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Original Research Article **Effects of co-infusion of fresh frozen plasma and nafamostat mesilate on its clotting activities**

ABSTRACT (ARIAL, BOLD, 11 FONT, LEFT ALIGNED, CAPS)

Aims: Nafamostatmesilate (NM), a protease inhibitor, is available for treating acute pancreatitis and disseminated intravascular coagulopathy, and it is used as an anticoagulant for hemodialysis in Japan. A plasmapheresis circuit using NM can easily be blockaded. Therefore, we investigated the influence of co-infusion of fresh frozen plasma (FFP) and NM on clotting activities mainly in the static condition.

Study design: *in vitro* study

Methodology: We investigated the effect of co-incubation of expired FFP and various concentrations of NM (0–0.1mg/mL). We measured the plasma fibrinogen level and activities of factor XIII, anti-thrombin III, protein C (PC), and protein S (PS). In addition, we examined the influence of NM on coagulation tests using blood from healthy volunteers.

Results: NM reduced PC and PS activities in FFP, although it did not affect plasma fibrinogen levels, or the activities of anti-thrombin III or factor XIII. While anti-thrombin III activity and plasma fibrinogen level increased in NM-containing blood, PC and PS activities decreased. Gabexate mesilate, sodium heparin, and citrate did not reduce the activities of PC or PS.

Conclusion: Co-infusion of NM and FFP reduces PC and PS activities, and it might lead to the obstruction of the plasmapheresis circuit when using NM as an anticoagulant.

Keywords: *co-infusion, protease inhibitor, protein C, protein S*

1. INTRODUCTION

Simultaneous infusion of blood components and drug solutions is contraindicated worldwide. Current transfusion guidelines forbid the addition of medications other than 0.9% sodium chloride solution (i.e., saline) to blood components [1-2]. Thus, the addition of any medication to an intravenous line through which blood components are infused is prohibited. Because of limited venous access, adherence to these guidelines may be compromised in certain clinical settings such as emergencies, bone marrow transplantation procedures, and intravenous chemotherapy treatment in pediatrics as well as in patients receiving opioids such as morphine for intravenous analgesia [3-5]. Co-infusion of heparin as an anticoagulant with red cell concentrate (RCC) does not induce hemolysis [6]. However, co-infusion of RCC and

other drugs results in unexpected adverse reactions in patients [7]. Moreover, we previously reported that co-infusion of RCC, nafamostat mesilate (NM), and gabexate mesilate (GM) induces hemolysis in packed erythrocytes [8]. NM is a synthetic protease inhibitor that inhibits coagulation and fibrinolysis by inactivating thrombin, plasmin, trypsin, kallikrein, coagulation factors XIIIa and Xa, and complements [9-10]. NM is used to treat acute pancreatitis and disseminated intravascular coagulation; it is also used as an anticoagulant during hemodialysis in patients with bleeding tendencies. NM was also recently used as an anticoagulant in cardiac surgery with cardiopulmonary bypass in Korea and Japan [11-12]. GM, a synthetic protease inhibitor, is also effective for treating patients with acute pancreatitis and disseminated intravascular coagulation, but is not used as an anticoagulant in hemodialysis [13].

In the present study, we examined the effects of various anticoagulant agents including heparin, sodium citrate, GM, and NM on clotting activities, particularly the anticoagulant factors protein C (PC) and protein S (PS) during the transfusion of fresh frozen plasma (FFP). Although co-infusion of heparin, sodium citrate, and GM with FFP did not reduce PC or PS activities, we report for the first time that NM induces the reduction of both PC and PS activities in FFP.

2. MATERIAL AND METHODS

NM was purchased from Torii Pharmaceutical Co. (Tokyo, Japan). GM was purchased from Ono Pharmaceutical Co. Ltd. (Osaka, Japan). NM and GM were readily soluble in 5% glucose solution (Fuso Pharmaceutical Industries Ltd., Osaka, Japan); this was mainly used as a vehicle in the experiments, because NM is not directly soluble in 0.9% saline. As a vehicle, 0.9% saline (Terumo Co. Ltd. Tokyo, Japan) was used in the experiments with anticoagulant citrate dextrose solution formula A (ACD-A solution) and sodium heparin.

2.1 Clotting activity evaluation

In the static test, aliquots of plasma from FFP (Japanese Red Cross Society, Tokyo, Japan) were placed in plain plastic tubes (Tokuyama Sekisui Co. Ltd., Yamaguchi, Japan). Immediately after, various volumes of anticoagulant agents including sodium heparin (Mochida Pharmaceutical Co. Ltd., Tokyo, Japan), sodium citrate (ACD-A solution, Terumo Co. Ltd., Tokyo, Japan), NM, and GM were added to the plasma from FFP. The co-infused plasma was analyzed 30 minutes after mixing at room temperature (20°C). The FFP (blood type: A) used in the experiments was expired. In Japan, FFP must be transfused within 1 year after phlebotomy; we used FFP within 3 months after the expiry date for the experiments. We also used whole blood from healthy volunteers (blood type: A). Whole blood was added to plain plastic tubes containing 0.2 mL NM (1 mg/mL; final concentration, 0.1 mg/mL) and heparin (Tokuyama Sekisui Co. Ltd., Yamaguchi, Japan).

Clotting tests (fibrinogen, anti-thrombin III [AT-III], factor XIII, PC, and PS) were measured by BML (Tokyo, Japan).

All study participants (i.e., normal healthy volunteers) provided informed consent, and the study design was approved by the ethics review board of our institution.

2.2 Statistical analysis

We compared differences among groups using Wilcoxon analysis. The data are expressed as mean $\pm$ standard error (SE). All statistical tests were conducted using JMP version 8.0 (SAS Institute, Inc., Cary, NC, USA). The level of statistical significance was set at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Effects of NM on clotting activities in FFP and plasma from whole blood

NM solution (0.5–1 mg/mL) was added to plasma in plastic tubes at a dilution ratio of 1:9. Thirty minutes after mixing at room temperature, co-infused plasma significantly reduced the activities of both PC and PS compared with the effect of 5% glucose solution (vehicle) (Table 1). However, NM did not affect plasma fibrinogen or factor XIII levels, or AT-III activity. Similarly, NM solution (0.1 mg/mL) co-incubated with whole blood for 30 minutes at room temperature reduced the activities of both PC and PS similar to that in the plasma co-infused with NM (Table 2). In addition, NM did not decrease plasma fibrinogen levels or plasma factor XIII levels in whole blood (Table 2). AT-III activity in plasma from whole blood anticoagulated with NM increased significantly compared to that with 5% glucose solution (Table 2).

Table 1. Effect of co-incubation of NM solution and FFP on clotting activities

Glucose solution (5%)	Nafamostat mesilate	Nafamostat mesilate
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	(Vehicle)	(0.05mg/mL)	(0.1mg/mL)
Fibrinogen (mg/dL)	314 (7)	320 (8)	311 (5)
Factor XIII (%)	122 (1)	118 (1)	119 (3)
Anti-thrombin III activity (%)	87 (1)	93 (2)	91 (1)
Protein C activity (%)	115 (1)	10*	10*
Protein S activity (%)	81 (1)	14 (1)*	10 (0.3)*

* $p < 0.05$ vs. group treated with 5% glucose solution, # $p < 0.05$ vs. NM solution

Data represent means with standard errors in parentheses (N = 3). The results are representative of 3 independent experiments. NM, nafamostat mesilate; FFP, fresh frozen plasma

Table 2. Influence of NM solution on the coagulation tests for whole blood from healthy volunteers

	Control # (sodium citrate)	Nafamostat mesilate (0.05mg/mL)	Nafamostat mesilate (0.1mg/mL)
Fibrinogen (mg/dL)	265 (1)	274 (14)	282 (3)*
Factor XIII (%)	113 (2)	95 (21)	117 (2)
Anti-thrombinIII activity (%)	88 (1)	103 (2)*	107 (1)*
Protein C activity (%)	105 (2)	10*	10*
Protein S activity (%)	117 (1)	10*	13 (1)*

98 * $p < 0.05$ vs. group treated with 5% glucose solution.
 99 # Control means the results from the clotting tests in the routine tube containing sodium citrate.
 100 Data represent means with standard errors in parentheses (N = 3). The results are representative of 3 independent
 101 experiments.
 102 NM, nafamostat mesilate

103
 104 On the other hand, co-infusion of NM and plasma from whole blood anticoagulated with sodium heparin also showed the
 105 same results as whole blood containing NM (Table 3). An increase in PS in plasma (with 5% glucose solution as a
 106 vehicle) was due to sodium heparin; this increase in PS activity was inhibited by NM (Table 3).
 107 However, the co-infusion of other anticoagulant agents such as ACD-A solution and sodium heparin, with plasma from
 108 FFP at a dilution ratio of 1:9 did not reduce PC activity (ACD-A solution: $113\% \pm 0.2\%$, N = 3; sodium heparin: $125\% \pm$
 109 6% , N = 3) or PS activity (ACD-A solution: $148\% \pm 1\%$, N = 3; 100IU/L sodium heparin: $1713\% \pm 24\%$, N = 3) compared
 110 to that with saline (PC activity: $117\% \pm 1\%$, N = 3; PS activity: $132\% \pm 1\%$, N = 3). Sodium heparin alone increased PS
 111 activity.
 112
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Table 3. Effects of co-infusion of NM and plasma from heparin-containing blood from healthy volunteers

	5% glucose solution	Nafamostat mesilate	Nafamostat mesilate
	(Vehicle)	(0.05mg/mL)	(0.1mg/mL)
Fibrinogen	248 (8)	246 (3)	230 (3)
(mg/dL)			
Factor XIII	89 (2)	91 (2)	96 (0.4)
(%)			
Anti-thrombin III activity (%)	130 (4)	130 (1)	132 (2)
Protein C activity			
(%)	71 (1)	10*	10*
Protein S activity			
(%)	422 (24)	255 (15)*	229 (16)*

114 * $p < 0.05$ vs. group treated with 5% glucose solution.
 115 Data represent means with standard errors in parentheses (N = 3). The results are representative of 3 independent
 116 experiments.
 117 NM, nafamostat mesilate

120 3.2 Effects of mesilates on PC and PS activities during co-infusion with FFP

121 The co-incubation of FFP and NM solution reduced the activities of PC and PS in a dose-dependent manner (Table 4).
 122 However, the co-incubation of FFP and GM solution did not reduce the activities of PC or PS (Table 4).
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Table 4 . Effects of mesilate compounds, NM and GM, on protein C and protein S activities

	Protein C activity (%)	Protein S
Glucose solution (5%)	119 (1)	63 (2)
(Vehicle)		

NM		
0.001 mg/mL	85 (2)*	75 (1)
0.01 mg/mL	16 (0.3)*	61 (4)
0.1 mg/mL	10*	10*
Gabexate mesilate		
1 mg/mL	121 (1)	64 (2)
10mg/mL	114 (2)	66 (1)

* $p < 0.05$ vs. group treated with 5% glucose solution

Data represent means with standard errors in parentheses (N = 3). The results are representative of 3 independent experiments.

NM, nafamostat mesilate; GM, gabexate mesilate

3.3 Discussion

Co-infusion of FFP and intravenous drugs is usually prohibited. However, misunderstandings about the transfusion route may lead to co-infusion. Iatrogenic accidents may consequently occur. This study demonstrates that co-incubation of FFP and NM solution but not GM solution decreases the activities of PC and PS. Matsuo et al. [14] report a decrease in PC activity in blood from the venous side of the dialysis circuit. Despite sufficient prolongation of activated partial thromboplastin time in the circuit, NM might be less effective in suppressing thrombin generation, resulting in the blockade of the circuit. NM may inhibit PC activity via a serine protease inhibitor [9]. The inhibitory effect of NM on PS activity has not been reported previously. PS is a vitamin K-dependent anticoagulant factor and acts as a cofactor in activated PC [15]. PS is not known to be a serine protease. We speculate that the mechanism by which NM decreases PS activity is the same as that for PC.

Red cell concentrates and FFP are usually preserved by citrate. The removal of citrate by washing with saline completely inhibits the NM solution-induced hemolysis in packed erythrocytes [9]. Therefore, co-infusion of plasma from heparin-containing whole blood without citrates and NM solution reduced the activities of both PC and PS (Table 3). We speculate that the presence of citrate during co-incubation might be unimportant in the decrease in the activities of PC and PS.

The co-incubation of FFP and NM solution reduced the activities of PC and PS in a dose-dependent manner (Table 4). However, the co-incubation of FFP and GM solution did not reduce the activities of PC or PS (Table 4). We speculate that NM exhibits specificity for PC as a serine protease inhibitor.

4. CONCLUSION

Co-infusion with NM but not GM reduces the activities of PC and PS in FFP. Thus, these results suggest that NM as an anticoagulant exerts an inhibitory effect on the activities of the anticoagulant factors, PC and PS, on the blockade in the plasmapheresis circuit.

A limitation of this study is that it did not clarify the mechanism by which PS activity decreases during co-infusion with NM solution and FFP.

COMPETING INTERESTS

None of the authors have any conflict of interest to declare.

CONSENT

All study participants (i.e., normal healthy volunteers) provided informed consent.

ETHICAL APPROVAL

The study design was approved by the ethics review board of our institution.

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