The pharmacokinetics and pharmacodynamics of dermatan sulphate MF701 during haemodialysis for chronic renal failure

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Single i.v. bolus doses of dermatan sulphate MF701 were administered before the onset of haemodialysis to patients with chronic renal failure, to prevent clotting in the extra-corporeal circuit. Six patients received 2 mg kg⁻¹; six were given 2.5 and 3 mg kg⁻¹; 13 received 4.5 and 6 mg kg⁻¹. Plasma MF701 concentrations (chromogenic assay), activated partial thromboplastin time (APTT) and plasma markers of coagulation and platelet activation (TAT and β -TG) were measured over 4 or 8 h from the onset of dialysis. The disposition of MF701 was described by a monoexponential function. $C(0)$ and AUC values increased proportionally with dose. Volumes of distribution (≈ 4 l) were dose-independent. Half-lives showed a non significant increase with dose (from 2.2 to 3.1 h) and were 2.5–3 times longer than those reported for healthy subjects. There was a significant correlation between plasma MF701 concentration and its effects in prolonging APTT and suppressing TAT and β -TG generation during dialysis.

Keywords dermatan sulphate antithrombotic pharmacokinetics pharmacodynamics haemodialysis kidney failure

Introduction

Dermatan sulphate is a natural glycosaminoglycan which potentiates heparin cofactor II, a selective physiological inhibitor of thrombin, and thereby acts in the coagulation system as a pure antithrombin agent (Tollefsen, 1989). Dermatan sulphate is an effective antithrombotic agent in animal models, but it is less haemorrhagic than heparin and has less influence on conventional coagulation tests and platelet function (Cofrancesco *et al.*, 1990; Fernandez *et al.*, 1986; Luzzatto *et al.*, 1989; Maggi *et al.*, 1987; Sié *et al.*, 1991; Van Ryn-McKenna *et al.*, 1989).

During haemodialysis, the exposure of blood to the dialysis circuit results in the activation of the coagulation system and eventually in visible clot formation. Therefore, substantial anticoagulation is required, which is usually achieved with heparin.

Uraemia is known to cause a complex haemostatic defect with a significant risk of bleeding (Ireland *et al.*, 1989). Owing to its reduced haemorrhagic potential, dermatan sulphate may represent a safer alternative to heparin for haemodialysis in patients with renal failure.

We have recently reported the results of dose-finding studies with dermatan sulphate in patients undergoing haemodialysis for chronic renal failure (Lane *et al.*,

1992). Using additional data from these studies, we now report the pharmacokinetics and pharmacodynamics of dermatan sulphate during dialysis. The pharmacodynamic response to the drug was assessed by *ex vivo* hypo-coagulation, as measured by activated partial thromboplastin time (APTT), and by suppression of *in vivo* thrombin formation and platelet activation during dialysis, as reflected by the appropriate plasma markers (thrombin-antithrombin complex [TAT] and β -thromboglobulin [β -TG], respectively).

Methods

Patients

A total of 22 patients undergoing maintenance haemodialysis for chronic renal failure were included in three consecutive studies. There were 9 males and 13 females, their mean age was 55 years (range 20–75 years) and their mean creatinine clearance was 6.2 ml min⁻¹ (range 2.9–12.7 ml min⁻¹). Three patients from Study 1 also participated in Studies 2 or 3. The protocol for each

study was approved by the ethics committee of the Academic Medical Centre, Amsterdam, The Netherlands and written informed consent was obtained from each patient.

Treatments

Dermatan sulphate was provided in the form of 100 mg/2 ml ampoules (MF701, batches 01.701.C and 21.701.C, Mediolanum Farmaceutici, Milan, Italy). MF701 was administered as a single intravenous bolus 2 min before the onset of dialysis. As reference treatment for Studies 2 and 3, heparin was administered in doses adapted to individual needs (a pre-dialysis bolus of 1000–6000 IU followed by an infusion of 500–2400 iu h⁻¹). Patients were dialyzed over 3 to 4 h using cuprophane membranes. A plate-type dialyzer was used in Studies 1 and 2, and a disposable capillary dialyzer in Study 3.

Study design

In Study 1, one dialysis session was investigated in six patients after a dose of 2 mg kg⁻¹ body weight MF701. Studies 2 and 3 were conducted according to a randomized crossover design. In Study 2, three dialysis sessions were investigated in each of 6 patients, who were given 2.5 and 3 mg kg⁻¹ MF701 and the heparin regimen. In Study 3, three dialysis sessions were investigated in 13 patients, who received 4.5 and 6 mg kg⁻¹ MF701 and the heparin regimen.

Blood sampling

Blood samples were collected immediately before treatment and 0.25, 0.5, 1, 2, 4, 5, 6, and 8 h after the onset of dialysis in Studies 1 and 2. In Study 3, blood samples were collected prior to treatment and at 0.25, 0.5, 1, 2, 3, and 4 h after the onset of dialysis.

Blood for the assay of plasma dermatan sulphate, APTT and TAT was collected in 3.2% w/v trisodium citrate (vol. 9/1). For β -TG, blood was collected in a plastic syringe preloaded with PECT (0.282 μ M PGE₁, 0.27 M Na₂EDTA 2H₂O, 1.9 mM Na₂CO₃ and 0.03 M theophylline). Plasma was obtained by centrifugation at 4° C for 20 min at 1600 g (citrate blood) or 60 min at 2600 g (PECT blood) and stored at -70° C.

Assays

Plasma dermatan sulphate concentrations were measured by a chromogenic substrate assay (Strachrom DS, Diagnostica Stago, France). The method is based upon the catalytic effect of dermatan sulphate on thrombin inhibition by heparin cofactor II (Dupouy *et al.*, 1988). Assays for Studies 1 and 2 were performed in the Department of Haematology, Charing Cross and Westminster Hospital, London, U.K. Samples from Study 3 were assayed on the study site in Amsterdam. A cross-laboratory validation study showed an interassay coefficient of variation ranging from 4% to 20% according to the laboratory, while the variability between laboratories was 13%, at a dermatan sulphate concentration of 2 μ g ml⁻¹ (Sié *et al.*, 1991).

APTT, plasma TAT and β -TG were only measured

in plasma samples collected up to 4 h. All of the assays were performed in Amsterdam. APTT was determined using Actin FS reagent (Baxter Dade, Switzerland). TAT and β -TG were determined by ELISA kits (Behringwerke and Boehringer Mannheim, Germany). Normal values were 24–36 s for APTT, <4.6 ng ml⁻¹ for TAT and <30 u ml⁻¹ for β -TG.

Pharmacokinetic analysis

A monoexponential function was fitted to the individual plasma drug concentration data using the TOPFIT program (Heinzel, 1982). This model was selected on the basis of the Imbimbo criterion (Imbimbo *et al.*, 1988). Values of $C(0)$, $t_{1/2}$, AUC, V , and CL were calculated by standard methods. AUC(0,4) values were calculated using the linear trapezoidal rule.

Statistics

APTT data were transformed by calculating individual differences from pre-treatment values and AUC (0,4) values calculated by the linear trapezoidal rule. AUC (0,4) values were also calculated for plasma TAT and β -TG data, using untransformed data.

Pharmacokinetic parameters and pharmacodynamic data obtained for the different treatments or doses were compared by analysis of variance. When appropriate, the data were log-transformed to obtain homoscedasticity (Bartlett, 1947). The relationship between pairs of variables was evaluated by linear regression. The level of significance was $P < 0.05$. Results are expressed as mean $\pm$ s.d.

Results

In Study 2, one patient did not receive the 2.5 mg kg⁻¹ MF701 dose and plasma concentrations after 3 mg kg⁻¹ could not be assayed in another patient. In Study 3, one patient did not receive the 4.5 mg kg⁻¹ dose of MF701. A total of 41 dialysis sessions were investigated with MF701; 19 sessions were investigated with the heparin regimen. For a few sessions, AUC (0,4) values for plasma dermatan sulphate or the haemostatic tests could not be calculated owing to missing values at 0 or 4 h.

Pharmacokinetics

Mean plasma dermatan sulphate concentrations after the five doses of MF701 are shown in Figure 1a. The pharmacokinetic parameters estimated for each dose are listed in Table 1. Mean values of $C(0)$, AUC (0,4) and AUC increased proportionally with the dose of MF701, with the exception that values of $C(0)$ and AUC (0,4) after 2.5 mg kg⁻¹ were marginally higher than after 3 mg kg⁻¹. Regression analysis showed a significant linear relationship between dose and individual log-transformed values of $C(0)$ ($r^2 = 0.597$, $P < 0.001$), AUC (0,4) ($r^2 = 0.638$, $P < 0.001$) and AUC ($r^2 = 0.542$, $P < 0.001$).

Values of V and CL were dose-independent. $t_{1/2}$ showed

Table 1 Pharmacokinetic parameters (mean $\pm$ s.d.) of MF701 after single pre-dialysis i.v. bolus doses

	Study 1 2 mg kg ⁻¹ n = 6	Study 2 2.5 mg kg ⁻¹ n = 5	Study 2 3 mg kg ⁻¹ n = 5	Study 3 4.5 mg kg ⁻¹ n = 12	Study 3 6 mg kg ⁻¹ n = 13
C(0) ($\mu\text{g ml}^{-1}$)	33.9 $\pm$ 9.1	50.4 $\pm$ 16.5	40.3 $\pm$ 11.5	66.5 $\pm$ 16.1	87.4 $\pm$ 24.2
CL (ml min ⁻¹)	24.7 $\pm$ 7.5	15.9 $\pm$ 4.0	20.3 $\pm$ 4.7	18.7 $\pm$ 6.4	19.4 $\pm$ 6.8
t _{1/2} (h)	2.2 $\pm$ 0.5	2.3 $\pm$ 0.5	2.7 $\pm$ 0.5	2.8 $\pm$ 0.8	3.1 $\pm$ 1.4
V (l)	4.44 $\pm$ 1.10	3.30 $\pm$ 1.46	4.70 $\pm$ 1.14	4.42 $\pm$ 0.91	4.60 $\pm$ 1.27
AUC (0,4) ($\mu\text{g ml}^{-1}$ h)	71 $\pm$ 22	109 $\pm$ 36	100 $\pm$ 39*	164 $\pm$ 38**	215 $\pm$ 78***
AUC ($\mu\text{g ml}^{-1}$ h)	104 $\pm$ 32	165 $\pm$ 50	164 $\pm$ 71	275 $\pm$ 93	401 $\pm$ 279

*n = 4; **n = 11; ***n = 12.

Figure 1 (a) Mean plasma dermatan sulphate concentrations after single pre-dialysis i.v. bolus doses of MF701. (b) Mean increase in APTT vs pre-treatment values (Δ APTT) after single pre-dialysis i.v. bolus doses of MF701. Pre-treatment values for the five doses ranged from 29 ± 2 s to 32 ± 5 s. $\square$ = 2 mg kg⁻¹; $\circ$ = 2.5 mg kg⁻¹; $\bullet$ = 3 mg kg⁻¹; Δ = 4.5 mg kg⁻¹; $\blacktriangle$ = 6 mg kg⁻¹.

a gradual but non-significant increase with dose, from 2.2 ± 0.5 h after 2 mg kg⁻¹ to 3.1 ± 1.4 h after 6 mg kg⁻¹.

Pharmacodynamics

Mean increases in APTT over pre-treatment values after the five doses of MF701 are shown in Figure 1b. The time-course of changes in APTT was similar to that of plasma dermatan sulphate. Individual AUC (0,4) values for APTT changes were linearly related to both dose ($r^2 = 0.636$, $P < 0.001$) and plasma dermatan sulphate AUC (0,4) ($r^2 = 0.687$, $P < 0.001$).

Despite a large inter-dialysis variability, log-transformed AUC (0,4) values of plasma TAT and β -TG were inversely related to log-AUCs of plasma dermatan sulphate ($r^2 = 0.256$ and 0.248 , respectively; $P < 0.01$).

In study 3, the AUC (0,4) value for APTT change induced by the heparin regimen was 552 ± 259 s h, as opposed to 107 ± 21 s h with the highest dose of MF701 ($P < 0.001$). In contrast, log-transformed AUC (0,4) values for TAT and β -TG with either treatment did not differ significantly ($P > 0.20$).

Discussion

In this study, a one-compartment model was used to estimate the pharmacokinetic parameters of dermatan sulphate during haemodialysis. Although drug concentration data for some patients seemed to suggest a biexponential decay after the highest doses (4.5 and 6 mg kg⁻¹), the confidence in parameters estimated using a two-compartment model was low. This reflected the limited number of data points and limited duration of sampling. We believe that the one-compartment model provides a reasonable description of the available data. Using the same assay method, a monoexponential decay of plasma dermatan sulphate concentrations was also found in healthy volunteers, with maximum plasma concentrations and distribution volumes consistent with those estimated in the present study (Agnelli *et al.*, 1990; Dawes *et al.*, 1991; Dol *et al.*, 1989).

The appearance of a possible biexponential decay of plasma dermatan sulphate with increasing doses, and the trend towards a dose-related increase in $t_{1/2}$, may

suggest a deviation from linearity of MF701 pharmacokinetics during dialysis. However, the proportionality found between dose and AUC (0,4) indicates that any deviation was of limited clinical relevance.

The clearance of the drug during dialysis was 2.5 to 3.5 times slower, and the $t_{1/2}$ was accordingly longer, than in healthy subjects given 2–3 mg kg⁻¹ MF701 as an i.v. bolus or 6 mg kg⁻¹ as a 10-h infusion (Agnelli *et al.*, 1990; Boneu *et al.*, personal communication, 1990; Dawes *et al.*, 1991; Dol *et al.*, 1989). This finding is consistent with previous studies showing that renal excretion is the main route of elimination for MF701 in humans (Dawes *et al.*, 1989), and that the clearance of the drug is impaired in nephrectomized rabbits (Dol *et al.*, 1988).

Plasma TAT and β -TG have been shown to provide an objective and sensitive estimation of coagulation and platelet activation during haemodialysis (Ireland *et al.*, 1991). MF701 inhibited the generation of these markers in a plasma concentration-dependent manner. Thrombin formation is the primary cause of platelet activation and release during dialysis (Ireland *et al.*, 1989). As MF701 is devoid of any anti-platelet activity other than on thrombin-induced aggregation (Luzzatto *et al.*, 1989; Cofrancesco *et al.*, 1990), its effect in suppressing β -TG generation was probably a consequence of its anti-thrombin activity.

A dose of 6 mg kg⁻¹ MF701 was equivalent to an individualized heparin regimen in suppressing TAT and β -TG generation during dialysis. This provides confirmation of dose-finding data obtained in the same patients by visual inspection of the dialysis circuit (Lane *et al.*, 1992). Effective suppression of coagulation activation by MF701 was accompanied by much less prolongation in APTT, as compared with that induced by heparin.

In conclusion, the elimination half-life of MF701 is significantly prolonged in end-stage renal failure. Therefore, MF701 can be administered in a single bolus for prevention of clotting of the haemodialysis circuit, when the dialysis is of short duration (≤ 4 h). MF701 blocks the *in vivo* generation of thrombin during dialysis, thereby confirming the physiological and pharmacological relevance of the heparin cofactor II pathway of thrombin inhibition.

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