The pharmacokinetics of dermatan sulphate MF701 in healthy human volunteers

J. DAWES¹, M. McLAREN², C. FORBES², J. J. F. BELCH², D. A. LANE³, B. BRAY³, J. McEWEN², G. HOUIN⁴ & F. GIANESE⁵

¹Heart Research Institute, Sydney, Australia, ²Ninewells Hospital and Medical School, Dundee, Scotland, ³Charing Cross and Westminster Medical School, London, England, ⁴Unité de Pharmacocinétique, Hôpital Purpan, Toulouse, France and

⁵Mediolanum Farmaceutici, Milan, Italy

- 1 The pharmacokinetics of dermatan sulphate MF701 were studied in 12 healthy males after administration of single intravenous bolus (200 mg), intramuscular (100 and 300 mg) and oral (1 g) doses. The study was conducted according to a within-subject crossover design in two paired blocks.
- 2 Plasma drug concentrations were measured using a competitive binding assay and a range of biological activity assays, including a sensitive catalysed thrombin inhibition test.
- 3 Following intravenous administration, plasma concentrations of dermatan sulphate determined by competitive binding assay were described by a two-compartment open model with an initial $t_{1/2, \text{in}}$ of 0.6 h and a $t_{1/2, \text{z}}$ of 7.5 h. Biological activity assays were insufficiently sensitive to detect the second phase, and therefore yielded apparent monoexponential kinetics.
- 4 After intramuscular injection the apparent bioavailability of dermatan sulphate was 16–20%. Plasma drug concentrations increased in proportion to dose when measured by competitive binding assay. Low concentrations persisted for more than 24 h at the higher dose, and these may prove therapeutically relevant on chronic administration.
- 5 We confirm that dermatan sulphate is the only glycosaminoglycan known to generate significant plasma concentrations following oral administration. Oral bioavailability was estimated to be 7%.

Keywords dermatan sulphate pharmacokinetics antithrombotic anticoagulant activity safety

Introduction

Dermatan sulphate is a naturally occurring glycosaminoglycan which inhibits blood clotting both by potentiating the inactivation of thrombin by heparin cofactor II (Tollefsen *et al.*, 1983) and by reducing thrombin generation in plasma (Ofosu *et al.*, 1984). Animal studies have indicated that it is an effective antithrombotic agent at concentrations which do not affect *in vitro* tests of anticoagulant activity (Buchanan *et al.*, 1985; Maggi *et al.*, 1987) and that it is also able to inhibit growth of established thrombi (Van Ryn-McKenna *et al.*, 1989). Despite possessing anti-platelet activity (Sié *et al.*, 1982) it appears to be less haemorrhagic than heparin at equivalent antithrombotic doses (Fernandez *et al.*, 1986). Thus, dermatan sulphate is a potential antithrombotic agent with

a different spectrum of pharmacological activity from that of heparin. It is currently undergoing clinical trial in man.

In a preliminary study using single 100 mg intravenous and subcutaneous doses and a 1 g oral dose (Dawes *et al.*, 1989a), we showed that concentrations of dermatan sulphate could be measured in plasma after administration to healthy human volunteers using a competitive binding assay, but that existing anticoagulant assays were insufficiently sensitive as reliable *ex vivo* markers of activity except after intravenous injection. Plasma drug concentrations following intravenous injection appeared to follow multiexponential kinetics, although Dol *et al.* (1989), using a new chromogenic assay, observed

monoexponential decay. The effect of assay methods on the measurement of pharmacokinetic parameters thus requires investigation.

We have now examined the pharmacokinetics and pharmacodynamics of dermatan sulphate MF701 following single intravenous, intramuscular and oral doses in a within-subject four-phase crossover study in healthy human volunteers. Dermatan sulphate was measured both by competitive binding assay and using a range of clotting function tests in an attempt to resolve the previously reported discrepancies.

Methods

Materials

Dermatan sulphate from porcine intestinal mucosa (MF701; 100 mg in 2 ml ampoules batch 01.701.C, and 250 mg capsules batch 01.701.CPS) was prepared by Mediolanum farmaceutici S.r.l., and was free of detectable traces of heparin, heparan sulphate and chondroitin sulphate. The active principle contained in both batches has been characterised by Dol *et al.* (1989) as follows. The mean molecular weight determined by high performance liquid chromatography was 35,700 daltons, with 80% of the product weight in the 15,000–45,000 dalton range. Its $\text{SO}_4 : \text{COOH}$ ratio was 1.22 and its anti-factor IIa activity *in vitro* was 2 u mg^{-1} , using a specific chromogenic assay and unfractionated heparin as a standard. The material was devoid of chromogenic anti-factor Xa activity.

Subjects

The protocol used in this study was approved by the University of Dundee Ethics Committee. Subjects were adult male volunteers aged 18–42 years who were within 15% of their ideal body weight according to the Metropolitan Life Insurance Company tables (1983). Normal findings were obtained following physical examination and routine screening procedures, including urinalysis, laboratory biochemistry and haematology, and electrocardiography. The subjects abstained from alcohol for 24 h preceding and 48 h after each treatment and did not receive any other drug for at least 1 week prior to receiving dermatan sulphate. Written informed consent was obtained, and each subject's General Practitioner was consulted.

Study design

A within-subject crossover design was used, in two paired blocks of two treatments 1 week apart with an interval of 1 month between blocks. In the first block a balanced crossover of intravenous (200 mg as a bolus) and oral (1000 mg as four capsules) administration was applied; in the second two intramuscular doses of 100 mg and 300 mg were injected deep into the gluteus maximus with the 6 ml volume for the 300 mg dose being divided into two 3 ml injections, one into each gluteus maximus. Subjects were fasted for at least 10 h prior to administration of dermatan sulphate.

Blood samples were collected into citrate anti-coagulant before and at 5, 10, 20, 40, 80, 160, 320, 640 min and 24 h following intravenous dosage, before and at 0.5, 1, 2, 3, 4, 5, 7, 12 and 24 h after intramuscular injection and before and 1, 2, 3, 4, 5, 6, 7, 12 and 24 h after oral administration. The concentrations and biological activities of dermatan sulphate were measured as described below. The bleeding time was measured once during the 4 weeks baseline and 4 h after each drug administration.

Urine was collected over the 4 h interval after each treatment to test for microscopic haematuria, and the morning stool sample 24 h after receiving dermatan sulphate was tested for occult blood. Subjects received a full routine haematology, and clinical chemistry screen and urinalysis before and 24 h after each treatment. In addition oral temperature, recumbent blood pressure and pulse were measured immediately preceding and 2, 4, 6, 12 and 24 h after administration of dermatan sulphate.

Assays

The concentration of dermatan sulphate MF701 in plasma was measured by competitive binding assay as described by Dawes *et al.* (1989a). The lower limit of the method was $0.20 \mu\text{g ml}^{-1}$ and at this concentration the interassay coefficient of variation was 16%. Activated partial thromboplastin time (APTT) and thrombin time were measured using kits for automated analysis (Instrumentation Laboratory). The thrombin time kit contained bovine thrombin. Thrombin generation was assessed using a microtitre plate method based on photoelectric measurement of the turbidity generated when fibrinogen is polymerised by the action of thrombin (McLaren *et al.*, 1989). Catalysed thrombin inhibition was measured essentially as described by Fernandez *et al.* (1987). The lower limit of this method was $4 \mu\text{g ml}^{-1}$ and the interassay coefficient of variation was up to 34% at this limit (Bray *et al.*, 1989). Samples from the intravenous treatment arm of the study as well as selected samples from the 300 mg intramuscular dose phase were also assayed using a chromogenic substrate assay (Dupouy *et al.*, 1988) with a lower limit of $0.10 \mu\text{g ml}^{-1}$ and an interassay coefficient of variation of less than 20% at this concentration. The results of both tests for catalysed thrombin inhibition were transformed into equivalent plasma MF701 concentrations using individual reference curves, established by adding known amounts of MF701 to the pre-treatment plasma of each subject. Bleeding time was determined by the Simplate II technique.

Calculation of pharmacokinetic parameters

Individual plasma dermatan sulphate concentrations measured by competitive binding assay after intravenous injection were described by a two-compartment open model. Plasma drug concentrations measured after intravenous injection by the catalysed thrombin inhibition test of Fernandez *et al.* (1987) were described by a one-compartment open model. Plasma concentrations of dermatan sulphate measured by the chromogenic assay were best fitted with a one-compartment open model in eight subjects and a two-compartment open

model in the remaining three cases. The half-lives of the initial elimination phase ($t_{1/2, \text{in}}$) and of the terminal elimination phase ($t_{1/2, \text{z}}$) were determined by compartmental analysis. Peak plasma drug concentration (C_{max}), area under the plasma drug concentration-time curve extrapolated to infinity (AUC), clearance (CL), mean residence time (MRT) and volume of distribution at steady state (V_{ss}) were derived by non-compartmental analysis. Data obtained by competitive binding assay following intramuscular administration were fitted by a one-compartment open model. Compartmental analysis yielded an apparent absorption half-life ($t_{1/2, \text{abs}}$) and an elimination half-life ($t_{1/2, \text{z}}$); other parameters were derived by non-compartmental analysis. Following oral administration only C_{max} , t_{max} , AUC and $t_{1/2, \text{z}}$ were determined as the values were too low to estimate other parameters with confidence. Bioavailability was calculated as the ratio of AUC obtained by the competitive binding assay following extravascular and intravascular administration. Pharmacokinetic parameters were calculated with a non-linear, unweighted, least-square regression programme (SIPHAR, Gomeni & Gomeni, 1978).

Differences between pharmacokinetic parameters obtained after intravenous and intramuscular (100 and 300 mg) administration were compared using a two-way analysis of variance. Simple contrasts were used to carry out multiple comparisons. Pre- and post-dose results of the biological activity tests were compared using the Wilcoxon signed rank test. Results are expressed as mean $\pm$ s.d. unless stated otherwise.

Results

Twelve subjects of mean age 24.7 (range 18–42) years and mean body weight 71.3 (range 58–107) kg were included in the study. Two subjects were withdrawn owing to intercurrent events unrelated to the study drug. A further subject did not receive one of the MF701 doses for reasons discussed below. As a result, all 12 volunteers received the oral MF701 dose whereas 11 received the intravenous dose, 10 the 100 mg intramuscular dose and 9 the 300 mg intramuscular injections.

Intravenous route

Plasma concentrations of dermatan sulphate MF701 measured by competitive binding assay were detectable

Figure 1 Mean ($\pm$ s.d.) plasma levels of dermatan sulphate after intravenous injection of 200 mg to 11 healthy subjects. Concentration (a) was measured by competitive binding assay, and biological activity (b) was measured by catalysed thrombin inhibition according to the method of Dupouy *et al.* (1988). Data are represented up to the last time point at which detectable plasma concentrations were obtained for all subjects.

up to 11 h after drug injection. The concentrations decayed bi-exponentially (Figure 1a) in all subjects and were described by a two-compartmental model. The half-life of the initial elimination phase ($t_{1/2, \text{in}}$) was 0.60 ± 0.10 h, while that of the terminal elimination phase ($t_{1/2, \text{z}}$) was 7.5 ± 3.3 h (Table 1). Using the method of Fernandez *et al.* (1987), MF701 activity was detectable only during the initial elimination phase up to 3 h and decayed monoexponentially with a half-life of 0.97 ± 0.33 h (Table 1). Using the method of Dupouy *et al.* (1988), MF701 activity was measurable only in the initial elimination phase (up to 6 h) in eight subjects with a half-life of 0.90 ± 0.13 h. In the other three subjects, the drug was detected in plasma for longer and decayed bi-exponentially with a $t_{1/2, \text{in}}$ of 0.86 ± 0.18 h during the initial elimination phase and a $t_{1/2, \text{z}}$ of 7.3 ± 5.2 h during the terminal elimination phase (Figure 1b; Table 1). Comparison of parameters obtained using the two catalysed thrombin inhibition tests showed that although the chromogenic substrate assay was more sensitive, the two assays gave similar pharmacokinetic results.

Other tests of biological activity were insufficiently sensitive to describe the pharmacokinetics accurately. The APTT was prolonged by a factor of 2.15 and the thrombin time by 1.22 at 5 min post-injection ($P = 0.001$

Table 1 Pharmacokinetic parameters (mean $\pm$ s.d.) describing the fate of dermatan sulphate after a 200 mg intravenous bolus

	Competitive binding assay	Catalysed thrombin inhibition (Fernandez) (Dupouy)		
Number of subjects	11	11	8	3
Number of compartments	2	1	1	2
$t_{1/2, \text{in}}$ (h)	0.60 ± 0.10	0.97 ± 0.33	0.90 ± 0.13	0.86 ± 0.18
$t_{1/2, \text{z}}$ (h)	7.5 ± 3.3			7.3 ± 5.2
C_{max} (mg l^{-1})	63 ± 8	72 ± 40	44 ± 7	37 ± 12
MRT (h)	2.75 ± 1.33	1.38 ± 0.53	1.25 ± 0.18	3.42 ± 2.02
AUC ($\text{mg l}^{-1} \text{ h}$)	78.0 ± 13.3	72.5 ± 27.1	55.5 ± 8.2	61.6 ± 1.7
CL (l h^{-1})	2.63 ± 0.41	3.15 ± 1.25	3.68 ± 0.60	3.25 ± 0.09
V_{ss} (l)	7.21 ± 3.80	4.22 ± 2.19	4.57 ± 0.71	12.00 ± 6.49

Figure 2 Mean ($\pm$ s.d.) plasma concentrations of dermatan sulphate measured by competitive binding assay following intramuscular administration of 100 mg ($\square$) and 300 mg ($\blacksquare$) dermatan sulphate to 10 and 9 healthy subjects respectively.

Figure 3 Mean ($\pm$ s.d.) plasma concentrations of dermatan sulphate measured by competitive binding assay following oral administration of 1 g dermatan sulphate to 12 healthy subjects.

for both tests), when the concentration measured by competitive binding assay was $63.5 \pm 8.2 \mu\text{g ml}^{-1}$, and the results of both tests returned to pre-dose values within 6 h. Thrombin generation was $40 \pm 16 \text{ u ml}^{-1}$ before administration of dermatan sulphate, and was significantly inhibited to $23 \pm 26 \text{ u ml}^{-1}$ ($P = 0.001$) at 5 min post-injection; at 40 min it was further decreased to $14 \pm 9 \text{ u ml}^{-1}$, but like the other measures of biological activity it had returned to normal by 320 min.

Intramuscular route

Peak plasma drug concentrations of $0.76 \pm 0.18 \mu\text{g ml}^{-1}$ and $1.43 \pm 0.58 \mu\text{g ml}^{-1}$ were measured by competitive binding assay 3 h after intramuscular doses of 100 mg and 300 mg respectively (Figure 2). Measurable concentrations persisted in the majority of subjects for 12 h at the lower dose and for 24 h at the higher dose, although there was considerable variation between subjects after this route of administration. Pharmacokinetic parameters are shown in Table 2. Although $t_{1/2,z}$ appeared to be slightly dose-dependent, the AUCs corrected for dose and bioavailability did not differ significantly. C_{max} also increased with the dose, but to a lesser extent than AUC. The apparent bioavailability was 16–20%.

After injection of 100 and 300 mg the APTT was slightly but significantly ($P \leq 0.004$) prolonged by factors

of 1.10 and 1.16 respectively at 2 h, returning to baseline values at 7 h. After 300 mg thrombin generation was lowered from 39 ± 14 to $26 \pm 4 \text{ u ml}^{-1}$ at 4 h ($P = 0.02$), and activity was detected by the chromogenic assay with peak values of $1\text{--}2 \mu\text{g ml}^{-1}$ at 3 h, although the limited number of samples tested by this method did not permit a full analysis. The thrombin time and catalysed thrombin inhibition test of Fernandez *et al.* (1987) were not significantly affected by either dose.

Oral route

After ingestion of 1 g dermatan sulphate MF701, the maximum plasma drug concentration, $0.56 \pm 0.12 \text{ mg ml}^{-1}$ as measured by competitive binding assay, was observed at 4 h (Figure 3; Table 2). The bioavailability was about 7% and measurable concentrations persisted in plasma for 12 h in 4 of the 12 subjects. There was a significant prolongation of the APTT from the baseline value of $31.7 \pm 0.9 \text{ s}$ to $34.0 \pm 0.9 \text{ s}$ ($P = 0.013$) at 3 h after drug ingestion but biological activity could not be detected by any of the other methods used.

Clinical safety

Following all four treatments, dermatan sulphate MF701 was subjectively well tolerated, and there was no

Table 2 Pharmacokinetic parameters (mean $\pm$ s.d.) describing the fate of dermatan sulphate after i.m. (100 mg and 300 mg) and oral (1 g) administration

	i.m. 100 mg (n = 10)	i.m. 300 mg (n = 9)	Orally 1 g (n = 12)
$t_{1/2,\text{abs}}$ (h)	0.64 ± 0.16	0.51 ± 0.18	
$t_{1/2,z}$ (h)	6.2 ± 1.2	9.7 ± 1.9	5.3 ± 2.9
C_{max} (mg l^{-1})	0.76 ± 0.18	1.43 ± 0.59	0.56 ± 0.11
t_{max} (h)	2.90 ± 0.57	2.89 ± 0.33	3.92 ± 0.29
MRT (h)	9.6 ± 1.7	14.4 ± 2.8	
AUC ($\text{mg l}^{-1} \text{ h}$)	7.8 ± 2.0	19.5 ± 4.0	4.9 ± 1.9
Apparent bioavailability (%)	19.4 ± 5.5	16.9 ± 3.5	6.8 ± 3.3

bleeding or bruising at the injection sites. Pulse and blood pressure remained stable, there were no treatment-related changes in routine biochemistry and haematology, and the bleeding time was unchanged throughout. One of the subjects showed a positive faecal occult blood reading after the intravenous injection and was withdrawn from the following treatment arm. He was subsequently negative on repeat testing and did not show any further sign of bleeding after his final MF701 administration (100 mg intramuscularly). In the absence of a pre-dose faecal occult blood determination and in view of the subsequent history, any relationship between drug treatment and this episode was considered unlikely.

Discussion and conclusions

Our findings confirm that standard coagulation tests are insufficiently sensitive to dermatan sulphate to serve as useful indicators of circulating concentrations, particularly at the low levels generated by extravascular administration. The thrombin time was even less sensitive than APTT, probably because of the use of bovine thrombin which is less readily neutralised by heparin cofactor II than the human enzyme (Pratt *et al.*, 1989).

Measurement of the plasma concentration of dermatan sulphate using a competitive binding assay indicated that the drug disappears from plasma according to a bi-exponential function. This is in agreement with earlier studies using lower doses of unlabelled (Dawes *et al.*, 1989a) and radioiodinated (Dawes *et al.*, 1989b) dermatan sulphate. However, as reported by Dol *et al.* (1989), measurement of biological activity by catalysed thrombin inhibition tests yielded a monoexponential decay in most subjects. Much of this discrepancy can be resolved on the basis that biological activity could not be measured beyond 6 h after injection in most subjects, and was not therefore detectable during the period of the terminal elimination phase as measured by competitive binding assay. In this study there was a good agreement between the initial half-lives measured using the competitive binding assay and the thrombin inhibition tests, and excellent correlation between the terminal half-life obtained with the competitive binding assay and that measured in three subjects with the chromogenic substrate assay of Dupouy *et al.* (1988). The low detection limit of the competitive binding assay which permits the routine identification of a second, slow elimination phase is also the main cause of differences in the other pharmacokinetic parameters when compared with those calculated from biological activity data. It should be pointed out that owing to the limited number of terminal data points, estimates of $t_{1/2,z}$ were of limited accuracy. The individual coefficients of variation were 325–3190% of the estimated values using the competitive binding assay and 310–705 using Dupouy's method.

There is disagreement regarding the dose-dependence of dermatan sulphate pharmacokinetics. In this study

plasma drug concentrations measured by competitive binding assay after intramuscular injection of 100 mg and 300 mg did not show dose-dependence, and we have previously reported similar kinetics after intravenous injections of radioiodinated dermatan sulphate with 5 mg and 100 mg unlabelled carrier (Dawes *et al.*, 1989b). However, Dol *et al.* (1989) found an increase in the $t_{1/2}$ of catalysed thrombin inhibition with increasing intravenous dose of dermatan sulphate, consistent with the prolonged values reported here after higher doses. As dermatan sulphate is a heterogeneous material composed of molecules with widely differing affinities for heparin cofactor II, through which its biological activity is expressed, and possibly also for cellular receptors which could mediate a clearance mechanism, the kinetics of the fractions may differ. The data currently available can be interpreted in terms of dose-dependent clearance of the most biologically active dermatan sulphate fraction, superimposed on dose-independent clearance of the majority of the material. This may also explain discrepancies in C_{max} values after intravenous injection when measured by the different assays. A similar dichotomy exists for heparin (Caranobe *et al.*, 1986).

Dermatan sulphate MF701 was administered by intramuscular rather than subcutaneous injection because at doses greater than 100 mg the injection volume was too large for the latter route. The AUC was linearly related to the dose and the apparent bioavailability of dermatan sulphate MF701 following intramuscular injection was 16–20%. This low recovery may reflect the limitation of assay sensitivity on the extrapolated AUC values. Plasma drug concentrations after intramuscular injection approached the assay sensitivity limit during the terminal elimination phase. Thus, owing to the availability of few data points the terminal half-life for the intramuscular route, and consequently the extrapolated AUC, are likely to be underestimated. Therefore reported bioavailabilities could also be underestimated. The low plasma concentrations of dermatan sulphate following extravascular administration probably reflect a slow absorption process, and this may lead to a significant increase in plasma concentrations of the drug during chronic administration.

Competitive binding assay and APTT measurements on plasma following ingestion of dermatan sulphate confirmed previous reports (Dawes *et al.*, 1989a,b) indicating that dermatan sulphate is the only glycosaminoglycan known to generate significant plasma concentrations after oral administration. Although these are normally too low for reliable *ex vivo* measurement of biological activity, potential therapeutic efficacy has not been excluded and remains to be tested. Moreover, as yet no effort has yet been directed towards developing formulations which enhance delivery of dermatan sulphate by this route; clearly this approach should be considered.

The authors would like to thank Dr Bruno Imbimbo for his useful comments on the manuscript.

References

- Bray, B., Lane, D. A., Freyssinet, J. M., Pejler, G. & Lindahl, U. (1989). Effect of saccharide chain length on thrombin inhibition by heparin cofactor II and by antithrombin. *Biochem. J.*, **262**, 225–232.
- Buchanan, M. R., Boneu, B., Ofosu, F. A. & Hirsh, J. (1985). The relative importance of thrombin and factor Xa inhibition to the antithrombotic effects of heparin. *Blood*, **65**, 198–201.
- Caranobe, C., Petitou, M., Dupouy, D., Gabaig, A. M., Sié, P., Buchanan, M. R. & Boneu, B. (1986). *Thromb. Res.*, **43**, 635–641.
- Dawes, J., Hodson, B. A., MacGregor, I. R., Pepper, D. S. & Prowse, C. V. (1989a). Pharmacokinetics and biological activities of dermatan sulphate (Mediolanum MF701) in healthy human volunteers. *Ann. N.Y. Acad. Sci.*, **556**, 292–303.
- Dawes, J., Hodson, B. A. & Pepper, D. S. (1989b). The absorption, clearance and metabolic fate of dermatan sulphate administered to man—studies using a radioiodinated derivative. *Thromb. Haemostas.*, **62**, 945–949.
- Dol, F., Houin, G., Rostin, M., Montastruc, J. L., Dupouy, F., Gianese, F., Sié, P. & Boneu, B. (1989). Pharmacodynamics and pharmacokinetics of dermatan sulphate in humans. *Blood*, **74**, 1577–1582.
- Dupouy, D., Sié, P., Dol, F. & Boneu, B. (1988). A simple method to measure dermatan sulfate at submicrogram concentrations in plasma. *Thromb. Haemostas.*, **60**, 236–239.
- Fernandez, F., van Ryn, J., Ofosu, F. A., Hirsh, J. & Buchanan, M. R. (1986). The haemorrhagic and antithrombotic effects of dermatan sulphate. *Br. J. Haematol.*, **64**, 309–317.
- Fernandez, F., Buchanan, M. R., Hirsh, J., Fenton, J. W. & Ofosu, F. A. (1987). Catalysis of thrombin inhibition provides an index for estimating the antithrombotic potential of glycosaminoglycans in the rabbit. *Thromb. Haemostas.*, **57**, 286–292.
- Gomeni, C. & Gomeni, R. (1978). IGPHARM: Interactive graphic package for pharmacokinetic analysis. *Comput. Biomed. Res.*, **11**, 345–361.
- McLaren, M., Forbes, C. D. & Belch, J. J. F. (1989). Measurement of thrombin generation using a microtitre plate method. *Thromb. Haemorrh. Disorders*, **1**, 73–75.
- Maggi, A., Abbadini, M., Pagella, P. G., Borowska, A., Pangrazzi, J. & Donati, M. B. (1987). Antithrombotic properties of dermatan sulphate in a rat venous thrombosis model. *Haemostasis*, **17**, 329–335.
- Ofosu, F. A., Modi, G. J., Smith, L. M., Cerskus, A. L., Hirsh, J. & Blajchman, M. A. (1984). Heparin sulfate and dermatan sulfate inhibit the generation of thrombin activity in plasma by complementary pathways. *Blood*, **64**, 742–747.
- Pratt, C. W., Whinna, H. C., Meade, J. B., Treanor, R. E. & Church, F. C. (1989). Physicochemical aspects of heparin cofactor II. *Ann. N.Y. Acad. Sci.*, **556**, 104–115.
- Sié, P., Fernandez, F., Caranobe, C., Gabaig, A. M. & Boneu, B. (1982). Inhibition of thrombin-induced platelet aggregation and serotonin release by antithrombin III and heparin cofactor II in the presence of standard heparin, dermatan sulphate and pentosan polysulfate. *Thromb. Res.*, **35**, 231–236.
- Tollefsen, D. M., Pestka, C. A. & Montafo, W. J. (1983). Activation of heparin cofactor II by dermatan sulfate. *J. biol. Chem.*, **258**, 6713–6716.
- Van Ryn-McKenna, J., Ofosu, F. A., Gray, E., Hirsh, J. & Buchanan, M. R. (1989). The effects of dermatan sulfate and heparin on inhibition of thrombus growth *in vivo*. *Ann. N.Y. Acad. Sci.*, **556**, 304–312.

(Received 23 October 1990,
accepted 8 April 1991)