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The effects of Plasma-Lyte 148 solution on blood coagulation: an in-vitro, volunteer study using rotational thromboelastometry

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The current study aimed to measure the effects of Plasma-Lyte 148 solution on the blood coagulation profile according to the hemodilution level using rotational thromboelastometry (ROTEM) tests. Venous blood was collected from 12 healthy volunteers and divided into four specimen bottles, which were diluted at different levels with Plasma-Lyte 148 (0, 20, 40, and 60%). Following this, ROTEM tests were performed on the study samples. We found that as the hemodilution level increased, the ROTEM values showed a hypocoagulable pattern. The change rate of the maximum clot firmness (MCF) of INTEM was greater in the 40 ($P = 0.015$) and 60% ($P < 0.001$) dilutions than it was in the 20% dilution. Greater lengthening of the clot formation time of EXTEM was observed in the 60% dilution than it was in the 20% dilution ($P < 0.001$). The alpha-angle of EXTEM showed a greater decrease in the 60% dilution than it did in the 20% dilution ($P < 0.001$). A larger change rate of the MCF of EXTEM was observed in the 40 ($P = 0.003$) and 60% ($P < 0.001$) dilutions than it was in the 20% dilution. A greater decrease in the MCF of FIBTEM was identified in the 40 ($P = 0.009$) and 60% ($P < 0.001$) dilutions than in the 20% dilution. All coagulation pathways exhibited hypocoagulable patterns as the hemodilution level increased. However, most of the mean values of ROTEM parameters were within the normal reference range, except for those of the 60% dilution.
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Introduction

Aggressive fluid resuscitation is essential for maintaining hemodynamic stability in patients with massive hemorrhaging due to severe trauma or surgery. Various fluids have been used for this purpose, and efforts have been made to establish an optimal fluid for reducing the unwanted adverse effects in critical situations.

On the contrary, despite the important role fluids play in volume replacement, resuscitation with large amounts of fluid may lead to coagulation derangement and/or acid-base imbalance depending on the type of fluid used [1,2]. Uncontrolled bleeding initially causes the loss of coagulation factors, red blood cells, and platelets. The vicious cycle of coagulation factor consumption, fluid resuscitation, and massive transfusion, which results in thrombocytopenia, can worsen the coagulopathy and perpetuate bleeding via deleterious effects on hemostasis, with altered fibrin polymerization and decreased platelet adhesive and aggregating properties [3–5].

Recently, various studies examining the utility of administering Plasma-Lyte 148 (Baxter, S.L., Valencia, Spain) solution in patients undergoing surgery or resuscitation have been conducted [6–8]. Plasma-Lyte 148 is a balanced crystalloid that contains sodium, potassium, chloride, and magnesium, but not calcium. Because of its 'physiologic' or 'balanced' formulation, Plasma-Lyte 148 is less likely than other crystalloid solutions, such as Hartmann's solution or isotonic saline (NaCl), to lead to dilutional or hyperchloremic acidosis [6–9].

Although several studies have reported the effects of hemodilution with crystalloids or colloids on hemostasis, to date, few studies have examined the effects of Plasma-Lyte 148 on blood coagulation. Therefore, we performed this in-vitro study to investigate the effects of Plasma-Lyte 148 on the blood coagulation cascade according to dilution level using rotational thromboelastometry (ROTEM) tests.

Methods

The current study was approved by the Institutional Review Board of Seoul National University Bundang Hospital, Seongnam-si, South Korea (approval obtained on 9 May 2017; B-1703/386-303), and registered at ClinicalTrials.gov (NCT03168087). Written informed consent was obtained from 12 healthy volunteers (seven

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men and five women, age range: 28–43 years) who had no history of taking medications known to interfere with hemostasis, such as anticoagulants, antiplatelets, or non-steroidal anti-inflammatory agents.

After collecting 10 ml of fresh venous blood from an antecubital vein, the blood samples were placed immediately in citrate-containing polypropylene tubes (Vacutainer; Becton Dickinson, Plymouth, UK). A two-syringe blood sampling method was used to reduce tissue thromboplastin contamination, that is, the initial blood sample (~5 ml) was discarded, and then the study blood sample was drawn in succession. The collected samples were divided into four bottles of 2.0 ml each. To generate the final dilution levels of 20, 40, and 60%, we added 400, 800, and 1200 μl of Plasma-Lyte 148 to three bottles, respectively, after the same volume of blood was discarded. One sample (0%) was used as a control (baseline value).

One investigator (H.-J.S.) performed the ROTEM analyses, and the following four ROTEM parameters were obtained: clotting time (CT), clot formation time (CFT), alpha angle (α-angle), and maximum clot firmness (MCF). We assessed the EXTEM, INTEM, and FIBTEM values, which indicate the status of the extrinsic, intrinsic, and fibrin polymerization pathways of the coagulation cascade, respectively, using the recommended reagents [ex-TEM: 20 μl of 0.2-mol/l $CaCl_2$ and 20 μl of tissue factor (TF); in-TEM: 20 μl of 0.2-mol/l $CaCl_2$ and 20 μl of thromboplastin phospholipid; fib-TEM: 20 μl of 0.2-mol/l $CaCl_2$ with cytochalasin D and 20 μl of TF].

Data were analyzed with SPSS for Windows (ver. 22; IBM Corp., Armonk, New York, USA) or Sigma Plot 10.0 (Systat Software, Inc., San Jose, California, USA), as appropriate. Repeated measures analyses of variance were used to compare the ROTEM values according to the hemodilution level. Categorical data were analyzed by Fisher's exact test. Data are expressed as the mean (SD) or a number (proportion). Statistical significance was set at P less than 0.05.

Table 2 Incidence of out of reference rage at each rotational thromboelastometry parameter

	Dilution level			
	0%	20%	40%	60%	*P* value
INTEM					
CT (s)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1.000
CFT (s)	0 (0%)	2 (17%)	3 (25%)	10 (83%)	<0.001
α (°)	0 (0%)	2 (17%)	4 (33%)	8 (67%)	0.001
MCF (mm)	0 (0%)	1 (8%)	1 (8%)	9 (75%)	<0.001
EXTEM					
CT (s)	0 (0%)	2 (17%)	2 (17%)	2 (17%)	0.499
CFT (s)	0 (0%)	0 (0%)	0 (0%)	5 (42%)	<0.001
α (°)	0 (0%)	0 (0%)	0 (0%)	6 (50%)	<0.001
MCF (mm)	0 (0%)	0 (0%)	0 (0%)	9 (75%)	<0.001
FIBTEM					
MCF (mm)	0 (0%)	3 (25%)	6 (50%)	7 (58%)	0.005

Data are expressed as number of patients (proportion). CFT, clot formation time; CT, clotting time; MCF, maximum clot firmness.

Results

We found that the ROTEM values showed a hypocoagulable pattern, including prolonged CFTs and decreased α-angles and MCFs, as the hemodilution level increased (Table 1). Only the α-angle of EXTEM in the 20% dilution was not statistically significant compared with 0% dilution. Nevertheless, most of the mean values of ROTEM parameters were within the normal reference range, except for those of the severe dilution specimen (60%).

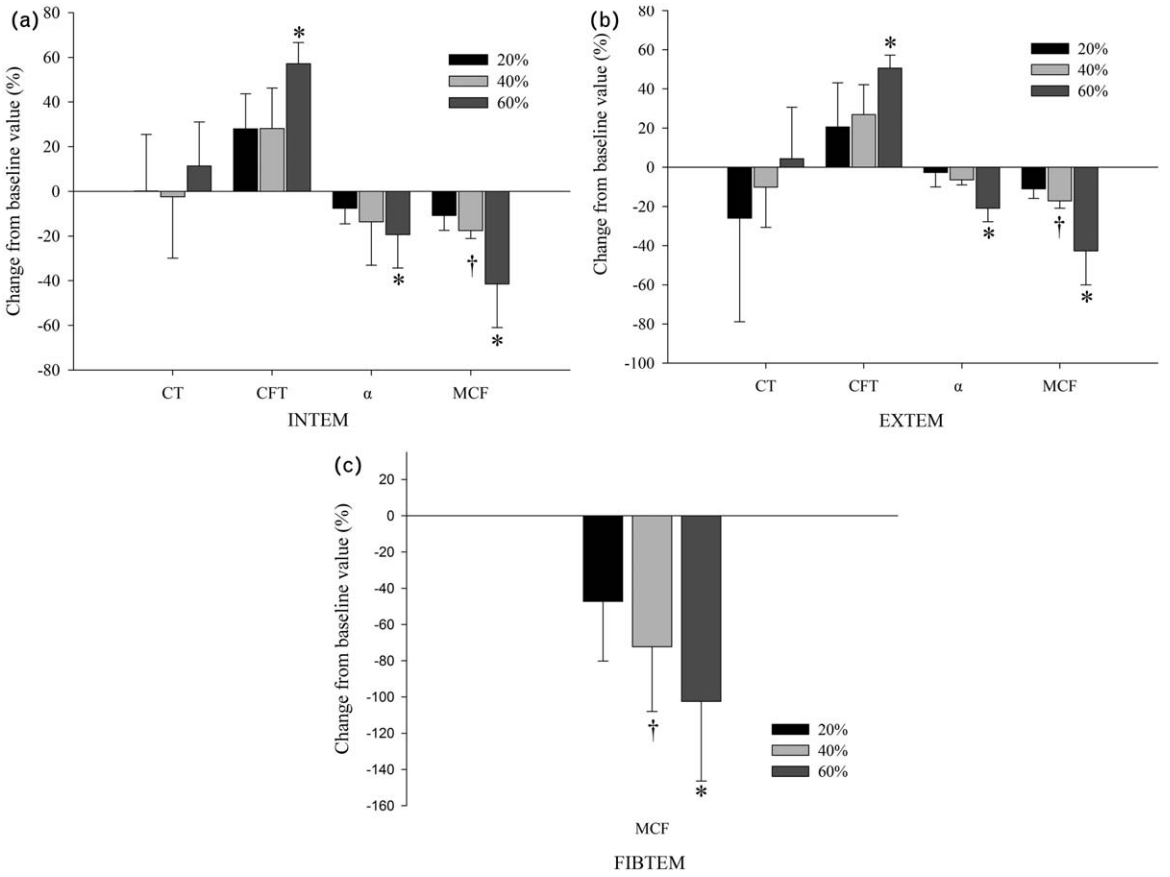
Percentage of samples outside reference ranges for each ROTEM parameters at each dilutional level was described in Table 2. In the CFT, α-angle, and MCF of INTEM, significant percentage differences were observed among the dilutional groups ($P < 0.001$, $P = 0.001$, and $P < 0.001$, respectively). In the CFT, α-angle, and MCF of EXTEM, only the 60% dilution caused blood coagulation impairment showing out of the reference range compared with other dilution groups ($P < 0.001$). Also, in the MCF of FIBTEM, number of patients whose values fall out of reference range was significantly different among the four dilutional groups ($P = 0.005$).

Table 1 Rotational thromboelastometry parameters ($n = 12$)

		Dilution level						
	Reference values	0%	20%	*P* value	40%	*P* value	60%	*P* value
INTEM								
CT (s)	100–240	166 (38)	170 (28)	0.794	165 (25)	0.951	188 (26)	0.068
CFT (s)	30–110	67 (11)	98 (27)	0.004	97 (22)	0.001	163 (39)	<0.001
α (°)	70–83	76 (2)	71 (5)	0.005	69 (9)	0.014	65 (7)	0.001
MCF (mm)	50–72	63 (3)	57 (4)	<0.001	54 (3)	<0.001	46 (6)	<0.001
EXTEM								
CT (s)	38–79	62 (7)	54 (13)	0.127	58 (10)	0.282	69 (16)	0.283
CFT (s)	34–159	80 (14)	106 (29)	0.010	113 (25)	<0.001	165 (36)	<0.001
α (°)	63–83	74 (3)	73 (6)	0.359	68 (4)	<0.001	62 (5)	<0.001
MCF (mm)	50–72	64 (3)	58 (5)	<0.001	55 (3)	<0.001	46 (6)	<0.001
FIBTEM								
MCF (mm)	9–25	15 (4)	11 (3)	<0.001	9 (3)	<0.001	8 (3)	<0.001

Data are expressed as mean (SD). CFT, clot formation time; CT, clotting time; MCF, maximum clot firmness.

Fig. 1



Percentage change from preoperative values for the following rotational thromboelastometry parameters: (a) INTEM, (b) EXTEM, and (c) FIBTEM for the 10% (black), 20% (pale grey), and 40% (dark grey) dilution groups. Values are the means (SDs). α, alpha angle (°); CFT, clot formation time (s); CT, clotting time (s); MCF, maximum clot firmness (mm). *,† Significantly different from the 20% dilution group ($P < 0.05$).

Figure 1a demonstrates the change rate (%) of the INTEM parameters from the baseline value in each dilution group. No significant differences were noted among the dilution groups regarding the CT change rate ($P = 0.662$). The CFT of INTEM was longer in the 60% dilution group (57%) than it was in the 20% dilution group (28%) ($P < 0.001$). The α-angle of INTEM was significantly decreased by 19% in the 60% dilution groups compared with 8% in the 20% dilution group ($P = 0.041$). The MCF change rate of INTEM was greater in the 40 and 60% dilution groups (18 and 41%, respectively) than it was in the 20% dilution group (11%) ($P = 0.015$ and $P < 0.001$, respectively).

Figure 1b demonstrates the change rate (%) of the EXTEM parameters from the baseline value in each dilution group. The CT change rates of EXTEM were not significantly different among the dilution groups ($P = 0.282$). Greater prolongation of the CFT of EXTEM was observed in the 60% dilution group (51%) than in the 20% dilution group (21%) ($P = 0.001$). The change rate of the α-angle of EXTEM was larger in the 60% dilution group (21%) than it was in the 20% dilution group (3%) ($P < 0.001$). A greater decrease in the MCF of EXTEM was identified in the 40 and 60% dilution groups (17 and 43%, respectively) than it was in the 20% dilution group (11%) ($P = 0.003$ and $P < 0.001$, respectively).

The MCF change rate of FIBTEM was greater in the 40 and 60% dilution groups (72 and 102%, respectively) than it was in the 20% dilution group (47%) ($P = 0.009$ and $P < 0.001$, respectively, Fig. 1c).

Discussion

In the current study, Plasma-Lyte 148 changed the coagulation pattern toward a hypocoagulable state, as explained by the results of our ROTEM analyses. Significantly, despite moderate hemodilution of up to 40%, most of the mean values ROTEM values were within the normal reference ranges. Furthermore, only mild coagulation impairment was observed in the 60% hemodilution.

The administration of intravascular fluid (hemodilution) is known to influence blood coagulation [10,11]. Roche *et al.* [12] reported that isotonic saline and lactated

Ringer's solution caused a hypercoagulable change at 20 and 40% hemodilution. At 60% hemodilution, isotonic saline, hydroxyethyl starches, and human albumin solutions all produced a hypocoagulable state. Similarly, using thromboelastography, Ekseth *et al.* [13] demonstrated increased coagulation activity *in vitro* at mild-to-moderate levels of hemodilution (up to 40%) with crystalloids and colloids. Otherwise, the hypercoagulable pattern decreased at dilutions more than 40% for all solutions, whereas a hypocoagulable pattern was induced with hydroxyethyl starches. In general, colloids have been found to exhibit more-pronounced deleterious effects on hemostasis [14].

Isotonic saline is one of the most commonly used resuscitation fluids [6]. However, infusing large volumes of saline produces metabolic acidosis by increasing the plasma chloride concentration relative to the plasma sodium concentration [6,15]. On the contrary, coagulopathy is confounded by acidosis, which worsens fibrin polymerization and strengthens the clot [16]. Although isotonic saline has been known to cause hypercoagulability in moderate hemodilutions, when infused at larger volumes that cause severe hemodilution, a hypocoagulable state can be induced combined with the derangement of acid-base homeostasis.

On the other hand, the electrolyte composition of Plasma-Lyte 148 more closely reflects the constituents of human plasma than isotonic saline, and is hence considered a more-physiologic solution [9]. Therefore, Plasma-Lyte 148 has important advantages over isotonic saline, namely, its ability to maintain the acid-base balance, even when administered in large volumes [6,8,17]. Furthermore, in the current study, Plasma-Lyte 148 caused mild coagulation impairment only in the 60% hemodilution. Collectively, these findings support that Plasma-Lyte 148 solution could be used for massive fluid resuscitation, without causing severe coagulation derangement.

Significantly, after hemodilution with Plasma-Lyte 148 solution, the CFT, α-angle, and MCF values were more sensitively affected than were the CT values in both the intrinsic and extrinsic coagulation pathways. Each parameter of the ROTEM analysis represents a different coagulation step. The CT indicates the beginning of clotting (speed of clot formation), which is mainly influenced by coagulation factors. Both the CFT and α-angle indicate the initial rate of fibrin polymerization, whereas the MCF indicates the maximal strength of a clot, which involves fibrinogen and platelets [18,19]. Therefore, Plasma-Lyte 148 seems to primarily affect the initiation of fibrin polymerization and clot strengthening.

The current study has several limitations that need to be considered. First, because of the inherent nature of in-vitro experiments, our results may be of limited relevance to clinical situations. Nevertheless, the strengths of an in-vitro study, namely, controllability and reproducibility, lend credibility to the results of the current study. Here, the dilution range was controlled from a clinically relevant level to an extreme level, enabling extensive evaluations of the potential effects of Plasma-Lyte 148 on coagulation. A study using the severe hemodilution would be practically impossible to perform in a clinical setting, as it would be a risk to the patients. Second, as this experimental study was designed to evaluate the hemodilution effects on coagulation, it is impossible to determine the effects that additional consumptive coagulopathy and associated hyperfibrinolysis related to the exposure of damaged tissue components would have in severe trauma or surgical patients. Finally, patient enrollment started (15 May 2017) prior to being posted on ClinicalTrials.gov (30 May 2017), which could increase the likelihood of publication bias. However, this short time disparity effect seems to be minimal on the present results.

In conclusion, this study showed that Plasma-Lyte 148 solution may influence the blood coagulation pathways (change toward a hypocoagulable pattern), as assessed by ROTEM. However, the degree of blood coagulation changes was minimal, which indicates that the use of Plasma-Lyte 148 in massive fluid resuscitation may be well tolerated in terms of coagulopathy.

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Conflicts of interest

There are no conflicts of interest.

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