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COMPARATIVE INVESTIGATION OF CLOTTING AND CHROMOGENIC ASSAYS FOR HEPARIN AND LOW MOLECULAR WEIGHT HEPARIN-(OID) IN PLASMA OF VOLUNTEERS AND PATIENTS.

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Two recently developed and simplified anti-Xa methods, a chromogenic assay and a clot-based assay (Heptest) were investigated on their accuracy and ex-vivo characteristics. The coefficients of variation (c.v.) for heparin, a heparin fragment (K 2165) and a LMW heparinoid (Org 10172) were respectively: Heptest, within assay c.v.: 5.4; 4.5; 5.0 % between-assay c.v.: 7.8; 5.8; 9.5 %. Chromogenic Anti-Xa assay, within assay c.v.: 3.7; 5.6; 4.6 %, between assay c.v.: 6.6; 8.2; 12.1 %.In plasma samples obtained from volunteers and patients who participated in clinical studies using heparin, K 2165 and Org 10172, the ex-vivo correlation between both assays were determined. Generally, heparin and K 2165 induced anti-Xa activities correlated very well ($0.83 < r < 0.99$) and the slopes of the regression lines approached the value of 1. K 2165 in haemodialysis patients produced much higher Heptest values compared to the chromogenic anti-Xa assay, for which no explanation is provided. For Org 10172 the anti-Xa assay was more sensitive than the Heptest, although both tests detected anti-Xa activities at very low levels (± 0.02 U/ml). After s.c. injection of Org 10172 a poor correlation was found ($r=0.49$), which may be due to inter-individual differences in bio-availability. In conclusion, the chromogenic anti-Xa assay and the Heptest are accurate and sensitive assays. However, the tests give substantial differences in results depending upon the heparin preparation and patient category treated.

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AMBULANT MONITORING OF HEPARIN AND HAEMOSTASIS DURING VASCULAR SURGERY.

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Heparin has been used to prevent thrombotic events in vascular surgery for more than 45 years. Heparin-activity monitoring has been advocated, but is not usually performed routinely. A direct method for measuring heparin anti-Xa activity with a chromogenic substrate is difficult to perform during surgery for logistic reasons or lack of suitable equipment. The use of this assay, however, could give us a better insight in the kinetics of heparin during vascular surgery. To use this heparin-activity assay during vascular surgery in combination with clotting assays and a antithrombin III-assay, one should be able to perform both kinetic and end-point methods in a rapid, simple and reproducable manner. Therefore, in this study we tested the FP-910 coagulation analyser (Labsystems), in which both methods can be assayed. It was used during 20 consecutive abdominal aorta reconstruction operations, in which a standard dose of 4-5,000 IU heparin was given intravenously. Bloodsamples were taken at several intervals. APTT, PT, ThT, fibrinogen, AT-III and heparin could be assayed within 30 min. after a bloodsample was taken, with good intra-variations (1.8%, 3.3%, 3.9%, 7.5%, 4.9% and 5.0%, ersp.) and intervariations (5.0%, 4.5%, 3.8%, 11%, 8.9% and 6.1%, resp.). Heparin activity alone could be measured within 10 min. The pre-operative samples showed no abnormalities. Heparin activity response 5 min. after injection showed a wide variation (0.2-2.8 IU/ml) and this was also seen in the individual heparin elimination rates. In one case a thrombotic complication occured during a period of low heparin activity (0.1 IU/ml). In another patient a combined decrease of fibrinogen and AT-III, to 30% and 37% resp. of the initial value was seen. These results showed that, during vascular surgery, a close monitoring of heparin activity with a anti-Xa activity assay, together with other haemostasis parameters is necessary, and possible in a rapid and simple way, using the FP-910 coagulation analyser.

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ACTIVATED PARTIAL THROMBOPLASTIN TIME MONITORING OF HEPARIN THERAPY: COMPARISON OF INSTRUMENTS AND REAGENTS.

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It is current clinical practice to monitor heparin therapy by maintainig the activated partial thromboplastin time (APTT) at 1.5-2.5 x control normal pool. There is however controversy regarding the choice of reagents with respect to their heparin sensitivity. Choice of instrumentation is also known to affect the results. We have compared two automatic coagulometers, the ACL (Instrumentation Laboratory), a laser-nephelometric centrifugal analyzer and the KOAGULAB 40A (Ortho Diagnostics), an optical automatic coagulometer, with the tilt tube technique for the performance of APTT. Seven commercial reagents have been used for APTT replicate determinations in 30 normals, 30 liver disease patients and 30 patients on heparin therapy (20-40,000 IU daily). The overall observed imprecision (C.V.) was 1.8% for ACL, 3.0% for KOAGULAB 40A and 2.3% for tilt tube technique. The F test for the two-way interaction of ratios was statistically significant ($p < 0.001$) for the large majority of reagent/technique combinations in normals, liver disease and heparin treated patients. The percentage of patients adequately, inadequately and excessively heparinazed obtained with the two automatic instruments and with the tilt tube technique were not significantly different when using the same reagent. However, major differences were observed when comparing the different reagents. For instance, inadequately heparinized patients were 90% according to one reagent (Cephotest, Nyegaard) as opposed to 17% according to another reagent (APTT, Instrumentation Laboratory). These results stress the need for a standardized APTT reagent to provide effective laboratory monitoring of heparin treatment.

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COMPARISON OF VARIOUS AMIDOLYTIC HEPARIN ASSAYS: REPORT OF A COLLABORATIVE STUDY.

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The authors performed a controlled study to compare commercially available amidolytic heparin assays based on anti-Xa or anti-IIa systems. All participants (see below) were provided with the same batch of all heparin preparations and reagents including homogenous plasma preparation. In addition plasma was spiked with the various unfractionated (n=2) as well as lmwh- (n=5) and heparinoid preparation (n=1). The four university laboratories analyzed all heparin preparations that are registered or used in clinical trials in FRG (n=8). The two testkit producers analyzed registered heparin preparations and the heparin producer their brand only. The study was recently completed and the data analysed and collected. The first evaluation revealed that the amidolytic anti-IIa assay is insensitive for lmwh and heparinoids. Determination of unfractionated heparins is not accurate below 0.5 units of the corresponding standard. The Xa-methods are more sensitive for lmwh and heparinoids, however these methods also reveal a high standard deviation. Estimation of concentrations below 0.5 units of all tested heparin preparations is questionable.

The authors are indepted for active cooperation to Prof. Dr. K. Andrassy (Med. Univ. Heidelberg), Dr. M. Biegholdt (MIDY LABAZ), Dr. G. Dertinger (Sandoz AG), Dr. W. Feller (Braun Melsungen AG), Dr. P. Friberger (Kabi Vitrum Stockholm), Dr. P. Wellstern (Univ. Klinik Homburg), Dr. D.G. Meulemann (Organon Scientific Development Group), Dr. K. Müller (Nordmarkwerke), Dr. Zimmer (Boehringer Mannheim GmbH).