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Differences in the adsorption of nafamostat mesilate between polyester-polymer alloy and polysulfone membranes

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Abstract We previously experienced severe clot formation in a polyester-polymer alloy (PEPA) dialyzer and hemodialysis (HD) circuit with nafamostat mesilate (NM) as an anticoagulant. The possibility of NM adsorption to the PEPA membrane was taken into consideration, but there was not enough information. In the present study, we evaluated differences in the adsorption of NM between a PEPA membrane (FDX-120 GW, Nikkiso, Tokyo, Japan) and two different polysulfone membranes (FX-140, Fresenius Medical Care, Tokyo, Japan; NV-15U, Toray Medical, Tokyo, Japan). We calculated the NM concentration by measuring absorbance at 241 nm using a spectrometer. NM adsorption was evaluated in three ways. First, we evaluated NM adsorption to hollow fibers. Then, we passed an NM solution through dialyzers and evaluated its adsorption in a single-pass examination. Finally, we circulated an NM solution in an HD circuit using a blood pump and evaluated NM adsorption. In all the experiments, NM adsorption to the PEPA membrane was greater than that to the polysulfone membranes examined. In the blood pump experiment, the estimated adsorption quantities of NM to the PEPA membrane and the FX-140 and NV-15U polysulfone membranes were 12.0 ± 0.1, 1.0 ± 0.1, and 4.1 ± 0.4 mg/m^2, respectively. NM adsorption was confirmed, especially in the early phase, and the PEPA membrane adsorbed greater amounts of NM than the polysulfone membranes. We should pay attention to the choice of dialyzer as well as the appropriate dose of NM administration during the preparation of HD circuits.

Keywords Nafamostat mesilate · Hemodialysis · Polyester-polymer alloy · Polysulfone

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Introduction

Nafamostat mesilate (NM) has been widely used as an anticoagulant during hemodialysis (HD) for patients with various hemorrhagic complications, and this agent has a lower risk of inducing further deterioration of hemorrhage because of its short half-life [1–4]. However, NM reportedly adsorbs to the membrane surface of some kinds of dialyzers, including polyacrylonitrile (PAN) membranes [5–7]; therefore, priming with natural saline containing 20 mg NM is recommended by manufacturers and the administered dose of NM for use as an anticoagulant must be adjusted during HD.

When using NM instead of heparin, clotting of dialyzers and HD circuits is occasionally observed. Recently, we experienced severe clot formation in an HD circuit of a HD patient who was dialyzed with a polyester-polymer alloy (PEPA) membrane when using NM as an anticoagulant. We had not experienced such a serious clotting event with a polysulfone (PS) membrane in HD therapy and the

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patient did not have other factors that could activate blood coagulation, except for being on HD. Based on our experience, there is a possibility of NM adsorption to PEPA membranes and a reduction in the anticoagulant efficacy of NM. However, there is insufficient information regarding NM adsorption to PEPA membranes.

Therefore, in the present study, we evaluated and compared the differences in the adsorptive capacity of NM between a PEPA membrane and two different PS membranes as controls.

Materials and methods

Membranes

In all experiments, a PEPA membrane dialyzer (FDX-120 GW, Nikkiso, Tokyo, Japan) and two different PS membrane dialyzers (FX-140, Fresenius Medical Care, Tokyo, Japan; NV-15U, Toray Medical, Tokyo, Japan) were examined.

Circuit

The HD circuit produced by Nikkiso was used in this experiment. The total circuit volume was 151 mL.

Measurement of NM concentration

NM was purchased from Torii Pharmaceutical, Tokyo, Japan. NM shows maximum absorbance at a wavelength near 241 nm, and its absorbance was measured at that wavelength using a spectrometer (Thermo Fisher Scientific NanoDrop™ 2000/2000c, Kanagawa, Japan). On the basis of these results, we calculated the NM concentration using a calibration curve of an NM solution.

We evaluated the adsorption of NM in three ways. First, we evaluated NM adsorption to cut hollow fibers (Experiment 1). Then, we passed an NM solution through the dialyzers and evaluated its adsorption rate in a single pass (Experiment 2). Finally, we circulated an NM solution in an HD circuit using a blood pump and evaluated the adsorption rate of NM to each dialyzer (Experiment 3).

For the measurement of the NM concentration, we first checked the absorbance of distilled water (blank solution) and regarded the limit of quantification (LOQ) as the average blank measurement + 10 times the SD, which gave an LOQ of 1.88 ng/μL. All measured absorbances of the solution without NM were under the LOQ in each experimental condition. Therefore, PVP and other chemicals eluted from the membranes had little influence on our measurement method.

Experiment 1: NM adsorption to cut hollow fibers

Hollow fibers in each dialyzer were removed and cut into 5-mm long sections. To make a 25 ng/μL NM solution, 100 μL of a 10 mg/mL NM solution was added to 40 mL distilled water. The concentration of the NM solution was checked before each experiment and the experiments were then performed as follows:

1. 3.2 g of cut hollow fibers were added to the prepared NM solution. The NM concentration was measured at 0, 5, 10, 20, and 30 min after the addition of the cut hollow fibers from each dialyzer.
2. To confirm the relationship between the NM adsorption capacity and the quantity of cut hollow fibers, double the amount (6.4 g) of hollow fibers was added to the prepared NM solution, and the NM concentration was measured in the same manner as in (1).

Experiment 2: single pass through a dialyzer

To make a 25 ng/μL NM solution, 100 μL of a 10 mg/mL NM solution was added to 40 mL distilled water. We used parts of the dialysis circuit and connected them to the arterial side and venous side of the dialyzer as shown in Fig. 1. Before this experiment, each dialyzer was washed 10 times with 20 mL of distilled water from the arterial side to the venous side. After checking the concentration of the NM solution, 20 mL of NM solution was passed 20 times from the arterial side and collected at the venous side. A total of 40 mL of NM solution, which was collected after the passage of 20 mL of NM solution twice, was regarded as one flow-through, and the NM concentration was consecutively measured from the first to the tenth flow-through. On the basis of these results, the adsorption rate and estimated adsorption quantity in flow-through were calculated using the following equations:

$$\begin{aligned}&\text{Adsorption rate in flow-through } [\%]\\&= (\text{pre-experiment NM concentration } [\text{ng}/\mu\text{L}]\\&\quad - \text{NM concentration } [\text{ng}/\mu\text{L}] \text{ of each flow-through}) /\\&\quad \text{pre-experiment}\\&\quad \text{NM concentration } [\text{ng}/\mu\text{L}] \times 100\end{aligned}$$

$$\begin{aligned}&\text{Estimated adsorption quantity in flow-through } [\text{mg}/\text{m}^2]\\&= (1 \text{ mg} \times \text{adsorption rate in flow-through } [\%]/100)/\\&\quad \text{surface area } [\text{m}^2]\end{aligned}$$

For the estimated total adsorption quantity, we summed the estimated adsorption quantity of each flow-through calculated using the above equation.

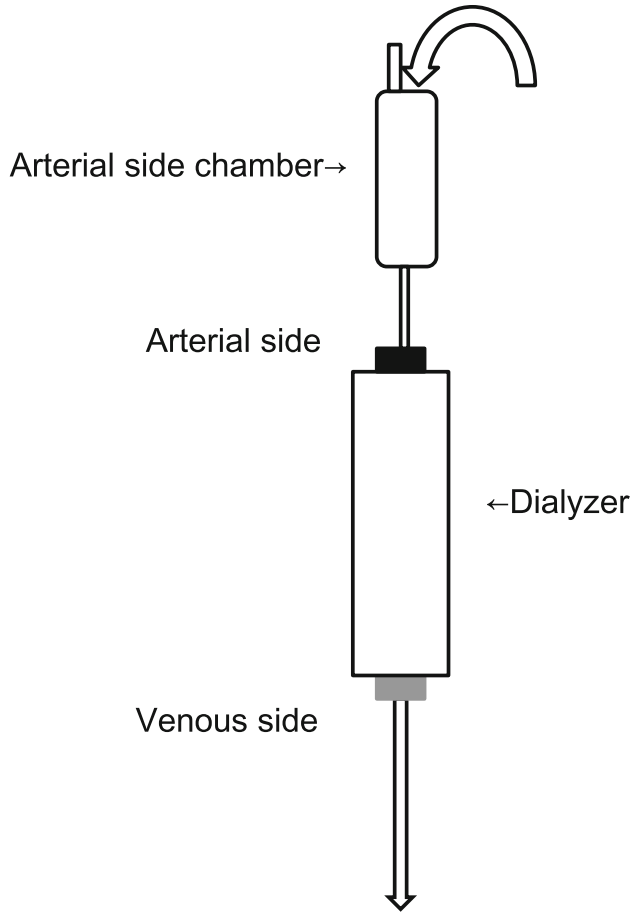

Fig. 1 Schema of the single-pass experiment to examine nafamostat mesilate adsorption to each dialyzer

Experiment 3: closed circuit on a pump

Before this experiment, the HD circuit, including each dialyzer, was constructed as for usual use, and the circuit was washed with 500 mL of distilled water as a priming procedure and then left filled with distilled water. To make a 25 ng/μL NM solution, 2 mL of a 10 mg/mL NM solution was added to 800 mL of distilled water. The concentration of NM in this solution was checked before each experiment.

1. We circulated the prepared NM solution in a closed circuit at a speed of 100 mL/min. We collected 0.5 mL sample from the arterial side port (pre-dialyzer) and the venous side port (post-dialyzer) at 0, 5, 10, 20, and 30 min after the initiation of this experiment and the NM concentration of each sample was measured.
2. To confirm the flow-dependency of NM adsorption capacity, the same experiment was performed, except the flow rate was set at 200 mL/min.

Based on these results, the adsorption rate and estimated adsorption quantity in circulation were calculated using the following equations:

$$\begin{aligned}&\text{Adsorption rate in circulation } [\%]\\&\quad = (\text{NM concentration } [\text{ng}/\mu\text{L}] \text{ of arterial side}\\&\qquad - \text{NM concentration } [\text{ng}/\mu\text{L}] \text{ of venous side})/\\&\qquad \text{NM concentration}\\&\qquad \times [\text{ng}/\mu\text{L}] \text{ of arterial side} \times 100\end{aligned}$$

$$\begin{aligned}&\text{Estimated adsorption quantity in circulation } [\text{mg}/\text{m}^2]\\&= \{\text{pre-experiment NM concentration } [\text{ng}/\mu\text{L}]\\&\times 800\,\text{mL}/1000 - \text{NM concentration } [\text{ng}/\mu\text{L}]\\&\text{of arterial side in } 30\,\text{min}\\&\times (800\,\text{mL} + 151\,\text{mL} + \text{inside dialyzer volume mL})/1000\}/\\&\text{surface area } [\text{m}^2].\end{aligned}$$

Statistical analysis

In this study, the same experiments were repeated five times in each dialyzer and the absorbance was measured three times in each sample. The results are shown as mean ± standard error (SE). Student's *t* test for unpaired values was used to compare results between the two conditions. Differences among the three different dialyzers were evaluated using one-way analysis of variance (ANOVA) with Tukey's test. Differences in the NM concentration and adsorption rate within each dialyzer were evaluated using repeated ANOVA with Tukey's test. p values <0.05 were considered statistically significant.

Results

In the experiment of NM adsorption to cut hollow fibers, the time courses of NM concentration changes with 3.2 and 6.4 g of each fiber were shown in Fig. 2. In all types and both amounts of hollow fibers, the NM concentration showed significant decreases from 5 min to the end of the

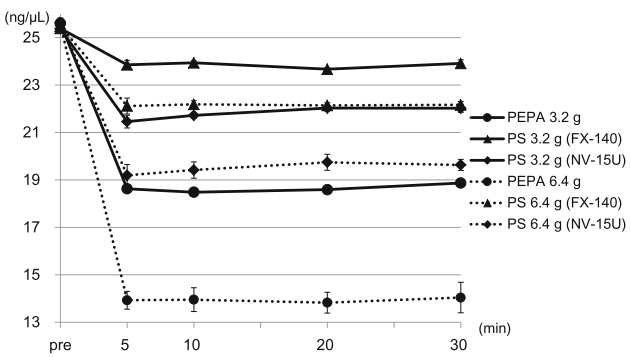

Fig. 2 Changes of the nafamostat mesilate concentration with 3.2 and 6.4 g hollow fibers ($n = 5$, respectively). *PEPA* polyester-polymer alloy, *PS* polysulfone

experiment (30 min) compared to those before the experiment ($p < 0.001$). For the comparison of NM concentration between the PEPA membrane (FDX-120 GW) and the two different PS membranes (FX-140, NV-15U), there were significant differences at each time point from 5 min to the end of the experiment (30 min) among the three membranes for both amounts of fibers (3.2 and 6.4 g) ($p < 0.01$). These results showed that the decrease in NM concentration in the PEPA membrane was significantly larger than for the PS membranes and that there were also significant differences in the decrease in NM concentration between the FX-140 and NV-15U PS membranes. As for dose dependency, the decrease in NM concentration was significantly larger for 6.4 g hollow fibers than for 3.2 g hollow fibers at each time point from 5 min to the end of the experiment (30 min) (Table 1).

In the single-pass experiment, as shown in Fig. 3a, NM was completely adsorbed from the first to the seventh flow-through and was still highly adsorbed from the eighth to the tenth flow-through in the PEPA dialyzer. Conversely, in the FX-140 PS dialyzer, the NM adsorption rate in flow-through decreased rapidly and significantly from the second flow-through ($p < 0.01$ vs. first flow-through). Thereafter, FX-140 did not show NM adsorption after approximately the seventh flow-through. The NM adsorption rate in flow-through in the NV-15U PS dialyzer gradually but significantly decreased from the third flow-through to the tenth flow-through ($p < 0.01$ vs. first flow-through). For the comparison of the NM adsorption rate in flow-through between the PEPA membrane dialyzer and the two different PS membrane dialyzers (FX-140, NV-15U), the NM adsorption rate in flow-through of the PEPA membrane was significantly higher than that of both PS membranes at all flow-through stages ($p < 0.01$ vs. PEPA membrane). Furthermore, the NV-15U dialyzer showed a significantly higher NM adsorption rate in flow-through than the FX-140 dialyzer ($p < 0.01$). In addition, changes of the estimated total adsorption quantity of NM in the flow-through are shown in Fig. 3b. The PEPA membrane showed a linear increase in adsorption quantity throughout the experiments, whereas the NV membrane showed a gradual increase. In contrast, in the FX-140 dialyzer, the adsorption quantity plateaued at around the fifth flow-through. The total estimated adsorption quantities in the final flow-through of PEPA, FX-140, and NV-15U were 8.2 ± 0.0, 1.4 ± 0.0, and 3.8 ± 0.1 mg/m^2, respectively ($p < 0.01$ vs. each other group).

In the closed-circuit pump experiment, as shown in Fig. 4, the NM adsorption rate in circulation of the PS membranes decreased significantly at 5 min ($p < 0.01$ vs. 0 min) and showed a further decrease to approximately 10% at 10 min, whereas, for the PEPA membrane, NM was completely adsorbed by 5 min from the start of the experiment and it took 20 min for the NM adsorption rate in circulation to fall below 10%. For the comparison of the NM adsorption rate in circulation between the PEPA membrane and the two different PS membranes (FX-140, NV-15U), there were significant differences at the early phase (5 and 10 min) between the PEPA membrane and each PS membrane (5 min: PEPA vs. FX-140: $p < 0.01$; PEPA vs. NV-15U: $p < 0.05$; 10 min: PEPA vs. FX-140 and NV-15U: $p < 0.01$). On the basis of these results, we calculated the quantity of estimated NM adsorption in circulation (Table 2). The estimated NM adsorption quantities in circulation of PEPA, FX-140, and NV-15U were 12.0 ± 0.1, 1.0 ± 0.1, and 4.1 ± 0.4 mg/m^2, respectively. When we performed the same experiments at a pump speed of 200 mL/min and compared NM adsorption quantity in circulation between 100 and 200 mL/min, there was no significant change in the estimated NM adsorption quantities in circulation of the PEPA membrane (FDX-120 GW) and FX-140 and NV-15U PS membranes (PEPA: 12.1 ± 0.5 mg/m^2; FX-140: 0.8 ± 0.1 mg/m^2; NV-15U: 3.6 ± 0.1 mg/m^2; $p = 0.77$, 0.20, and 0.19 vs. values at a pump speed of 100 mL/min in each dialyzer, respectively).

Table 1 Comparisons of nafamostat mesilate concentration (ng/μL) between 3.2 g ($n = 5$) and 6.4 g ($n = 5$) hollow fibers

(min)	Pre	5	10	20	30
Polyester-polymer alloy					
3.2 g	25.6 ± 0.3	18.6 ± 0.3	18.5 ± 0.2	18.6 ± 0.1	18.9 ± 0.2
6.4 g	25.5 ± 0.3	13.9 ± 0.9	14.0 ± 1.1	13.8 ± 1.0	14.1 ± 1.4
p	0.72	<0.01	<0.01	<0.01	<0.01
Polysulfone (FX-140)					
3.2 g	25.4 ± 0.4	23.9 ± 0.4	23.9 ± 0.2	23.7 ± 0.1	23.9 ± 0.3
6.4 g	25.5 ± 0.6	22.1 ± 0.7	22.2 ± 0.3	22.1 ± 0.3	22.2 ± 0.3
p	0.76	<0.01	<0.01	<0.01	<0.01
Polysulfone (NV-15U)					
3.2 g	25.4 ± 0.3	21.5 ± 0.6	21.7 ± 0.3	22.0 ± 0.3	22.0 ± 0.4
6.4 g	25.6 ± 0.5	19.2 ± 1.0	19.4 ± 0.8	19.8 ± 0.8	19.6 ± 0.5
p	0.3	<0.01	<0.01	<0.01	<0.01

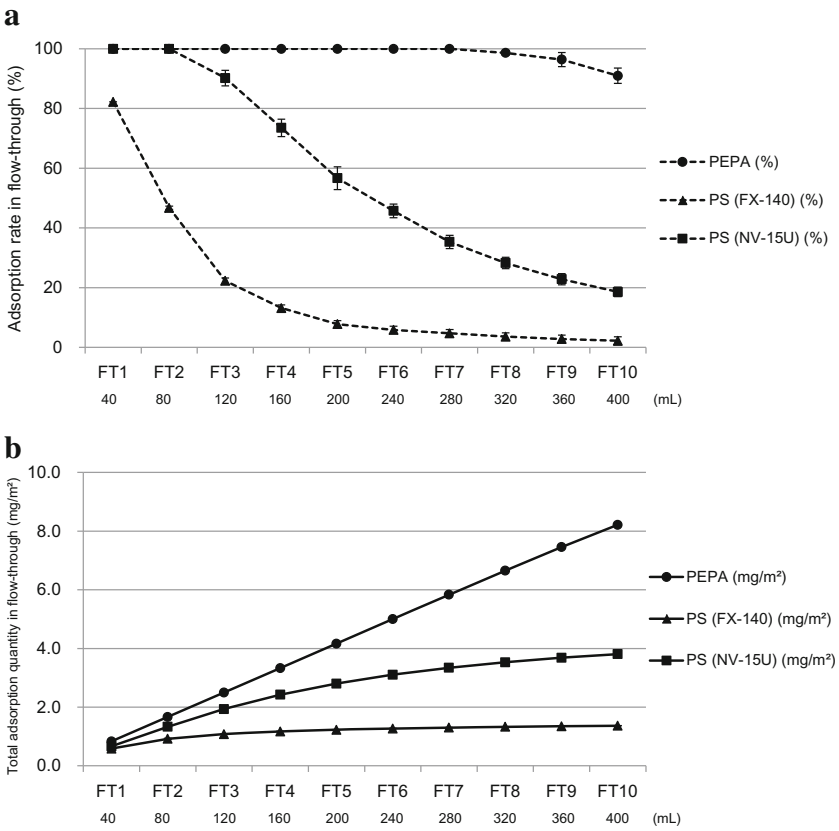

Fig. 3 **a** Changes of the nafamostat mesilate adsorption rate and **b** the estimated total adsorption quantity in flow-through in the single-pass experiment in each dialyzer ($n = 5$, respectively). *PEPA* polyester-polymer alloy, *PS* polysulfone, *FT* Flow-through

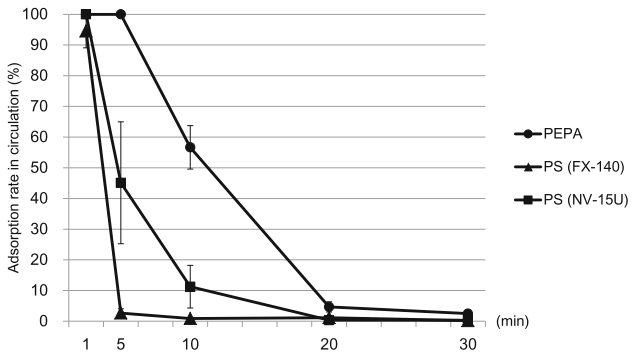

Fig. 4 Changes of the nafamostat mesilate adsorption rate in circulation in each dialyzer ($n = 5$, respectively). *PEPA* polyester-polymer alloy, *PS* polysulfone

Discussion

In patients with chronic kidney disease, upregulation of coagulant activity and defects in fibrinolysis are well-known events and both are reportedly enhanced by HD [8]. Furthermore, the activation of tissue factor coagulation and resistance to activated protein C have been confirmed in both patients with non-dialyzed renal insufficiency and with maintenance HD [8]. The thrombin-antithrombin complex, which is a plasma-coagulation marker, was also reported to be elevated in the early phase after starting HD [9]. Therefore, the abnormalities of coagulation and fibrinolysis associated with HD can lead to the formation of clots in dialyzers and HD circuits. In the clinical setting,

Table 2 Comparisons of estimated nafamostat mesilate adsorption in circulation (mg/m^2) among each dialyzer at pump speeds of 100 and 200 mL/min

	Polyester-polymer alloy	Polysulfone (FX-140)	Polysulfone (NV-15U)	*p*
100 mL/min ($n = 5$)	12.0 ± 0.1	1.0 ± 0.1	4.1 ± 0.4	<0.01 vs. each other group
200 mL/min ($n = 5$)	12.1 ± 0.5	0.8 ± 0.1	3.6 ± 0.1	<0.01 vs. each other group
p	0.77	0.20	0.19	–

heparin sodium has been used widely during HD to prevent clot formation. However, in patients with hemorrhagic disorders, heparin sodium administration may worsen their tendency to bleed. Thus, NM has been used widely as an anticoagulant in these patients because of its short half-life and lower risk of hemorrhage [1–4].

The adsorption of an anticoagulant to the surface of a dialyzer sometimes reduces its effect and causes clot formation during HD. It is well-known that NM itself, which is positively charged, is adsorbed to the surface of PAN membranes, which are negatively charged [5, 6]. Furthermore, Yoshimoto et al. examined NM adsorption to various continuous hemofilter membranes and reported that the amount of NM adsorption was 55.3 mg/m^2 to a PAN membrane, 38.0 mg/m^2 to a PS membrane, and 7.7 mg/m^2 to a polymethyl methacrylate membrane at 24 h after continuous circulation of an NM solution [7]. In the present study, the PEPA membrane showed quicker and larger amounts of NM adsorption than the PS membranes. It was reported previously that NM adsorption to a PEPA membrane was three times larger than to a PS membrane in a single-pass examination [10], which was consistent with our results. Regarding the mechanism of NM adsorption to PEPA membranes, the surface of PEPA membranes reportedly has a weak negative electric charge [11] and there is a possibility that electrostatic attraction would be associated with NM adsorption to PEPA membranes. Not only electrostatic attraction, but also other factors such as hydrophobic binding, membrane thickness, and surface roughness of hollow fibers could be associated with differences in adsorption [7, 12]. As for hydrophobic binding, polyvinylpyrrolidone (PVP) is used to hydrophilize the membrane surface, and there are differences in the original embedded amounts of PVP. These differences might contribute to the differences in NM adsorption among the different membranes. Furthermore, Matsuda et al. reported that PVP was eluted more from PEPA membranes than from PS membranes because of differences in the manufacture of PEPA and PS membranes [13–16]. PVP retention on PEPA membranes was less than that on PS membranes [13] and its retention could possibly be associated with NM adsorption on PEPA membranes from the viewpoint of hydrophobic binding.

Furthermore, in this study, the NV-15U membrane showed greater NM adsorption than the FX-140 membrane, even though they are both manufactured with the same PS materials. However, there are differences in the PVP-containing ratio and types of PVP and membrane thickness between the FX-140 and NV-15U membranes [15, 16]. A thicker membrane has higher adsorption ability because a hollow fiber has a number of membrane pores and a thicker membrane has a larger effective contact area. Therefore, PVP and membrane thickness may contribute to this difference in the adsorption of NM between the FX-140 and NV-15U membranes. Moreover, NV-15U contains different hydrophilizing agents besides PVP. The hydrophilizing agent is called as the "NV polymer" and it could reduce the adsorption of platelet and proteins in blood to the dialyzer membrane. Although the relationship between the NV polymer and NM adsorption to the dialyzer membrane remains uncertain, there is a possibility that the NV polymer affects NM adsorption.

It was reported previously that NM adsorption was 24% at 5 min, 18% at 10 min, and nearly 0% at 20 min after starting circulation of an NM solution with a regenerated cellulose membrane in vitro [1]. Our results from the pump experiment also showed that NM adsorption occurred in the early phase from 0 to 10 min. On the basis of our results, NM adsorption to the dialyzer membrane in the early phase of HD has the possibility to cause a reduction in anticoagulant efficacy and lead to clot formation. Therefore, when NM is used as an anticoagulant during HD for patients with hemorrhagic disorders, it is necessary to pay attention to the choice of dialyzer and the addition of NM into the priming solution used to prepare the HD circuit. The NM package insert recommends the addition of 20 mg of NM to the priming solution; however, there are some differences in the level of NM adsorption among dialyzers. Inadequate addition of NM to the priming solution may lead to clot formation, whereas an excess of NM is an unnecessary burden and may result in serious side effects. Therefore, we have to use an appropriate dose of NM for preparing an HD circuit according to the amount of NM adsorption in each dialyzer.

This study had several limitations that merit consideration. First, the examination of the NM adsorption ability of dialyzer membranes was only performed using a PEPA membrane and the two different PS membranes as controls in this study. Many different dialyzer membranes are used in the clinical setting, and the NM adsorption ability of some membranes remains unclear. Second, the results of this study were generated using in vitro experiments. However, in vivo, NM, which is metabolized and adsorbed to the membrane surface during HD, will be influenced by its interaction with plasma proteins. Therefore, in addition to in vitro experiments, further studies will be necessary to clarify the relationship between NM adsorption and each dialyzer membrane in vivo.

Conclusion

NM adsorption to dialyzer membranes, particularly a PEPA membrane, was confirmed during the early phase of the experiments in the present study. Therefore, we should pay attention to the choice of dialyzer, as well as the

appropriate dose of NM during the preparation of HD circuits and during HD.

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Compliance with ethical standards

Conflict of interest The authors declare that we have no conflict of interest.

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