Intermittent saline flushes during haemodialysis do not alleviate coagulation and clot formation in stable patients receiving reduced doses of dalteparin

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Abstract

Background. Heparin-free haemodialysis (HD) with intermittent saline flushes (ISF) in patients with bleeding risk is widely used. The aim of this study was to investigate if ISF reduce coagulation and clotting in stable patients receiving reduced doses of dalteparin.

Methods. Inclusion criteria were stable chronic HD patients ≥18 years of age and haemoglobin ≥11 g/dl. Exclusion criteria were use of warfarin and acetylsalicylic acid. Six HD sessions were evaluated per patient. Dalteparin was given as one bolus dose at start of HD (50% of the conventional dose). In HD number 1, 3 and 5, 100 ml saline solution was flushed through the filter each 30 min. In HD 2, 4 and 6, no ISF were given. Potential clotting in the bubble trap was visually observed each hour and graded on a 4-point scale: 1 = normal, 2 = fibrinous ring, 3 = clot formation and 4 = coagulated system. The dialyser was visually inspected at the end of each session: 1 = normal, 2 = a few blood stripes (affecting less than 5% of the surface fibres), 3 = many blood stripes (more than 5% of the fibres) and 4 = coagulated filter. The coagulation marker PF1+2, the platelet activation marker β-TG and anti-FXa activity were repeatedly measured during HD.

Results. Six men and two women were included. In four cases (four different patients), HD was stopped due to a coagulated system, all cases on days with ISF performed. Multiple linear regression analyses with repeated measurements showed that ISF adjusted for dalteparin dose/kg significantly increased mean clot in the bubble trap, estimate (B) = 0.717, P = 0.0001 and also showed that ISF increased PF1+2, B = 0.16, P = 0.001 when adjusted for anti-FXa activity and hours of dialysis, whereas β-TG was only borderline increased, B = 0.09, P = 0.055.

Conclusions. ISF during HD does not alleviate visible clotting or intravascular coagulation activity in stable patients receiving reduced doses of dalteparin and polysulphone dialysers. Whether this applies to unstable patients with increased bleeding risk not receiving any anticoagulation remains to be shown.

Keywords: anticoagulation; clotting; dalteparin; haemodialysis; prothrombin fragment 1+2; saline flushes

Introduction

Haemodialysis (HD) in patients with increased bleeding risk is a challenge. Various solutions have been attempted to solve this problem. Regional heparinization with protamine neutralization involves the problem with bleeding due to a delayed heparin effect (rebound anticoagulation) and is not superior to limited-dose heparinization [1,2]. Low-dose systemic heparin anticoagulation still involves a risk of bleeding [3]. Regional citrate anticoagulation is described as a safe and effective method, and is reported to cause less clotting during HD than does anticoagulation with low molecular weight heparin or unfractionated heparin [4,5]. However, this method requires two accurate infusion pumps of citrate and calcium, and there is a risk for severe metabolic alkalosis and hypocalcaemia [6,7]. HD with prostacyclin is another approach, but this method is often associated with side effects like
hypotension, flushing, headache and gastrointestinal complaints [8].

Finally, heparin-free HD with intermittent saline flushes has been described as a safe and effective method [9].

The aim of this study was to investigate if intermittent saline flushes during HD really reduce clotting in the bubble trap and the dialyser. We investigated chronic stable HD patients to avoid interference of haemodynamic instability, inflammation and other uncontrolled factors in the ICU setting. The patients were also investigated at a constant blood flow throughout all HD sessions (only patients with well-functioning arterio-venous fistulas were included). For ethical reasons, the dalteparin bolus dose could not be withdrawn, but was reduced to 50% of the conventional dose to obtain some degree of clotting events. Prior to start of the study, our hypothesis was that the number of clinical clotting events would be 50% lower in HD with saline flushes as compared to HD with no saline flushes. The study was designed to assess the impact of saline flushes not only on clinical clotting events, but also on prothrombin fragment 1+2 (PF1+2), a sensitive marker of intravascular thrombin formation that directly reflects an activation of the haemostasis system, and on β-thromboglobulin (β-TG), a sensitive marker of platelet activation. Previously, we have shown that both these markers increase significantly during 4 h of HD, and PF1+2 significantly correlated to the degree of clinical clotting [10].

The experience with saline flushing in stable HD patients receiving reduced doses of dalteparin may create basis for further investigations in unstable patients at bleeding risk.

Material and methods

Patients

Stable patients of 18 years of age or more on chronic HD for at least one month and dialysis time at least 4 h 3 times per week were recruited. A stable haemoglobin value above 11 g/dl during the last week was mandatory. No patient received infusion of blood products the last week before and during the study.

Patients in need of oral warfarin or acetylsalicylic acid were excluded. Other exclusion criteria were clinical signs of infection and disseminated malignant disease.

The protocol was approved by the Regional Ethics Committee and written informed consent was obtained from all the patients according to the Helsinki declaration.

Dialysis procedure

A polysulphone hollow fibre dialyser (F6 HPS, Fresenius, Germany) and bicarbonate dialysate were used in all patients the last week before and during the study. The priming fluid consisted of 0.9% NaCl without anticoagulant. A blood flow rate of at least 200 ml/min was kept as stable and constant as possible within each patient. Dialysate flow was kept constant at 500 ml/min. All patients had a well-functioning arterio-venous fistula. Dalteparin was given as a single bolus dose at the start of HD.

Study design

Six HD sessions over 2 weeks were studied in eight patients, i.e. there were altogether 48 sessions. In three of these sessions (HD session 1, 3 and 5) at least 100 ml of 0.9% sodium chloride was infused via the arterial line and rapidly flushed through the extracorporeal system and filter every 30 min of HD while occluding the blood inlet line. The flushing fluid was added to the ultrafiltration volume required to gain dry weight. In HD session 2, 4 and 6, no saline flush was performed. With this design, the patients were their own controls.

In the first HD session, the regular dalteparin dose was reduced to 50% in each patient and maintained at that level throughout the study if no or only a minor degree of clotting (grade 1 and 2) was observed. With severe degree of clotting or stop in HD (grade 3 and 4), the dalteparin dose was increased to 75% of the regular dose in the next HD session.

Clinical clotting was evaluated by visual inspection after blood draining of the air trap (venous drip chamber) every hour (1 = no clotting in the air trap, 2 = fibrinous ring, 3 = clot formation and 4 = coagulated system) and by visual inspection of the dialyser at the end of each session [1 = clean filter, 2 = a few blood stripes (affecting less than 5% of the surface fibres), 3 = many blood stripes (affecting more than 5% of the fibres) and 4 = coagulated filter], as previously described [11].

During HD sessions with saline flushes, visual inspection of the bubble trap was performed immediately before the next saline flush.

Blood sampling

All blood specimens were taken after lowering the blood flow to 100 ml/min for 1 min. During HD sessions with saline flushes, blood specimens were drawn immediately before the next saline flush. At the start of dialysis, before start of ultrafiltration, the following blood specimens were taken from the arterial line: prothrombin fragment 1+2 (PF1+2) and β-thromboglobulin (β-TG). After 3 and 4 h of dialysis, these measures were repeated from the arterial line. Also anti-FXa activity was taken from the arterial line after 1, 3 and 4 h of dialysis. Blood for β-TG, PF1+2 and anti-FXa activity was collected into Diatube H® tubes and immediately cooled in a crushed ice-water mix for 15 min. The blood sample was prepared by centrifugation at 2500 g for 30 min at 4°C within 1 h of collection.

Laboratory methods

After centrifugation, the middle third of the plasma supernatant of the liquid portion was collected and stored at −70°C for later determination of β-TG, PF1+2 and anti-FXa activity. β-TG and PF1+2 were measured with an ELISA (EIA) procedure (Asserachrom® β-TG kit and Enzygnost® PF1+2 micro). Anti-FXa activity was measured with a chromogenic assay (Coatest®, Chromogenix AB, Mölndal, Sweden).
Sample size calculations

From observations previously reported, a total number of 36 clinical clotting events of degree 2 or more was expected in the bubble trap during the 24 HD sessions without saline flushes and 18 during the sessions with saline flushes [11]. Power calculations were performed using the nomogram described in Altman [12]. The study had a power of 90% (α = 0.05) to detect a 50% reduction of clinical clotting events in the bubble trap during HD sessions with saline flushes performed.

Statistics

For non-parametric distributions, paired comparisons were performed by the Wilcoxon matched-pairs signed ranks test. In order to estimate the effect of saline flushes and dalteparin dose on clotting in the air trap, a linear regression model of repeated measurements was adopted. In this analysis, the dependent variable was mean degree of clot score (grade) in the air trap per dialysis session. To estimate the effect of saline flushes, anti-FXa activity and time on PF1+2 and β-TG, linear regression analyses with repeated measurements were performed. PF1+2 and β-TG were not normally distributed and were logarithmically transformed before linear regression analysis.

A possible limitation to a linear regression method is that this method assumes additive effect, for instance that the increases in mean clot score (dependent variable) between hours 1 and 2 and between hours 3 and 4 are equal (Table 2).

In all analyses, a two-sided P-value of <0.05 was considered statistically significant.

The statistical software SPSS (SPSS 11, Inc., Chicago, IL) and SAS (SAS 6.12, SAS Institute Inc., Cary, NC) were used for the calculations.

Results

General outcome

Eight stable patients on chronic HD were included in the study, 6 men and 2 women. Mean (range) age was 67 (37–83) years and mean (range) duration on HD was 17.5 (6–34) months. The primary kidney disease causing chronic renal failure was nephrosclerosis in four patients, chronic glomerulonephritis in one, polycystic kidney disease in one, diabetic nephropathy in one and bilateral renal cancer in one. Mean ± SD body dry weight at study start was 76.5 ± 11.4 kg. None of the patients had bleeding disorders.

Six patients were treated with HD for 4 h three times per week. In one patient, the HD sessions lasted 5 h and in one 4.5 or 5 h dependent on the degree of fluid overload, all were treated three times per week.

The mean (range) applied dalteparin dose at inclusion in the study was 6875 (2500–12 500) IU or 91 (32–161) IU/kg. Mean (range) dalteparin bolus dose during the study was 4087 (1250–9375) IU or 53 (16–109) IU/kg. Two of the patients with long dialysis time received high conventional dalteparin doses of 12 500 and 10 000 IE, respectively. To avoid clotting, both patients usually received two bolus doses of dalteparin during the dialysis session. When included into the study, only one bolus dose at the start of dialysis was given, 6250 and 5000 IE, respectively. In one of these patients, clot score degree 4 (full stop) occurred on study day 5. These two patients obviously needed high doses of anticoagulation, and when reducing the conventional dalteparin dose to 50%, high clot scores were obtained.

None of the patients needed blood transfusions the last month before or during the study. Mean (range) ultrafiltration and filtration rate in the eight patients studied were 1745 (0–3900) ml and 417 (0–950) ml/h, respectively; only one of the patients had no ultrafiltration. Mean (range) blood flow was 295 (150–320) ml/min; the value of 150 ml/min was measured in one of the patients 4 h after start of dialysis, immediately before stop in dialysis due to a clot grade 4 in the bubble trap.

Six of the patients received darbepoetin alfa in a mean (range) dose of 47.5 (15–80) µg per week. One patient received 8000 units of erythropoietin per week.

Altogether 48 HD sessions were evaluated. There were four cases (in four patients) with clotting degree 4 (=full stop in HD); all these cases occurred on dialysis when saline flushes were given. Two of these patients lost about 200 ml of extracorporeal blood. In the other two cases, the patients could be reinfused with the blood from the extracorporeal system. We could not record any precipitating factor in any of these four cases (hypotension or reduced blood flow rate).

The number of clinical clotting degree 3 in the bubble trap was 80, 54 with saline flushes and 26 without P < 0.001. The number of clinical clotting events degree 1 and 2 was not different with and without flushing.

Mean degree of clinical clotting in the dialyser in HD with saline flushes versus without saline flushes were 2.96 vs 2.58, respectively, P = 0.01 (Wilcoxon matched-pairs signed ranks test). Mean degree of clinical clotting in the bubble trap at the end of 6 dialysis sessions was calculated for each patient and correlated to mean clot in the filter, r = 0.87, P = 0.004.

Mean haemoglobin (Hb, g/dl) during 48 HD sessions in eight patients at baseline and after 4 h of HD were 11.8 ± 1.0 and 12.5 ± 1.1, respectively, and mean haematocrit (HCT, %) were 34.5 ± 3.5 and 36.6 ± 3.5, respectively.

The coagulation markers PF1+2 and AT and the platelet activation marker β-TG both increased significantly during the dialysis sessions, and the increases in PF1+2 and in β-TG are far greater than what could be expected merely from the increased HCT (Table 1). On the other hand, the increase in AT may be attributable to water deprivation.

Risk factors for coagulation

A multiple linear regression analysis with repeated measurements was performed to evaluate the effect of
Saline flushes in haemodialysis

**Table 1.** Markers of coagulation during 48 HD sessions [median (p25–p75)]

<table>
<thead>
<tr>
<th>Biological markers</th>
<th>Baseline</th>
<th>After 4 h</th>
<th>Normal range</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coagulation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PF1+2, nmol/l</td>
<td>1.96 (1.40–2.60)</td>
<td>3.33 (2.42–4.18)</td>
<td>0.4–1.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AT%</td>
<td>92 (82–100)</td>
<td>103 (94–115)</td>
<td>80–120</td>
<td>0.01</td>
</tr>
<tr>
<td>Platelet activation</td>
<td>β-TG, IU/ml</td>
<td>147.24 (110.91–177.54)</td>
<td>292.85 (201.23–418.82)</td>
<td>10–40</td>
</tr>
</tbody>
</table>

*Baseline vs 4 h of dialysis. The statistical test used is the Wilcoxon signed ranks test.

**Table 2.** Effect of saline flushes and dalteparin-dose/kg on mean clot score in the air trap

<table>
<thead>
<tr>
<th></th>
<th>B</th>
<th>SE</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline flush</td>
<td>0.717</td>
<td>0.120</td>
<td>0.0001</td>
</tr>
<tr>
<td>Dalteparin dose, IU/kg</td>
<td>−0.011</td>
<td>0.005</td>
<td>0.03</td>
</tr>
</tbody>
</table>

B = linear regression coefficient; SE = standard error. The effect of saline flushes and dalteparin-dose/kg on mean clot in the air trap estimated by a linear regression model with repeated measurements. This table shows that when saline flushes are given, the mean clot score in the air trap increases with 0.717 units when adjusted for dalteparin dose compared with no flushing. Similarly, when the dalteparin dose increases with 1 IU/kg, the mean clot score decreases (as expected) with 0.011 units, when adjusted for saline flush.

**Table 3.** Effect of saline flushes, anti-FXa activity and time on PF1+2

<table>
<thead>
<tr>
<th></th>
<th>B</th>
<th>SE</th>
<th>P</th>
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<tbody>
<tr>
<td>Saline flush</td>
<td>0.156</td>
<td>0.047</td>
<td>0.001</td>
</tr>
<tr>
<td>Anti-FXa activity, IE/ml</td>
<td>−0.843</td>
<td>0.168</td>
<td>0.0001</td>
</tr>
<tr>
<td>4 h of HD (compared to 3 h of HD)</td>
<td>0.181</td>
<td>0.049</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

B = linear regression coefficient; SE = standard error. The effect of saline flushes, anti-FXa activity and time on PF1+2 estimated by a linear regression model with repeated measurements. In this analysis only parameters after 3 and 4 h of dialysis are evaluated. This table shows that when saline flushes are given, PF1+2 (logtransformed) increase with 0.16 units, when adjusted for anti-FXa activity and time. Similarly, when anti-FXa activity increases with 1 IE/ml, PF1+2 decreases with 0.84 units, when adjusted for saline flush and time. Finally, when going from 3 hours to 4 hours after start of HD, PF1+2 increases with 0.18 units when adjusted for saline flush and anti-FXa activity.

**Table 4.** Effect of saline flushes, anti-FXa activity and time on β-TG.

<table>
<thead>
<tr>
<th></th>
<th>B</th>
<th>SE</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline flush</td>
<td>0.09</td>
<td>0.04</td>
<td>0.055</td>
</tr>
<tr>
<td>Anti-FXa activity, IE/ml</td>
<td>−0.31</td>
<td>0.18</td>
<td>0.1</td>
</tr>
<tr>
<td>4 h of HD (compared to 3 h of HD)</td>
<td>0.14</td>
<td>0.05</td>
<td>0.004</td>
</tr>
</tbody>
</table>

B = linear regression coefficient; SE = standard error. The effect of saline flushes, anti-FXa activity and time on β-TG estimated by a linear regression model with repeated measurements. In this analysis only parameters after 3 and 4 h of dialysis are evaluated.

*Antithrombin in dialysis sessions with saline flushes versus no saline flushes*

There was no difference in the levels of antithrombin (AT) measured 3 h after start of dialysis in sessions with saline flushes vs no saline flushes (mean AT 101 vs 100%, Wilcoxon matched-pairs signed rank test). The same result applies to the AT levels measured 4 h after start of dialysis (mean AT 107 vs 103%, respectively).

*Elimination half-life of dalteparin*

The elimination of the anti-FXa activity (dalteparin) follows mono-exponential first-order kinetics and thus the half-life is assumed to be dose independent [13,14]. The elimination half-life ($T_{1/2} = \ln(2)/k$) was calculated from the formula $C = C_0e^{-kt}$ ($k$ = elimination constant, $t$ = hours of dialysis and $e = 2.718$). In one of the patients, a 37-year-old man, the elimination half-life of dalteparin was extremely high (range 5 to 12 h). In this patient, a rebound phenomenon was observed; the anti-FXa activity increased with time on two occasions. This patient was omitted from the calculation of mean half-life. For the other seven patients, mean ± SD dalteparin half-life was 2.6 ± 0.9 h.

*Discussion*

Heparin-free haemodialysis with intermittent saline flushes is reported to be a safe and effective method
both in maintenance HD patients with increased bleeding risk and in patients with acute renal failure and bleeding risk [9,15–21]. However, in none of these studies was heparin-free dialysis with saline flushes compared to appropriate controls comprising heparin-free dialysis without saline flushes.

The major consistent finding in our study of stable patients receiving reduced doses of dalteparin and polysulphone dialysers was that saline flushes promote coagulation as measured by PF1+2 and clinical clot formation in the bubble trap and filter. We demonstrated that saline flushes significantly increase clotting also when adjusting for dalteparin dose per kg body mass in multiple linear regression analysis. Moreover, when also adjusting for dialysis time and anti-FXa activity in multiple linear regression analysis, saline flushes significantly increased PF1+2, a marker of intravascular thrombin formation. Thus, saline flushes are unfavourable in stable patients receiving reduced dalteparin doses at start of HD.

This finding was surprising and contradictory to our hypothesis and despite a study design to test the opposite effect. However, the study design prohibits extrapolation of the results to unstable patients with bleeding risk who do not receive anticoagulation. Nevertheless, the results from our study are in accordance with a previous study indicating that saline flushes are unfavourable also in such a patient population [15]. They found a slight tendency towards larger fiber-bundle losses in the saline flush groups. Actually, in that study, the authors recommend heparin-free dialysis without saline flushes to be the first strategy employed in patients at high risk of bleeding.

It has been shown that haemodilution with saline induces a hypercoagulable state both in vitro and in vivo [22–24]. The mechanism is not clearly shown, but a reduction in the concentrations of natural anticoagulants such as AT, α1-antitrypsin and α2-macroglobulin has been suggested as a possible explanation [23,24]. When saline flushes are performed, any dilution takes place in the arterial line of the extracorporeal circulation before the filter and may last for a very short period of time. Some of the fluid infused with the saline flushes is subsequently ultrafiltrated, and ultimately when the blood is mixed in the systemic circulation any dilution can no longer be detected. Antithrombin was measured in this study and, as expected, no difference in AT levels after 3 or 4 h of dialysis could be demonstrated in HD sessions with saline flushes compared to dialysis with no saline flushes.

Similarly, in this study, the saline flushes may also have led to a markedly extracorporeal dilution of the anti-FXa activity, which has contributed to the increased coagulation. However, when adjusting for anti-FXa activity in multiple analysis, saline flushes significantly increased PF1+2, indicating that dilution of the anti-FXa activity cannot fully explain the procoagulant effect.

The patients in the present study were maintenance haemodialysis patients in clinically stable condition with no increase in bleeding risk. In this way, it was possible to keep blood flow, blood pressure and haemoglobin values stable. All the patients had well-functioning arteriovenous fistulas, and the risk of hypotension during dialysis treatment was minimal. All these factors may influence the degree of coagulation and clinical clotting scores in the extracorporeal circulation. Furthermore, to limit confounding factors, only F6 HPS was used in this study, a biocompatible polysulphone hollow fibre dialyser commonly used at our centre also for studies of coagulation in HD [10,11]. However, the importance of biocompatibility of dialyser membranes in stable HD patients remains to be elucidated [25].

The present study was not literally designed as 'heparin-free'. For ethical reasons, we had to apply half normal dose of dalteparin. The question is if the results can be extrapolated to unstable patients with increased bleeding risk in the ICU. Stable HD patients with chronic renal failure have an activated coagulation system and platelet activation both during dialysis treatment and before the next dialysis session [10,26,27]. However, in the ICU setting patients may be even more procoagulant than stable patients.

In conclusion, intermittent saline flushes do not alleviate coagulation, but rather promote clot formation during HD in stable patients receiving reduced doses of dalteparin. However, it remains to be shown in future studies whether these results apply to unstable patients receiving no anticoagulation.

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Conflict of interest statement. None declared.

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