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Original Article

Application of citric acid in continuous veno-venous hemofiltration of severe acute pancreatitis patients in ICU

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Abstract: Objective: This study aimed to investigate the effect of citric acid on anticoagulation and kidney functions in the continuous veno-venous hemofiltration (CVVH) of severe acute pancreatitis (SAP) patients in ICU as compared with the low molecular weight (LMW) heparin. Methods: 40 SAP patients admitted to ICU of our hospital from January 2017 to December 2019 were included for retrospective analysis and divided into the control group (CG, n=20) and the observation group (OG, n=20) by double-blind randomization. All patients were treated with CVVH. The difference lied in the anticoagulation that citric acid was used in the OG, and LMW heparin was used in the CG. The two groups were compared for anticoagulation effects and kidney functions. Results: (1) The APTT level was higher in the OG as compared with the CG ($P<0.05$) after 2 h, 6 h and 9 h of treatment. At 1 h after treatment, the APTT level changed insignificantly in the OG as compared with that before treatment ($P>0.05$) but elevated sharply in the CG ($P<0.05$). (2) PLT levels remained almost the same in the OG ($P>0.05$) but reduced in the CG ($P<0.05$) as compared with those before treatment, after treatment and at 1 d after treatment. (3) After treatment, the OG yielded lower levels of inflammatory factors, i.e., IL-6 and TNF-α, and kidney function indices, including Bun and Scr ($P<0.05$) than the CG. (4) No statistical difference was found between the two groups in terms of proportions of grades I, II and III for coagulation and bleeding at the puncturing and insertion sites ($P>0.05$). Conclusion: The application of citric acid in CVVH of SAP patients in ICU could achieve better anticoagulation effect and improve patients' kidney functions, which deserves popularizing.

Keywords: ICU, SAP, continuous veno-venous hemofiltration, citric acid, anticoagulation, kidney functions

Introduction

Acute pancreatitis (AP) is a kind of disease with high incidence in the gastroenterology department. Without prompt and effective treatment, up to 10% of the cases may develop to severe acute pancreatitis (SAP) [1]. SAP is more severe than AP, with a high incidence of complications, poor prognosis and a mortality rate of around 25% [2].

A variety of mechanisms contribute to the development of SAP, of which, inflammatory reaction plays a leading role. At the site of inflammation, inflammatory cells are involved and over activated to promote the release of a large number of pro-inflammatory mediators and cell factors, which further move into blood and result in systemic inflammatory response syndrome or multiple organ failure in severe cases [3]. Therefore, active control of inflammatory level is of extreme importance in the treatment of SAP. Hemofiltration is the main treatment for SAP, and continuous veno-venous hemofiltration (CVVH) helps alleviate inflammatory reaction and control the progression of the disease [4]. However, the selection of anticoagulation method during treatment is very important as it may directly affect the hemofiltration effects. Any mistake in the selection of anticoagulant may aggravate bleeding and directly threaten the life safety of patients [5].

General anticoagulants include citric acid and low molecule weight (LMW) heparin, and their application effects have been studied by different authors with different conclusions [6, 7]. This study aimed to compare the effects of cit-

ric acid and LMW heparin for anticoagulation in CVVH, so as to find more safe and effective methods for the treatment of patients with SAP.

Materials and methods

Materials

40 SAP patients admitted to the ICU of our hospital from January 2017 to December 2019 were included for retrospective analysis and divided into the control group (CG, n=20, with the age range of 35-63) and the observation group (OG, n=20, with the age range of 37-62) by double-blind randomization. The causes of SAP were alcoholic, biliogenic, or related to hyperlipoidemia, bulimia, *etc*. (1) Inclusion criteria: patients who met the SAP diagnosis criteria [8] due to AP with organ functional failure over 48 h, and admitted to the ICU for treatment were included. They had normal cardiac, hepatic and coagulation functions, and hemofiltration indications. This study has been approved by the Ethics Committee of the First Affiliated Hospital of Gannan Medical University. All study participants provided written informed consent before participating in the study. (2) Exclusion criteria: patients were excluded due to pregnancy, drug allergy history, concurrent and severe disorders in important organs such as heart, liver, kidneys and lungs, or contractions of anticoagulation, or failure to provide the informed consent.

Methods

Both groups were treated according to the same CVVH procedures with instruments including a hemofiltration instrument (PrismsfexM100Set, Sweden) and a hemofiltration machine (Prismaflex, Sweden). A double-channel catheter with single needle head was inserted into the femoral vein to establish the vascular pathway. All filters were disposable. The flow rate was controlled between 150 and 200 ml/min for blood, and around 1500 ml/h for displacement liquid. The infusion continued for 10 h. All filters and blood pathways were thoroughly soaked and rinsed in heparin saline prior to use.

For the CG, LMW heparin was selected for anticoagulation during CVVH (Changshan Biochemical Pharmaceutical Co. Ltd., Hebei, with SFDA approval number: H20063910). At the beginning of hemofiltration, heparin sodium injection was infused through the artery at the dose of 0.5 to 1.0 mg/kg. Subsequently, the added dose was controlled at 0.05~0.1 mg/(kg·h). Furthermore, nucleoprotamine was infused through the vein to neutralize heparin according to the principle of 1 mg for 100 U heparin.

For OG, citric acid (Nangle Biotechnology Co. Ltd., Szechwan, with SFDA approval number: H20058912) was selected for anticoagulation during CVVH. 4.0 mmol/L sodium citrate solution was infused into the patients through the artery at the flowrate of 180 ml/h, and 10% calcium gluconate solution was pumped into the vein at the flow rate of 5.5 mmol/L. Every 2 h after CVVH began, arterial blood gas and serum calcium level (SCL) were measured. The infusion amount and flow rate of sodium citrate and calcium gluconate were properly adjusted.

Observation indices

(1) Activated partial thromboplastin time (APTT): APTT was measured in both groups before treatment, after 2 h, 6 h and 9 h of treatment and at 1 h after treatment.

(2) Platelet count (PLT): PLT levels in both groups were measured before treatment, after treatment, and at 1 d after treatment.

(3) Inflammatory factors: the levels of interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) were measured in both groups before and after treatment.

(4) Kidney function: the levels of serum creatinine (Scr) and blood urea nitrogen (BUN) were measured in both groups before and after treatment.

(5) Coagulation: the two groups were compared for coagulation in pipelines and filterers before the end of treatment. The coagulation results were classified into 3 grades [9], grade 0 with no coagulation in the pipeline and filters, grade I with coagulation only in the filters, and grade II with coagulation only in the pipeline.

(6) Bleeding: after treatment, the bleeding at the puncture and insertion sites was evaluated for both groups [10], which was classified into 3 grades: grade 0: no subcutaneous bleeding at

Table 1. Comparison of general materials between the OG and the CG ($\bar{x}$ ± sd)/[n (%)]

Materials		OG (n=20)	CG (n=20)	t/X^2	*P*
Gender	M	13 (65.00)	12 (60.00)	0.107	0.744
	F	7 (35.00)	8 (40.00)		
Age (year)		52.38±6.93	51.76±6.96	0.282	0.779
Height (cm)		168.59±10.25	170.42±11.40	0.534	0.600
Weight (kg)		62.38±5.49	63.49±5.58	0.634	0.530
Cause of disease	Alcoholic	4 (20.00)	6 (30.00)	1.058	0.162
	Biliogenic	5 (25.00)	5 (25.00)		
	Hyperlipoidemia	5 (25.00)	4 (20.00)		
	Bulimia	6 (30.00)	5 (25.00)		

the puncture and insertion site; grade I: obvious ecchymoma at the puncture and insertion site; grade II: extensive ecchymoma at the puncture and insertion site, and capillary hemorrhage at the puncture site.

Statistical analysis

Statistical analysis was performed with SPSS 22.0. In case of numerical data expressed as mean ± standard deviation, comparison studies were carried out through independent-samples T test; in case of nominal data expressed as [n (%)], comparison studies were carried out through X^2 test for intergroup comparison. Multiple-point intragroup comparison was done with ANVOA and F test. For all statistical comparisons, significance was defined as P<0.05.

Results

Comparison of general materials in both groups

No significant difference was found between the OG and the CG in terms of proportions of male and female patients, mean age, height, weight, and composition of various causes (*P*>0.05, **Table 1**).

Comparison of APTT in both groups

The APTT was 40.23±1.16 s, 78.42±3.61 s, 71.49±4.29 s, 73.59±5.28 s and 41.19±3.29 s in the OG, and 40.19±1.22 s, 53.62±2.59 s, 59.34±3.59 s, 55.34±3.64 s and 41.19±3.29 s in the CG before treatment, after 2 h, 6 h and 9 h of treatment and at 1 h after the treatment. Before treatment, the APTT was almost the same in the two groups (*P*>0.05), but was elevated gradually after 2 h, 6 h, and 9 h of treatment to a level much higher in the OG as compared with the CG (*P*<0.05). At 1 h after treatment, the OG reported insignificant difference of the APTT level as compared with that before treatment (P>0.05), but the APTT level of the CG still remained at a high level (*P*<0.05, **Figure 1**).

Comparison of PLT in both groups

No significant difference was observed between the two groups in terms of PLT before treatment. The PLT levels of the OG remained almost the same with those of 210.23±17.63 $\times10^9$/L at the end of the treatment and 211.28±10.42 $\times10^9$/L at 1 d after treatment (*P*>0.05). At the same time points, the CG experienced a sharp reduction from 189.34±14.34 $\times10^9$/L to 180.34±12.29 $\times10^9$/L (*P*<0.05). Therefore, the PLT level in the OG was higher than that of the CG at the end of the treatment and at 1 d after treatment (*P*<0.05) (**Figure 2**).

Comparison of inflammatory factor level in both groups

The IL-6 and TNF-αlevels reduced in both groups after treatment (*P*<0.05) as compared with those before treatment which were almost the same (*P*>0.05). After treatment, the IL-6 and TNF-α levels were significantly lower in the OG as compared with the CG (*P*<0.05, **Table 2**).

Comparison of kidney functions in both groups

Before treatment, the levels of BUN and Scr were 18.42±6.38 mmol/L and 328.42±72.19 μmol/L in the OG, and 19.02±6.34 mmol/L and 324.52±73.38 μmol/L in the CG, which were not significantly different (*P*>0.05). After treatment, reduction was achieved to 5.43±1.49

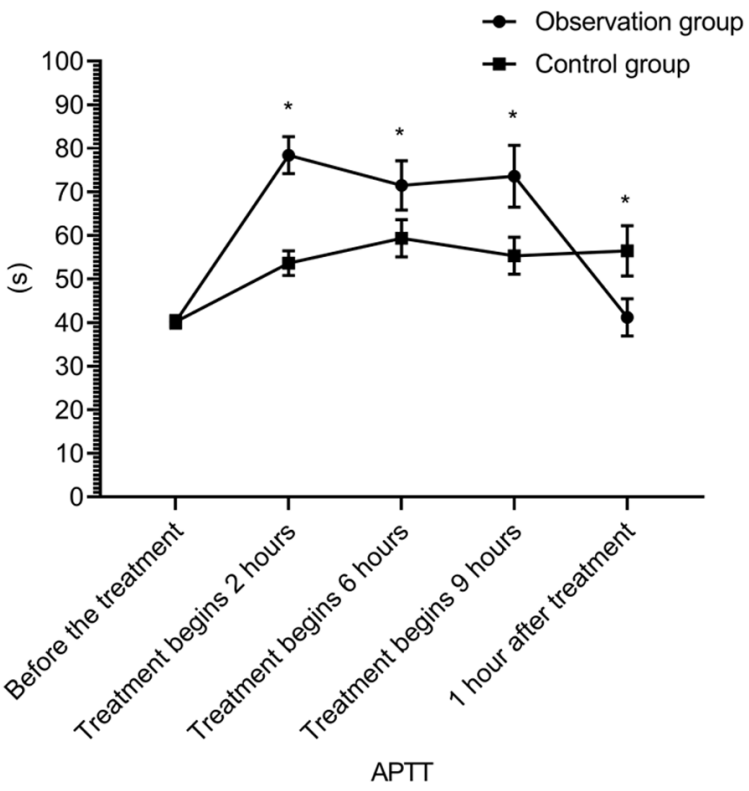

Figure 1. Comparison of APTT level between the OG and the CG. The APTT level in the OG was not significantly different from that of the CG before treatment ($P>0.05$), but was elevated to a higher level after 2 h, 6 h and 9 h of treatment. At 1 h after treatment, the APTT level reduced again in the OG to a lower value as compared with the CG ($P<0.05$). * indicates $P<0.05$ as compared between the two groups at the same time point.

mmol/L and 64.28±23.19 μmol/L in the OG, and 7.52±2.31 mmol/L and 93.58±27.48 μmol/L in the CG ($P<0.05$). The BUN and Scr levels in the OG were significantly lower than those of the CG after treatment ($P<0.05$) (**Figure 3**).

Comparison of coagulation in both groups

Before the end of treatment, 15 (75.00%) patients in the OG and 10 (50.00%) in the CG were classified as grade 0, 5 (25.00%) in the OG and 7 (35.00%) in the CG as grade I, and 0 (0.00%) in the OG and 3 (15.00%) in the CG as grade II in terms of coagulation, which were not significantly different ($P>0.05$, **Table 3**).

Comparison of bleeding in both groups

The two groups demonstrated no statistical difference in the proportions of bleeding grades 0, I and II at the puncture and insertion sites ($P>0.05$, **Table 4**).

Discussion

Most of the domestic and foreign studies on the nosogenesis of SAP focused on cell factors, for example, Jacob et al [11] and Muniraj T [12] verified that cell factors played a vital role in the systematic inflammatory reaction caused by SAP. In SAP patients, inflammatory cell factors interacted and eventually lead to vassal leakage and dysfunction of organs, which directly threaten their life safety [13]. The pancreatic tissue of SAP patients is a kind of inflammatory stimulant which can activate macrophages, promote the synthesis and secretion of inflammatory mediators, destroy the immune function of the body, and even cause systemic inflammatory response syndrome [14].

In China, clinical treatments of SAP include both operational and non-operational conservative methods. However, studies have found that the early operation could not satisfactorily control the progress of SAP because it failed to improve patients' inflammatory state but led to inflammatory stimulation afterward, which may cause the dysfunction of multiple organs [15]. Blood purification is a non-operational treatment for SAP in clinic. As one of its member, CVVH can not only ensure high filtration rate but also eliminate the impact on the specific microenvironment. It is helpful to obviously reduce the blood trypsin level in the body of SAP patients since tissues and organs may be damaged if the level remains high [16, 17]. In addition, CVVH is capable of removing small molecular substances and inflammatory factors in the body to interrupt the chain reaction caused by inflammation, so as to control the risk of systemic inflammatory reaction syndrome, the rates of illness and deaths related to the SAP [18] and effectively prevent the multiple organ dysfunction syndrome.

Clinically it is also found that the success of CVVH depends on good anticoagulation effects.

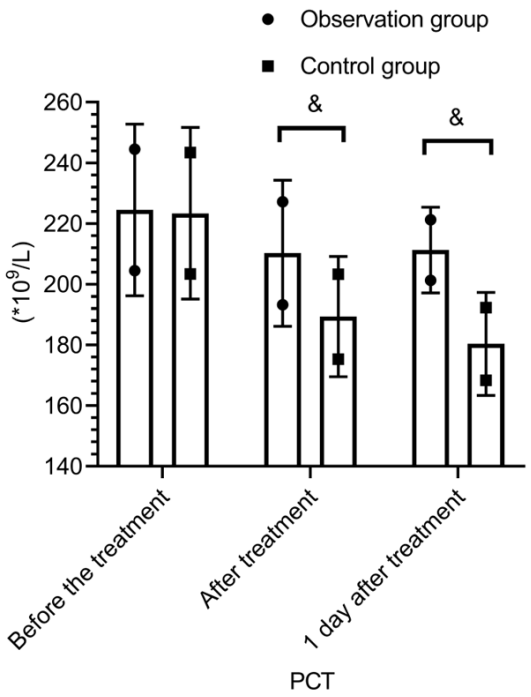

Figure 2. Comparison of PLT level between the OG and the CG. No significant difference was found in the PCT level between the two groups before treatment (P>0.05). The PCT level was significantly higher in the OG as compared with the GC upon the end of the treatment and at 1 d after treatment (P<0.05). & indicates P<0.05 as compared between the two groups at the same time points.

Therefore, proper selection of anticoagulants is necessary to prevent bleeding [19]. In this study, sodium citrate was applied in the OG for anticoagulation, and the results were compared with those of the patients in the CG who were treated with LMW heparin sodium. After 2 h, 6 h and 9 h of treatment, APTT levels rose gradually in both groups, but more sharply in the OG (P<0.05). However, at 1 h after the treatment, APTT level was reduced in the OG, but remained at a high level in the CG as compared with that before treatment, indicating that the application of citric acid in the CVVH could achieve better anticoagulation effects which were removed rapidly to eliminate the impact on patients. Through analysis, the reasons shall be that sodium citrate could bind to the formed clots to improve anticoagulation effects, while its resistance against calcium ion eliminates the obvious impact that may exist on the coagulation pathway [20]. In this study, at the end of the treatment and at 1 d after treatment, the PLT levels of the OG were not significantly different from those before treatment (P>0.05), while the PLT levels of the CG experienced a large reduction (P<0.05), indicating that the application of LMW calcium heparin in anticoagulation could affect the platelet count more obviously than the sodium citrate.

After treatment, the OG reported lower levels of IL-5 and TNF-α (inflammatory factors), BUN and Scr (kidney function indices) than the CG (P<0.05), indicating that compared with the LMW heparin, the selection of sodium citrate for anticoagulation in CVVH could better control the inflammatory level and better protect the kidney functions of SAP patients to avoid more severe damages. The anticoagulation effect of heparin is given to play by enhancing the activity of AT-III. LMW heparin sodium can be used to inhibit the synthesis and secretion of the proinflammatory factor caused by endotoxin, so that the expression of the adherence factor is reduced, followed by the generation of the inflammatory medium for the purpose of anti-inflammation [21]. Sodium citrate binds to the calcium ion in the blood to reduce the level of calcium ion therein, inhibit the process of prothrombin transforming to thrombin, inhibit the activation pathway of complements, improve the biofilm compatibility and give good play to anticoagulation effect [22, 23]. The studies of Urwin et al [24] also revealed that citric acid could reduce the level of calcium ion in the blood and the expression of inflammatory reaction mediated by the film to control the secretion of inflammatory cell factors and alleviate the inflammatory reaction. Furthermore, in this study, patients classified to coagulation grades 0, I and II, and bleeding grades 0, I and II at the puncture and insertion sites were 75.00%, 25.00% and 0.00%, 80.00%, 20.00% and 0.00% in the OG, and 50.00%, 35.00% and 15.00%, 60.00%, 30.00% and 10.00% in the CG (P>0.05). It is observed that the proportions of patients in each coagulation and bleeding grade in the OG were slightly lower than those of the CG, and in the OG, there were no patients with coagulation and bleeding at grade II, indicating that sodium citrate was safer for coagulation than LMW heparin sodium, but the results were not statistically different due to less included objects.

In conclusion, the application of citric acid in the CVVH of SAP patients in ICU could achieve

Table 2. Comparison of inflammatory level between the OG and the CG before and after treatment ($\bar{x} \pm sd$)

Group	n	IL-6 (pg/ml)		TNF-α (pg/ml)	
		Before treatment	After treatment	Before treatment	After treatment
OG	20	208.43±10.16	104.26±5.37	156.38±12.16	92.45±8.37
CG	20	206.37±10.42	138.45±6.92	155.29±11.37	130.28±10.16
t		0.633	17.456	0.293	12.852
P		0.531	0.000	0.771	0.000

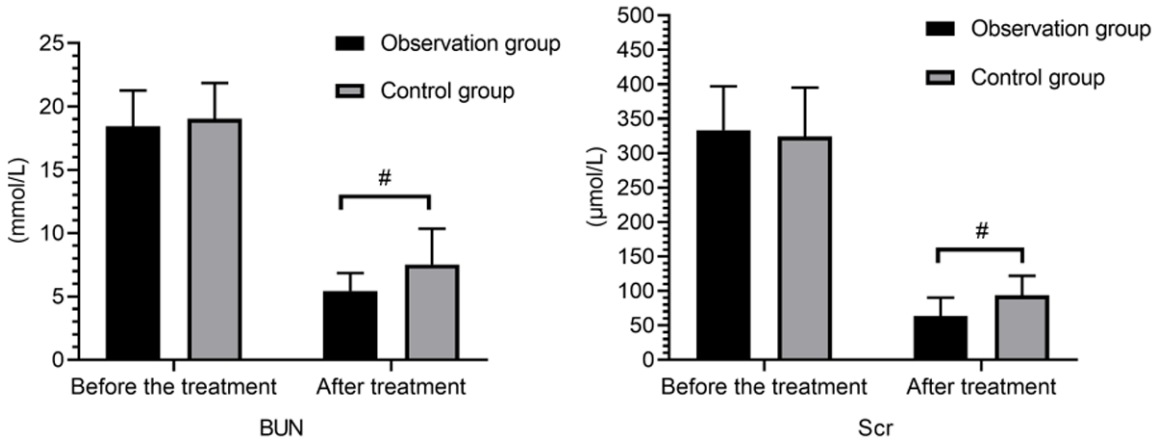

Figure 3. Comparison of kidney functions between the OG and the CG. Before treatment, the two groups demonstrated no significant difference in BUN and Scr levels ($P>0.05$). After treatment, the BUN and Scr reduced to lower levels in the OG as compared with the CG ($P<0.05$). # indicates $P<0.05$ as compared between the two groups at the same time points.

Table 3. Comparison of coagulation grading between the OG and the CG [n (%)]

Group	Level 0	Level I	Level II
OG (n=20)	15 (75.00)	5 (25.00)	0 (0.00)
CG (n=20)	10 (50.00)	7 (35.00)	3 (15.00)
X^2	2.667	0.476	3.243
P	0.102	0.490	0.072

Table 4. Comparison of grades of bleeding at the puncture and insertion sites between the OG and the CG [n (%)]

Group	Grade 0	Grade I	Grade II
OG (n=20)	16 (80.00)	4 (20.00)	0 (0.00)
CG (n=20)	12 (60.00)	6 (30.00)	2 (10.00)
X^2	1.905	0.533	2.105
P	0.168	0.465	0.147

better anticoagulation effects, including significant reduction of inflammatory level and protection of kidney functions, which deserves popularizing. However, the study included less objects and failed to comprehensively analyze the study results. In addition, the follow-up time was short and the results were somehow subjected to bias. The future studies shall be based on larger sample sizes and carried out from multiple aspects to be forward-looking and to obtain more scientific and representative conclusions for the purposes of providing more guidance for the clinical safe treatment of SAP patients.

Disclosure of conflict of interest

None.

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