Investigations on plasma activity of low molecular weight heparin after intravenous and oral administrations

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This study investigated the absorption of low molecular weight heparin (LMWH) after oral administration in man. Six healthy subjects received 5,000 anti-Xa units of LMWH (Kabi 2165) by both i.v. and oral administration. The oral formulations were prepared in pH 4.0 and pH 7.0 buffered solutions. Multiple blood samples were collected after each dose for measurements of anti-Xa, anti-IIa and APTT plasma heparin activities. Pharmacokinetic parameters based on the anti-Xa activity measurements after the i.v. dose were as follows (mean $\pm$ s.d.): $t_{1/2}$, 1.82 ± 0.23 h; V , 4.34 ± 0.65 l; and CL , 28.0 ± 6.2 ml min⁻¹. Half-life values based on the anti-IIa and APTT activities were 1.63 ± 0.43 and 1.09 ± 0.51 h, respectively. Considerable prolongations in APTT were observed, with APTT at 30 min averaging 55.7 ± 4.1 s (1.84 ± 0.27 times baseline values). After oral administration, no measurable plasma heparin activities were observed with either LMWH preparation. The results of this investigation indicate that LMWH does not have detectable plasma activity after oral administration, and that after i.v. administration it has significant anti-IIa and APTT activities in addition to its anti-Xa activity.

Keywords heparin absorption pharmacokinetics

Introduction

Early studies on the enteral absorption of heparin, involving introduction of heparin into the alimentary tracts of mice, dogs and rabbits, did not reveal any detectable absorption (Loomis, 1959; Fisher & Astrup, 1939). However, absorption was observed when heparin was instilled into the stomach of mice (Sue *et al.*, 1976). Its absorption was enhanced when it was diluted in an acidic buffer at pH 4.0 (Koh & Bacharucha, 1972), in weak acids, e.g., citric acid or tartaric acid, or in hydrochloric acid (Sue *et al.*, 1976). Thus, ionic suppression may represent one means of enhancing the absorption of heparin from the gastrointestinal tract. Studies on pharmaceutical modifications of heparin have shown that its absorption can also be enhanced when it is coupled with EDTA (Windsor & Cronheim, 1961), mixed with sulphated or sulphonated surfactants (Engel & Riggi, 1969),

or when heparinic acid is mixed with weak nitrogenous bases or amino acids, e.g., glycine (Koh & Bacharucha, 1972). Another potential method of enhancing absorption is to take advantage of its molecular weight dispersity. Studies on differing molecular weight fractions of heparin, which were instilled in 3% citric acid into the small intestine of mice, demonstrated that the extent of absorption increased with decreasing molecular weight (Sue *et al.*, 1976). More recent studies in rabbits and rats have provided evidence for absorption of low molecular weight heparin species (Doutremepuich *et al.*, 1984; Larsen *et al.*, 1986).

Heparin fractions of decreasing molecular weight exhibit a progressive loss of activated partial thromboplastin time (APTT) activity and anti-IIa activity, but not of anti-Xa activity (Anderson *et al.*, 1976; Holmer *et al.*, 1981).

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Thus, the anti-Xa to anti-IIa heparin activity ratio increases with decreasing molecular weight of heparin. This is thought to be a desirable property, resulting in greater therapeutic index, with greater antithrombotic effect and lower risk of haemorrhagic complications (Holmer, 1987). The purpose of the present study was to investigate plasma low molecular weight heparin (LMWH) activity after i.v. and oral administrations to healthy human subjects. The low molecular weight heparin used in this study (Kabi 2165; KabiVitrum) contains molecules with high and low affinity for antithrombin III and has an average molecular weight of 4,000 to 6,000, with 80% of the material between 3,000 and 8,000 (Holmer, 1987).

Methods

Materials

The LMWH used was Kabi 2165 (Fragmin®, KabiVitrum, Inc.; ampules of 10,000 anti-Xa units ml^{-1} ; batch number 5758751), with the following specifications: molecular weight 4,000–6,000 D; specific activities 144.5 anti-Xa units mg^{-1} and 43.3 APTT units mg^{-1} , and an anti-Xa/APTT activity ratio of 3.34. The chromogenic substrates S-2222 and S-2238, thrombin, Factor Xa and antithrombin III were obtained from KabiVitrum. The APTT reagent (Automated APTT®) and the prothrombin time (PT) reagent (Simplastin A®) were from General Diagnostics (Morris Plains, NJ). The intravenous preparation was used as supplied. The oral LMWH preparations consisted of 5,000 anti-Xa units of LMWH diluted in 100 ml of cold citrate buffer (0.05 M, pH 4.0) or in 100 ml of cold phosphate buffer (0.05 M, pH 7.0) solutions. The salt concentration was adjusted to 0.9% (w/w) by the addition of sodium chloride.

Study design

This was a balanced, randomised, three-way cross-over study involving six healthy volunteers (five males and one female). Their mean ($\pm$ s.d.) body weight was 74 ± 11 kg, ranging from 55 to 89 kg, and their age averaged 26 ± 5.9 years, ranging from 22 to 37 years. The study was approved by the University's Institutional Review Board, and the participating volunteers gave their informed consent. Prior to the study, all the volunteers had normal blood laboratory values, platelet counts, coagulation times and bleeding times. For 14 days before and during the study they refrained from smoking and

from taking any drugs known to interfere with haemostasis. All subjects fasted for 12 h prior to the study. The oral doses were immediately followed by 25 ml of deionized water. The volunteers received the alternate treatments following 1 week washout periods. Blood samples were collected through indwelling catheters or by venepuncture at 0, 15, 30, and 45 min, and 1, 1.5, 2, 3, 4, 5, 6 and 8 h after each dose. The samples were collected into polystyrene tubes containing 1 ml of 3.8% sodium citrate solution per 9 ml of collected blood. Plasma was prepared by centrifugation at 2,000 g for 20 min at 4° C and kept on ice until analysed. All samples were analysed on the day of the study.

Plasma heparin assays

Plasma heparin activity in each sample was determined by anti-Xa, anti-IIa and APTT assays. The anti-Xa assay was performed according to the method of Teien *et al.* (1976), with minor modifications. Briefly, 10 μl plasma, 10 μl antithrombin III (1 unit ml^{-1}) and 80 μl Tris buffer (pH 8.4) were incubated with 50 μl Factor Xa (1 unit ml^{-1}) for 30 s at 37° C. S-2222 (1.0 mM; 100 μl) was then added, and the reaction was stopped after 180 s by the addition of 100 μl of glacial acetic acid. Plasma LMWH equivalents were determined from standard curves constructed for each treatment in each volunteer by adding various amounts of LMWH (in anti-Xa units) to that subject's plasma drawn prior to drug administration. Two calibration curves were always performed; one for concentrations ranging from 0.05 to 0.4 anti-Xa unit ml^{-1} , and the other for the concentrations ranging from 0.4 to 1.2 anti-Xa unit ml^{-1} . The lower limit of sensitivity of the assay is 0.05 anti-Xa unit ml^{-1} .

Anti-IIa activity was measured according to the method described by Larsen *et al.* (1978). Briefly, 2 μl of plasma, 78 μl Tris buffer and 20 μl antithrombin III (1 unit ml^{-1}) were incubated with 50 μl thrombin (1.76 nkat) for 30 s at 37° C. Then, S-2238 (0.72 mM; 100 μl) was added and the reaction stopped after 60 s by the addition of 100 μl of glacial acetic acid. As with the anti-Xa assay, standard curves were constructed for each treatment in each subject. Lower limit of sensitivity is 0.05 anti-IIa unit ml^{-1} .

The APTT bioassay was performed as previously described (Bjornsson *et al.*, 1982). Plasma APTT units were determined from standard curves which were constructed for each treatment in each subject by adding various amounts of LMWH (in APTT units) to plasma

collected before drug administration. These curves exhibited log-linear relationships as previously observed for standard heparin (Bjornsson & Wolfram, 1982). Note that APTT refers both to APTT activity in unit ml^{-1} and APTT in s. The former is based on the APTT activity of LMWH reported by the manufacturer and the individual standard curves, whereas the latter is the observed coagulation time.

Kinetic and statistical analyses

The pharmacokinetic parameters elimination half-life ($t_{1/2}$), apparent volume of distribution (V), total clearance (CL), and mean residence time (MRT) were computed using conventional expressions following linear least-squares regression analysis of the monoexponential decay curve of plasma heparin levels with time (Bjornsson & Wolfram, 1982; Gibaldi & Perrier, 1982). The average coefficients of determination (r^2) for the linear regressions were 0.983 ± 0.010 , 0.962 ± 0.040 and 0.918 ± 0.077 for the anti-Xa, anti-IIa and APTT-heparin levels vs time curves, respectively.

The statistical significance of differences among individual pharmacokinetic parameters obtained after the different heparin assays was assessed using Wilcoxon signed-rank test (Snedecor & Cochran, 1980). Linear regression analysis was used to determine the statistical significance of relationships between different parameters. Statistical significance was accepted when two-tailed $P < 0.05$. Unless otherwise indicated the results are expressed as mean $\pm$ s.d.

Results

Pharmacokinetic parameters

Plasma activities of LMWH declined monoexponentially after i.v. injection of 5,000 anti-Xa units. Figure 1 shows the time courses of average plasma anti-Xa levels (upper panel) and anti-IIa and APTT levels (lower panel) for the six subjects studied. The average plasma anti-Xa activity at 15 min after the i.v. dose was 1.00 ± 0.12 anti-Xa unit ml^{-1} . The pharmacokinetic parameters based on the anti-Xa levels were as follows: $t_{1/2}$, 1.82 ± 0.23 h; V , 4.34 ± 0.65 l; CL, 28.0 ± 6.2 ml min^{-1} ; and MRT 2.63 ± 0.34 h. Adjusted for body weight, the anti-Xa-based V averaged 59.3 ± 10.5 ml kg^{-1} and CL averaged 0.382 ± 0.087 $\text{ml kg}^{-1} \text{min}^{-1}$. No detectable anti-Xa levels were observed after either of the

two oral formulations of LMWH, indicating no detectable oral absorption.

The $t_{1/2}$ values based on the anti-IIa and APTT levels, averaged 1.63 ± 0.43 and 1.09 ± 0.51 h, respectively; these were not significantly different from each other. However, the $t_{1/2}$ estimate based on the APTT levels was significantly shorter than that based on the anti-Xa levels ($P = 0.023$). Also, the V and CL estimates based on the anti-IIa levels differed from those based on the anti-Xa levels. The estimate for CL was 43.1 ± 17.9 ml min^{-1} (0.583 ± 0.223 $\text{ml min}^{-1} \text{kg}^{-1}$), which is 1.5 times as large as that based on the anti-Xa levels ($P = 0.014$). The estimate for V was 6.31 ± 4.00 l (84.9 ± 49.8 ml kg^{-1}), which is also 1.5 times as large as that based on the anti-Xa levels, although the difference did not reach statistical significance ($P = 0.086$). No detectable anti-IIa or APTT activities were observed after the oral doses.

Anticoagulant activity

Figure 2 shows the observed APTT values in s after the three administrations of LMWH. Significant increases in APTT were observed up to

Figure 1 a) Mean plasma anti-Xa activity after i.v. injection of 5,000 anti-Xa units of LMWH (Kabi 2165) in six normal volunteers. b) Mean plasma heparin activity after i.v. injection of 5,000 anti-Xa units of LMWH (Kabi 2165) in six normal volunteers. Heparin activity was determined by two different assays, APTT (◆) and chromogenic substrate S-2238 (□) assays. The vertical bars represent $\pm$ s.e. mean.

Figure 2 Mean APTT after i.v. injection ($\square$) and oral administration of 5,000 anti-Xa units of LMWH (Kabi 2165). The oral preparations were made in buffered solutions at pH 4 ($\blacklozenge$) and pH 7 ($\triangle$). The vertical bars represent $\pm$ s.e. mean.

3 h after i.v. administration of LMWH, with APTT at 30 min averaging 55.7 ± 4.1 s (1.84 ± 0.27 times baseline value). No changes were observed in APTT values after either of the two oral preparations of LMWH. No prolongation was observed in prothrombin time after either i.v. or oral LMWH. No changes were observed in platelet counts after any of the doses, and stool guaiac test performed on the day after the oral doses were negative in all volunteers.

Discussion

The first published attempt to administer heparin orally to laboratory animals was in 1939 (Fisher & Astrup, 1939), which was less than 2 years after the publication of the first reports of its successful use for the prevention of thrombosis in patients (Crafoord, 1937; Murray & Best, 1938). While initial attempts were unsuccessful, several subsequent reports have indicated that three different approaches may be useful to enhance the absorption of heparin after oral administration, i.e., ionic suppression (Sue *et al.*, 1976; Koh & Bacharucha, 1972), pharmaceutical modification (Koh & Bacharucha, 1972; Windsor & Cronheim, 1961; Engel & Riggi, 1969), and fractionation into smaller molecular weight species (Sue *et al.*, 1976; Douthremepuich *et al.*, 1984; Larsen *et al.*, 1986). Two recent studies have indicated that low molecular weight fractions of heparin were absorbed into the systemic circulation. The first study reported that only doses as high as 17,500 anti-Xa units kg^{-1} caused significant prolongation of the thrombin time when LMWH was injected into the duodenum of rabbits, whereas the lowest

dose which produced an increase in plasma anti-Xa activity was about 8,750 anti-Xa units kg^{-1} (Douthremepuich *et al.*, 1984). The second study demonstrated the appearance of radiolabelled heparin fragments in the plasma after the injection of 15,000 USP units kg^{-1} of standard heparin into the stomach of the rat (Larsen *et al.*, 1986). Neither of these studies, however, provided any estimate of the fraction absorbed of the administered doses.

The present investigation on the oral absorption of LMWH in man using a dose of 5,000 anti-Xa units, or approximately 70 anti-Xa units kg^{-1} , did not reveal any detectable absorption. Given the lower limit of sensitivity of the anti-Xa assay of 0.05 anti-Xa unit ml^{-1} , we would have detected absorption of $\geq 10\%$ of the administered dose. Thus, although this study has not demonstrated that there is absolutely no absorption of LMWH, any absorption in man is at best likely to involve only a few per cent of the administered dose. However, studies using higher doses should be undertaken with caution because of the possibility of adversely affecting sub-clinical gastro-intestinal bleeding. In this regard, it is worth noting that the studies by Douthremepuich *et al.* (1984) and Larsen *et al.* (1986) in animals used doses that were 100–200 times as high as that used in our study. The low *in vivo* levels observed in their studies thus suggest that only a small fraction of the administered dose was absorbed in the animals.

The pharmacokinetic parameters of LMWH Kabi 2165 following the i.v. injection of 5,000 anti-Xa units based on the plasma anti-Xa levels involved $t_{1/2}$ of almost 2 h, V of about 4.5 l and CL of about 30 ml min^{-1} . These parameter values are similar to those reported earlier (Bratt *et al.*, 1985, 1986). In contrast to the pharmacokinetics of standard heparin which exhibit distinct dose-dependent characteristics (Bjornsson *et al.*, 1982), the pharmacokinetics of LMWH have not displayed significant dose-dependent behaviour. However, as with standard heparin (Bjornsson *et al.*, 1982), LMWH pharmacokinetics also exhibit assay-dependent characteristics. When its pharmacokinetic parameters were based on the anti-IIa assay, both its CL and V estimates were about 1.5 times as large as those based on the anti-Xa assay, while there was essentially no difference in the $t_{1/2}$ estimates. A possible explanation for these differences is that the larger molecular weight species of LMWH are more bound to tissues, e.g., the vascular endothelium, and are cleared more rapidly than the smaller molecular weight species. This is because the anti-IIa/anti-Xa activity ratio of heparins of different molecular weights increases with

molecular weight (Holmer, 1987), and increasing molecular weight is associated with greater binding to vascular endothelial cells (Barzu *et al.*, 1984).

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