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Effects of Co-infusion of Plasma, Whole Blood and Anticogulants on Their Clotting Activities

Hiroshi Fujita[1*], Yumiko Ishihama[2] and Shigeko Nishimura[1]

[1]Department of Transfusion Medicine, Tokyo Metropolitan Bokutoh Hospital 4-23-15, Koutoubashi, Sumida-ku, Tokyo 130-8575, Japan.
[2]Clinical Laboratory, Tokyo Metropolitan Bokutoh Hospital 4-23-15, Koutoubashi, Sumida-ku, Tokyo 130-8575, Japan.

Authors' contributions

This work was carried out in collaboration between all authors. Author HF designed the study, performed the statistical analysis and wrote the first draft of the manuscript. Author YI managed the analyses of the study. Author SN managed the experiments and the literature searches. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: Nafamostat mesilate (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), whole blood and NM on clotting activities mainly in the static condition compared with other anticoagulants including heparin sodium and gabexate mesilate.
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 whole 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 whole blood, PC and PS activities decreased. Gabexate mesilate, sodium heparin and citrate did not reduce the

**Corresponding author: E-mail: yuketsuka@bokutoh-hp.metro.tokyo.jp;*

activities of PC or PS.
Conclusion: Co-infusion of FFP, whole blood and NM reduces PC and PS activities and we speculate that it may 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 XIIa 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 (DIC) but is not used as an anticoagulant in hemodialysis [13].

In the present study, we examined the effects of NM on clotting activities, particularly the anticoagulant factors protein C (PC) and protein S (PS) during the transfusion of fresh frozen plasma (FFP), compared with the other anticoagulant agents including sodium heparin, sodium citrate, GM, in order to clarify the reason why a plasmapheresis circuit using NM can easily be blockaded. 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. MATERIALS 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 at a dilution ratio of 1:9 (solution: plasma or WB) was analyzed 30 minutes after mixing at room temperature (20ºC). 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, because un-expired FFP derived from Japanese Red Cross Society cannot be easily used for basic research in certain Japanese hospital. We checked the clotting activities in the un-expired FFP from healthy normal volunteers among our hospital staffs (blood type: A). There were no differences of the clotting activities in this study between un-expired FFP and expired FFP, as shown in Table 1.

We also phlebotomied whole blood from healthy volunteers among the hospital staffs(blood type: A). The 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 activities in the plasma from the whole blood anti coagulated with NM, or sodium heparin were measured, as shown in Tables 2 and 3, respectively.

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

The study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki (Korea, 2008). 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 ± 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 anti coagulated 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%) (Vehicle)	Nafamostat mesilate (0.05mg/mL)	Nafamostat mesilate (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*
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)*

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

The clotting activities in the un-expired FFP from healthy normal volunteers among our hospital staffs was checked (fibrinogen: 288±10 mg/dL, factor XIII: 123% ±1%, AT-III activity: 92% ± 1%, PC activity: 92% ± 1%, PS activity: 85% ± 1%, N=4, respectively)

On the other hand, co-infusion of NM and plasma from whole blood anti coagulated with sodium heparin also showed the same results as whole blood containing NM (Table 3). An increase in PS in plasma (with 5% glucose solution as a vehicle) was due to sodium heparin; this increase in PS activity was inhibited by NM (Table 3).

However, the co-infusion of other anticoagulant agents such as ACD-A solution and sodium heparin, with plasma from FFP at a dilution ratio of 1:9 did not reduce PC activity (ACD-A solution: 113% ± 0.2%, N = 3; sodium heparin: 125% ± 6%, N = 3) or PS activity (ACD-A solution: 148% ± 1%, N = 3; 100IU/L sodium heparin: 1713% ± 24%, N = 3) compared to that with saline (PC activity: 117% ± 1%, N = 3; PS activity: 132% ± 1%, N = 3). Sodium heparin alone increased PS activity.

3.2 Effects of Mesilates on PC and PS Activities during Co-infusion with FFP

The co-incubation of FFP and NM solution reduced the activities of PC and PS in a dose-dependent manner (Fig. 1). However, the co-incubation of FFP and GM solution did not reduce the activities of PC or PS (Fig. 1).

Table 3. Effects of co-infusion of NM and plasma from heparin-containing blood from healthy volunteers

	5% glucose solution (Vehicle)	Nafamostat mesilate (0.05mg/mL)	Nafamostat mesilate (0.1mg/mL)
Fibrinogen (mg/dL)	248 (8)	246 (3)	230 (3)
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)*

**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

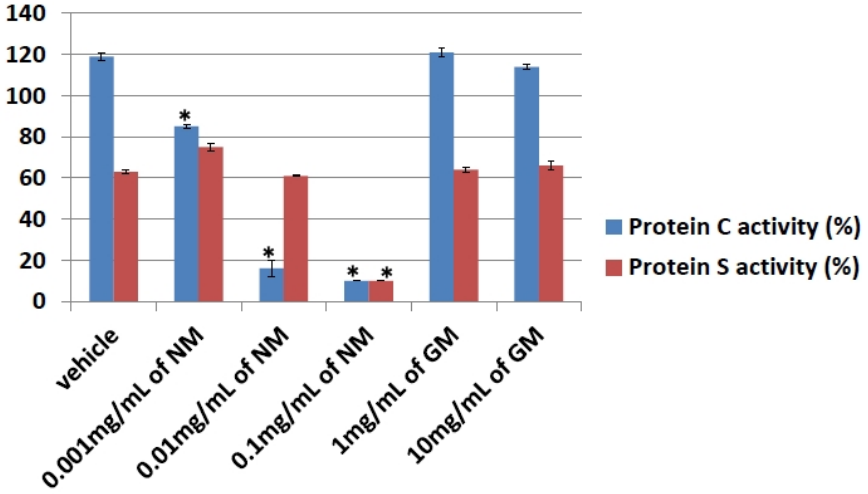

Fig. 1. Effects of mesilate compounds, NM and GM, on protein C and protein S activities

**p< 0.05 vs. group treated with 5% glucose solution.*
Data represent means with standard errors (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 through unknown inhibitory factors caused by NM.

AT-III activity in plasma from whole blood anti coagulated treated with NM for thirty minutes increased significantly compared to that with 5% glucose solution (Table 2), although co-infusion of FFP and NM did not affect AT-III activity (Table 1). The mechanism by which NM increased AT-III activity in the whole blood remains still unclear, because there are no papers about the relationship between AT-III activity in the whole blood and NM. However, NM has been reported to inhibit the leukocyte elastase release [16]. Therefore, we speculated that leukocyte-derived protease including elastase may break down the AT-III activity and NM may inhibit its action.

In the plasmapheresis circuit, the whole blood firstly contacts the surface of blood foreign with NM, and then it mixes with the infused FFP after the removal of plasma from patient`s whole blood. Therefore, we firstly checked the effects of NM on the clotting activities in both the whole blood and FFP.

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 (Fig. 1). However, the co-incubation of FFP and GM solution did not reduce the activities of PC or PS (Fig. 1). The effects of GM on DIC have been known to be similar to those of NM, but there were significant differences on PC and PS activities between GM and NM (Fig. 1). We newly showed that GM did not affect the PC and PS activities in the plasma.

Prevalence of thrombophilia in the patients with PC deficiency or PS deficiency is not extremely high [17]. Therefore, other episodes of pregnancy, infection, operation have been known to trigger the thrombophilia. However, we speculate that the decreased PS and PC activities by NM may induce the blockade the plasmapheresis circuit due to the contact with its blood foreign surface as a trigger.

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.

CONSENT

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

ETHICAL APPROVAL

The study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki (Korea, 2008). The study design was approved by the ethics review board of our institution.

COMPETING INTERESTS

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

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