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DEFINITION AND EVALUATION OF A THERAPUETIC RANGE FOR THE CONTROL OF ORAL ANTICOAGULANT TREATMENT BY CHROMOGENIC ASSAYS. R.M. Bertina and E.A. Loeliger. Haemostasis and Thrombosis Research Unit, Leiden University Hospital, Leiden, The Netherlands.

The evaluation of the usefullness of chromogenic assays in the control of oral anticoagulant treatment, often is hampered by the rather poor correlation between the new parameter and the reference one (Prothrombin Time determination) within the therapeutical range. This means that before large scale double blind prospective studies can be started, it should be checked that the use of the experimental method will not result in unacceptable deviations in prescribed dosage.

From 150 patients (each within therapeutic zone for more than 3 months) blood/plasma was analysed by at least 2 different chromogenic assays (FII, FX) and by the reference method (PT determination); the selection of patients was such to get an equal distribution over the total therapeutic range (2.0-5.0 in terms of proposed International Calibrated Ratio - ICR); on basis of these results a therapeutic range was defined for each of the experimental methods.

Next it was checekd to what extent the use of the experimental method would result in differences in prescribed dosages (when compared with the reference method) a. in 100 of the abovementioned patients at subsequent control periods, b. in previously stable patients with a PT < lower limit or > upper limit of the therapeutic zone, and c. in patients starting oral anticoagulant treatment (outclinic patients and hospitalized patients).

During the study the precision of the different chromogenic assays was checked using two lyophilized pooled patient plasmas.

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PROSPECTS FOR CHROMOGENIC AND FLUOROGENIC SUBSTRATE USE IN BIOCHEMISTRY AND BLOOD PROTEASE ASSAY. Craig M. Jackson. Department of Biological Chemistry, Washington University School of Medicine, St. Louis, MO.

Kinetic parameters describing the hydrolysis of peptide p-nitroanilide substrates by thrombin, Factor Xa, and activated Protein C indicate that a high degree of selectivity for each of these proteases can be achieved by using appropriate substrates. Determination of kcat (the maximum velocity per mole of enzyme) for peptide p-nitroanilide substrates indicates that sensitivity sufficient to detect pM concentrations of these proteases can be obtained. The use of fluorogenic substrates should increase sensitivity, although the absence of data for maximum velocities of hydrolysis of peptide fluorogenic substrates precludes quantitative statements about the extent of increase in sensitivity that may actually be obtained. The use of more than one substrate provides an opportunity to selectively assay individual enzymes present in a mixture of proteases. The selectivity of the various assays can be enhanced by use of competitive inhibitors such as those developed in several laboratories for use as potential antithrombotic agents. Although manual methods may be too tedious to be practical in complex situations, the automated methods which handle multiple samples can make such multiple-substrate based assay methods practical. The fact that a single function of the protease coagulation factors, namely the peptide bond hydrolysis function is being assessed by peptide chromogenic and fluorogenic substrates will permit more specific information to be obtained about these multifunctional molecules.