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The Application of Regional Citrate Anticoagulation in Protein A Immunoadsorption: A Single-Center Retrospective Cohort Study

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Keywords
Regional citrate anticoagulation · Protein A immunoadsorption · Bleeding · Ionized calcium

Abstract

Introduction: Protein A immunoadsorption (IA) is proving to be an effective treatment method for autoimmune diseases and other disorders. Regional citrate anticoagulation (RCA) prevents clotting in extracorporeal circuits without increasing hemorrhage risk in high bleeding risk patients, but there are no specific guidelines for its application in IA. We aimed to evaluate the safety and adverse effects of RCA used in IA therapy. ***Methods:*** We conducted a retrospective cohort study of forty-five RCA-IA sessions in 14 HLA-incompatible kidney transplant recipients with focus on the safety and adverse effects of RCA in IA. The extracorporeal circuit was equipped with 4% trisodium citrate solution as an anticoagulant and 10% calcium gluconate solution to compensate for calcium loss. The adverse events, including coagulation and blood biochemical indexes, especially calcium level, were recorded. ***Results:*** Our study found that 93.33% of the sessions were without circuit clotting or other significant complications. A slight decrease in fibrinogen level was observed, but without significant variations in other coagulation indexes or platelet count. There was a slight elevation in the potential of hydrogen, bicarbonate, and base excess after 2 h and 6 h posttreatment relative to prior treatment, but these returned to normal levels within 24 h posttreatment. ***Conclusion:*** RCA is a feasible, effective, and safe anticoagulation option for IA treatment in HLA-incompatible kidney transplant recipients. Electrolyte disturbances, especially alkalosis, hypocalcemia, hypomagnesemia, and fluid status, should be closely monitored and managed.

Introduction

Protein A immunoadsorption (IA) is a technique that removes pathogenic molecules and antibodies without relying on plasma or albumin (A) supplementation. IA has been shown to be effective in eliminating autoantibodies by using a protein-A-coated column [1, 2]. Several studies have suggested the effectiveness of IA in removing immunoglobulin or autoantibodies in autoimmune diseases such as antineutrophil cytoplasmic antibodies-associated vasculitis with kidney involvement, anti-

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glomerular basement membrane diseases, and lupus nephritis, and in desensitizing human leukocyte antigen (HLA)-incompatible kidney transplants [3–5]. Compared to plasmapheresis, one advantage of IA is that supplementation with plasma or A is not required. IA decreases the excessive loss of coagulation factors and reduces the risk of allergy and disease transmission because no blood products are transfused [6].

Low molecular weight heparin (LMWH) or unfractionated heparin (UFH) is used as anticoagulants in IA therapy for patients with certain autoimmune diseases [7]. But to perform IA in patients who are at risk of bleeding or have a systemic anticoagulation contraindication, LMWH and UFH are not recommended. However, regional citrate anticoagulation (RCA) is an excellent anticoagulant option that can prevent clotting without increasing the risk of bleeding [8]. It aids in the smooth, safe, and efficient operation of extracorporeal circuits, thereby extending the lifespan of both adsorbent and catheter. Therefore, it is collectively reducing the loss of blood and plasma, decreasing the cost, and reducing the workload of medical personnel [9]. It has been gradually used as an anticoagulant in blood purification technology, including continuous renal replacement therapy (CRRT), hemodialysis (HD), therapeutic plasma exchange, and double-filtration plasmapheresis [10].

Citrate becomes calcium-citrate complex (CCC) after combining with calcium in the extracorporeal circuit, enters into the systemic circulation, and is rapidly metabolized to bicarbonate (HCO_3^-) via the citric acid (Krebs) cycle in the liver, muscle, and renal cortex. Although the body's capacity to metabolize it is saturable, and its half-life is approximately 5 min in normal conditions, which may reduce toxic effects. If citrate dose exceeds this capacity, the presence of residual citrate, in the form of CCC, in blood can be detected only through indirect signs of increased total calcium (TCa)/ionized calcium (iCa) (Ca/calcium ion [Ca^{2+}]) ratio, hypocalcemia, high anion gap metabolic acidosis, and increased serum lactate levels. The tricarboxylic acid cycle or Krebs cycle is responsible for these anomalies, which reduce citrate metabolism and limit pyruvate metabolism, resulting in lactate generation [11]. Therefore, the administration of RCA in individuals with normal liver function may potentially mitigate the incidence of systemic adverse events. On the other hand, LMWH has a longer half-life of around 2–3 h while UFH up to 1–1.5 h [12]. Half-life of both is dose-dependent and increases with increasing doses. It may be slightly prolonged in anephric patients or patients with severe renal impairments [13, 14]. In contrast, a recent study on the pharmacokinetics of citrate in patients with renal failure found that the liver is the primary source of citrate clearance [15]. While some published studies have described the use of RCA during IA [16, 17], there are no specific guidelines to follow when applying it during IA. In addition, RCA can also cause metabolic complications such as hypocalcemia, alkalosis, and hypomagnesemia, which require proper investigation and management.

Table 1. Baseline clinical characteristics of all patients (n = 14)

Parameter	Value
Age, years	53.25±13.67
Female, n (%)	8 (57.14)
Primary kidney disease (n = 14), n (%)	
Primary glomerulonephritis	9 (64.28)
Hypertensive nephropathy	2 (14.29)
Obstructive nephropathy	2 (14.29)
Polycystic kidney disease	1 (7.14)
Hypertension, n (%)	12 (85.71)
Vascular access type (n = 14), n (%)	
AVF	12 (85.71)
Catheter	2 (14.29)
BMI (IQR), kg/m^2	23.86 (18.34, 27.61)
BUN, mmol/L	21.23±7.27
SCr, μmol/L	721.30±254.23
T, g/L	65.68±10.15
A, g/L	35.26±7.24
G, g/L	30.56±5.82

AVF, arteriovenous fistula; BMI, body mass index; BUN, blood urea nitrogen; SCr, serum creatinine; T, total protein; A, albumin; G, globulin; IQR, interquartile range.

Material and Methods

Inclusion and Exclusion Criteria

Our study included highly HLA-sensitized renal transplantation patients with an age range of 18–75 years, who have normal liver and lung function and are at higher risk of bleeding with LMWH or UFH. The criteria for high risk of bleeding were determined as described previously [18], including active bleeding within 3 days or surgical or traumatic wounds within 3 days. The criteria for exclusion were (1) patients who were aged under 18 or above 75 years; or (2) patients who had used drugs that could impact coagulation function within 7 days; (3) patients with severe cardiovascular diseases; (4) patients with acute systemic infections; (5) patients with impaired liver function or lung function, or patients using drugs that can decrease citrate-metabolizing capacity as described previously [11, 15, 19].

Study Population

We conducted a retrospective cohort study at our center that involved 14 patients (6 males and 8 females) in forty-five IA sessions using RCA as an anticoagulant from June 2019 to April 2023. Patients with HLA-incompatible renal transplantation underwent IA sessions that were associated with a higher risk of

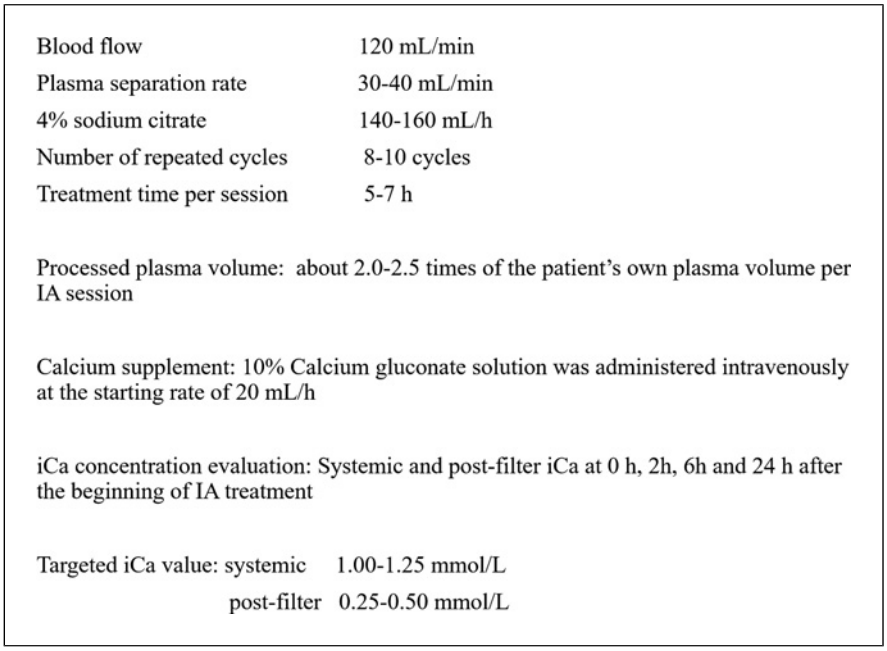

Fig. 1. Treatment protocol of RCA-IA. RCA, regional citrate anticoagulation; IA, protein A immunoadsorption; iCa, ionized calcium.

bleeding. As all patients (100%) were in the perioperative period of renal transplantation (Table 1), we included them in the criteria of high bleeding risk as described previously [18]. The study was approved by the Medical Ethics Committee of Xiangya Hospital of Central South University for Human Studies (No. 202008097) and was conducted in accordance with the Declaration of Helsinki. All patients provided written informed consent.

The Baseline Features of Patients

The baseline clinical characteristics of patients are summarized in Table 1. The average age of the subjects was 53.25 ± 13.67 years, and 8 (57.14%) were females. Among 14 patients, 12 (85.71%) had hypertension. Twelve (85.71%) patients used arteriovenous fistula as vascular access, while the other 2 (14.29%) cases used central venous catheter.

IA Treatment

In this study, the IA was conducted as previously described [3]. All sessions were performed using a genetically engineered recombinant regenerative protein A adsorption column (KCIA08; Guangzhou Koncen Bioscience, China) on the blood purification machine (JUN55X; Junken Medical, Japan), after separating the plasma through a membrane plasma separator (P2; Fresenius Medical Care, Germany). The rate of blood flow from the plasma separator was 120 mL/min, while plasma was separated at a rate of 30–40 mL/min before passing through protein A adsorption column, through which the filtered fluid repeated 8–10 cycles before returning to the body (Fig. 1). Depending on the severity of the disease, each therapy session lasted approximately 5–7 h and was performed intermittently 2–5 times per case. The plasma volume processed during an IA session was approximately 2.0–2.5 times the total plasma volume of the patient. The estimated plasma volume was evaluated according to the following equation [20]. Estimated plasma volume = [0.065 × weight (kg)] × [1 − hematocrit]. All patients with impaired renal function waiting for renal transplantation required HD, so the IA procedure was routinely conducted on non-HD days.

Regional Citrate Anticoagulation

From our experience, 4% trisodium citrate solution ($Na_3C_6O_7H_5$; Sichuan Nigale Biotechnology, China) was capable of preventing clotting in filtered blood when given at a starting dose from 2.83 to 3.02 mmol/L (e.g., 150–160 mL/h of 4% trisodium citrate solution with the blood flow of 120 mL/min) to become CCC after combining with the divalent calcium ions due to its high affinity. We decided to keep the iCa within the target range of 0.25–0.50 mmol/L in the circuit according to the protocol used to prevent clotting, as Ca^{2+} is a compulsory cofactor for the coagulation cascade. We administered intravenously 10% calcium gluconate solution at the end of the extracorporeal circuit, with a starting dose of 20 mL/h, to maintain the systemic iCa level of 1.00–1.25 mmol/L as a compensatory measure for calcium loss in the form of CCC. The levels of systemic and post-filter iCa were monitored at the commencement of treatment, 2 h subsequent to the commencement of treatment, 6 h subsequent to the commencement of treatment, and 24 h subsequent to the conclusion of treatment, respectively.

Clinical Assessment

BMI, blood urea nitrogen, serum creatinine, total protein, A, and globulin were recorded as given in Table 1. The evaluation of heart rate (HR), systolic blood pressure, diastolic blood pressure, and mean arterial pressure was done before and after the treatment. Venous blood samples were collected before and after the therapy for peripheral blood count, including white blood cells, red blood cells, blood platelets (PLTs), hemoglobin, and hematocrit, as indicated in Table 2. Table 2 also showed the recording of blood electrolytes including magnesium ions (Mg^{2+}), sodium ions (Na^+), chloride ions (Cl^-), potassium ions (K^+), and phosphate ions before and after therapy. Prothrombin time (PT), thrombin time (TT), activated partial thromboplastin time (APTT), fibrinogen (FIB), and international normalized ratio (INR) were measured and analyzed as described in Table 3. The blood potential of Ca^{2+}, TCa, potential of hydrogen (pH), HCO_3^-, and base excess (BE) were also compared at the beginning of therapy, 2 h after the beginning of therapy, 6 h after the beginning of therapy, and 24 h after treatment, respectively, as given in Table 4.

Table 2. Change in blood routine indexes, electrolyte (Na, Cl, K, Mg, P), HR, and BP before and after RCA-IA treatment in each session (n = 45)

Parameter	Before therapy	After therapy
WBC, $\times 10^9$/L	6.12±1.72	5.95±1.03
RBC, $\times 10^9$/L	2.68±0.54	2.37±0.43
PLT, $\times 10^9$/L	164.3+67.9	170.5+82.1
Hb, g/L	84.32+20.12	81.92+18.57
HCT, %	24.32±3.73	22.80±4.33
Na^+, mmol/L	138.55±6.02	140.24±5.11
Cl^-, mmol/L	97.89±4.32	99.15±4.45
K^+ (IQR), mmol/L	4.16 (3.69, 4.63)	4.35 (3.88, 4.82)
Mg^{2+}, mmol/L	0.91±0.07	0.76±0.05*
P^{3-} (IQR), mmol/L	1.94 (1.65, 2.23)	1.86 (1.55, 2.15)
HR, times/min	85.29±10.34	83.36±12.78
SBP, mm Hg	141.58±15.54	145.70±18.23
DBP, mm Hg	92.80±10.44	94.32±13.17
MAP, mm Hg	107.72±12.45	109.35±14.01

Compared with before therapy. RCA, regional citrate anticoagulation; IA, protein A immunoadsorption; WBC, white blood cell; RBC, red blood cell; PLT, platelet; Hb, hemoglobin; HCT, hematocrit; Na, natrium; Cl, chloride; K, kalium; Mg, magnesium; P, phosphate; HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; IQR, interquartile range. *$p < 0.01$.

Circuit Clotting and Adverse Events

Alterations in transmembrane pressure (TMP) at different time points, including 1 h, 2 h, 3 h, 4 h, 5 h, and 6 h after the commencement of the IA procedure, were measured. The clotting scores of the plasma filter and venous expansion chamber were determined by a semiquantitative analysis with a score of 0–3 (Table 5) [21].

In all patients during the RCA-IA sessions, all adverse events were recorded. Electrolyte disturbance parameters included were hypocalcemia (TCa <2.0 mmol/L or iCa <1.0 mmol/L) and severe hypocalcemia (TCa <1.9 mmol/L or iCa <0.8 mmol/L) [22]; hypercalcemia (TCa >2.5 mmol/L or iCa >1.4 mmol/L), severe hypercalcemia (TCa >3.0 mmol/L, iCa >1.5 mmol/L); metabolic alkalosis (HCO_3^- >28 mmol/L), severe metabolic alkalosis (HCO_3^- >35 mmol/L); metabolic acidosis (HCO_3^- <22 mmol/L); hyponatremia (Na^+ <135 mmol/L), hypernatremia (Na^+ >145 mmol/L); hypomagnesemia (Mg^{2+} <0.7 mmol/L), severe hypomagnesemia (Mg^{2+} <0.5 mmol/L) [23]; and others.

Statistical Analysis

SPSS software 26.0 was used to perform statistical analysis. Paired t tests were used to assess continuous variables with a normal distribution, which were expressed by mean ± standard deviation. The Mann-Whitney U test was used to evaluate continuous parameters with skewed distributions that were expressed as medians and interquartile range (25th percentile, 75th percentile). There were two ways to express categorical parameters: frequencies (n) and percentages (%). A repeated measure analysis of variance was used to compare groups within groups at various intervals. A p value of less than 0.05 was deemed statistically significant.

Table 3. Changes in coagulation indexes before and after RCA-IA sessions (n = 45)

Parameter	Before therapy	After therapy
PT (IQR), s	12.05 (10.07, 13.92)	12.73 (10.26, 14.78)
TT, s	20.23±6.71	19.89±5.24
APTT (IQR), s	29.02 (23.63, 35.11)	30.57 (24.54, 36.15)
FIB (IQR), g/L	3.67 (2.12, 5.03)	3.05 (1.84, 4.53)#
INR	0.98±0.36	1.04±0.29

Compared with before therapy. RCA, regional citrate anticoagulation; IA, protein A immunoadsorption; PT, prothrombin time; TT, thrombin time; APTT, activated partial thromboplastin time; FIB, fibrinogen; INR, international normalized ratio; IQR, interquartile range. #$p < 0.05$.

Results

All RCA-IA sessions were completed successfully without any significant clotting or systemic adverse events. In some sessions, there were slight changes in circuit clotting, coagulation function, FIB levels, electrolytes, pH, and metabolic alkalosis, but they did not cause any significant manifestations.

Change in TMP Value and Circuit Clotting

As shown in Figure 2a, TMP increases with prolongation of treatment time, but it remains within the normal range of 0–20 mm Hg. The blood clotting scores of plasma separator, and arterial and venous expansion chamber during the extracorporeal circulation are illustrated in Figure 2b. Clotting in the plasma separator (score = 3) occurred in one session (2.22%). However, this therapy-interrupted session was completed after replacing the plasma separator. Clotting in the venous expansion chamber (score = 3) occurred in two sessions (4.44%), which might be due to either high post-filter iCa level or vascular access dysfunction. Thus, blood clotting scores between 0 and 2 in the plasma separator, arterial expansion chamber, and venous expansion chamber after RCA-IA sessions were 97.78%, 100%, and 95.56%, respectively. The protein A column did not form clotting because it did not come in contact with blood cells. Overall, 93.33% RCA-IA sessions were successfully performed without any cessation due to circuit coagulation.

Change in Coagulation Function before and after Treatment

Coagulation values and PLT count were evaluated pre- and post-RCA-IA sessions. There was a slight decrease in the PLT count, but it was not statistically significant (p > 0.05, Table 2). As shown in Table 3, the coagulation values, including PT, TT, APTT, and INR, did not show any significant changes pre- and post-therapy (all p > 0.05). Nevertheless, a reduction in FIB levels was observed post-RCA-IA therapy ($p < 0.05$, Table 3), which may be due to the procedure itself. These indicated that, except

Table 4. Changes in calcium and pH values at different time points (n = 45)

Parameter	Before therapy	2 h after therapy	6 h after therapy	24 h after therapy
iCa, mmol/L	1.15±0.16	1.10±0.12	1.08±0.15	1.13±0.13
TCa, mmol/L	2.19±0.21	2.16±0.20	2.13±0.18	2.17±0.15
pH value	7.38±0.09	7.46±0.07#	7.49+0.05#	7.40+0.07
HCO_3^-, mmol/L	23.23±6.01	27.35+4.34#	29.65+5.33*	24.12+3.89
BE, mmol/L	−0.72±2.31	3.11±3.62*	5.62±3.86*	0.54±2.12

2 h after therapy, 2 h after the beginning of therapy; 6 h after therapy, 6 h after the beginning of therapy; TCa, total calcium; iCa, ionized calcium; pH, potential of hydrogen; HCO_3^-, bicarbonate; BE, base excess. Compared with before therapy, #$p < 0.05$. *$p < 0.01$.

Table 5. Clotting score criteria of plasma separator, arterial or venous expansion chamber during extracorporeal circulation of IA

Clotting score	Plasma separator	Arterial or venous expansion chamber
0	Clear or no coagulation of plasma separator	Clear or no coagulation of expansion chamber
1	Streaky separator area <1/3 of the total	Thrombotic area <1/3 of expansion chamber
2	Streaky separator area <2/3 of the total	Thrombotic area <2/3 of expansion chamber
3	Streaky separator area >2/3 of the total	Thrombotic area >2/3 of expansion chamber

IA, protein A immunoadsorption.

for a slight decrease in FIB level post-RCA-IA treatment, RCA exhibited no obvious effects on the coagulation function of patients in this study.

Comparison of Electrolytes, pH, and Adverse Effects in Blood before and after Therapy

As illustrated in Table 4, compared to the values at the pretreatment period and 24 h posttreatment period, the average levels of iCa and TCa decreased at 2 h and 6 h after therapy, but there was no statistical significance ($p > 0.05$). The average post-filter iCa level during the therapy was 0.40 ± 0.12 mmol/L at 2 h and 0.38 ± 0.07 mmol/L at 6 h, respectively. Hypocalcemia was observed during five sessions, including one with severe hypocalcemia, but without serious symptoms or significant electrocardiogram changes. The hypocalcemia was managed by increasing the infusion dose of 10% calcium gluconate solution to compensate for calcium loss in the circuit. Mild hypercalcemia also occurred in two sessions subsequent to RCA-IA sessions; however, it did not necessitate any treatment.

Interestingly, the value of Mg^{2+} was reduced relative to prior treatment level ($p < 0.01$) in six sessions, but it was not severe. As displayed in Table 2, there was no statistically significant difference in Na^+, Cl^-, K^+, and phosphate ion prior and post-therapy (all $p > 0.05$), but there were slight differences in hyponatremia in two sessions and hypernatremia in other two sessions. The levels of pH, HCO_3^-, and BE were higher at 2 h and 6 h posttreatment compared to pretreatment ($p < 0.05$ or $p < 0.01$) (Table 4). Metabolic alkalosis and metabolic acidosis were observed in six sessions and one session, respectively. It is noteworthy that there were no sessions exhibiting severe metabolic acidosis or metabolic alkalosis. It is interesting to note that all of these values returned to their pretreatment levels ($p > 0.05$) within 24 h after the treatment.

Due to the transfusion of fluids such as citrate and the intake of water or food during the RCA-IA session, the mean weight gain after therapy was approximately 1.28 (1.11–1.43) kg in patients (Table 6). As the treatment time required for tandem IA and HD on the same day was too long, and different machines are used for IA and HD procedures at our center, therefore, fluid overload was managed via HD in all renal failure patients 1 day after the RCA-IA session. Pruritus was observed in two sessions, and nausea and vomiting were observed in other two sessions. Musculoskeletal pain was presented during one session. Furthermore, slight tachycardia was observed in one session and chest tightness was also experienced in other two sessions. These symptoms were rapidly alleviated without any special management.

Comparison of Blood Routine Indexes, HR, and Blood Pressure before and after Therapy

As illustrated in Table 2, the changes in routine blood indexes, HR, and blood pressure before and after treatment were not statistically significant (all $p > 0.05$). In two

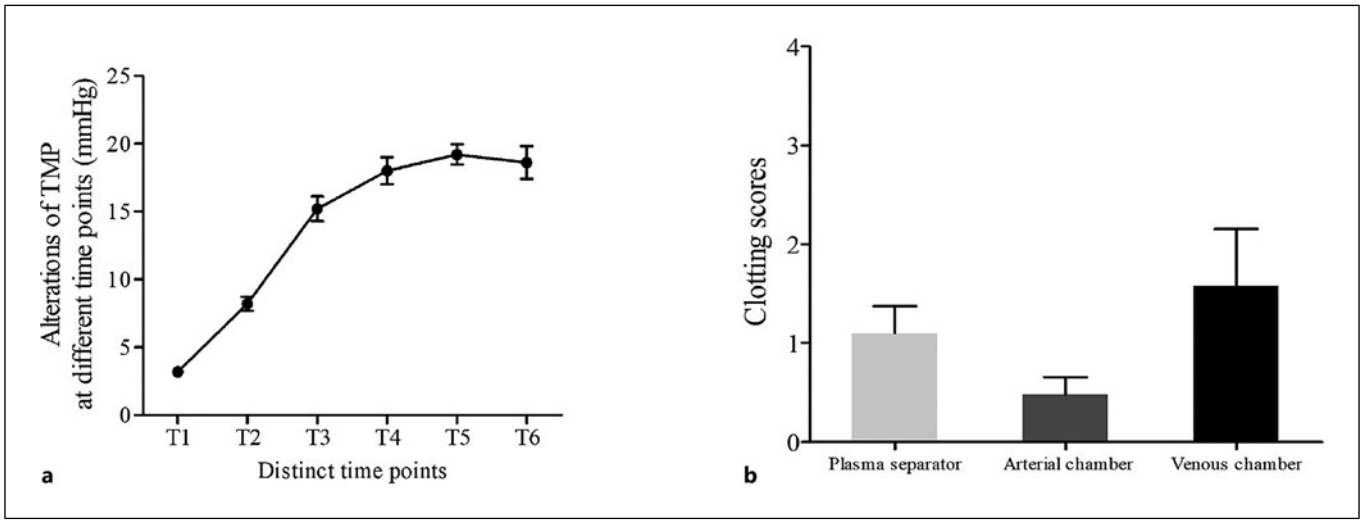

Fig. 2. Alterations of TMP, clotting scores of plasma separator, arterial expansion chamber, and venous expansion chamber. **a** Alterations of TMP at different time points of treatment. T1: 1 h after the beginning of therapy; T2: 2 h after the beginning of therapy; T3: 3 h after the beginning of therapy; T4: 4 h after the beginning of therapy; T5: 5 h after the beginning of therapy; T6: 6 h after the beginning of therapy. **b** Clotting scores of plasma separator, arterial expansion chamber, and venous expansion chamber during extracorporeal circulation of IA. TMP, transmembrane pressure.

sessions, hypotension (BP <90/60 mm Hg) occurred within 30 min after therapy, but it was handled with proper saline supplementation and did not affect the treatment process. Three sessions resulted in hypertension (BP >180/110 mm Hg) that was managed with antihypertensive drugs.

Discussion

Here, we have introduced a feasible, effective, and safe RCA protocol for therapeutic IA therapy in HLA-incompatible kidney transplant recipients. Our study revealed that most IA sessions were carried out without any circuit clotting or other significant systemic adverse events. Nonetheless, a slight decrease in FIB level and PLT count was observed following therapy in a few sessions; however, there were no significant manifestations. In a few sessions, the electrolytes were also slightly fluctuated, including hypocalcemia, mild hypercalcemia, hypomagnesemia, mild hyponatremia, and mild hypernatremia. A few sessions also showed slight changes in pH, HCO_3^-, and BE.

The lack of citrate clearance from filters in RCA-IA necessitates a lower citrate dose for effective anticoagulation than in CRRT. But there is a higher risk of citrate accumulation if its metabolism is impaired, compared to CRRT, which filters citrate up to 60% of the total dose administered [11]. Therefore, 4% trisodium citrate solution was infused at a rate of 150–160 mL/h (2.83–3.02 mmol/L) in RCA-IA sessions. Based on our experience in RCA-CRRT, we adjusted the systemic and post-filter calcium levels in RCA-IA sessions to maintain levels between 1.00–1.25 mmol/L and 0.25–0.50 mmol/L to avoid hypocalcemia and clotting, respectively. Our study demonstrated that this anticoagulation strategy was adequate in patients undergoing RCA-IA sessions, without any significant adverse effects.

Clotting in the extracorporeal circuit and replacement of the filter system not only interrupt the effective treatment time for blood purification, but also raise the treatment cost and cause more blood loss [24]. The venous expansion chamber and filter circuit serve as vulnerable sites for developing clotting [25]. Clotting rarely occurs in the protein A column (secondary filter), which might be due to the lack of PLTs and red blood cells after plasma separation. Thus, we focused on the coagulation status of the plasma separator (first filter) and the venous expansion chamber. Our findings revealed that the mean level of post-filter iCa in extracorporeal circulation during the therapy was 0.40 ± 0.12 mmol/L and 0.38 ± 0.07 mmol/L at 2 h and 6 h, respectively, which reduced the clotting risk in the plasma separator and venous expansion chamber. The clotting of the venous expansion chamber (with a score of 3) was observed in two sessions, and the clotting of plasma separator (with a score of 3) was occurred in one session. However, these therapy sessions interrupted by coagulation were completed after replacing the blood pipeline or plasma separator. The few clotting events observed in the

Table 6. Adverse events of RCA-IA sessions (n = 45)

Parameter	RCA-IA sessions, n = 45
Pruritus, n (%)	2 (4.44)
Nausea/vomiting, n (%)	2 (4.44)
Chest tightness, n (%)	2 (4.44)
Tachycardia, n (%)	1 (2.22)
Musculoskeletal pain, n (%)	1 (2.22)
New bleeding events, n (%)	0
Hypertension (BP >180/110 mm Hg), n (%)	3 (6.67)
Hypotension (BP <90/60 mm Hg), n (%)	2 (4.44)
Hypocalcemia (iCa <1.0 mmol/L or TCa <2.0 mmol/L), n (%)	5 (11.11)
Severe hypocalcemia (iCa <0.8 mmol/L or TCa <1.9 mmol/L), n (%)	1 (2.22)
Hypercalcemia (iCa >1.4 mmol/L or TCa >2.5 mmol/L), n (%)	2 (4.44)
Severe hypercalcemia (iCa >1.5 mmol/L or TCa >3.0 mmol/L), n (%)	0
Metabolic acidosis (HCO_3^- <22 mmol/L), n (%)	1 (2.22)
Metabolic alkalosis (HCO_3^- >28 mmol/L), n (%)	6 (13.33)
Hyponatremia (<135 mmol/L), n (%)	2 (4.44)
Hypernatremia (>145 mmol/L), n (%)	2 (4.44)
Hypomagnesemia (<0.7 mmol/L), n (%)	6 (13.33)
Severe hypomagnesemia (<0.5 mmol/L), n (%)	0
Change in weight (IQR), kg	1.28 (1.11, 1.43)

RCA, regional citrate anticoagulation; IA, protein A immunoadsorption; IQR, interquartile range.

extracorporeal circuit may be attributable to a high post-filter iCa level or vascular access dysfunction. The TMP was in a stable state with a normal range of 0–20 mm Hg. The above results indicated that RCA had adequate anticoagulant effect and could efficiently maintain the patency of the extracorporeal circulation system during IA treatment.

RCA has the potential to decrease the likelihood of adverse events by avoiding systemic effects on anticoagulation [8]. In our study, all patients completed their treatment successfully with stable vital signs, and no new bleeding-related complications occurred during RCA-IA sessions. Our results showed no differences regarding coagulation values, including PT, TT, APTT, and INR, before and after RCA-IA sessions. It is noteworthy that PLT levels decreased during some sessions of RCA-IA therapy, but there was no significant difference, indicating that the plasma separator may have had an impact on PLT levels. Researchers have suggested that therapeutic plasma exchange and double-filtration plasmapheresis could lead to a greater depletion of FIB than IA treatment [26]. Tryptophan or phenylalanine-conjugated columns can decrease the level of FIB in IA therapy [27]. Consistently, we found a slight reduction in blood FIB levels after IA therapy in our study, but it did not affect bleeding frequency or the requirement for FIB supplementation. Similar to our study, an IA therapy using a column containing protein A from *Staphylococcus aureus* exhibited only a weak impact on FIB levels has been reported [28]. This indicates that except for a slight reduction in FIB, RCA-IA sessions displayed no obvious influence on the coagulation function of patients in this study.

The RCA-IA therapy resulted in several sessions exhibiting electrolytes and acid-base disturbances, including hypocalcemia, hypomagnesemia, and metabolic alkalosis, which were mild, similar to the previous studies using RCA [29]. Compared to the values before and after 24 h, the average levels of iCa and TCa at 2 h or 6 h showed a downward trend; however, such values exhibited no statistical significance. Among these sessions, one session occurred with severe hypocalcemia, but the patient had no serious manifestations and improved after receiving additional intravenous calcium supplements. Three sessions were observed with mild hypercalcemia post-therapy, but did not require any intervention or management. This possible mechanism could be the binding of citrate with iCa in the circuit to form CCC, and this iCa returns to the blood after separating from CCC during metabolism. Therefore, it is necessary to monitor blood calcium level, because the effect of citrate metabolism on calcium balance may vary among different individuals due to different rates of citrate metabolism and there may be variable supplemental calcium demand. The magnesium (Mg) level was reduced after the therapy compared with the value before the therapy, which may be due to the binding of Mg with the citrate, as it is bivalent and shows affinity for citrate [15]. Hypomagnesemia has been reported during RCA in CRRT, as Mg may interact with citrate in a manner similar to calcium. Certain RCA sessions may necessitate Mg supplementation to prevent hypomagnesemia [30, 31].

In contrast to the baseline condition, the average levels of pH, HCO_3^-, and BE were elevated at 2 h and 6 h after the commencement of therapy, owing to the release of HCO_3^-

by citrate catabolism, thereby elevating the BE and pH. Within 24 h after treatment, the levels of pH, HCO_3^-, and BE gradually returned to the normal range. Furthermore, the metabolic alkalosis observed in our study was asymptomatic, which was similar to previous research [32]. Bianchi et al. [33] observed severe metabolic alkalosis in RCA during CRRT therapy in a few patients. However, we did not detect any severe metabolic alkalosis, which may be attributable to a low dose of citrate administration and normal liver function for citrate metabolism. We did not observe severe metabolic acidosis or fluctuation in Na^+, Cl^-, and K^+ at distinct time points posttreatment in any session.

The RCA-IA procedures were well tolerated without any significant adverse clinical reactions or technical issues during our research. Only a few patients showed common transient symptoms such as mild pruritus, nausea/vomiting, tachycardia, chest tightness, or musculoskeletal pain, but did not require discontinuation of the procedure. Other adverse events that have been reported in the literature regarding immunoadsorption, such as fever, chills, dizziness, bronchospasm, hemolysis, arrhythmia, or leukocytoclastic vasculitis, were not observed in this study [34, 35]. We believe that this is due to the improved quality of the recombinant genetic engineering protein A column used in our research. Furthermore, our experience has demonstrated that the adequate pre-flushing of the adsorption column prior to the commencement of each session was advantageous in mitigating undesirable side effects, as it eliminated the protective fluid utilized to store the protein A column. It is imperative to repeat the treatment cycles as HLA antibodies are equally distributed in both the intravascular and extravascular compartments. It is therefore necessary to remove these antibodies, by repeating and adequately processing cycles. The prolonged duration of RCA-IA sessions may have adverse effects pertaining to vascular access [36]. In addition to drinking water or eating food, patients received the transfusion of treatment fluids such as citrate and calcium gluconate, which need to be removed from the body. Contrary to HD or CRRT, the supplementary fluids present during the RCA-IA sessions cannot be removed through ultrafiltration. Since all patients were in renal failure and were waiting for kidney transplantation, HD was required for all patients during the study period to maintain any fluctuation in electrolytes, pH, metabolic wastes, or fluid overload. We routinely conducted the RCA-IA sessions on days that were not designated for HD. We think a more concentrated citrate solution would be preferable for such patients. Hence, it is noteworthy that ensuring a proper balance between fluid overload, metabolism, and electrolytes through HD was of utmost significance during the RCA-IA sessions.

The alterations in routine blood indexes, HR, systolic blood pressure, diastolic blood pressure, and mean arterial pressure during and after treatment were not statistically significant. Nonetheless, alterations in transient blood pressure were observed during multiple treatment sessions, which may be attributed to the diminution of plasma colloid osmotic pressure resulting from immunoglobulin clearance, or extracellular volume change and dysregulation of sympathetic nervous system. After proper therapy, transient blood pressure alterations did not affect the treatment process for IA.

Our study was characterized by several limitations. Initially, the experiences with RCA in this study were restricted to IA treatment subsequent to plasma separation, and the sample size in our study was relatively small. A larger cohort study will provide more reliable findings about the application of RCA in IA treatment. Moreover, given the single-center, retrospective study limitation, it is inevitable that it may encounter confounding factors; hence, it is imperative to conduct a prospective randomized controlled trial in the future. In this study, it was not possible to compare the impact of RCA with other anticoagulation measures due to the lack of a control group. As our inclusion criteria solely pertain to patients with renal impairment, it is possible that certain patients in hospitals requiring RCA-IA may have other systemic diseases, such as liver or lung impairment or current infections that are contradiction to our protocol. We were unable to establish a target for fluctuations in electrolytes, pH, metabolic alkalosis, or acidosis that RCA should refrain from administering due to its adverse effects. The absence of a direct measurement of blood citrate level in the event of adverse events resulting from citrate as an offending agent also hinders its application in IA therapy.

In brief, with careful monitoring and adjustment, our regimen with RCA is considered to be a feasible, effective, and safe anticoagulation method for patients with a high bleeding risk who underwent an IA procedure. It is imperative to closely monitor and manage electrolytes and pH imbalances, particularly hypocalcemia, hypomagnesemia, FIB level, metabolic alkalosis, and fluid overload. The RCA strategy may offer an alternative anticoagulation approach to maintain circuit patency in IA therapy for patients with high bleeding risk or heparin contraindications. However, further research is required to establish its appropriate application guidelines.

Statement of Ethics

The study was approved by the Medical Ethics Committee of Xiangya Hospital of Central South University for Human Studies (No. 202008097) and followed the Declaration of Helsinki. All subjects provided the written informed consent.

Conflict of Interest Statement

The authors declare that there were no conflicts of interest.

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Author Contributions

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Data Availability Statement

All data included in this study are available upon request from the corresponding author.

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