Chemical and microbiological stability, anticoagulant efficacy and toxicity of 35 and 90 mM trisodium citrate solutions stored in plastic syringes

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ABSTRACT

Background Trisodium citrate is an interesting alternative to heparin for the prevention of circuit clotting during extracorporeal procedures, but some protocols require non-commercially available citrate concentrations. Little published information is available about the stability of diluted citrate solutions.

Objectives To evaluate the long-term stability, efficacy and toxicity of 35 mM and 90 mM trisodium citrate solutions prepared by diluting a commercially available sterile solution, stored in plastic syringes and used as an anticoagulant during citrate bag changes in the coupled plasma filtration adsorption (CPFA) technique in the COMPACT-2 clinical trial.

Methods The chemical stability of trisodium citrate solutions was evaluated by high-performance liquid chromatography after 7, 14, 21 and 28 days of storage. Sterility tests were performed both immediately after preparation and after 28 days of storage.

Results After 28 days of storage, the concentration of trisodium citrate had not changed in comparison with day 1, and both solutions passed the sterility test. A preliminary test indicated that a 35 mM solution is insufficient to ensure an effective anticoagulant action on an extracorporeal circuit, but the 90 mM solution was successfully used for 7 CPFA treatments in 2 patients, without clinical signs of toxicity.

Conclusions Both the 35 mM and 90 mM solutions are chemically and microbiologically stable for 28 days when stored at room temperature in 50 mL syringes protected by light. The 90 mM solution is an effective and safe regional anticoagulant in the CPFA protocol.

Trial registration number NCT01639664.

INTRODUCTION

The haemodialysis procedure requires the maintenance of an adequate blood flow into the extracorporeal circuit, so the use of an anticoagulant is essential to avoid the intraluminal thrombus formation that could decrease the hemofiltration efficiency. Heparin is a systemic anticoagulant commonly used as thrombolytic agent in different pathological conditions and in the prevention of thrombus formation during a number of extracorporeal medical procedures.¹ It exerts its anticoagulant activity through inhibition of factor Xa and thrombin II, leading to blocking of the coagulation cascade before the activation of fibrin from fibrinogen.¹ One of the most important drawbacks to the use of heparin as anticoagulant during haemodialysis is its longlasting action. The biological half-life of heparin is in the range of hours,

and this potentially exposes the patients to systemic anticoagulant action, with increased risk of thrombocytopenia, bleeding and haemorrhage.² This risk is particularly relevant in uraemic, septic or critically ill patients, as they have an increased likelihood of bleeding due to recent surgery, trauma, mucosal lesions or coagulopathy.^{3–5} Moreover, binding of endothelial antithrombin to administered heparin inhibits its anti-inflammatory actions and prevents local prostacyclin formation, which, in turn, may aggravate sepsis-mediated disorders.^{3, 6}

Trisodium citrate is an interesting alternative to heparin when anticoagulant action is required only locally (eg, central venous catheter or extracorporeal circuit during haemofiltration or haemodialysis).^{3–5, 7} The regional anticoagulant action of trisodium citrate is due to its ability to chelate the free ionised calcium in the blood, leading to blockage of calcium-dependent clotting formation in the coagulation cascade. By chelating calcium, citrate acts regionally as an anticoagulant when administered pre-filter, and thereby reduces the risk of bleeding compared with systemic anticoagulation.⁴ During continuous renal replacement therapy (CRRT), an optimal regional anticoagulant activity occurs when the ionised calcium concentration in the extracorporeal circuit is <0.35 mmol/L.⁸ The remaining citrate, free and calcium bound, is partially removed by haemofiltration (from 20% to 80%, depending on the blood and effluent flow rate, and CRRT modality), and partially enters the systemic circulation. When citrate reach the bloodstream, its anticoagulant action is rapidly reverted because the calcium citrate chelates are efficiently metabolised by liver and muscles, and free calcium ions again become available in the blood.^{3, 9, 10} Furthermore, the normal patient's systemic calcium level is commonly restored by post-haemofilter replacement infusion of calcium solution.¹¹

Recent reviews of clinical studies, comparing regional citrate anticoagulation with systemic heparinisation in critically ill patients treated with CRRT, indicate that citrate is safer, more efficacious and more cost-effective, and allows better filter survival times with higher delivered CRRT doses.^{3–5, 7, 8, 10, 11} Consequently, the most recent Clinical Practice Guidelines for Acute Kidney Injury recommend the use of citrate as anticoagulant in patients requiring CRRT.¹²

Different methods of regional citrate anticoagulation have been reported: in some protocols, citrate is administered in the extracorporeal circuit as a separate solution, whereas, in other protocols,

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citrate is added to a calcium-free predilution replacement fluid.^{7 8 10 11 13 14}

The administration of citrate may cause serious adverse effects, such as metabolic alkalosis and acidosis, hypernatraemia and hyponatraemia, and hypocalcaemia. Thus, it is important to design citrate anticoagulation protocols carefully, and preferably, to incorporate an appropriate citrate module in the CRRT device, in order to continuously monitor the citrate concentration in the prefilter. Finally, the patient's level of electrolytes and calcium, and the acid–base status should be frequently monitored, in order to minimise the risk of toxicity.^{15 16}

Septic shock is a systemic imbalance of the proinflammatory and anti-inflammatory response that can be induced by the endotoxin of Gram-negative bacteria. Therefore, treatment strategies should pursue a twofold objective: to counteract the infection and to rebalance the immune response. The first objective is achieved by the administration of antibiotics, and the second, can be accomplished by various blood purification techniques, including coupled plasma filtration adsorption (CPFA), which can unselectively remove the majority of soluble inflammatory mediators involved in sepsis pathogenesis.^{17 18} The efficacy of CPFA in the treatment of critically ill patients with septic shock has been compared with standard care in the COMPACT multicentre randomised controlled clinical trial (NCT00332371). However, that trial was prematurely terminated owing to the high number of protocol violations in the CPFA arm—namely, the low volume of plasma treated each day.¹⁹ The prescribed volume of plasma was not treated mainly owing to clotting of the circuit, despite the use of heparin for anticoagulation. In an attempt to overcome this problem, the extracorporeal circuit has been modified, in order to support regional anticoagulation with citrate, and a new multicentre randomised controlled clinical trial, COMPACT-2 (NCT01639664) has been started.

The aim of our study was to evaluate the long-term stability, efficacy and toxicity of two different concentrations of trisodium citrate (35 and 90 mM) stored in plastic syringes, suitable for use in the CPFA procedure. Little published information is available about the chemical stability of diluted citrate solutions, and studies have reported data only on the 136 mM solution.^{20 21} In addition, no information about maintenance of the sterility of these solutions during storage has been reported.

The solutions were prepared by diluting commercially available sterile trisodium citrate concentrate with sterile 0.9% sodium chloride solution under sterile conditions, and were stored in 50 mL syringes for up to 28 days at room temperature. The solutions were then used as regional anticoagulant during CPFA therapy in the COMPACT-2 clinical trial. The immediate availability of citrate syringes ready-to-use for the nephrology department and intensive care unit (ICU) is very important in the treatment of patients with acute kidney failure or septic shock, as the prompt start of CRRT or CPFA procedures is critical for the chances of a successful treatment.^{22 23}

METHODS

Chemicals, reagents and materials

Commercially available trisodium citrate sterile concentrate solution (46.7%) was purchased from Monico (Mestre/Venice, Italy), sterile 0.9% sodium chloride solution was provided by Baxter (Rome, Italy), sterile 50 mL polypropylene (PP) syringes UV-protected with luer lock caps were purchased from Artsana (Como, Italy). Monobasic potassium phosphate, phosphoric acid, standard trisodium citrate, sodium hydroxide, hydrochloric

acid, 30% hydrogen peroxide and methanol high-performance liquid chromatography (HPLC) grade were provided by Sigma-Aldrich (Milan, Italy).

Preparation of trisodium citrate solutions

Two different concentrations of trisodium citrate were prepared: 35 mM (1.029%) and 90 mM (2.65%). Trisodium citrate solutions were prepared by diluting commercially available sterile concentrate (1.59 M, 46.7%) with sterile 0.9% sodium chloride solution under sterile conditions, in accordance with the European Pharmacopoeia.²⁴ The procedure was identical to that usually employed in our hospital pharmacy for the preparation of similar solutions for parenteral administration. This standard procedure has been validated and is regularly monitored. In order to assure aseptic filling of the syringes, the whole procedure were performed in a glove box isolator (Isoclean Healthcare Platform Isolator HPI-G3, manufactured by Esco Pharma, Rotherham, UK), avoiding direct exposure of solutions and syringes to the room environment or to compounding personnel. The filled syringes were then capped with a sterile luer lock tip, delivered to the nephrology unit and ICU, and stored at room temperature ($21 \pm 2^\circ\text{C}$) protected from direct light.

HPLC analysis

Trisodium citrate concentration was determined by isocratic reverse-phase HPLC by a method adapted from the official method 986.13 of the Association of Official Analytical Chemists.²⁵ The HPLC system consisted of a YL9300 HPLC (Young Lin Instruments, Anyang, Korea) equipped with a vacuum degasser, a quaternary pump, a manual injector with a 200 μL loop and a UV-Vis detector set at 200 and 215 nm. The analytical column was a Symmetry C18 (250×4.6 mm internal diameter, particle size 5 μm), equipped with a Symmetry C18 guard column, supplied by Waters (Vimodrone, Milan, Italy). The mobile phase consisted of a mixture of 0.08 M potassium dihydrogen phosphate buffer (adjusted to pH 2.6 with phosphoric acid) and methanol (98:2 v/v), and was delivered at 1 mL/min at room temperature ($21 \pm 2^\circ\text{C}$). Peak heights and areas were recorded on a PC and processed with YL-Clarity software (Young Lin Instruments). With these conditions, the retention time of trisodium citrate was about 8 min. A standard curve was calculated by least-squares linear regression from six trisodium citrate standard concentrations ranging from 10 to 500 $\mu\text{g/mL}$ (10, 20, 50, 100, 250 and 500 $\mu\text{g/mL}$), with each point consisting of six independent measurements. Concentrations of 20, 100 and 250 $\mu\text{g/mL}$ were used to determine accuracy and precision. Accuracy was reported by calculating the bias expressed as (measured concentration–nominal concentration)/(nominal concentration) $\times 100$, and precision by the coefficient of variation expressed as (SD/mean) $\times 100$.

Trisodium citrate concentrations of solution stored in the syringes were determined from the peak area ratios versus the standard curve.

Forced degradation of trisodium citrate

In order to verify the specificity of the HPLC method of analysis, degradation of citrate was forced by exposing citrate solution to acidic, alkaline or oxidising conditions. For the acidic or alkaline degradation, concentrated hydrochloric acid or 6N sodium hydroxide was added to 50 mL aliquots of 35 mM or 90 mM citrate solution in a 100 mL flask, in order to adjust the pH to about 1 or 12, respectively. For the oxidising condition, 25 mL of 30% hydrogen peroxide was added to 50 mL of

35 mM or 90 mM citrate solution in a 100 mL flask. The samples were incubated at 50°C for 24 hours, and then each flask was made up to volume with deionised water. Three aliquots (0.5 mL) of each flask were centrifuged at 15 000 g for 5 min in order to precipitate any insoluble material eventually formed. Samples were then analysed by HPLC after 1:25 v/v (for 35 mM) or 1:50 v/v (for 90 mM) dilution in deionised water, and the chromatograms were compared with that of fresh trisodium citrate solution at the same concentration.

Stability study

Syringes containing trisodium citrate solutions were stored at room temperature and kept away from direct exposure to light. For each concentration, three syringes were randomly taken for determination of the amount of trisodium citrate on days 1, 7, 14 and 28. Samples from selected syringes were diluted with deionised water (1:50 v/v for the 35 mM solution, or 1:100 v/v for the 90 mM solution), and analysed in triplicate by HPLC.

Three syringes containing 35 or 90 mM trisodium citrate solutions were stored at 4°C, and the amount of trisodium citrate was evaluated after 28 days of storage.

Sterility test

The sterility of the solutions was tested by the chemical laboratory of CCIA (Turin Chamber of Commerce) according to the European Pharmacopoeia assays.²⁴ The laboratory is certified ISO 9001, and accredited in accordance with the recognised International Standard of ISO/IEC 17025.

For each concentration, sterility tests were carried out on four syringes, from a batch of 40, both immediately after the preparation, and after 28 days of storage at room temperature.

Use of trisodium citrate solutions as anticoagulant agent in the CRRT procedure

The trisodium citrate solutions were used as regional anticoagulant during CPFA therapy in the COMPACT-2 clinical trial (NCT01639664). CPFA was performed with a four-pump modular treatment (AMPLYA, Bellco, Mirandola, Italy) consisting of a plasma filter (0.45 m² polyethersulfone) and a following absorption on an unselective hydrophobic resin cartridge (140 mL for 70 g, with a surface of about 700 m²/g) and a final passage of the reconstituted blood through a high-permeability 1.4 m² polyethersulfone haemofilter, in which convective exchanges may be applied in a postdilution mode. The 10/2 citrate solution is administered through a fifth pump in predilution 25%. During the citrate bag changes, the fifth pump is stopped for 3–7 min to avoid air entrance into the CPFA circuit, but this procedure increases the risk of circuit clotting. For this reason, the circuit has been modified by adding an alternative citrate inlet through which a citrate solution, stored in a 50 mL external syringe, is pumped at 300 mL/h, so ensuring the right citrate concentration in the CPFA circuit.

According to the available clinical evidence, CPFA should be repeated daily, for at least 10 h each time, so that an average of 0.15 L/kg predicted body weight of plasma should have been treated each day.¹⁹

RESULTS

Validation of the HPLC assay

The method was found to be linear over the range 10 to 500 µg/mL, with an $r^2 \geq 0.999$ and a mean absolute percentage deviation of standards from their nominal concentration of <4%. Intraday accuracy ranged from -1.06% to 3.11% and

Figure 1 Chromatograms of a trisodium citrate sample (35 mM, $\lambda=215$ nm) treated by forced degradation at oxidising condition (A) and control (B). Arrows indicates presumed degradation products. I=injection; T=trisodium citrate.

Table 1 Stability of 35 mM and 90 mM trisodium citrate solutions stored at room temperature. Data are the mean of three different samples, each analysed in triplicate

Trisodium citrate solution	Days of storage				
	1	7	14	21	28
35 mM	100.0	103.7±3.2	99.7±2.2	106.3±1.9	105.7±2.1
% of day 1 concentration residual					
90 mM	100.0	96.9±3.5	95.4±2.2	98.6±2.6	95.9±2.7
% of day 1 concentration residual					

Results are shown as mean±SD.

interday accuracy from −1.45% to 4.05%. Intraday precision was <4%, while interday precision was <5%. The limit of quantification of the method was 10 µg/mL.

Forced degradation of trisodium citrate solutions

The results of forced degradation of trisodium citrate solutions showed that the incubation in oxidising conditions for 24 h causes the formation of almost two new peaks, with a retention time of about 4.5 and 6.2 min, with only 89.7% of citrate remaining unchanged (figure 1). The solutions exposed to acidic or alkaline condition for 24 hours showed no sign of degradation, with a mean amount of 98.8% and 99.2% of unmodified trisodium citrate, respectively. No difference was found in trisodium citrate degradation between the 35 or 90 mM concentrations.

Stability study

During a storage period of 28 days at room temperature in 50 mL PP syringes, the trisodium citrate solutions remained clear, colourless and without the formation of any precipitate. The concentrations of citrate, determined by HPLC after 7, 14, 21 and 28 days of storage, were unchanged in comparison with that of day 1, resulting in a range of 100±10% of the initial concentration (table 1). Similar results were obtained from syringes stored for 28 days at 4°C, with a mean percentage of day 1 concentration residual of 99.6 for 35 mM and 102.4 for 90 mM solutions.

Sterility test

Both trisodium citrate solutions (35 mM and 90 mM), passed the sterility test both immediately after the preparation and after storage for 28 days at room temperature in 50 mL PP syringes.

Efficacy of trisodium citrate solutions as anticoagulant in the CRRT procedure

The use of the external syringe prepared with 35 mM citrate solution was abandoned early because a preliminary test indicated that this concentration is insufficient to ensure an effective anticoagulant action during the change of the citrate bags. Conversely, the external syringe containing 90 mM citrate solution was used during seven complete CPFA treatments in two patients admitted to the ICU with septic-shock diagnosis (table 2). Three sessions of CPFA were administered to patient number 1 and four to patient number 2. Both patients were treated with a mean of ≥0.22 L/kg predicted body weight per session. In both patients it was possible to overcome 10 hours of CPFA treatment without clotting (table 2). Moreover, after the CPFA session CRRT continued without the need for circuit substitution before its normal duration.

Neither patient developed clinical signs of citrate toxicity during CPFA/CRRT treatments.

Table 2 Clinical characteristics of patients at ICU admission and characteristics of CPFA sessions

Characteristics	Patient 1	Patient 2
Clinical characteristics of patients		
Sex	Male	Female
Age (years)	64	71
Predicted body weight (kg)	63	46
SAPS 2	54	68
Systolic blood pressure (mm Hg)	95	105
Lactate (mmol/L)	5.8	3
Cardiac frequency (bpm)	113	118
Systemic vascular resistance index (dynes.s/cm ⁵)	110	1400
Cardiac index (L/min/m ²)	4	2.8
Temperature (°C)	35.8	36.3
Central saturation O ₂ (%)	71	66
Platelets (×1000/mL)	237 000	186 000
White blood cells (×1000/L)	13 500	18 000
Bilirubin (mg/dL)	1.1	0.5
Creatinine (mg/dL)	1	1.3
Procalcitonin (ng/mL)	59	44
pH	7.43	7.29
PT INR	1.4	2.5
Haematocrit (%)	37	31
Noradrenaline dosage (µg/kg/min)	0.2	0.3
Characteristics of CPFA sessions		
Mean treated plasma (L)	14	11
Target plasma treated (L)	13	9
Mean treated plasma/kg PBW (L/kg)	0.22	0.24
CPFA sessions (n)	3	4
Mean session duration (hours)	12	12

CPFA, coupled plasma filtration adsorption; ICU, intensive care unit; INR, international normalised ratio; PBW, predicted body weight; PT, prothrombin time; SAPS, Simplified Acute Physiology Score.

DISCUSSION

The administration of trisodium citrate solution in the extracorporeal circuit proved to be an effective means of regional anticoagulation in patients treated with CPFA, but the commercially available solutions are not suitable for this application. The aim of this study was to evaluate the chemical and microbiological stability of two different concentrations of trisodium citrate solutions, 35 mM and 90 mM, prepared in a hospital pharmacy and stored at room temperature in 50 mL syringes 'ready-to-use' in ICU.

The chemical stability was evaluated by HPLC, and the specificity of the method was proved by the separation of degradation products from unmodified trisodium citrate in the analysis of a sample treated in oxidising conditions. Both the 35 mM and 90 mM trisodium citrate solutions are chemically stable for

28 days when stored at room temperature in 50 mL syringes protected by light, as demonstrated by the high percentage of citrate still present (at least 95%) in comparison with day 1.

Results of the sterility test demonstrated the microbiological stability of solutions during storage.

A preliminary test indicated that 35 mM trisodium citrate concentration is insufficient to ensure an effective anticoagulant action in the extracorporeal circuit during change of the citrate bag, and for this reason only the 90 mM concentration was used for CPFA/CRRT treatment. The efficacy of 90 mM trisodium citrate solution as regional anticoagulant was demonstrated by the absence of circuit clotting, premature haemofilter change or loss of plasma treated in a total of seven sessions administered to two different patients. Moreover, no signs of citrate toxicity were seen during treatments, demonstrating the safety of this anticoagulation procedure.

In conclusion, our results demonstrate that both the 35 mM and 90 mM trisodium citrate solutions are chemically and microbiologically stable for 28 days when stored at room temperature in 50 mL PP syringes protected by light. The 90 mM solution was as effective and safe as a regional anticoagulant during the citrate bags changes in the CPFA protocol, and will be used for the treatment of all patients enrolled in the COMPACT-2 clinical trial.

What this paper adds

What is already known on this subject

- Recent reviews of clinical studies in critically ill patients treated with continuous renal replacement therapy (CRRT) indicate that regional citrate anticoagulation is safer, more efficacious and more cost-effective than systemic heparinisation, and allows better filter survival times with higher delivered CRRT doses.
- The clinical trial COMPACT, which aimed to compare the efficacy of coupled plasma filtration adsorption (CPFA) with standard care in the treatment of critically ill patients with septic shock, was terminated prematurely because of the high number of protocol violations, mainly due to clotting of the circuit despite the use of heparin for anticoagulation. For this reason, the extracorporeal circuit has been modified in order to support regional anticoagulation with citrate, and a new clinical trial (COMPACT-2) has been started.
- Some protocols require non-commercially available citrate concentrations, and very little published information about the stability of diluted citrate solutions is available. In addition, advance preparation and storage in the nephrology department and intensive care unit (ICU) is very important, as the prompt start of CRRT or CPFA procedures is critical for the treatment of patients with acute kidney failure or septic shock.

What this study adds

- Syringes 'ready-to-use' containing 50 mL of 35 mM or 90 mM sterile solutions of trisodium citrate have a shelf life of almost 1 month if stored at room temperature protected by light. Solutions at suitable concentration may be prepared by the hospital pharmacist and stored in the nephrology department and ICU in order to be immediately available at any time.
- The use of 90 mM trisodium citrate as regional anticoagulant during the citrate bag changes in CPFA is safe and more effective than heparin for the prevention of circuit clotting.

Contributors PM planned and conducted the high-performance liquid chromatography analysis, MLV prepared the citrate solutions, SM conducted the continuous renal replacement therapy procedure and SA supervised the work. All authors contributed to the drafts and final version of the manuscript.

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