Clinical Image

Hydrophilic Polymer-Coated Polysulfone Membrane Improves Endothelial Function of Hemodialysis Patients: A Pilot Study

Hidaka S1*, Kobayashi S1, Maesato K1, Mochida Y1, Ishioka K1, Oka M1, Moriya H1, Ohtake T1 and Nomura S2
1Department of Nephrology, Immunology, and Vascular Medicine, Shonan Kamakura General Hospital, Japan
2Department of Internal Medicine, Kansai Medical University, Japan

Abstract

Most polysulfone hemodialysis membranes are hydrophilized to improve biocompatibility; however, platelets are still activated by adhering to the membrane surface. We evaluated a newly developed polysulfone membrane in terms of platelet activation and endothelial function. Twenty-four patients underwent dialysis with a traditional polysulfone membrane (n = 12) or a new polysulfone membrane coated with hydrophilic polymer (n = 12) for 3 months. We analyzed the level of platelet-derived microparticles and flow-mediated dilatation of the brachial artery before and after 3 months. Our results showed that treatment with the traditional membranes did not significantly affect the level of platelet-derived microparticles (11.8 ± 2.2 to 12.1 ± 3.2 U/mL, p = 0.76) or flow-mediated dilatation (2.3 ± 1.5% to 2.7 ± 0.9%, p = 0.41). However, treatment with the new polysulfone membrane significantly decreased the level of platelet-derived microparticles (16.7 ± 8.1 to 15.0 ± 6.1 U/mL, p = 0.03) and increased flow-mediated dilatation (3.0 ± 1.8% to 4.1 ± 1.9%, p = 0.04). A newly developed polysulfone membrane coated with hydrophilic polymer improves the level of platelet-derived microparticles and endothelial function.

ABBREVIATIONS

PDMPs: Platelet-Derived MicroParticles; HD: Hemodialysis; FMD: Endothelium-Dependent Vasodilatation; PVP: Polyvinylpyrrolidone; PS: Polysulfone.

INTRODUCTION

Accelerated atherosclerosis is a major risk factor for long-term survivors receiving maintenance hemodialysis (HD); in particular, cardiovascular disease is a leading cause of morbidity and mortality in HD patients [1,2]. Atherosclerosis results from complex processes accompanied by endothelial dysfunction and inflammation. HD patients have 3 types of risk factors for atherosclerosis: traditional, uremia-related, and dialysis-related [3]. Dialysis-related risk factors result from bio-incompatibility between the blood and medical equipment, including the dialyzer. Therefore, it is extremely important to reduce dialysis-related risk factors to prevent the progression of accelerated atherosclerosis.

Platelet-derived microparticles (PDMPs) are released from activated platelets during HD [4]. PDMPs contain platelet granular proteins, such as P-selectin (CD62P), and various platelet surface membrane glycoproteins (GPs), such as GP I/II (CD42) and GP IIb/IIIa (CD41) [5,6]. They possess procoagulant activity themselves. The level of PDMPs is significantly increased in many prothrombotic diseases, including diabetes, hypertension, acute coronary syndromes, peripheral artery disease, and uremia [4,6-8]. When uremic patients undergo HD, shear stress and the contact between blood and non-human materials could be the mechanisms of PDMPs generation [4]. Because PDMPs contribute to the development of thrombotic complications and atherosclerosis, lower levels of PDMPs are desirable.

Biocompatibility characteristics and solute clearance of HD...
membranes are the most important criteria for successful long-term HD [9,10]. The polysulfone (PS) membrane is the mainstay of HD treatment because of its high performance. Most PS membranes are hydrophilized by polyvinylpyrrolidone (PVP) to improve biocompatibility; however, platelets still adhere to the surface of the membrane and activate platelets.

In 2011, a new PS HD membrane coated with a new hydrophilic polymer was developed, which focused on the mobility of adsorbed water close to the membrane surface. Recently, Yamaka et al. reported that platelet activation and adhesion to this membrane were lower than with a traditional PS membrane [11]. Using 2 different types of PS membrane, the traditional and a new, we evaluated differences in biocompatibility by analyzing the level of PDMPs and observing endothelial dysfunction via flow-mediated dilatation (FMD) of the brachial artery [12,13].

**MATERIALS AND METHODS**

**Study design**

Twenty-four stable chronic-maintenance HD patients who were receiving dialysis for 4 hours 3 times a week were enrolled. Inclusion criteria were age 50–85 years and clinically stable health condition; hence, all patients had a fistula, and none had a catheter. The dialyses flow rate was 500 mL/min. Patients with malignancy, chronic inflammatory diseases, hematological disorders, or severe liver or lung diseases were excluded.

All patients underwent HD with an APS-SA membrane (Asahi Kasei Medical, Tokyo, Japan), which is composed of a PS membrane for 6 months as a baseline. Then, the patients were randomized to one of 2 groups: HD for an additional 3 months with an APS-SA membrane or an NV-U membrane (Toray Medical, Tokyo, Japan), a new PS membrane coated with a new hydrophilic polymer. Randomization was conducted using a table of random numbers. Table 1 shows the clinical data of the patients. Age, sex, HD duration, comorbidities, ankle-brachial index, percentage of habitual smoking, and blood pressure were all similar between the 2 groups.

During the study, with the exception of the dialysis membrane, we did not change any other HD condition such as dry weight, blood and dialysate flow rate, dosage or type of anticoagulant used, or the type of dialysate. We also fixed the medications for hypertension, hyperlipidemia, and diabetes mellitus. Blood samples were taken from a peripheral vein at the first HD session of the week at baseline and after 3 months in both groups. The level of PDMPs and FMD were also examined at these same time points.

All subjects gave informed consent, and this study was performed in accordance with the Declaration of Helsinki.

**Measurement of PDMP levels**

An enzyme-linked immunosorbent assay (ELISA) kit (JIMRO, Tokyo, Japan) was used for the detection of PDMPs described previously [14]. Briefly, the blood was drawn directly from the vascular access and collected in vacuous tubes containing citrate/ethylenediaminetetraacetic acid (Nipro, Osaka, Japan). The samples were gently mixed by inverting the tubes upside down once or twice and then kept at room temperature for 2–3 hours, followed by centrifugation at 8000 g for 5 minutes at room temperature. Thereafter, 200 µL of the upper layer of supernatant was collected from a 2-mL sample to avoid contamination by platelets. The collected samples were stored at -40°C until analysis. The kit employs 2 monoclonal antibodies directed against platelet GPs, CD42b, and CD42a (GP Ib and IX). The levels of PDMPs were measured twice and mean values were recorded. One U/mL of PDMPs was defined as the amount of PDMPs obtained from 24,000 solubilized platelets/mL in this ELISA system. The performance of this kit has obtained suitable reproducibility such as simultaneous CV (1.1 – 4.0 %) and daily CV (5.2 – 8.8%).

**Endothelium-dependent vasodilatation (via FMD)**

FMD of the brachial artery was assessed noninvasively using high-resolution ultrasound as described previously in detail [15]. Using a 10-MHz linear-array transducer probe (UNEX, Nagoya, Japan), longitudinal images of the brachial artery on the arm opposite the vascular access were recorded at baseline, and artery diameter was measured after rest in the supine position for >5 minutes. Then, suprasystolic compression (50 mmHg higher than systolic blood pressure) was performed at the same side forearm for 5 minutes, and measurements of artery diameter were performed continuously from 30 seconds until >2 minutes after cuff release. Maximum vasodilatation was evaluated from the change in artery diameter after release of occlusion (%FMD).

**Statistical analyses**

Normally distributed data are expressed as the mean and standard deviation (SD). Non-normal data are expressed as the median and inter-quartile range (IQR). Categorical variables were compared using the chi-square test or Fisher exact test. The Wilcoxon test was used to compare the 2 groups. Comparisons between data at baseline and after 3 months were performed with the paired Student t test or Wilcoxon signed-rank test. All statistical analyses were performed with JMP program version 10 (SAS Institute Inc., Cary, NC). A p value < 0.05 was considered statistically significant.

**RESULTS**

A total of 24 patients were treated after the observation period, and all of them completed the study for 3 months without any side effects.

**Baseline laboratory data and dialysis efficiency**

Table 2 shows the laboratory data, PDMPs levels, and %FMD at baseline. The hemoglobin level was higher in the NV-U group, and the platelet count was higher in the APS-SA group. The other data showed no significant differences between the 2 groups. Laboratory data were not significantly different after 3 months (data not shown).

**Change in PDMPs level at baseline and after 3 months**

Figure 1a shows the change in PDMPs level in the NV-U and APS-SA groups. The PDMPs level was significantly reduced by dialysis with the NV-U membrane, from 16.7±8.1 to 15.0±6.1 U/mL after 3 months of treatment (p = 0.03), whereas in the APS-SA group, no significant change in PDMPs level was observed.
(11.8±2.2 to 12.1±3.2 U/mL, p = 0.76). The difference in PDMPs level was defined as the PDMPs level after 3 months minus the baseline level. It was -1.80±2.59 U/mL in the NV-U group, whereas in the APS-SA group, 0.30±3.30 U/mL. This value was lower in the NV-U group (p = 0.049) (Figure 1b).

Change in percentage FMD before and after 3 months

Figure 2 shows the change in %FMD in the NV-U and APS-SA groups. Percentage FMD significantly increased by dialysis with the NV-U membrane, from 3.0±1.8% to 4.1±1.9% after 3 months of treatment (p = 0.04). When the APS-SA dialyzer was used, no significant change in %FMD was observed (2.3±1.5% to 2.7±0.9%, p = 0.62). There were no significant differences in %FMD at baseline and after 3 months between the 2 groups.

DISCUSSION

In this study, we demonstrated that use of an NV-U membrane for 3 months was associated with a significant decrease in PDMPs and a significant increase in FMD; in contrast, HD with the traditional PS membrane did not show these favorable changes. Differences in biocompatibility might account for the reduction in PDMPs associated with the NV-U membrane.

Oxidative stress is generated when blood components adhere to the surface of HD membranes [3,16]. The NV-U membrane was developed with the goal of reducing the adherence of blood components to the PS membrane [11]. As a result of the new hydrophilic polymer coating, platelets are much less able to adhere to the surface. Therefore, the NV-U membrane is a biocompatible HD membrane with a high membrane performance that considerably prevents fibrinogen and platelets from adsorbing to the surface.

PDMPs promote the expression of adhesion molecules via monocytes and endothelial cells and contribute to the development and progression of atherosclerosis [17]. High shear stress can initiate both platelet aggregation and shedding of procoagulant-containing PDMPs, suggesting that the generation of PDMPs occurs in small diseased arteries and arterioles. The PDMPs levels of HD patients in this study were higher than those of patients with angina (10.8 ± 8.0 U/mL), according to the study by Namba[18], indicating that patients undergoing HD have a higher risk of thromboembolic and atherosclerotic events than patients with angina. Namba et al. revealed that a high level of PDMPs was an independent predictor for secondary thrombotic events. Because the NV-U membrane activates platelets to a lesser extent than the APS-SA membrane, the level of PDMPs is reduced; therefore, using this HD membrane might be one salient method of preventing thromboembolic conditions.

Endothelial dysfunction, assessed by %FMD of the brachial artery, is thought to be a marker of vascular damage and/or a predictor of further cardiovascular events [12,13]. Kosch et al. reported a decrease in %FMD after dialysis with a cellulosic cuprophane membrane, but not with a synthetic PS membrane [19]. They mentioned that a reduction in serum vitamin E level affected the result. In this study, we did not measure oxidative stress, but the improvement in %FMD associated with use of the NV-U membrane might be associated with a reduction in PDMPs level.

There were several important limitations to our study. First, this was a single center, open-label, and small-scale study. We performed this study as a pilot study. Therefore, a degree of

![Figure 1a](image1.png) Change in plasma PDMP level at baseline and after 3 months. Plasma PDMPs level was significantly reduced by dialysis with the NV-U membrane (p = 0.03). When the APS-SA dialyzer was used, no significant change in plasma PDMPs level was observed (p = 0.76).

![Figure 1b](image2.png) The difference in PDMPs levels after using each dialyzer for 3 months. The difference in PDMP level (defined as the PDMP level after 3 months minus the baseline level) was significantly lower in the NV-U group (p = 0.49).

![Figure 2](image3.png) Change in %FMD at baseline and after 3 months. %FMD was significantly increased by dialysis with the NV-U membrane (p = 0.04). When the APS-SA dialyzer was used, no significant change in %FMD was observed (p = 0.62).
patient selection bias might have occurred. Second, exercise and diet therapy as well as several drugs often affect FMD. We did not directly assess exercise habits and lifestyle in this study, but we met the patients thrice weekly, and their laboratory data, such as serum HDL-cholesterol levels, did not change significantly during the study period (data not shown). Therefore, the possibility of these factors influencing FMD could be ruled out.

**CONCLUSION**

A newly developed PS membrane coated with hydrophilic polymer improves the level of PDMPs and endothelial function. These improvements might have desirable effects on preventing the development and progression of atherosclerosis in HD patients.

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**REFERENCES**


