Calcium and potassium changes during haemodialysis alter ventricular repolarization duration: \textit{in vivo} and \textit{in silico} analysis

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Abstract

Background. Alterations of ventricular repolarization duration, as measured by the QT interval, are frequently observed in haemodialysis (HD) patients. The nature and the sign of these changes are not yet fully understood.

Methods. Different dialysate K$^+$ and Ca$^{2+}$ levels, leading to different end-HD plasma concentrations in the patient, have been tested in the present study in terms of their impact on QTc. A model of the human cardiomyocyte action potential (AP) has been used to assess \textit{in silico} whether the changes in Ca$^{2+}$ and K$^+$ were able to justify at the cellular level the observed alterations of QTc.

Results. QTc was prolonged in HDs with low (1.25 mM) versus high (2 mM) Ca$^{2+}$ (424 ± 33 versus 400 ± 28 ms, $P < 0.05$) and in HDs with low (2 mM) versus high (3 mM) K$^+$ (420 ± 35 versus 399 ± 36 ms, $P < 0.05$). These alterations were confirmed at the cellular level by computational analysis showing prolongation of ventricular AP at low K$^+$ and low Ca$^{2+}$ at the same extent of the measured QTc variations. Numerical simulation predicted a critically long AP (and QT) when considering low K$^+$ and Ca$^{2+}$ simultaneously, suggesting the concurrent lowering of Ca$^{2+}$ and K$^+$ as a potential arrhythmogenic factor.

Conclusions. Numerical simulations of the ventricular AP may be useful to quantitatively predict the complex dependence of AP duration on simultaneous changes in Ca$^{2+}$ and K$^+$. Moreover, Ca$^{2+}$ content in the dialysate should be designed not to critically lower serum Ca$^{2+}$, especially in sessions at risk of end-dialysis hypokalaemia.

Keywords: calcium; electrolytes; electrophysiology; haemodialysis; ventricular repolarization

Introduction

Mortality from cardiac arrest and sudden death is unusually high in patients receiving maintenance haemodialysis (HD). Electrocardiographic alterations are frequently observed and the incidence of ventricular arrhythmias increases during and immediately after the HD session [1–3]. The QT interval is a recognized ECG marker of the ventricular repolarization and its prolongation has been associated with increased risk of sudden death in both pathological [4–6] and healthy [7] populations. For this reason, several studies addressed the analysis of QT, which is often significantly changed by the end of the dialysis treatment. Nevertheless, the results on the effect of HD on QT are not univocal. In some studies, the QTc interval increased during the dialysis session [8–14]. In other studies the QTc interval did not change, or decreased or showed a variable response [15–25]. Discrepancies between the studies may be attributed to population selection, methods of QT measurement [26,27] and variables related to the dialysis technique.

Apart from the above-mentioned confounding factors, HD therapy per se introduces several factors that can influence the duration of the QT interval. HD impacts body fluid composition, tissue hydration, electrolyte equilibrium and adrenergic activation. All these factors are known to have considerable effects on the excitability of the cardiac cells and potentially contribute to the observed QT changes. Since these factors act simultaneously and exert a complex influence on cardiac activity, it is difficult to assess their relative importance. In fact, mechanisms underlying QT alterations during haemodialysis are not completely understood, nor it is clear the clinical relevance.

Electrolyte disorders are one of the main HD-related factors that can cause QT interval alterations and cardiac arrhythmias, because of their involvement in the genesis, duration, morphology and propagation of the cellular action potential. The electrolytes that mostly influence the ventricular repolarization are K$^+$ and Ca$^{2+}$ [28]. In fact, in patients undergoing HD, prolongation of QTc was inversely...
To assess the effect of serum Ca\textsuperscript{2+} or intravenous intake of K\textsuperscript{+} and diabetic neuropathy. During the treatments any oral mental disease, presence pacemaker or cardiac stimulator variable doses of digitalis. Further exclusion criteria were eligible for the study if on HD three times a week for at least

Inclusion criteria

correlated with variations in plasma Ca\textsuperscript{2+}, suggesting that patients with the greatest reduction in Ca\textsuperscript{2+} had the greatest increases in QTc at the end of the HD session [14,16,18,20]. On the other hand, several authors pointed out the role of K\textsuperscript{+} removal as a pivotal arrhythmia-inducing factor associated with the dialysis treatment [1,22,29,30]. Since a HD session induces changes in both K\textsuperscript{+} and Ca\textsuperscript{2+} plasma concentrations, which mainly depend on the dialysate composition, an increasing interest has been devoted to a more rational design of dialysate K\textsuperscript{+} and Ca\textsuperscript{2+} contents, possibly time-profiled or targeted to the needs of specific subgroups of HD patients [16,19,22,30–32]. Matching the patient’s haemodynamic and biochemical profile to an individualized dialysis prescription has been indicated as the most effective strategy to reduce the risk of cardiac arrest during HD [3]. In this perspective it seems crucial to deepen the knowledge on the specific effects of HD-induced Ca\textsuperscript{2+} and K\textsuperscript{+} changes on cardiac electrical activity.

The aim of the present study was to explore the impact of HD-induced changes in plasma Ca\textsuperscript{2+} and K\textsuperscript{+} concentrations on the duration of the ventricular repolarization by using a computational model of the human ventricular cardiomyocyte [33]. To this purpose, we tested two different levels of Ca\textsuperscript{2+} and K\textsuperscript{+} concentration in the dialysis bath and compared measured QTc interval variations to simulated data on action potential duration (APD). Trough simulation the impact of each individual ion concentration change on the human ventricular AP was assessed and the effect of concurrent K\textsuperscript{+} and Ca\textsuperscript{2+} variations was predicted.

**Subjects and methods**

**Inclusion criteria**

End-stage renal disease (ESRD) patients were considered eligible for the study if on HD three times a week for at least 6 months and aged between 50 and 90 years. None of them had been receiving anti-arrhythmic or anti-hypertensive treatment with beta-blockers. None had been receiving variable doses of digitalis. Further exclusion criteria were mental disease, presence pacemaker or cardiac stimulator and diabetic neuropathy. During the treatments any oral or intravenous intake of K\textsuperscript{+} and Ca\textsuperscript{2+} had to be avoided. The study was performed at the Nephrology and Dialysis Units of the Malpighi Hospital in Bologna and the Infermi Hospital in Rimini. Informed patient consent was obtained.

**Alternative dialysate Ca\textsuperscript{2+} HDs**

To assess the effect of serum Ca\textsuperscript{2+} levels on QTc 23 HD patients (5 women and 18 men, 71.1 ± 9.9 years old) were enrolled in a crossover design. The causes of ESRD were chronic glomerulonephritis (n = 8), diabetic nephropathy (n = 2), nephritis caused by abuse of analgesics (n = 2), vascular nephropathy (n = 4), polycystic kidney disease (n = 2) and nephrosclerosis (n = 5).

Each patient underwent two acetate-free biofiltration (AFB) dialysis sessions. AFB is a mixed diffusive–convective dialysis therapy characterized by the total absence of any buffer in the dialysis bath. Patient’s acid–base balance is restored by infusing in postdilution mode a sterile solution of sodium bicarbonate at a concentration ranging from 145 to 167 mEq/L. The infusion flow rate was set between 2 and 2.4 L/h depending on individual prescription. The two study sessions were identical (time of dialysis, blood, dialysate and infusion flow rate, bath conductivity and size of the polyacrylonitrile haemofilter) except for Ca\textsuperscript{2+} concentration in the dialysate. Namely, the dialysate (in mM: Na\textsuperscript{+} 139, K\textsuperscript{+} 3, Mg\textsuperscript{2+} 0.37, Cl\textsuperscript{−} 145 and glucose 5.55) Ca\textsuperscript{2+} concentration was set at 1.25 or 2 mM. The ultrafiltration rate was set depending on patient needs; it was 0.80 ± 0.22 L/h in 1.25 mM Ca\textsuperscript{2+} HD and 0.79 ± 0.20 L/h in 2 mM Ca\textsuperscript{2+} HD (NS).

**Alternative dialysate K\textsuperscript{+} HDs**

Twelve patients (6 women and 6 men with a mean age of 71.3 ± 9.0 years) were studied to investigate the impact of different K\textsuperscript{+} levels. The causes of ESRD were chronic glomerulonephritis (n = 2), diabetic nephropathy (n = 1), nephritis caused by abuse of analgesics (n = 1), vascular nephropathy (n = 2), polycystic kidney disease (n = 4) and nephrosclerosis (n = 2).

Each patient underwent two identical AFB dialysis sessions except for K\textsuperscript{+} concentration in the dialysate. Namely, the dialysate bath (in mM: Na\textsuperscript{+} 139, Ca\textsuperscript{2+} 2, Mg\textsuperscript{2+} 0.37, Cl\textsuperscript{−} 146 and glucose 5.55) contained 2 or 3 mM K\textsuperscript{+}. The ultrafiltration rate was set depending on patient needs; it was 0.76 ± 0.24 L/h in 2 mM K\textsuperscript{+} HD and 0.83 ± 0.21 L/h in 3 mM K\textsuperscript{+} HD (NS).

**QT interval analysis**

Twelve-lead electrocardiogram (ECG) was continuously recorded (H-12 holter, Mortara Instruments Inc., Milwau-kee, WI, USA). ECGs were sampled at 180 Hz. At the end of the recording session, the ECGs were transferred to the hard disk of a personal computer. Sections of ECGs containing five consecutive sinus beats were extracted for all the recordings at t = 0 min and at the end of the treatment. For each section, QT and RR intervals of the three central sinus beats were calculated by a semiautomatic procedure.

The computer-automated technique identified the end of the T wave by the intersection between the baseline and the slope of the T wave between 30 and 70% of peak amplitude [34]. The only analyst intervention was the identification of the baseline. T-wave amplitude was measured from T-wave peak to TP baseline. T waves with an amplitude less than 100 µV were excluded.

For each beat, the QT interval was obtained as the mean of eight independent leads. QT intervals were corrected for heart rate with Bazzet’s formula: QTc = QT/√RR. Repeatability of the QTc measurement from one session to the next independent of any changes in electrolyte composition was assessed on a small subset of patients (16 haemodialysis sessions, 4 patients). No significant difference was found between measurements obtained from the same patient (3.5 ± 19.2 ms, with a coefficient of variation of 4.5%).

**Statistical analysis**

All the results are expressed as mean ± standard deviation. Repeated measures (Geisser–Greenhouse correction)
ANNOVA and Tukey–Kramer Multiple-Comparison Test were applied to every measured parameter for which normal distributions were found (Skewness, Kurtosis and Omnibus tests). Wilcoxon Rank-Sum nonparametric test was applied to parameters for which the normal distribution assumption was not met. Multiple regression analysis was used to determine significant correlations with QTc changes. All the tests were performed with the NCSS statistical tool (NCSS, Kaysville, UT, USA).

**Action potential model**

The ventricular action potential was simulated using the Ten Tusscher et al. [33] model of human ventricular cell. The model describes all the main membrane currents and active transport mechanisms and the regulation of intracellular Ca2+ concentrations.

The model has been validated against a wide set of experimental data [33]. With respect to the original model formulation, intracellular Na+ and K+ concentrations were kept constant during the simulations. [K+]i was set to 138.3 mM. [Na+]i was kept at 11.6 mM in the pre-dialysis simulations and to 12.9 mM in end-dialysis simulations. In addition, the Ca2+-dependent inactivation gate of I_{Ca}, fca, was modified according to the following formula on the basis of recent studies demonstrating that the Ca2+ channel inactivation process depends more strongly on local Ca2+ than on membrane potential [35–37]:

\[
f_{Ca\infty} = \frac{\alpha_{fca} + \beta_{fca} + \gamma_{fca}}{1.3156}
\]

where

\[
\alpha_{fca} = \frac{1}{1 + \left(\frac{[Ca^{2+}]}{0.000600}\right)^8}
\]

\[
\beta_{fca} = \frac{0.1}{1 + e^{\frac{(5[Ca^{2+}]-0.00009)}{0.0001}}}
\]

\[
\gamma_{fca} = \frac{0.3}{1 + e^{\frac{(5[Ca^{2+}]-0.00075)}{0.0001}}}
\]

Model differential equations were implemented in Simulink (Mathworks Inc., Natick, MA, USA).

The computer simulator was used to reproduce the pre- and end-dialysis conditions. The average plasma Ca2+, Na+ and K+ concentrations recorded at the beginning and at the end of HD sessions were set as extracellular concentrations in the model. Heart rate recorded at the beginning of treatment was used as model input for both (pre- and end-dialysis) simulations in order to exclude the heart rate adaptation of APD and to make comparisons with the heart rate-corrected QT.

Simulations were performed considering an in silico M-cell and lasted until a steady state AP was reached (typically 100 s). The action potential duration was measured as the interval between the action potential upstroke and the 90% repolarization level of the action potential (APD90).

**Results**

**Electrolyte changes during HD**

As expected, HD with 2 mM Ca2+ dialysate led to a significant increase in the serum Ca2+ level at the end of the treatment, whereas 1.25 mM Ca2+ HD significantly decreased the plasma Ca2+ (Table 1) because of the significant convective removal during AFQ [38]. The final Ca2+ levels were different between the two HD protocols (1.38 ± 0.11 mM versus 1.03 ± 0.10 mM, \( P < 0.001 \)). Plasma pH was higher in 1.25 mM Ca2+ HD. No differences were observed in K+, Na+ and HCO3− between the two HDs.

Two different levels of K+ in the dialysis bath led to different end dialysis plasma K+ concentrations. Three millimolar K+ HD caused a significantly less pronounced decrease in the serum K+ level with respect to 2 mM K+ HD (3.43 ± 0.51 mM versus 2.86 ± 0.42 mM, \( P < 0.01 \)). No other differences were observed between the two protocols (Table 2).

**Effect of HD-induced Ca2+ and K+ changes on the QTc interval**

The QTc interval changes during the HD sessions were not univocal and their trend (prolongation or shortening) was dependent on the level of Ca2+ and K+ in the dialysate. Indeed, none of the four tested protocols was able to induce a significant post- versus pre-dialysis alteration of the QTc. Nevertheless, despite the same initial values, QTc at the end of the sessions was found significantly different both between 1.25 and 2 mM Ca2+ HDs and between 2 and 3 mM K+ HDs (Tables 3 and 4). The end-dialysis QTc interval was longer with lower Ca2+ concentration in the dialysate

**Table 1.** Pre- and end-dialysis laboratory data measured on the haemodialysis procedures with alternative Ca2+ dialysate

<table>
<thead>
<tr>
<th></th>
<th>1.25 mM Ca2+ HD</th>
<th>2 mM Ca2+ HD</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>End</td>
</tr>
<tr>
<td>Ca2+ (mM)</td>
<td>1.14 ± 0.09</td>
<td>1.03 ± 0.10^p</td>
</tr>
<tr>
<td>K+ (mM)</td>
<td>5.36 ± 0.81</td>
<td>3.68 ± 0.41^a</td>
</tr>
<tr>
<td>Na+ (mM)</td>
<td>140 ± 3</td>
<td>143 ± 2^a</td>
</tr>
<tr>
<td>HCO3− (mM)</td>
<td>21.2 ± 2.4</td>
<td>25.8 ± 3.1^a</td>
</tr>
<tr>
<td>pH</td>
<td>7.38 ± 0.04</td>
<td>7.47 ± 0.04^a</td>
</tr>
</tbody>
</table>

End versus Pre: ^P < 0.001.
Ca2^+ 2 mM versus Ca2^+ 1.25 mM: ^bP < 0.01, ^cP < 0.001.
traces of APs corresponding to 1.25 and 2 mM Ca\(^{2+}\) HDs are depicted in Figure 3. As expected, K\(^{+}\) removal during HD induced membrane hyperpolarization independently on the simulated external Ca\(^{2+}\) concentration (Figure 3, end-dialysis APs). The lower Ca\(^{2+}\) content in the dialysate led to prolongation of AP under the end-dialysis condition (+5.3%), whereas the higher Ca\(^{2+}\) content in the dialysate shortened AP (−2.7%, Figure 4, upper panel). Similarly, the lower K\(^{+}\) concentration increased APD\(_{90}\) (+2.1%), whereas the higher K\(^{+}\) decreased it (−2.3%, Figure 4, lower panel).

We compared HD-induced ventricular AP variations with experimental data from previous clinical studies. When using the experimental electrolyte values by Nappi et al. [16], who also performed HDs with different dialysate Ca\(^{2+}\) concentrations, the simulated ventricular APDs qualitatively reproduced the in vivo results on the QTc interval, as shown in Figure 5. In particular, also in this case both QTc and APD\(_{90}\) were inversely correlated to the dialysate Ca\(^{2+}\) level. Covic et al. [18] compared patients with increasing QTc and patients with decreasing QTc during HD. The different experimental QTc trends were reproduced by simulations at the cellular level (QTc: +5.8% versus −3.4%, APD\(_{90}\): +3.4% versus −1.8%). Similarly, the QTc prolongation found by Morris et al. [11] when patients experienced a HD-induced Ca\(^{2+}\) decrease was reproduced (QTc: +3.5%, APD\(_{90}\): +3.9%) as well as the QTc shortening found by Flocchini et al. [20] who observed a HD-induced Ca\(^{2+}\) increase accompanied by a limited K\(^{+}\) decrease (QTc: −1.4%, APD\(_{90}\): −1.9%).

### Combined effect of Ca\(^{2+}\) and K\(^{+}\) on APD

The impact of concurrent K\(^{+}\) and Ca\(^{2+}\) variations on APD\(_{90}\) was further analysed (Figure 6). A typically large HD-induced K\(^{+}\) decrease (from 6 mM to 3 mM) was tested with three different concurrent Ca\(^{2+}\) pre- versus end-dialysis variations (from 1.1 mM to 0.9, 1.1 and 1.3 mM, respectively). When a low end-dialysis K\(^{+}\) plasma concentration was accompanied by a decreased Ca\(^{2+}\) concentration the AP was largely prolonged (APD\(_{90}\): +16%). By observing the time course of the total membrane current, the AP prolongation can be mainly ascribed to a less rapidly activated and less pronounced positive repolarizing current under the end-dialysis condition with respect to the pre-dialysis (Figure 6, lower panel). The AP prolongation was markedly reduced when the HD-induced K\(^{+}\) reduction was accompanied by

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### Table 2. Pre- and end-dialysis laboratory data measured on the haemodialysis procedures with alternative K\(^{+}\) dialysate

<table>
<thead>
<tr>
<th></th>
<th>2 mM K(^{+}) HD</th>
<th></th>
<th>3 mM K(^{+}) HD</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>End</td>
<td>Pre</td>
<td>End</td>
</tr>
<tr>
<td>Ca(^{2+}) (mM)</td>
<td>1.06 ± 0.11</td>
<td>1.26 ± 0.24(^{a})</td>
<td>1.06 ± 0.16</td>
<td>1.34 ± 0.14(^{c})</td>
</tr>
<tr>
<td>K(^{+}) (mM)</td>
<td>4.77 ± 0.65</td>
<td>2.86 ± 0.42(^{c})</td>
<td>4.87 ± 0.83</td>
<td>3.43 ± 0.51(^{d})</td>
</tr>
<tr>
<td>Na(^{+}) (mM)</td>
<td>137 ± 4</td>
<td>140 ± 5</td>
<td>137 ± 4</td>
<td>141 ± 5</td>
</tr>
<tr>
<td>HCO(_{3}^{-}) (mM)</td>
<td>21.9 ± 2.5</td>
<td>27.2 ± 3.0(^{c})</td>
<td>20.1 ± 2.4</td>
<td>24.1 ± 6.7(^{a})</td>
</tr>
<tr>
<td>pH</td>
<td>7.38 ± 0.05</td>
<td>7.47 ± 0.04(^{c})</td>
<td>7.36 ± 0.03</td>
<td>7.44 ± 0.07(^{b})</td>
</tr>
</tbody>
</table>

End versus Pre: \(^{a}\)P < 0.05, \(^{b}\)P < 0.01, \(^{c}\)P < 0.001.
K\(^{+}\) 3 mM versus K\(^{+}\) 2 mM: \(^{d}\)P < 0.01.

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### Table 3. Effect of haemodialysis with alternative Ca\(^{2+}\) dialysate on HR, QT and QTc

<table>
<thead>
<tr>
<th></th>
<th>1.25 mM Ca(^{2+}) HD</th>
<th>2 mM Ca(^{2+}) HD</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>End</td>
<td>Pre</td>
<td>End</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>76 ± 9</td>
<td>78 ± 11</td>
<td>81 ± 12</td>
<td>84 ± 14</td>
</tr>
<tr>
<td>QT (ms)</td>
<td>359 ± 32</td>
<td>361 ± 33</td>
<td>358 ± 25</td>
<td>317 ± 83(^{b})</td>
</tr>
<tr>
<td>QTc (ms)</td>
<td>407 ± 32</td>
<td>424 ± 33</td>
<td>408 ± 26</td>
<td>400 ± 28b</td>
</tr>
</tbody>
</table>

End versus Pre: \(^{a}\)P < 0.05.
Ca\(^{2+}\) 2 mM versus Ca\(^{2+}\) 1.25 mM: \(^{b}\)P < 0.05.

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### Table 4. Effect of haemodialysis with alternative K\(^{+}\) dialysate on HR, QT and QTc

<table>
<thead>
<tr>
<th></th>
<th>2 mM K(^{+}) HD</th>
<th></th>
<th>3 mM K(^{+}) HD</th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>End</td>
<td>Pre</td>
<td>End</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>64 ± 13</td>
<td>83 ± 17(^{a})</td>
<td>66 ± 13</td>
<td>75 ± 18</td>
</tr>
<tr>
<td>QT (ms)</td>
<td>401 ± 45</td>
<td>363 ± 49</td>
<td>394 ± 43</td>
<td>361 ± 41</td>
</tr>
<tr>
<td>QTc (ms)</td>
<td>409 ± 27</td>
<td>420 ± 35</td>
<td>409 ± 25</td>
<td>399 ± 36(^{a})</td>
</tr>
</tbody>
</table>

End versus Pre: \(^{a}\)P < 0.01.
K\(^{+}\) 3 mM versus K\(^{+}\) 2 mM: \(^{a}\)P < 0.05.

(424 ± 33 ms versus 400 ± 28 ms, P < 0.05) and with lower K\(^{+}\) dialysate (420 ± 35 ms versus 399 ± 36 ms, P < 0.05).

In Figure 1, QTc values at the beginning and at the end of sessions with alternative dialysate Ca\(^{2+}\) or K\(^{+}\) concentrations are reported for each patient. QTc was prolonged in 18 out of 23 patients by dialysis with 1.25 mM Ca\(^{2+}\), whereas it was shortened in 14 patients by dialysis with 2 mM Ca\(^{2+}\). QTc was prolonged in 8 out of 12 patients by dialysis with 2 mM K\(^{+}\), whereas it was shortened in 10 patients by dialysis with 3 mM K\(^{+}\).

The change in the QTc interval induced by the study HD sessions correlated inversely with change in serum Ca\(^{2+}\) (P < 0.01, Figure 2). In multiple regression analysis, where the changes in Na\(^{+}\), Ca\(^{2+}\), K\(^{+}\), pH and HCO\(_{3}^{-}\) were assumed as independent variables, this was the only independent correlation with QTc change.

### Effect of HD-induced Ca\(^{2+}\) and K\(^{+}\) changes on APD

Simulated changes in ventricular APs induced by HD with alternative dialysate compositions were in good agreement with our in vivo results on the QTc interval. Representative
Individual values of QTc before and at the end of HD sessions with different dialysate Ca\textsuperscript{2+} (upper panels) or K\textsuperscript{+} (lower panels) concentrations.

Ventricular repolarization and Ca\textsuperscript{2+}

Lengthening of the QTc interval due to prolongation of phase 2 of the AP is a known electrocardiographic manifestation of hypocalcaemia and it might trigger afterdepolarizations leading to dysrhythmias and torsades de pointes. The known effect of hypercalcaemia on the electrocardiogram is the opposite of hypocalcaemia with the hallmark of abnormal shortening of the QTc interval, but clinically significant rhythm disturbances associated with hypercalcaemia are rare. The significant inverse correlation between QTc prolongation and variations in plasma Ca\textsuperscript{2+} that we found is in agreement with previous reports on patients undergoing haemodialysis [14,16,18,20]. Nappi et al. [16] also performed HDs with different dialysate Ca\textsuperscript{2+} concentrations and measured the QT intervals by means of
Impact of dialysis-induced Ca and K changes on QT

Fig. 2. Scatter plot and regression line showing the significant inverse correlation between QTc interval duration and serum Ca\(^{2+}\) concentration changes during HD sessions (35 patients, 70 sessions).

Fig. 3. Simulated ventricular action potentials (APs) under the conditions of pre- and end-dialysis with different dialysate Ca\(^{2+}\). Simulations were performed by using as input to the ventricular cell model the average measured K\(^{+}\), Ca\(^{2+}\) and Na\(^{+}\) concentrations as reported in Table 1. The lower Ca\(^{2+}\) content in the dialysate leads to prolonged AP under the end-dialysis condition, whereas the higher Ca\(^{2+}\) content in the dialysate shortens AP.

Fig. 4. Comparison of percent variations in measured QTc (mean ± SD) and simulated APD in HD with alternative dialysate Ca\(^{2+}\) (upper panels) or K\(^{+}\) (lower panels). According to experimental results, APD increases at lower dialysate K\(^{+}\) or Ca\(^{2+}\), whereas it decreases at higher K\(^{+}\) or Ca\(^{2+}\). Both QTc and APD were normalized with respect to their average pre-dialysis value.

A tangent-based method. They found QTc changes similar to those we presented in this study and in our simulations APs of the digital cell well reproduced the calcium dependence of the alterations they observed (Figure 5). Genovesi et al. [14] showed the same QTc sensitivity to plasma Ca\(^{2+}\) variations we observed, but greater QTc prolongations. In fact, when analysing the linear regression between QTc and Ca\(^{2+}\) variations, they found a similar slope (63 versus 56 ms/mM) but different intercept (34 versus 10 ms) with respect to our results. Such systematic difference is likely due to differences in methodology and population characteristics.

**Ventricular repolarization and K\(^{+}\)**

K\(^{+}\) plays an important role in maintaining the electrical potential across the cellular membrane, as well as in depolarization and repolarization of the cardiac myocytes. Alterations in serum K\(^{+}\) levels such as the HD-induced K\(^{+}\) removal may lead to electrocardiographic changes and severe arrhythmias. Previous studies failed to find a significant correlation between QTc prolongation and variations in plasma K\(^{+}\) [14,16,18,20]. To the best of our knowledge, the present study compared for the first time the effect on QTc of two dialysate formulations designed to have different end-dialysis K\(^{+}\) plasma levels (e.g. identical bath composition except for K\(^{+}\) concentration). We found that the duration of ventricular repolarization at the end of dialysis was significantly related to K\(^{+}\), being longer in sessions with lower K\(^{+}\) (Table 4). Buemi et al. [22] recently tested two different protocols of K\(^{+}\) removal leading to differences in plasma K\(^{+}\) during dialysis but to equal end-dialysis values. Coherently with our results they found prolonged QTc in the first half of the sessions with faster K\(^{+}\) removal, when lower plasma K\(^{+}\) concentrations were induced.
Fig. 5. Comparison of percent variations in QTc measured by Nappi et al. and simulated APD in HD with alternative dialysate Ca\(^{2+}\). Simulations were performed by using as input to the ventricular cell model the average measured K\(^{+}\), Ca\(^{2+}\), Na\(^{+}\) and heart rate reported in [16]. According to experimental results, APD is inversely correlated to the dialysate Ca\(^{2+}\) level.

Fig. 6. Simulated ventricular APs (upper panel) and total membrane current (lower panel, positive values correspond to outward currents, the y-scale was zoomed to appreciate differences in the repolarization phase) under a typical pre-dialysis condition (K\(^{+}\) = 6 mM and Ca\(^{2+}\) = 1.1 mM, thin line) and under three end-dialysis conditions with different plasma Ca\(^{2+}\) concentrations. When a low end-dialysis K\(^{+}\) plasma concentration (3 mM) is accompanied by a decreased Ca\(^{2+}\) concentration (thick line), the AP is prolonged by 16%. Such prolongation is markedly reduced when the dialysis-induced K\(^{+}\) reduction is accompanied by a constant (dashed line) or increased (dotted line) Ca\(^{2+}\) concentration at the end of dialysis with respect to the pre-dialysis.

Combined effect of Ca\(^{2+}\) and K\(^{+}\) changes
Results from previous studies on QTc in HD indicated that when both Ca\(^{2+}\) and K\(^{+}\) change during the treatment, the overall result on QTc is not easily predictable. We pointed out that numerical simulation of the ventricular AP allows quantitative predictions on the complex dependence of APD on simultaneous changes in Ca\(^{2+}\) and K\(^{+}\). In silico analysis showed that K\(^{+}\) removal can lead to APD\(_{90}\) increase even if performed under conditions of constant plasma Ca\(^{2+}\) (dashed versus thin line in Figure 6) or increasing plasma Ca\(^{2+}\) (dotted versus thin line in Figure 6). On the other hand, when the HD-induced plasma K\(^{+}\) decrease is not large, its prolonging effect can be overwhelmed by a concomitant shortening effect of increasing plasma Ca\(^{2+}\), as it was the case in both the 2 mM Ca\(^{2+}\) and 3 mM K\(^{+}\) HDs. Under those conditions, the experimental observation of QTc shortening was well reproduced by simulated APs (Figure 4). Notably, this sort of analysis was successfully applied to data from other clinical studies reporting either HD-induced shortening or prolongation of QTc (see Figure 6 and the Results section).

Importantly, our in silico analysis predicted a critically long QT when simulating a low end-dialysis K\(^{+}\) plasma concentration accompanied by a decreased Ca\(^{2+}\) concentration (Figure 6). It is worth to note that the Ca\(^{2+}\) and K\(^{+}\) concentrations we used for that simulation are within the range of the measured values. By considering the average pre-dialysis QTc (410 ms, Tables 3 and 4) and a percent HD-induced increase equal to those obtained for APD (+16%, Figure 6) an end-dialysis critically prolonged (>440 ms [7,39]) QTc value of 476 ms was predicted. The HD-induced QT prolongation related to Ca\(^{2+}\) and K\(^{+}\) removal could be further exacerbated in uraemic patients receiving any drug that is capable of inhibiting K\(^{+}\) channels, so leading to the clinical manifestation of the acquired long QT syndrome, as recently discussed by Gussak and Gussak [40].

In silico analysis
In this study, in silico analysis has been performed by means of a mathematical model of the human ventricular AP. This sort of computational approach has proven valuable to link information obtained at the molecular level to the whole cell and to the clinical phenotype in a number of inherited [41,42] and acquired [43,44] cardiac diseases. For the first time in this study, it has been applied to analyse HD-induced modifications in ventricular electrical activity. The application of computational cell biology to the analysis of ECG changes associated to HD can contribute to deepen the knowledge on the cardiac impact of HD therapy. On the other hand, HD represents an unique model to study the effects of ionic changes on the ventricular repolarization process under relatively stable and controlled conditions, and it may contribute to clarify the mechanisms involved in the genesis of electrocardiographic repolarization abnormalities related to acquired prolonged QT.

Limitations
The two experimental protocols were designed to separately analyse the effects of Ca\(^{2+}\) and K\(^{+}\); a more complete
analysis could be performed by a factorial design on a larger patient population. Actually, we also found a difference in end-dialysis pH between 2 mM Ca\(^{2+}\) and 1.25 mM Ca\(^{2+}\) HDs. Nevertheless, regression analysis excluded a direct influence of pH on QTc, as already reported in previous studies [16].

The model we used has been developed to describe a physiological condition [33], it does not take into account the known electrical remodelling effects of uraemic cardiomyopathy and it does not incorporate a reliable description of intracellular Na\(^{+}\) dynamics under large non-physiological variations of extracellular electrolytes. Thus, we set intracellular Na\(^{+}\) and K\(^{+}\) concentrations to constant values. A detailed model of human ‘uraemic cardiomyocyte’ should be identified for a more quantitative description of the cardiac electrical activity in uraemic patients.

Conclusions

The present study showed that the impact of HD on ventricular repolarization duration strictly depends on the level of Ca\(^{2+}\) and K\(^{+}\) in the dialysate. In silico analysis proved to be a valuable tool to gain insights into the impact of Ca\(^{2+}\) and K\(^{+}\) changes at the cellular level and predicted a critical QTc prolongation when a low end-dialysis K\(^{+}\) plasma concentration is accompanied by a decreased Ca\(^{2+}\) concentration. Our results suggest that Ca\(^{2+}\) content in the dialysate should be designed so as not to lower serum Ca\(^{2+}\), especially in sessions at risk for end-dialysis hypokalaemia. This aspect has to be carefully considered when designing new profiled and/or personalized dialysates.

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