days, suggesting that carboxylase activity is expressed in alveolar epithelium independent of culture-induced changes in cellular differentiation. Carboxylase activity in type II cells was enhanced 2-fold when cells were cultured for 24 hours in the presence of 50 µM warfarin. Type II cells, therefore, resemble hepatocytes with regard to their response to coumarin drugs. Our data suggest that macrophages and type II cells may act in a cooperative manner to contribute to extravascular coagulation in the lung. Supported by NIH Grant HL-32070-02 and by The American Heart Association.

1520

SPECIFICITY OF INCREASED DES-GAMMA-CARBOXYPROTHROMBIN AS A MARKER OF HEPATOCELLULAR CARCINOMA AFTER VITAMIN K INJECTION. J.J. Lefrère (1), D. Gozin (1), J.P. Soulier (1), P. Mavier (2), L. Bettan (2), D. Dhumeaux (2). Centre National de Transfusion Sanguine, 6, rue A. Cabanel,75015,Paris (1), département d'hépatologie, CHU H. Mondor, 94010 Créteil ; Service de gastro-entérologie, Hôpital de Villeneuve -Saint-Georges), (2), FRANCE.

An elevation of des-gamma-carboxyprothrombin (DCP) has been observed in about 70 % of cases of hepatocellular carcinoma (HCC). Howewer, an increased DCP is not specific of HCC. Oral anticoagulant therapy increases the DCP level by preventing the gamma-carboxylation of prothrombin : thereafter an increased DCP can not be used as an HCC marker before three weeks have elapsed after stopping antivitamin K therapy. Furthermore, since vitamin K is necessary for the gamma-carboxylation of vitamin K dependent factors, a vitamin K deficiency increases the DCP level long before the modification of the prothrombin time. It is thus imperative to eliminate an underlying vitamin K deficiency before attributing an increased DCP to a HCC. We used a method of DCP assay using staphylocoagulase. We studied the effect of an intravenous injection of 20 mg of vitamin K1 on DCP level in 7 patients with histologically proven HCC and in 10 patients with various disorders (5 alcoholic cirrhosis, 1 chronic hepatitis, 4 pancreatic cancer). All these 17 patients had increased DCP before vitamin K injection. In a second sampling obtained 15 days or more after injection, only the 7 patients with HCC kept increased DCP level. In patients of both categories in whom we obtained intermediary samplings, we observed that the DCP level decreased in all cases. The normalisation of the DCP level was lasting only in those patients without HCC, confirming the hypothesis of an underlying vitamin K deficiency ; this decrease was very transitory in those patients with HCC, suggesting that the elevated DCP came from a yet unexplained (but not linked to a vitamin K deficiency) mechanism. We may conclude that an increased DCP level 15 days after vitamin K injection may constitute a specific marker of HCC.

1521

DEFECTS OF VITAMIN K-DEPENDENT FACTORS IN CA(II)-STABILIZED STRUCTURE. S.R. Poort, C. Krommenhoek-van Es, I.K. van der Linden, N.H. van Tilburg and R.M. Bertina. Haemostasis and Thrombosis Research Unit, Leiden Univerversity Hospital, Leiden, The Netherlands.

Vitamin K-dependent (anti)coagulation factors (factor II, VII, IX, X protein C and S) undergo a conformational transition upon binding of Ca(II), which is a prerequisite for their normal function. Abnormalities in these properties occur during vitamin K deficiency or treatment with antivitamin K drugs and in some genetic variants of coagulation factors. Immunological assays utilizing antibodies against the Ca(II)-stabilized structure are useful to detect such abnormalities.

Starting from specific rabbit antisera antibody populations specific for the Ca(II)-dependent conformation of factor II, VII, IX, X and protein C and S were isolated using immunoaffinity procedures. Subsequently immunoradiometric assays specific for the Ca(II)-dependent (Ca(II)Ag) and Ca(II)-independent (NonCa(II)Ag) conformations of the different proteins were developed. These assays were used for the analysis of plasmas of patients stably treated with oral anticoagulants; Ca(II)Ag, NonCa(II)Ag and their ratio were measured as function of the intensity of the treatment (INR 2.4 to 4.8). The same parameters were measured in plasmas of patients with hereditary coagulation disorders. After treatment with oral anticoagulation with an antivitamin K drug reduced ratios of Ca(II)Ag/-NonCa(II)Ag were observed for factor II, VII, IX, protein C and protein S. However, the actual degree of reduction and its dependence on the intensity of treatment varied for the different vitamin K-dependent proteins. In general Ca(II)Ag levels correspond nicely with the procoagulant activity of the concerning proteins. These data provide indirect evidence for the existence of abnormal (non and/or subcarboxylated) forms of the vitamin K-dependent proteins during oral anticoagulant treatment.

Genetic variants with a mutation in one of the sites involved in the formation of the Ca(II)-stabilized structure could be detected for factor IX, factor VII and factor II. However, the extent of reduction of the ratio Ca(II)Ag/-NonCa(II)Ag differed considerably in those variants.