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SUBCUTANEOUS PHARMACOKINETICS/PHARMACODYNAMICS OF A LOW MOLECULAR WEIGHT DERIVATIVE (RD 11885) AND UNFRACTIONATED HEPARIN (PM 16885) IN PRIMATES. R. Nonn, J. Fareed, I. Silber, A. Bello, *R. Fenichel and *R. McGuire. Loyola University Medical Center, Maywood, IL 60153, *Wyeth Laboratories, Philadelphia, PA 19101.

Subcutaneous pharmacokinetics/pharmacodynamics of a depolymerized low molecular weight heparin (RD 11885) and an unfractionated porcine mucosal heparin (PM 16885) were studied in primates (Macaca mulatta) at 0.5, 1.0 and 2.5 mg/kg/24 hr for 10 days after repeated administration. Ex vivo actions were determined using partial thromboplastin time (APTT), thrombin time (TT), Heptest^{R} time (HT), anti-factor Xa and anti-factor IIa assays at various time periods. Platelet counts and bleeding times were also measured. The cumulative bioavailability of RD 11885 calculated ex vivo was found to be 2-3 fold higher than PM 16885. The RD 11885 treated group exhibited a clear dissociation of the anti-factor Xa and anti-factor IIa activities. The biological half-life of RD 11885 was significantly greater than PM 16885 in all assays. No staircasing phenomenon was observed with either agent. A desensitization of the PM 16885 effects was observed. Neither agent produced any effect on the bleeding time or platelet count at any time. The pharmacokinetics/pharmacodynamics of RD 11885 were uniform and allowed the calculation of various pharmacologic parameters, whereas inconsistent results were obtained with PM 16885. These results demonstrate that this low molecular weight heparin exhibits better and more predictable bioavailability, in contrast to unfractionated heparin.

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CIRCADIAN CHANGES IN THE ANTICOAGULANT EFFECT OF HEPARIN. PHARMACODYNAMIC AND PHARMACOKINETIC EFFECT. H.A. Decousus, M.F. Scully*, J. Reynaud, E. Arnaud-Crozat, C. Boissier, X. Barral, P. Queneau, P. Girard, C. Parker* and V.V. Kakkar*. Hopital de Bellevue, 42023, Saint Etienne, France., *Thrombosis Research Unit, King's College School of Medicine & Dentistry, Denmark Hill, London SE5 8RX, UK.

Six patients, with thromboembolic arterial disease, were prospectively studied to assess the influence of the time of injection of a constant dose of calcium heparin (Choay), given subcutaneously, on the level of heparin measured by APTT and anti-Xa assay. For each patient, the initial dose of heparin was adjusted by APTT 6h after a morning injection to 1.5 and 2.5 times control. Dose was then kept constant. Four randomized times of injection were tested (8am, 4pm, 8pm & 12pm), in each patient acting as his own control. Blood was sampled via a cannula, at 0h, 2h, 3h, 4h, 5h, 6h, 8h, 10h & 12h after injection. The mean APTT and anti-Xa values for the evening injections (8pm & 12pm) were higher than for the morning injection (8am), at 2h until 10h after injection. These differences were significant (analysis of variance:$p<0.001$) and reached almost 30 seconds for mean APTT values measured 4h, 5h and 6h after injection. For the afternoon injection (4 pm) the mean APTT and anti-Xa values were intermediate but significantly different from all the other times of injection (analysis of variance: $p<0.01$).

Blood was sampled also on two consecutive days at 12am and 12pm from eight patients receiving heparin subcutaneously for treatment of DVT (administered at 6am and 6pm respectively). Heparin levels by APTT, TT and three antifactor Xa methods (Heptest, Hepaclot, Chromogenic) were significantly higher at night than morning (analysis of variance $p< 0.005$). Cosinor analysis confirmed these results are consistent with circadian variation as we have previously reported after continuous infusion of UF heparin (Br. Med. J., 1985, 290, 341-344). The observed variation was found to be a resultant of a pharmacodynamic effect (circadian variation in assay response to heparin) and pharmacokinetic effect (circadian variation in ^{99m}Tc-heparin clearance). Such circadian variation should be taken into account when deciding heparin dosage.

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KINETICS OF LOW MOLECULAR WEIGHT HEPARIN IN MAN DETERMINED BY PROTAMINE CHLORIDE. V. Bode (1), R. Franz (1), D. Welzel (2), H. Wolf (2) and J. Harenberg (3). Institute of Pharmacy, University, 8400 Regensburg, FRG, (1), Sandoz AG, 8500 Nürnberg, FRG, (2), and Department of Internal Medicine, Medical University Clinic, 6900 Heidelberg, FRG (3).

Low molecular weight (LMW) heparin is characterized by a higher affinity to antithrombin III, less inhibition of thrombin and increased inhibition of factor Xa. The half life of the antifactor Xa activity of LMW heparin is doubled compared to normal heparin. However, these parameters reflect the pharmacodynamics rather than the kinetic of the compound. We, therefore, analyzed the kinetics of LMW heparin after i.v. injection in man using protamine chloride for gravimetric evaluation of LMW heparin in the plasma samples.

Six healthy adults received 100 units per kg bodyweight normal heparin or 100 anti Xa units per kg LMW heparin (Sandoz AG, Nürnberg, FRG). To serial samples of venous blood protamine chloride was added in serial dilutions until the thrombin inhibition was antagonized. Since factor Xa inhibition of LMW heparin cannot be abolished completely by protamine chloride, two amounts of protamine chloride were added to the plasma samples ex vivo, until factor Xa was inhibited up to 0,2 and 0,04 units/ml. The following maximal plasma concentrations (C max) and half lives (T/2) were calculated (average values):

	C max µg/ml			T/2 min		
	TCT	aXa 0,2/ 0,04		TCT	aXa 0,2/ 0,04	
heparin	82	-	87	60	-	58
LMW heparin	28	88	180	45	70	120

The pharmacokinetics of normal heparin show no differences on thrombin and factor Xa interaction. LMW heparin, however, interacts to 30 % with thrombin and to 100 % with factor Xa; the half life on factor Xa is twice as long as on thrombin; releases endogenous compounds with antifactor Xa activity, which are neutralized only hardly by protamine chloride;and these endogenous compounds mediate in part the longer half life.

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BLEEDING EFFECTS ASSOCIATED WITH HEPARIN CONTAMINANTS. J. Pangrazzi (1), P. Oreste (2), G. Torri (2), A. Maggi (1), M.B. Donati (1) and B. Casu (2). Mario Negri Institute (1) and "G. Ronzoni Institute (2) Milano, Italy.

Clinical use of heparin as an antithrombotic drug is limited by the risk of excessive bleeding, generally ascribed to the anticoagulant activity of this mucopolysaccharide.

Unexpectedly, some low molecular weight heparins with reduced anticoagulant activity are reported to cause bleeding in clinical and experimental studies. To approach this problem, a number of heparin preparations with various molecular weights were characterized by analysis of their ^{13}C-NMR spectra. The bleeding potential of the same heparins was tested by measuring the "template" bleeding time (BT, a method exploring the mechanisms of primary haemostasis) in the rat tail, 15 min after i.v. administration of 0.75 mg/kg b.w. of the drug.

Besides major NMR signals associated with known units of the regular and irregular regions of heparins, some of the preparations display ^{13}C-NMR "extra-signals", which have a higher degree of relative intensity in the spectra of samples with higher haemorrhagic potential; indeed, all the samples exceeding in "extrasignals" a conventional intensity level of 2 had at least 70% prolongation of the BT over controls.

These results suggest that specific contaminants, responsible for these ^{13}C-NMR "extra-signals", may also be responsible for the unusually high bleeding expressed by some heparin preparations. Work is in progress to identify these components and to evaluate which mechanism of primary haemostasis is involved in their haemorrhagic effect.