Citrate anticoagulation does not correct cuprophane bioincompatibility as evaluated by the expression of leukocyte surface molecules

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Abstract

Background. Citrate, used for the anticoagulation of the extracorporeal dialysis circuit, reduces ionized calcium by chelation and has been claimed to attenuate dialyser membrane bioincompatibility. Dialysis with complement-activating cuprophane membranes is associated with leukopenia which has been related to an increase in adhesion molecule expression on the surface of circulating leukocytes.

Methods. The effect of citrate anticoagulation on the expression of CD11b, CD11c and CD45 on the surface of granulocytes and CD14 on monocytes during haemodialysis with cuprophane membranes, was evaluated by flow cytometric analysis. A comparison of standard heparin versus citrate was performed in 14 chronic haemodialysis patients. During citrate anticoagulation a calcium-free dialysate was used and citrate was infused to obtain a concentration of 4.3 mmol/l blood. The unchallenged ‘baseline state’ expression of the surface molecules and the increase after ex vivo stimulation with phorbol 12-myristate 13-acetate (delta-PMA) or formyl-methionyl-leucyl-phenylalanine (delta-fMLP) was studied.

Results. With heparin, as well as with citrate, a sharp fall in granulocyte and monocyte count was observed after 15 min of dialysis, followed by a recovery at the end of the session. The expression of CD11b, CD11c and CD45 on granulocytes increased markedly during cuprophane dialysis with a peak at 15 min; there were no differences in response between heparin and citrate anticoagulation. Delta-PMA and delta-fMLP for CD45, CD11c and CD14 showed a decrease during cuprophane dialysis vs t0; again there were no differences between heparin and citrate.

Conclusion. We conclude that use of citrate was not associated with reduced leukocyte activation as measured by the expression of surface molecules during cuprophane dialysis and that no effect on dialysis leukocytopenia could be registered.

Key words: adhesion molecule; biocompatibility; calcium; citrate; cuprophane; haemodialysis

Introduction

Locoregional citrate anticoagulation has been proposed as a treatment for haemodialysis patients who, during conventional anticoagulation, are subjected to enhanced bleeding risk, due to major surgery, trauma, massive haemoptysis, gastrointestinal bleeding or intracranial hemorrhage [1]. At least two studies observed an attenuation of the complement activation currently seen during dialysis with both cuprophane and cellulose acetate membranes, when citrate anticoagulation was applied [2,3]. Complement activation has been held responsible for intradialytic leukopenia since the very first studies in the field of biocompatibility [4]. However, citrate anticoagulation does not consistently result in an attenuation of intradialytic leukopenia [1–3,5–7].

The leukopenia in dialysis has been attributed to an enhanced expression of adhesion molecules on the leukocyte membrane, leading to an increase in adhesion to the vascular endothelium [8]. The effect of citrate anticoagulation on the expression of surface molecules on the leukocyte membrane has, to our knowledge, not yet been evaluated. Therefore, in the present paper, the effect of citrate anticoagulation on the expression of CD11b, CD11c (leukocyte integrins) and CD45 (leukocyte common antigen) on granulocytes and CD14 (receptor for lipopolysaccharides) on monocytes during haemodialysis with complement-activating cuprophane was compared to that of standard heparin anticoagulation. In addition to the unchallenged ‘baseline state’ of the leukocytes, the ex vivo response to challenges with phorbol-myristate-acetate (PMA), a direct protein kinase C activator, or with formyl-methionyl-leucyl-phenylalanine (fMLP), a chemotactic peptide, was evaluated. Sodium citrate can be administered at different doses during haemodialysis, but high concentrations have been associated with electrolyte disturbances [7] unless specific modifications in the...
Analytical techniques

Haemodialysis and anticoagulation

nephropathy (n = 2), chronic glomerulonephritis (n = 1), chronic interstitial nephritis (n = 6) and renovascular disease (n = 5). Parenteral nutrition, iron or mannitol were not administered during the study. All patients received erythropoetin to maintain a haematocrit of ≥ 33%. Before the start of the study, all patients underwent conventional haemodialysis with a low-flux polysulphone membrane (Rapido BLS 643, 1.36 m², Sorin Biomedica Bellco, Mirandola, Italy) or F8, 1.8 m², Fresenius, Bad Homburg, Germany) three times a week for either 4 or 4.5 h per session.

In all patients, two consecutive dialysis sessions were studied; both were performed with a low flux cuprophane membrane (Spiraffo NT 1375, 1.35 m², Sorin Biomedica Bellco, Mirandola, Italy). In a random order, either standard heparin or locoregional citrate anticoagulant was used. Approval of the local ethical committee was obtained.

Patients and methods

Patients

This study was undertaken in 14 well-equilibrated patients on maintenance haemodialysis (6 men, 8 women, mean age: 70 ± 12 years) with a mean body weight of 71.5 ± 17.9 kg. The patients had no infections and had not received antibiotic or immunosuppressive treatment for at least 3 months preceding the study. Patients suffering from auto-immune disorders were excluded. Informed consent was obtained from all patients. Causes of end-stage renal disease included diabetic nephropathy (n = 2), chronic glomerulonephritis (n = 1), chronic interstitial nephritis (n = 6) and renovascular disease (n = 5). Parenteral nutrition, iron or mannitol were not administered during the study. All patients received erythropoetin to maintain a haematocrit of ≥ 33%. Before the start of the study, all patients underwent conventional haemodialysis with a low-flux polysulphone membrane (Rapido BLS 643, 1.36 m², Sorin Biomedica Bellco, Mirandola, Italy). In a random order, either standard heparin or locoregional citrate anticoagulant was used. Approval of the local ethical committee was obtained.

Haemodialysis and anticoagulation

During each experimental dialysis session, blood flow, dialysate flow and dialysate temperature were maintained at 200 ml/min, 500 ml/min and 37°C respectively.

Haemodialysis with heparin anticoagulation was performed according to standard procedures with a starting dose of 2000 IU, followed by a continuous infusion of 1932 ± 578 IU/h. The degree of anticoagulation obtained by heparin was monitored by measuring activated clotting time (ACT, Hemochron® 801, International Technidyne Corporation, Edison, NJ, USA). The normal range of ACT is 105–167 s. The ACT obtained at inlet blood line of the dialyzer was targeted at ≥ 150 s.

A standard dialysate composition was used: bicarbonate 38.5 mmol/l, sodium 138 mmol/l, chloride 104 mmol/l, acetate 4 mmol/l, calcium 1.25 mmol/l and magnesium 0.5 mmol/l. The dialysate potassium concentration was adapted to the needs of the individual (range: 1–3 mmol/l). Routine control of dialysate bacterial content revealed < 5 CFU/ml.

Citrated anticoagulation was performed with a calcium-free dialysate; all other dialysate constituents were present in concentrations identical to those in the heparin sessions. Trisodium citrate at 1.2 mol/l (Sterop SA, Brussels, Belgium) was infused into the inlet blood line of the dialyser at a starting rate of 43 ml/h, corresponding to 4.3 mmol citrate per litre of blood passing through the extracorporeal circuit. In order to restore the calcium concentrations in the blood before returning to the patient, calcium chloride [1 g CaCl₂ :4 aq. (Ca²⁺ 11 meq), aqua ad 10 ml, Sterop] was infused in the outlet blood line, at a starting rate of 20 ml/h.

The degree of anticoagulation obtained by citrate was monitored by measuring ACT in the inlet blood line (upstream the citrate infusion) and in the outlet blood line (downstream the dialyser and upstream the CaCl₂ infusion). Ionized calcium, sodium and pH were measured in blood collected from the inlet line: blood samples were drawn into syringes containing heparin (Ciba Corning, Rapidlylyte® arterial blood sampler), transported on ice and immediately analysed on a Ciba Corning 288 blood gas system (Ciba Corning Diagnostics Corp, Medfield, MA, USA). Citrate and calcium chloride infusion rates were adjusted to the outlet ACT and inlet ionized calcium; an outlet ACT between 180 and 250 s was sought; the inlet ionized calcium concentration was targeted between its starting value and 0.9 mmol/l. Only minor adaptations were necessary.

Leukocyte counting and expression of surface molecules

Sample collection. At the start of dialysis and before heparin or citrate administration, blood was collected into EDTA tubes. At 15, 60 and 180 min of dialysis, blood samples were drawn from the inlet blood line, upstream of the citrate or heparin infusion port. Blood samples were processed immediately.

Analytical techniques. Total leukocyte number was counted in each sample, using a Coulter Counter® M530 (Coulter Electronics Ltd, Bedfordshire, UK). Cell differentiation was based on light scatter using a flow cytometer (FACScan® (Becton Dickinson, San Jose, CA, USA)); lymphocytes, granulocytes, and monocytes were selectively gated by low forward-angle light scatter, which is a measure of cell size, and by 90° light scatter, which is a measure of granularity.

The expression of CD45, CD11b and CD11c on granulocytes and of CD14 on monocytes was evaluated flow cytometrically by direct immunofluorescence. Leukocytes were studied in the ‘baseline state’, without extra ex vivo stimulation, as well as after ex vivo stimulation with PMA or with fMLP. For this purpose, PMA (Sigma, St Louis, MO, USA) was dissolved in dimethylsulphoxide (DMSO, Sigma) to obtain a stock solution (1 mg/ml). Before the experiment, the latter solution was immediately diluted 1:100 in sodium acetate buffer. One hundred microlitres of this PMA-solution were added to 500 μl whole blood to a final concentration of 1.7 pg/ml. fMLP (Sigma) was dissolved in DMSO to obtain a stock solution of 5 mg/ml, which was diluted 1/2000 in sodium acetate buffer immediately before the experiment. Ten microlitres of this fMLP-solution were added to 500 μl whole blood (final concentration of 49 ng/ml). Samples were incubated for 10 min at 37°C.

The expression of surface molecules on leukocytes was measured using the following monoclonal antibodies (Becton Dickinson Immunocytometry Systems, San Jose, CA, USA): (i) phycoerythrin (PE) conjugated anti-CD11b (Leu®-15), (ii) PE conjugated anti-CD11c (Leu®-M5), or (iii) a mixture of fluorescein isothiocyanate (FITC) conjugated anti-CD45 (Anti-Hle-1) together with PE conjugated anti-CD14 (Leu®-M3) (Simultest®, LeucoGATE®). Twenty microlitres of these antibodies were then placed into Falcon® polystyrene test tubes (Becton Dickinson Labware, Lincoln Park, NJ, USA), to which 100 μl of whole blood were added. After mixing at low speed, the samples were incubated with the respective monoclonal antibodies for 20 min at 4°C in the dark. FACS® Lysing Solution, 2 ml, diluted 10 x, was added to each tube, followed by incubation for 5 min. After washing the cells twice with Dulbecco’s phosphate-buffered saline (GIBCO BRL Life Technologies, Paisley, UK), the cell pellet
was resuspended in Hanks’ balanced salt solution (GIBCO BRL), and immediately analysed on a FACScan®. Data on 10 000 cells were collected in list mode files, the detector threshold being held on FSC-H:200. FacsFlow® was used as sheath fluid. CaliBRITE® Beads and AutoCOMP® Software were applied for setting photomultiplier tube voltages, adjusting fluorescence compensation and checking detector sensitivity. The mean channel of fluorescence intensity was measured.

Antibodies were used in saturating concentrations. Non-specific labelling was excluded by Simulset® Control γ1/γ2a (IgG, FITC/IgG2a, PE) (Becton Dickinson). Data analysis and statistics. Compared to the ‘baseline’ condition, ex vivo PMA or fMLP-stimulation of leukocytes in whole blood resulted in an enhanced expression of CD45, CD11b, CD11c and CD14 on their cell membranes. Hence, the following parameters of leukocyte surface molecule expression are given: (1) ‘baseline state’ expression, (2) the increase in expression after stimulation with PMA vs ‘baseline state’ (delta-PMA) and (3) the increase in expression after stimulation with fMLP (delta-fMLP). However, ex vivo CD11b upregulation after PMA stimulation was so pronounced that fluorescence intensity became too high to be measured correctly. Therefore, activation of CD11b was only studied with fMLP.

Data are expressed as means ± standard deviation (SD), and were analysed using repeated measures analysis of variance (ANOVA), in the case of significance followed by the Mann–Whitney U or Wilcoxon signed rank test where appropriate. Significance was accepted if $P \leq 0.05$.

Results

Anticoagulation methodology

The dialysis procedures were well tolerated with both anticoagulation modalities. One patient complained of muscular cramps during citrate dialysis.

During citrate dialysis, inlet sodium concentrations and pH remained within the physiological range with starting values of $137 \pm 2$ mmol/l and $7.36 \pm 0.05$, and values at $t_{180}$ of $140 \pm 2$ mmol/l and $7.43 \pm 0.04$, respectively. Ionized calcium blood levels were $1.19 \pm 0.12$ mmol/l at $t_0$ and $1.11 \pm 0.06$ at $t_{180}$.

No systemic anticoagulation occurred during citrate administration as inlet ACT remained within the normal range with a mean of $121 \pm 18$ s at $t_0$, $119 \pm 24$ at $t_{60}$, $106 \pm 19$ at $t_{120}$ and $115 \pm 31$ at $t_{180}$.

Leukocyte counts

Figure 1 shows the evolution of the peripheral granulocyte (A) and monocyte (B) counts. With both heparin and citrate, a sharp and similar fall in granulocyte and monocyte counts was observed after 15 min of dialysis ($P \leq 0.01$ vs $t_0$), followed by a recovery at the end of the session. ANOVA revealed no differences between heparin and citrate.

Expression of surface molecules

The evolution of ‘baseline state’ expression of CD45, CD11b and CD11c on granulocytes is illustrated in Figure 2 A, B and C respectively, and also in Table 1. The increase in expression of CD45, CD11b and CD11c after stimulation with PMA or fMLP, is displayed in Table 2. The expression of CD14 on monocytes is shown in Table 3.

CD45 expression on granulocytes

The ‘baseline state’ expression of CD45 on granulocytes is displayed in Figure 2A and Table 1A for both heparin and citrate. During heparin dialysis ‘baseline state’ CD45 expression increased from $99 \pm 25$ at $t_0$ to $204 \pm 36$ at $t_{15}$ ($P \leq 0.01$ vs $t_0$) and remained above the starting value at $t_{60}$ and $t_{180}$ with values of $150 \pm 29$ and $146 \pm 19$, respectively ($P \leq 0.01$ vs $t_0$). A similar enhanced expression was observed during citrate dialysis from $115 \pm 25$ at $t_0$ to $187 \pm 55$ at $t_{15}$, $144 \pm 24$ at $t_{60}$ and $138 \pm 20$ at $t_{180}$ ($P \leq 0.01$ vs $t_0$ at all time points). ANOVA revealed no differences between heparin and citrate.

The evolution of delta-PMA and delta-fMLP CD45 expression is detailed in Table 2A. A substantial decrease of delta-PMA vs $t_0$ was observed during both

![Fig. 1. (A) Granulocyte counts, (B) monocyte counts. Citrate dialysis is illustrated by triangles and full lines and heparin by squares and broken lines. There were no significant differences between heparin and citrate. Statistical significance vs $t_0$ is indicated for heparin by: *: $P \leq 0.05$ and **: $P \leq 0.01$. Statistical significance vs $t_0$ is indicated for citrate by: ***: $P \leq 0.01$ vs $t_0$.](image-url)
Table 1. ‘Baseline state’ expression of CD45, CD11b and CD11c on granulocytes

<table>
<thead>
<tr>
<th></th>
<th>$t_0$</th>
<th>$t_{15}$</th>
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<tr>
<td>A: CD45</td>
<td></td>
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<tr>
<td>heparin</td>
<td>99 ± 25</td>
<td>204 ± 36**</td>
<td>150 ± 29**</td>
<td>146 ± 19**</td>
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<td>citrate</td>
<td>115 ± 25</td>
<td>187 ± 55**</td>
<td>144 ± 24**</td>
<td>138 ± 20**</td>
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<tr>
<td>B: CD11b</td>
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<tr>
<td>heparin</td>
<td>505 ± 268</td>
<td>1339 ± 479**</td>
<td>1133 ± 314**</td>
<td>1069 ± 290**</td>
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<tr>
<td>citrate</td>
<td>662 ± 244</td>
<td>1118 ± 434**</td>
<td>1000 ± 373**</td>
<td>1014 ± 276**</td>
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<td>C: CD11c</td>
<td></td>
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<tr>
<td>heparin</td>
<td>148 ± 57</td>
<td>264 ± 47**</td>
<td>216 ± 36**</td>
<td>202 ± 34**</td>
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<tr>
<td>citrate</td>
<td>183 ± 55</td>
<td>244 ± 61**</td>
<td>224 ± 41*</td>
<td>206 ± 29*</td>
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</tbody>
</table>

*P<0.05 vs $t_0$. **P<0.01 vs $t_0$ (Wilcoxon signed rank test). Heparin vs citrate: not significant.

Table 2. Delta-PMA and delta-fMLP expression of CD45, CD11b and CD11c on granulocytes

<table>
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<tr>
<th></th>
<th>$t_0$</th>
<th>$t_{15}$</th>
<th>$t_{60}$</th>
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<tr>
<td>A: CD45</td>
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<td>delta-PMA</td>
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<tr>
<td>heparin</td>
<td>126 ± 40</td>
<td>38 ± 54**</td>
<td>79 ± 33**</td>
<td>96 ± 32**</td>
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<tr>
<td>citrate</td>
<td>110 ± 38</td>
<td>46 ± 50**</td>
<td>84 ± 42**</td>
<td>96 ± 30*</td>
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<td>delta-fMLP</td>
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<tr>
<td>heparin</td>
<td>158 ± 28</td>
<td>76 ± 28**</td>
<td>109 ± 23**</td>
<td>116 ± 14**</td>
</tr>
<tr>
<td>citrate</td>
<td>153 ± 33</td>
<td>90 ± 49**</td>
<td>115 ± 35**</td>
<td>118 ± 22**</td>
</tr>
<tr>
<td>B: CD11b</td>
<td></td>
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<tr>
<td>delta-fMLP</td>
<td></td>
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<tr>
<td>heparin</td>
<td>1768 ± 672</td>
<td>1492 ± 678</td>
<td>1620 ± 400</td>
<td>1513 ± 384</td>
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<tr>
<td>citrate</td>
<td>1886 ± 434</td>
<td>1834 ± 675</td>
<td>1854 ± 392</td>
<td>1633 ± 319</td>
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<tr>
<td>C: CD11c</td>
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<tr>
<td>delta-PMA</td>
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<tr>
<td>heparin</td>
<td>265 ± 87</td>
<td>83 ± 69**</td>
<td>103 ± 60**</td>
<td>132 ± 64**</td>
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<tr>
<td>citrate</td>
<td>225 ± 50</td>
<td>120 ± 75**</td>
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<td>147 ± 40**</td>
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<tr>
<td>delta-fMLP</td>
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<tr>
<td>heparin</td>
<td>323 ± 76</td>
<td>115 ± 64**</td>
<td>159 ± 54**</td>
<td>180 ± 45**</td>
</tr>
<tr>
<td>citrate</td>
<td>291 ± 37</td>
<td>157 ± 91**</td>
<td>180 ± 32**</td>
<td>197 ± 31**</td>
</tr>
</tbody>
</table>

*P<0.05 vs $t_0$. **P<0.01 vs $t_0$ (Wilcoxon signed rank test). Heparin vs citrate: not significant.

CD11b expression on granulocytes

The ‘baseline state’ expression of CD11b on granulocytes is displayed in Figure 2B and Table 1B. CD11b expression increased markedly during both heparin and citrate dialysis with a maximum at 15 min: for heparin from 505 ± 268 at $t_0$ to 1339 ± 479 at $t_{15}$, 1133 ± 314 at $t_{60}$ and 1069 ± 290 at $t_{180}$ ($P<0.01$ vs $t_0$ for each time point); for citrate from 662 ± 244 at $t_0$ to 1118 ± 434 at $t_{15}$, 1014 ± 276 at $t_{60}$ and 207 ± 73** at $t_{180}$ ($P<0.01$ vs $t_0$ for each time point). ANOVA revealed no differences between heparin and citrate.

The evolution of delta-fMLP CD11b expression is detailed in Table 2B: no significant changes were observed during cuprophane dialysis vs $t_0$. In addition, there were no significant differences at each time point between heparin and citrate.
CD11c expression on granulocytes

The ‘baseline state’ expression of CD11c on granulocytes is displayed in Figure 2C and Table 1C for both heparin and citrate. The evolution paralleled the results observed with CD45 and CD11b. During heparin dialysis ‘baseline state’ CD11c expression increased from 148±57 at \( t_0 \) to 264±47 at \( t_{15} \) (\( P < 0.01 \) vs \( t_0 \)) and remained elevated at \( t_{60} \) and \( t_{180} \) with values of 216±36 and 202±34 respectively (\( P < 0.01 \) vs \( t_0 \)). A similar increase was observed during citrate dialysis from 183±55 at \( t_0 \) to 244±61 at \( t_{15} \) (\( P < 0.01 \) vs \( t_0 \)), 224±41 at \( t_{60} \) and 206±29 at \( t_{180} \) (\( P < 0.05 \) vs \( t_0 \)). ANOVA revealed no differences between heparin and citrate.

The evolution of delta-PMA and delta-fMLP CD11c expression is detailed in Table 2C. A substantial decrease was observed in delta-PMA vs \( t_0 \) during both heparin and citrate dialysis. No differences were observed between heparin and citrate. Delta-fMLP also showed a pronounced decrease during cuprophane dialysis with both heparin and citrate. Again, no significant differences were observed between heparin and citrate.

CD14 expression on monocytes

The expression of CD14 on monocytes during heparin and citrate dialysis is summarized in Table 3. After an initial decrease in ‘baseline state’ CD14 expression during both heparin and citrate dialysis at \( t_{15} \) and \( t_0 \), the CD14 expression rose again to reach a slightly increased value at the end of the session. No significant differences were observed between heparin and citrate dialysis.

A marked decrease was observed in delta-PMA vs \( t_0 \) during cuprophane dialysis with both heparin and citrate. After 15 min of heparin dialysis delta-PMA decreased and remained suppressed during the whole dialysis session (\( P < 0.01 \) vs \( t_0 \) for each time point). After 15 min of citrate dialysis a non-significant decrease in delta-PMA was observed; this parameter was significantly suppressed at the other time points: \( P < 0.05 \) vs \( t_0 \) for \( t_{60} \) and \( t_{180} \). ANOVA revealed no differences between heparin and citrate.

Delta-fMLP showed a decrease during cuprophane dialysis vs \( t_0 \), with both heparin and citrate. No significant differences were observed between the two anticoagulation regimens.

Discussion

In the present study we evaluated the expression of the surface molecules CD45, CD11b and CD11c on circulating granulocytes and of CD14 on monocytes, during cuprophane dialysis, with different anticoagulation regimens, comparing heparin with citrate. The evolution of this expression was studied, either on leukocytes which were not submitted to ex vivo stimulation (‘baseline state’), or on PMA- or fMLP-activated leukocytes. The main conclusions are: (i) leukocytes induced by cuprophane dialysis with a nadir at 15 min, was profound with both anticoagulation regimens; (ii) the ‘baseline state’ expression of CD45, CD11b and CD11c on granulocytes showed a similar increase during cuprophane dialysis with a peak value at 15 min, irrespective of the anticoagulation regimen; (iii) delta-PMA and delta-fMLP for CD45 and CD11c (granulocytes) and for CD14 (monocytes), were suppressed during cuprophane dialysis, but again to a similar extent with both heparin and citrate. It is concluded that no significant differences in leukocyte surface molecule expression were observed between heparin and citrate.

It has repeatedly been demonstrated that cuprophane haemodialysis is associated with leukopenia, complement activation and an up-regulation of granulocyte surface molecules such as CD11b [8–11], CD11c [10,12] and CD45 [11,13,14], CD11b and CD11c being adhesion molecules. There is a striking parallelism in the timing of these phenomena, suggesting a causal relationship: the up-regulation of leukocyte adhesion molecules is considered to be a consequence of complement activation and leads to an increased adhesion of circulating leukocytes to the vascular endothelium, resulting in sequestration and leukopenia. In addition, in vivo and in vitro experiments demonstrated an up-regulation of CD11b/CD18 after injection of C5a [15] or incubation with C5a [8,9,16]. Also, in the present study, dialysis with the complement activating cuprophane membrane resulted in an up-regulation of the ‘baseline’ expression of CD45, CD11b and CD11c (Figure 2).

The up-regulation of surface molecules on leukocytes during dialysis has been attributed to the recruitment to the cell surface of already synthesized molecules stored in intracellular granules. This theory is based on the rapidity of the up-regulation, which cannot be accomplished in the same time frame by protein synthesis. Moreover, in vitro up-regulation was not inhibited by protein synthesis inhibitors [17]. Storage in specific granules has been demonstrated for CD11b [18].

There are at least two mechanisms whereby citrate anticoagulation might influence the expression of surface molecules: (i) inhibition of degranulation by lowering the calcium concentration in the extracorporeal circuit, and (ii) attenuation of complement activation by lowering the calcium (classical pathway) and magnesium (alternate pathway) concentrations.

Leukocyte degranulation is calcium dependent as it can be inhibited by lowering extracellular ionized calcium in vitro [19,20] and in vivo [2]. The depletion of ionized calcium within the artificial kidney, due to complexation by citrate and the use of calcium-free dialysate, could inhibit the degranulation of leukocytes and the up-regulation of surface molecules. A blunted up-regulation of CD11b/CD18 and CD45 was observed in in vitro experiments, after fMLP stimulation, when calcium was depleted [21,22].

The second mechanism whereby citrate could act, is the blunting of complement activation. The main route
of complement activation in cuprophane haemodialysis is through the alternate pathway [4,23], which is magnesium dependent. It is well known that citrate complexes not only calcium but also magnesium. In addition, ionized calcium is required for the stabilization of the C1 complex (C1q–C1r–C1s) in the classical pathway. At least two studies suggest that the classical pathway is also involved in intradialytic complement activation [16,24]. Whatever the mechanism, an attenuation of complement activation can be expected with citrate, and has been demonstrated during haemodialysis [2,3] as well as during plasma exchange procedures [25,26].

In spite of the theoretical benefit of citrate, the present study was not able to demonstrate a reduced leukopenia or blunted up-regulation of surface molecules. Citrate can be administered at different concentrations. Data in the literature suggest that high citrate concentrations (> 7.4 mmol/l) may be associated with a blunted leukopenia [1,2,7] in contrast to low citrate concentrations [3,5,6]. It could be hypothesized that high citrate concentrations give rise to lower ionized calcium concentrations and that, in the present study, this ionized calcium might be lowered insufficiently within the dialysate to block leukocyte degranulation. However, the blood calcium concentration was apparently low enough to inhibit blood coagulation. In addition, during a number of citrate dialysis sessions, the ionized calcium in the outlet blood line, proximal to the CaCl₂ infusion, was determined and found to be immeasurably low (detection limit 0.2 mmol/l, data not shown).

Another possible explanation is that degranulation could occur in spite of low calcium concentration depending on the type and strength of the activating stimulus. This hypothesis is supported by a study demonstrating that calcium depletion did not blunt in vitro CD11b/CD18 up-regulation after LPS stimulation, whereas it did blunt the up-regulation observed after fMLP [22]. Complement suppression by citrate is incomplete [2,3], so that the threshold value for induction of the surface molecule expression might still be attained.

Finally, it is of note that ex vivo challenge with PMA and fMLP, which normally leads to an up-regulation of surface molecules [17,18], results in a blunted up-regulation in the presence of cuprophane dialysis. The present data confirm earlier studies, where in parallel with the blunting of respiratory burst response [27], a decrease in CD45 and CD14 up-regulation after PMA [13] or fMLP [14] stimulation, was observed for granulocytes collected during cuprophane dialysis. However, in the present study, citrate anticoagulation induced no improvement of this blunted surface molecule up-regulation. These data might be related to the enhanced susceptibility to infection of dialysed patients, although other factors such as toxin retention, anaemia and hypovitaminosis D may also be involved. An increased susceptibility to infection has been observed in patients treated with complement-activating dialysers [27,28].

The leukocyte surface molecules evaluated in the present study, play a pathophysiological role in the defence mechanisms against infection. CD11b and CD11c (alpha subunits), associated non-covalently with CD18 (beta subunit), are leukocyte integrins. Numerous physiological functions have been ascribed to these integrins, including activation of neutrophils and monocytes and enhancement of adhesion, transmigration and phagocytosis [29]. The importance of this integrin subfamily is illustrated by the severe infections that develop in patients with a congenital deficiency of leukocyte integrins.

CD45 leukocyte common antigen is a glycoprotein expressed on the cell surface of all nucleated haematopoietic cells. Its intracellular domain has a tyrosine phosphorylation motif and regulates chemotactic response [30].

CD45 is a glycosylphosphatidylinositol-anchored glycoprotein, expressed chiefly on monocytes and macrophages. CD14 functions as a receptor for bacterial lipopolysaccharides (LPS). It transduces activation signals for the production of tumour necrosis factor-alpha (TNF-α), IL-6 and IL-8 [31], and plays a pivotal role in the immunological reaction against bacterial infection and in sepsis. In conclusion, despite its theoretical benefits, no correction of the bioincompatibility phenomena, as measured by leukocyte count and expression of leukocyte surface molecules was obtained when citrate anticoagulation was used in a concentration of 4.3 mmol/l.

It was the aim of the present study to determine the effect of citrate dialysis on the expression of surface molecules, using citrate at concentrations without potential for electrolyte disturbances. Further study with different dosages of citrate should be considered in the future.

References


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