2008

THE DIRECT DEPLETION AND RECOVERY OF HUMAN COAGULATION FACTOR IX FROM PLASMA USING IMMOBILISED MONOCLONAL ANTIBODY

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An immobilised monoclonal antibody (ESN1) was repeatedly used for the preparation of artificial factor IX (FIX) deficient plasma (AND), and subsequent recovery of bound FIX. Column depletion removed >99% of the FIX from plasma, without affecting other clotting factors. Results of FIX assays performed with AND showed excellent correlation (r=0.981) with those using congenital FIX deficient plasma for plasma obtained from normal subjects, from patients on warfarin treatment, and congenitally FIX deficient subjects. Moreover, 70-90% of bound FIX was each time recovered from the ESN1 column in active form by elution with 7 M urea. After concentration and absorbtion of the eluate with protein A Sepharose CL4B, western blotting of non-reduced and reduced FIX showed a single major band which co-migrated with conventionally purified FIX. Repeated use of the ESN1 column (>9 times) has not hindered AND preparation or the recovery of bound FIX in active form.

2009

IRREVERSIBLE INHIBITION OF THE THROMBIN-MEDIATED SIGNAL TRANSFER.

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The inhibition of the thrombin-mediated signal transfer by a common irreversible inhibitor Z of the factor Xa complex (X_a^c) and thrombin has been analysed for the two-step process of the X_a^c-triggered formation of thrombin and the consecutive splitting of a thrombin-specific substrate S. Assuming that both proteolytic processes follow simple Michaelis-Menten kinetics, that the inhibition reactions are second-order and that the prothrombin and irreversible inhibitor are in excess it can be shown that:

1. clotting time (t_c) is inversely proportional to the time-averaged thrombin concentration
2. the endpoint of the conversion of the thrombin specific substrate S reached at exhaustion of thrombin and the X_a^c is inversely proportional to the square of the inhibitor concentration
3. the continously monitored thrombin generation inhibition is a more sensitive assay than the classical two-stage thrombin generation inhibition assay
4. the shift in the effective concentration range of the continuously monitored thrombin generation inhibition assay relative to the continuously monitored anti-Xa assay and to that of the continuously monitored anti-IIa assay, depends on the initial rate of formation of thrombin with the thrombin generation inhibition assay and the original enzyme concentrations of the anti-enzyme assays.

It can further be shown that the above conclusions still hold when the Z-mediated (with Z = antithrombin III e.g.) inhibitions are potentiated by heparin(oid)s.

2010

PHOTOMETER-LINKED MICROCOMPUTER SYSTEM ENABLES PRECISE CHROMOGENIC PROTHROMBIN TIME ASSAYS.

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In coagulation diagnostics photometric assay procedures are in widespread use. Due to the availability of new specific chromogenic peptide substrates, automated instruments play an important role in clinical routine laboratory diagnosis. For research work, the benefit of expensive industrial photometric coagulation systems is questionable, especially as the program cannot be adapted by the user. For this purpose we developed an inexpensive microcomputer-controlled measuring system as well as a suitable photometric assay which allows to determine chromogenic clotting times with any conventional spectrophotometer.
Absorbance data were taken from the analog chart-recorder output of a double-beam spectrophotometer, digitized by a 12 bit analog-to-digital converter and read by the computer via an interface. Menu driven, user orientated / user dialogue based compiled BASIC software was written to enable data acquisition and processing.
During the chromogenic assay procedure, automatically collected absorbance data were displayed, stored, analyzed immediately, saved on disk for later kinetic analysis and printed.
Preliminary results with our chromogenic PT-assay indicate excellent reproducability of the test. The clotting time itself is defined as the interval from the beginning of the test to the moment when a preselected absorbance change occurred. Standard curves can automatically be calculated by regression routines after measurement of reference values.
It must be emphasized that the occurence of fibrinogen-generated turbidity during the chromogenic assay sometimes influences the total absorbance significantly. For this reason the reaction time has to be limited by a low optical endpoint setting.

2011

STUDIES ON PLATELET AGGREGATION BY IMPEDANCE AGGREGOMETRY AND ATP SECRETION IN NON-ANTICOAGULANT BLOOD.

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Investigations in vitro on the time-dependent increase in thrombin activity and platelet function have been used to characterize the kinetics of the clotting process in non-anticoagulated blood. The test procedures described involve great effort and expense and therefore are not suitable for routine tests in pharmacology and clinical pharmacology. The present contribution describes the determination of clotting times in ATP secretion in the Chrono-Log Whole Blood Aggregometer.

Blood was taken from healthy donors who had not used any drug in the two weeks before the trial. 0.5 ml blood were immediately transfered into siliconized glass cuvettes containing 0.4 ml saline and 0.1 ml luciferin-luciferase cocktail prewarmed to 37°C. Impedance and luminescence were recorded continuously. Clotting at the electrodes is indicated by an immediate steep rise in both impedance and luminescence. Clotting time is defined as the time from diluting the blood in the cuvettes until the point at which marked elevation of these variables begins.

In the blood of twelve subjects the mean clotting time was 3.8 min and intersubject variation (SD) was 0.45 min. Drastic interindividual differences in response to collagen and ADP in citrated whole blood were observed in the study group.

In vitro addition of 20 µl Fibraccel (Behringwerke AG, Marburg, FRG), a platelet factor 3 containing platelet extract decreased clotting time by 35 % (n=10). In the presence of 0.2 U heparin a slow and long-lasting increase in impedance was seen. 1 g oral Aspirin® did not influence clotting time measured ex vivo.

The results indicate that whole blood aggregometry is a simple, fast, and precise method of determining blood clotting and the effects of drugs in a medium reflecting almost physiological conditions.