1363

EXPERIMENTAL THROMBUS FORMATION AND HAEMOSTASIS OF DIFFERENT LOW MOLECULAR WEIGHT HEPARINS AND DOSAGES. R.Zimmermann, C.Rieger,C.W.Hübner,J.Harenberg,W.Kübler, Medizinische Universitätsklinik, 6900 Heidelberg 1, GFR

Low molecular weight heparin induces a higher anti factor Xa (a-Xa) and a lower antithrombin activity in plasma in comparison to conventional heparin.From this constellation a more pronounced antithrombotic effect and a minor incidence of bleeding complications has been suggested.

Therefore the antithrombotic activity of heparins was studied in a standardized experimental thrombosis model in rabbits. Three low molecular weight heparins with a mean molecular weight of 4.200 (heparin I),4.000 (heparin II),4.600 Dalton (heparin III) and standard heparin were tested at different dosages in 120 experiments.In the first series the dose of 60 anti Xa units (a-Xa U) given initially and 60 a-Xa U/kg/h induced a reduction of the thrombus size by 40 % (heparin I),37 % (heparin II) and 53 % (heparin III) and a prolongation of the aPTT to 45 (heparin I),66 (heparin II) and 79 sec (heparin III). The a-Xa activity was minor than 0.1 U/ml.In the second series heparins were given to aim at an a-Xa activity of 0.2-0.3 U/ml.Thereby the thrombus formation could be reduced by 84 % (heparin I), 62 % (heparin II) and 39 % (heparin III). aPTT and a-Xa activity were measured at 65.5 sec and 0.22 a-Xa U/ml (heparin I),67.3 sec and 0.3 a-Xa U/ml (heparin II) and 67.5 and 0.31 a-Xa U/ml (heparin III),respectively. In the third series the increase of the a-Xa activity to more than 0.3 U/ml showed no further reduction of the thrombus formation by heparin I, while heparins II and III already at this level reached the antithrombotic activity of heparin I.

Our data on three different low molecular weight heparins demonstrate that already a heparin level ranging at a minimal a-Xa activity induces a clear and statistically significant antithrombotic effect. A higher heparin dosage with higher a-Xa activity increases the antithrombitic effect.At a level of 0.2-0.3 a-Xa U/ml an obvious and maximum effect could be reached, but the further elevation of the a-Xa activity produced no further antithrombotic action.

1364

PHARMACOLOGIC INEQUIVALENCE OF LOW MOLECULAR WEIGHT HEPARINS. J. Fareed, J.M. Walenga, D. Hoppensteadt, R.M. Emanuele and A. Racanelli. Loyola University Medical Center, Maywood, IL 60153.

Compared to unfractionated heparin, low molecular weight heparins (LMWHs) have been found to exhibit marked variations in in vitro effects due to variations in molecular weight and structure. Moreover, when the in vitro potency of these agents is equally adjusted by pharmacopeial assay (current and proposed) wide variations in the in vivo responses have been noted. These variations were strongly dependent on the route of administration. Utilizing defined animal models, a systematic comparative study of the in vivo responses of seven commercial LMWHs was undertaken. Choay Fraxiparine (CY 216), Choay CY 222, Novo LHN, Kabi Fragmin, Opocrin 2123 (OP), Hepar RD 11885 (RD), Pharmuka Enoxaparin (PK) and Choay porcine mucosal heparin (PMH) were tested in identical settings at equigravimetric dosages. The graded results are given in the following.

ANTITHROMBOTIC ACTIONS (Rabbit stasis thrombosis model)

PCC/RVV (IV):	PMH>CY 222>NOVO>CY216>PK>KABI>OP>RD
PCC/RVV (SC):	KABI>RD>PMH>CY 216>PK>OP>NOVO>CY 222
FEIBA® (IV):	PMH>CY 216=KABI=OP=NOVO>CY 216>PK>RD
FEIBA® (SC):	CY 216=PK=KABI=OP=NOVO=CY 222>PMH>RD

CUMULATIVE BIOAVAILABILITY (Macaca mulatta model)

AXa/AIIa/Heptest®: CY 222>CY 216>PK>KABI>NOVO>OP>RD>PMH

HEMORRHAGIC INDEX (Rabbit ear and rat tail models)

Rabbit ear:	NOVO>KABI>RD>PK>OP>CY 216>PMH>CY 222
Rat tail:	PMH>NOVO=KABI>RD>OP>PK>CY 216>CY 222

Wide variations in the in vivo pharmacologic and toxicity responses were noted suggesting that different LMWHs are not bioequivalent at equigravimetric levels. When these responses were expressed in anti-factor Xa or pharmacopeial potency, these differences were further magnified. The clinically reported dosimetric and safety problems may be minimized by profiling LMWHs in defined in vivo test systems to optimize their safety/efficacy ratio.

1365

STUDIES ON THE ANTITHROMBOTIC ACTION OF A LOW MOLECULAR WEIGHT HEPARIN OBTAINED FROM BEEF MUCOSAL ORIGIN AND PEROXIDATIVE CLEAVAGE. P. Bianchini (1), R. Nonn, J. Fareed, J.M. Walenga, and A. Kumar. (1) Research Laboratories, OPOCRIN, Corlo, Italy, and Loyola University Medical Center, Maywood, IL 60153 USA.

We have studied a low molecular weight heparin (LMWH) obtained by a controlled peroxidative depolymerization of beef mucosal heparin (OP 2123, Opocrin, Corlo, Italy). This product was found to be significantly different from other LMWHs in that it exhibits the same COO^-/SO_3^- ratios as unfractionated heparin, contains reducing end groups composed of 2-sulfated iduronic acid or 6-disulfated glucosamine and retains an identical structural integrity as that of native heparin. As opposed to most other LMWHs the oligosaccharide components of OP 2123 consist of homogeneous progressive units. In addition, the relative amount of AT-III affinity components in OP 2123 were 20-30% less than other LMWHs. OP 2123 has a mean molecular weight of 6200 daltons with a potency of 90 anti-factor Xa U/mg and 68 USP U/mg. This agent produced strong antithrombotic actions in a rabbit stasis model against both an activated prothrombin complex and a prothrombin complex concentrate/Russell's viper venom combination (ED50:(IV) 30-70 ug/kg; (SC) 0.6-1.5 mg/kg). The antithrombotic effects were comparable to other LMWHs in normal rabbits; however, in AT III depleted rabbits (immunodepleted and γ thrombin depleted), OP 2123 produced stronger antithrombotic effects than most other LMWHs. The in vitro systems in contrast to other LMWHs, OP 2123 produced stronger inhibitory effects in AT III depleted plasma as measured by fibrinopeptide A generation and amidolytic anti-factor Xa and anti-factor IIa methods. The relative heparin cofactor II activity as measured by amidolytic method was also found to be higher than with most LMWHs. These results suggest that OP 2123, unlike most LMWHs, non AT III mediated actions play a major role in the mediation of the antithrombotic actions.

1366

MEASUREMENT OF HEPARIN IN PLASMA: INFLUENCE OF INTER-SUBJECT AND CIRCADIAN VARIABILITY IN HEPARIN SENSITIVITY ACCORDING TO METHOD. M.F. Scully, H.A. Decousus*, V. Ellis, P. Girard*, C. Parker and V.V. Kakkar. Thrombosis Research Unit, King's College School of Medicine & Dentistry, Denmark Hill, London SE5 8RX, UK. *Hopital de Bellevue 42023, Saint Etienne, France.

Heparin was measured, with respect to standard curves prepared with normal pooled plasma, by five methods, APTT, (Auto-APTT), thrombin time (Thromboquik), one (Heptest) and two-stage (Hepaclot) coagulation, anti-factor Xa and chromogenic anti-factor Xa after addition at three concentrations (0.2, 0.4, 0.6iu/ml) to plasma prepared from three groups of ten individuals: normal young volunteers, hospitalized patients with malignancy and hospitalized geriatric patients. By the APTT and TT, differences in sensitivity were observed, for example, at 0.4iu heparin/ml corresponding to an apparent difference in heparin level of 10- and 14-fold between high and low responding individuals. All low responders, by APTT and TT, were in the older hospitalized group, tending to overheparinization when monitoring by these methods. Such large differences were not apparent by any of the anti-factor Xa assays. A circadian difference (1.9 and 1.8 fold) in sensitivity was also observed in the patient group such that in samples taken at night (12 pm), heparin levels were 30-50% higher than those taken during the morning on average when measured in the APTT and TT ($p<0.001$, analysis of variance). Again, such large differences were not apparent by anti-factor Xa methods. In light of recent findings about the usefulness of anti-factor Xa methods for efficient monitoring of heparin, it is suggested that this conclusion may arise from the tendency for anti-factor Xa methods to determine actual concentrations of heparin.