Individualized anticoagulation with dermatan sulphate for haemodialysis in chronic renal failure

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Abstract

Background. Dermatan sulphate (DS) is a selective thrombin inhibitor with antithrombotic properties and low bleeding potential. In preliminary studies it was reported to be effective for preventing clot formation in the haemodialysis circuit.

Methods. Ten patients on maintenance haemodialysis for chronic renal failure underwent three consecutive investigation phases. In phase 1 (individual dose titration), repeated dialyses were performed with increasing doses of DS until successful dialysis was obtained in two sessions at the same dose. In phase 2, individualized DS doses were validated by a randomized crossover comparison with the individual heparin dose of each patient. In phase 3, each patient underwent 24 consecutive dialyses with DS over 8 weeks. Successful dialysis was defined as completion of the procedure without visible clot formation in the bubble traps and lines or a greater than 20% decrease in dialyser capacity. Dialysis efficiency (decrease in serum urea and creatinine, Kt/V), APTT prolongation, bleeding time, and DS plasma concentrations were also assessed.

Results. Phase 1: successful dialysis was achieved in nine patients with 4 mg/kg DS as a predialysis intravenous bolus followed by continuous infusion of 0.65 mg/kg/h. One patient required 5 mg/kg plus 1.3 mg/kg/h. Phase 2: no statistically significant differences were found between DS and heparin in any of the investigated variables. Residual dialyser capacity and dialysis efficiency indexes indicated equivalent efficacy. Phase 3: residual dialyser capacity and dialysis efficiency did not change with time. There was no accumulation of DS in plasma. No bleeding or thrombocytopenia were observed.

Conclusions. The dose of DS can be individually titrated to suppress clot formation during haemodialysis as efficiently as with individualized heparin. Such an individualized DS regimen maintains its anticoagulant efficacy and is safe in prolonged use.

Key words: anticoagulation; clinical trial; dermatan sulphate; haemodialysis; heparin

Introduction

Rapid and effective anticoagulation is required during haemodialysis in order to prevent clotting in the extracorporeal circuit. This is usually achieved with intravenous heparin. Heparin-induced bleeding, thrombocytopenia, and osteoporosis [1,2] may, however, represent significant problems in dialysed uraemic patients, as they may exacerbate the haemorrhagic diathesis and osteodystrophy caused by the underlying disease [2]. Moreover, individual adjustment of heparin doses is complicated by non-linear pharmacokinetics and variability of anticoagulant response [1,3].

Dermatan sulphate is a natural glycosaminoglycan with a unique mechanism of action on the coagulation system, as it selectively inhibits thrombin through potentiation of endogenous heparin cofactor II [4]. Unlike heparin, dermatan sulphate is effective on both soluble and fibrin-bound thrombin [5]. In animal models dermatan sulphate inhibits both thrombus formation and extension, while exerting a reduced haemorrhagic effect as compared with heparin [6]. The lack of interaction of dermatan sulphate with platelets and platelet function [6,7] may partly explain its low bleeding potential and may reduce the risk of thrombocytopenia [8]. Osteoporosis may be less likely with dermatan sulphate than with heparin as binding of zinc ions, a possible mechanism for this adverse effect [9], does not occur with the former [10]. Dermatan sulphate has been found clinically effective and safe in the prophylaxis of postoperative deep vein thrombosis [11–13].

Short-term clinical studies have been conducted in haemodialysis for chronic renal failure, testing fixed intravenous doses of dermatan sulphate against individualized heparin regimens [14–16]. Dermatan sulphate suppressed both visible clot formation in the dialysis circuit and the generation of plasma markers of coagulation and platelet activation during the
procedure [14–17]. It also induced a moderate prolongation of activated partial thromboplastin time (APTT) [15–17]. These effects were related to dermatan sulphate doses and plasma concentrations, which followed linear pharmacokinetics [15–17]. Effective doses ranged from 6 to 10 mg/kg body weight per dialysis session, depending on the type of dialysate and duration of the procedure [14–18].

As an extension of the above experience, the present study was aimed at: (i) finding the lowest effective individual dose of dermatan sulphate for haemodialysis in each of 10 uraemic patients; (ii) validating the individualized doses by comparison with the individualized heparin regimens currently used in the same patients; (iii) assessing the continued efficacy and safety of dermatan sulphate over a period of 8 weeks.

**Subjects and methods**

**Patients**

Patients with chronic renal failure, undergoing maintenance haemodialysis at the Nephrology and Dialysis Division of Ospedali Riuniti, Bergamo, were eligible for the study if meeting the following criteria: age ≥18 years; dialysis frequency, three times a week; haematocrit between 25 and 32%; blood flow in the extracorporeal circuit ≥200 ml/min; no recent or current history of cardiac or cerebral ischaemia, uncontrolled arterial hypertension, symptomatotic hypotension, intradialytic blood transfusions, or pregnancy; no current treatment with drugs known to affect haemostasis. Written informed consent was obtained in each case. Ethical and administrative approvals were obtained from the Commissione Consultiva per la Sperimentazione Clinica, Regione Lombardia, and Direzione Sanitaria, Ospedali Riuniti, Bergamo.

**Anticoagulation and dialysis procedure**

Dermatan sulphate (MF701, 200 mg/2 ml ampoules, Mediolanum Farmaceutici, Milan, Italy) was injected intravenously by an electrical pump as a slow bolus (2 min) immediately before dialysis onset, followed by a constant rate infusion interrupted 30 min before dialysis end. Sodium heparin (Eparina Vister, 5000 IU/ml multi-dose vials, Parke–Davis, Lainate, Italy) was administered by repeated intravenous bolus injections [19]. An individually adjusted pre-dialysis dose was followed by 60% of the same dose at 1 h, 40% at 2 h and, if dialysis lasted longer than 3.5 h, 30% at 3 h. Before dialysis the circuit was primed with dermatan sulphate, 300 mg/l saline, or with heparin, 500 IU/l saline.

Patients were dialysed over 3–4 h on a parallel-flow kidney with cellulosic membrane (CA210, Baxter Renal Division). Extracorporeal blood flow was maintained between 250 and 300 ml/min. Dialysate flow was 500 ml/min. The dialysate formula was kept unchanged throughout the study. On each session, the bubble traps and lines were visually inspected for clot formation at hourly intervals. At the session end, the blood compartment of the dialyser was washed with saline through the arterial line. The arterial and venous lines were clamped before disconnecting the dialyser. The venous line clamp was then removed and the volume of effluent saline was measured. A manual pump was used to ensure complete emptying of the blood compartment. Dialyser capacity was expressed as percentage of the nominal capacity specified by the manufacturer. The dialysate compartment was kept full during the procedure.

**Study design. Criteria for clinical efficacy and safety**

Each patient underwent three consecutive investigation phases. In phase 1 (individual dose titration), repeated dialyses were performed with dermatan sulphate until successful dialysis was obtained in two consecutive sessions at the same dose. The starting dose was 4 mg/kg as a bolus and 0.65 mg/kg/h as an infusion. In case of failure, the dose was increased stepwise to 5 mg/kg plus 0.65 mg/kg/h, 5 mg/kg plus 1 mg/kg/h, and 5 mg/kg plus 1.3 mg/kg/h. Successful dialysis was defined as regular completion of the procedure without visible clot formation in the bubble traps and lines or >20% decrease in dialyser capacity as an index of fibrin deposition. Pre- to post-dialysis changes in serum urea and creatinine were also assessed.

Phase 2 was a randomized crossover comparison of two consecutive dialysis sessions, using the dermatan sulphate dose established in phase 1 and the patient’s individual heparin dose as established before the study. Anticoagulation efficacy was assessed according to residual dialyser capacity, changes in serum urea and creatinine, and post-dialysis ratio of total clearance to distribution volume of urea (Kt/V, calculated according to Daugirdas [20]). Laboratory personnel were blind to treatment allocation.

In phase 3, patients underwent 24 consecutive dialyses with dermatan sulphate over a period of 8 weeks. Residual dialyser capacity was assessed for each dialysis; changes in serum urea and creatinine and Kt/V were determined on the first and last sessions. Patients were followed for adverse clinical events over the study period. Haemoglobin, haematocrit, leukocyte and platelet count, ALT, and AST were also monitored.

**Effects on haemostatic function and pharmacokinetics**

**Phase 1: APTT** was determined immediately before dialysis, 1, 2 and 3 h from its onset and at dialysis end (if longer than 3 h), using IL–Test liophilized silica reagent (Instrumentation Laboratory) and expressing the result as ratio to control plasma.

**Phase 2: APTT** and plasma concentration of fibrinopeptide A (FpA), a marker of fibrin generation, were determined at the above time-points. On the heparin session, the relevant blood samples were taken immediately before heparin injections. FpA was assayed on frozen plasma at Istituto Mario Negri, Bergamo, using a radioimmunoassay kit (Byk-Sangtec, Diagnostica Gmbh), after addition of bentonite to remove potentially cross-reactive fibrinogen and fibrinogen degradation products. *In vivo* platelet adhesion from whole blood to collagen-coated surfaces under flowing conditions was tested on a sample taken 15 min from dialysis onset, according to the method of Aleviadrou et al. [21] modified as follows. A flow chamber thermostated to 37 °C was used, in which one surface of the perfusion channel was a glass disc coated with collagen type II or gelatin. The system was perfused with TRIS buffer (pH 7.3) for 1 min, then with the test blood without added anticoagulant for 2 min. The shear rate was 1500 s⁻¹. Platelet thrombi adherent to the collagen-coated slide were fixed and stained with May–Grunwald–Giemsa. Platelet adhesion was assessed at Istituto Mario Negri with a computer image analysis system connected to a video camera and a light-microscope, and
expressed as percentage of the surface covered by thrombi. Ten microscope fields per slide were measured using systematic sampling. Bleeding time was measured pre- and immediately postdialysis as average from two forearm skin incisions, using Simplate II device (General Diagnostic) under a counterclockwise pressure of 40 mmHg. The reference range was 180–420 s. Dermatan sulphate plasma concentration was monitored on the session performed with this agent. The relevant assays were performed on frozen plasma in the Mediolanum Farmaceutici laboratory according to the chromogenic substrate method of Dupouy et al. [22] and using a commercial kit (Stachrom DS, Diagnostica Stago), with a detection limit of 1.0 μg/mL. All laboratory determinations were performed blindly.

Phase 3: APTT was monitored on the first and last dialysis sessions. Pre-dialysis dermatan sulphate plasma concentration was determined on sessions 1, 9, 18 and 24.

Statistical analysis

Areas under the curves (AUC) of APTT ratio and FpA levels over dialysis time were calculated by the trapezoidal rule. Data from successful dialyses of phase 1 and from first and last sessions of phase 3 were summarized by descriptive statistics. Analysis of variance for crossover designs was applied on data from phase 2, including treatment, carry-over and period effects in the model. Analysis of AUCs of FpA was conducted on log-transformed values to obtain homogeneous variances. Significance level was set at 0.05 (two-sided). 95% confidence intervals were calculated for the estimated differences between dermatan sulphate and heparin. Statistical analysis was performed with SAS package, version 6.10 (SAS Institute, Cary, NC, USA). Unless otherwise specified, data are reported as mean (SD).

Results

Ten patients (4 females) were included in the study. The age range was 46–78 years (mean 65.5) and body weight 49–102 kg (mean 66.7). They had been on maintenance haemodialysis for 1.5–84 months (median 15 months). Chronic renal failure was due to polycystic kidney disease in two patients, lupus nephritis in one, and to unknown causes in seven. Three patients were on chronic treatment with recombinant erythropoietin. One patient had chronic thrombocytopenia. All patients completed the three investigation phases. A total of 275 dialysis sessions were investigated (23 in phase 1, 20 in phase 2 and 232 in phase 3).

Phase 1

Successful dialysis was achieved in nine patients with the starting dose of dermatan sulphate (4 mg/kg plus 0.65 mg/kg/h), while the remaining patient required 5 mg/kg plus 1.3 mg/kg/h. After the first and second sessions performed with the effective dose, residual dialyser capacity was 90.4 (4.4)% and 90.0 (4.1)% of the nominal value. Pre- to post-dialysis decrease in serum urea and creatinine indicated normal dialysis efficiency in all patients. APTT was prolonged to a mean ratio of 2.4–2.7 during the first successful session and to 2.4 at the session end (2.3–2.6 and 2.1 respectively, in the second session).

Phase 2

Individual dialysis time (mean 3.7 h) was the same for both sessions. Using regimens established in phase 1, the total per dialysis dose of dermatan sulphate was between 5.6 and 6.3 mg/kg in nine patients and 8.9 mg/kg in the remaining one. Total heparin dose ranged from 65 to 134 IU/kg (median 117). P values for carry-over and period effects were >0.10 for all investigated variables. No statistically significant differences were found between dermatan sulphate and heparin in any of the investigated variables. Residual dialyser capacity, urea and creatinine decreases and Kt/V obtained with dermatan sulphate and heparin indicated equivalent efficacy within narrow confidence limits (Table 1). AUCs of plasma FpA levels were also compatible with equivalence although with a much wider confidence interval, due to the large inter-patient variability observed with both drugs (Table 1).

The time course of APTT ratio is shown in Figure 1. The resulting AUCs, reported in Table 1, showed a smaller coefficient of variation with dermatan sulphate (26.2%) than with heparin (44.2%). Ex vivo platelet adhesion to collagen was almost identical with either treatment (Table 1). Three patients had a pre-dialysis bleeding time >420 s on both sessions. Post-dialysis changes (n=8; a reliable measurement could not be obtained in two patients) are reported in Table 1. Mean dermatan sulphate plasma concentrations (Figure 1) showed a minor peak at 15 min (41 μg/mL) as a result...
of the initial bolus injection and then stabilized around 30 μg/ml.

Phase 3

One patient was switched from thrice- to twice-weekly dialysis when beginning phase 3. The same dialysis time and dermatan sulphate dose used in phase 2 were maintained for each patient. Residual dialyzer capacity after the initial and final sessions was unchanged, as shown in Table 2. On the intervening 22 dialyses, mean capacity never dropped below 86%, the relevant SD never exceeding 5%. Urea and creatinine changes and Kt/V also remained stable over the 8-week period (Table 2). Some decrease in APTT response to dermatan sulphate (n = 8) was apparent in the final session (Table 2). Predialysis bleeding time (n = 9) was 431 (48) and 417 (62) s on the initial and final sessions respectively. The corresponding postdialysis changes are shown in Table 2.

In two patients, residual dermatan sulphate was repeatedly detected in plasma before dialyses 9, 18 and 24. Drug concentrations (1.3–2.7 µg/ml) were low as compared with on-dialysis levels and did not increase with time. Four additional patients had residual drug levels before dialyses 1 (carried over from phase 2) or 9 or 18 (1.1–3.9 µg/ml), which, however, disappeared before dialysis 24. Predialysis APTT of the patients with measurable drug levels was ≤1.0.

### Table 2. Phase 3. Data from the initial and final sessions in 10 patients undergoing consecutive dialyses with dermatan sulphate over 8 weeks

<table>
<thead>
<tr>
<th></th>
<th>Dialysis 1</th>
<th>Dialysis 24</th>
</tr>
</thead>
<tbody>
<tr>
<td>Residual dialyser capacity (%)</td>
<td>89.1 (7.8)</td>
<td>87.9 (3.8)</td>
</tr>
<tr>
<td>Urea change* (mmol/l)</td>
<td>−21.1 (7.0)</td>
<td>−18.4 (5.0)</td>
</tr>
<tr>
<td>Creatinine change* (µmol/l)</td>
<td>−537 (176)</td>
<td>−463 (159)</td>
</tr>
<tr>
<td>Post-dialysis Kt/V</td>
<td>1.30 (0.29)</td>
<td>1.36 (0.27)</td>
</tr>
<tr>
<td>AUC of APTT (ratio·min)</td>
<td>25.2 (20.1)</td>
<td>22.6 (14.3)</td>
</tr>
<tr>
<td>Platelet adhesion to collagen (%)</td>
<td>16.8 (4.1)</td>
<td>17.0 (4.3)</td>
</tr>
<tr>
<td>Bleeding time change* (s, n = 8)</td>
<td>−31 (71)</td>
<td>-31</td>
</tr>
</tbody>
</table>

Data are expressed as mean (SD). *Pre- to post-dialysis change.

### Safety

No bleeding episode was observed in any investigation phase, nor other clinically overt adverse reactions. Mean predialysis haematology and liver enzyme data remained stable over the study period, and the corresponding mean postdialysis changes were minimal with either treatment. Individual data showed a drop in platelet count (104 to 51 × 10^9/l) in one patient after heparin dialysis in phase 2. In the patient with chronic thrombocytopenia, platelet count over the study period fluctuated between 40 and 100 × 10^9/l and did not decrease with time.

### Discussion

It is well known that heparin requirement for haemodialysis varies according to dialysis technique, membrane type, and individual patient characteristics. Inter-patient variability was also suggested by earlier dialysis studies using fixed doses of dermatan sulphate [14–18]. With this background, an individual dose titration approach was chosen for the present study, based on a simple assessment of clot formation in the dialysis circuit. Dermatan sulphate regimens were modelled from available pharmacokinetic and clinical data [15–18]. The aim was to keep potentially effective plasma drug concentrations as steady as possible over the procedure, thereby minimizing total dose requirement. A combination of bolus injection and continuous infusion was therefore adopted.

Nine of the 10 study patients could actually be dialysed with the lowest scheduled dose of dermatan sulphate, whereas the range of effective heparin doses for the same patients was relatively wide. Whether this represents a less variable anticoagulant response to dermatan sulphate than to heparin is not certain, as the possibility remains that some of our patients might have been dialysed with an even lower dermatan sulphate dose. Steadiness of dermatan sulphate plasma levels was successfully achieved, as shown in Table 2. The titrated doses were then tested for efficacy over single dialyses against the current heparin regimen of
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Each patient, and for consistency of effect in prolonged use.

Equal anticoagulant efficacy was shown for derma-
tan sulphate and heparin on clot formation in the dialyser, as reflected by dialysate capacity, and on the resulting overall efficiency of dialysis, as reflected by urea and creatinine changes and Kt/V. FpA generation, reflecting systemic activation of the coagulation system, was extremely variable without any detectable trend in favour of either drug. APTT response to dermatan sulphate was less variable than to heparin, consistently with the narrower dose range of the former drug, but it was not significantly reduced as found in earlier comparative studies [15–17]. Two likely explanations are that present heparin doses were slightly lower than in previous studies and that the AUC of APTT with heparin was underestimated, as peaks following repeated bolus injections could not be detected with pre-injection blood sampling.

As concerns primary haemostasis, neither dermatan sulphate nor heparin produced a clear-cut change in bleeding time over a single dialysis session. Platelet adhesion to collagen at high blood flow rate is dependent on the interaction of platelet glycoprotein Ib with von Willebrand factor adsorbed onto collagen [21,23]. This process has been shown to be unaffected by heparin both in vitro [21] and ex vivo in humans given a 12 000 IU bolus [23]. Therefore the observed lack of difference between the study drugs indicates that dermatan sulphate also does not interfere with the adhesion process.

No decline in dermatan sulphate’s efficacy was detected in its continued use over 8 weeks, for a total of 232 investigated sessions. In view of this, and in the absence of concurrent controls, the apparent decrease in APTT response at the end of the study may probably be considered a random fluctuation. Bleeding time remained stable over this period. The elimination half-life of dermatan sulphate is prolonged in uraemic patients as compared with healthy subjects [17]. Nevertheless, no pharmacologically relevant accumulation of dermatan sulphate in plasma occurred over time, in agreement with findings from an earlier study [18]. Residual pre-dialysis plasma levels of dermatan sulphate, when occasionally present, were low and had no influence on APTT. Safety of dermatan sulphate was excellent over the entire study. There were no bleeding complications nor thrombocytopenic reactions.

Two main findings emerge from the present study. First, dermatan sulphate dose can be titrated to suppress clot formation during haemodialysis as efficiently as does individualized heparin. Doses established in the present study do not necessarily extrapolate to patients dialysed with membranes other than cellulosic, as dermatan sulphate requirement may vary according to the type of dialyser used [15,16]. However, individual titration may be facilitated by injecting approximately 60% of the total dose as a bolus and the remaining as a continuous infusion and by monitoring APTT response. Second, such an individualized derma-
tan sulphate regimen maintains its anticoagulant efficacy and is safe in prolonged use.

These findings confirm that dermatan sulphate is a suitable alternative to heparin for anticoagulation in haemodialysis. Dermatan sulphate has pharmacological advantages with potential relevance to dose–response predictability and to the risk of bleeding, thrombocytopenia and osteoporosis. Long-term comparative trials are warranted to assess whether these translate into significant clinical benefits for uraemic patients on maintenance haemodialysis.

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