ANTITHROMBIN III

ANTITHROMBIN III AND HEPARIN COFACTOR II IN PATIENTS WITH CHRONIC RENAL FAILURE. P. Toulou, C. Jacob, N.D. Friedman, D. Vignon, M. Arut, Hopital Broussais, Paris, France.

Antithrombin III (AT III) and heparin cofactor II (HC II) were measured in 77 blood donors, 82 patients with chronic renal failure (CRF) undergoing regular hemodialysis and 36 undialyzed patients with CRF. AT III was measured as heparin cofactor and HC II as dermavate cofactor using amidolytic assays.

The results (mean ± SD expressed in percentage of the in pooled normal plasma) are summarized in the table.

<table>
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<tr>
<th>Control group</th>
<th>Dialyzed patients</th>
<th>Undialyzed patients</th>
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<tr>
<td>n = 77</td>
<td>n = 52</td>
<td>n = 22</td>
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AT III (%): 102 ± 10 (NS) 99 ± 13 (NS) 100 ± 9 (NS)

HC II (%): 106 ± 20 (p < 0.001) 85 ± 16 (p < 0.001) 106 ± 20 (NS)

Comparison were made using Student's t test. NS = non significant.

Subnormal AT III levels were found in both dialyzed and undialyzed patients with CRF, while HC II was significantly (p < 0.001) reduced in dialyzed patients (12 of them were found to have HC II levels below to lowest value founded in our control group).

In order to explain this decrease of HC II level in dialyzed patients with CRF, we compared both AT III and HC II activities before and after a dialysis session in 24 patients (12 with a low and 12 with normal before dialysis HC II activity). AT III and HC II increased significantly (p < 0.05) in all patients after dialysis. When related to total plasma proteins in order to suppress the influence of hemococoncentration induced by dialysis, AT III decreased significantly (p < 0.01) in the 24 patients while HC II increased significantly (p < 0.01) only in patients with low before dialysis HC II levels (the increase in HC II activity was found significantly in the pooled 24 patients).

EVIDENCE FOR A HOMOLOGY BETWEEN THE HEPARIN AND HEPARAN SULFATE BINDING REGIONS TO ANTITHROMBIN III. A.M. Plachy (1), J. Tapon-Brotaudière (1), M.D. Deutsenberg (1), S. Stenber (1) and J. Choay (2). Laboratoire d'Hématologie, Faculté Necker, Paris, France (1) and Institut Choay, Paris, France (2).

As we have previously demonstrated, heparan sulfate, a glycosaminoglycan physiologically present on the endothelial wall, is, like heparin, unable to potentiate the inhibitory effect against Factors IIa and Xa of two abnormal type 3 antithrombin III (AT III) variants. We report here that a synthetic pentasaccharide which constitutes the sequence of the heparin binding site to AT III is also unable to potentiate these two AT III variants in an anti-Factor Xa assay. According to these data, we speculated the existence of a homology between the heparin and heparan sulfate binding regions to normal AT III. We thus studied the competitive inhibition by the pentasaccharide of heparin and heparan sulfate in their potentiation of AT III activity. Such a competitive inhibition can be observed in an AT III anti-Factor IIa assay because the pentasaccharide which exhibits a high anti-Factor Xa activity is devoid of any anti-Factor IIa activity. In the absence of pentasaccharide, with both heparin and heparan sulfate, a plateau is reached in AT III potentiation for concentrations of respectively 2.5 μg and 17.2 μg (corresponding to the same anti-Factor IIa activity of 0.4 u/mI for both glycosaminoglycans). In the presence of 5.5 μg of pentasaccharide, the inhibition of heparan cofactor activity is 70%. With heparan sulfate, the inhibition by the same amount of pentasaccharide is less pronounced, being only 30%. These results strongly suggest the existence of a partial homology between heparin and heparan sulfate binding sites to AT III. For heparan sulfate, the exact sequence of this site remains to be identified.

ANTITHROMBIN III ACTIVITY AND ANTITHROMBIN III-THROMBIN COMPLEXES BINDING TO CULTURED CELLS BY MONOCLONAL ANTIBODIES AGAINST ANTITHROMBIN III.

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Two monoclonal antibodies (mAb's) against antithrombin III (ATIII) were characterized with respect to their ability to interfere with ATIII activity. ATIII activity was measured by its ability to inhibit the amidolytic activity of thrombin on ATIII: Its binding to ATIII induces conformational changes between inhibition and enhancement of thrombin binding to ATIII and of ATIII-Th complexes binding to cells by the two mAb's. These mAb's may provide a new tool to control the activity of ATIII and to identify the cellular binding site on the ATIII-Th complex.

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Test for pyrogenicity in human blood products using rabbit has been authorized as one of the security test, but LAL (Limulus Amebocyte Lysate) test has not been approved in Japan. We have studied the problem by using the in vitro LAL test (endotoxin test) for the rabbit pyrogen test of blood products especially human serum albumin (commercial HSA) and ATIII products. It is well known that the LAL inhibitory reaction has been detected, although slightly, in most human serum albumin products, and therefore, the factors participating in this inhibition were investigated using commercial HSA and PFP products, and also preparations without heating in their production process. ATIII activity was assayed by two methods clotting and androgenic substrate method: the LAL activity was assayed by gelatin method (Toximenter, Mako-junyaku Kogyo Co.). We found that the HSA activity in non-heated PFP preparation (NHFPP) was determined to be significantly lower than heated PFP preparation (PFP product) from one manufacturer, but not from others. The ATIII activity of this particular NHFPP was extremely high as compared with the other PFP. Addition of ATIII to the PFP caused a significant decrease of the LAL activity in reciprocal proportion to its increase. The LAL inhibitory activity and ATIII activity of the NHFPP were inactivated by heating at 60° C for 2.5 hrs. We also found that ATIII products from the two manufacturers possessed remarkable LAL inhibitory activity which also induced a decrease by heat treatment at 56° C for 30 min. The ATIII activity was considered to be one of the LAL inhibitory factors from the correlation between LAL reactivity and ATIII activity.