

RESEARCH

Open Access



Suppression of platelet reactivity during dialysis by addition of a nitric oxide donor to the dialysis fluid

Shunichiro Urabe¹, Kenichi Kokubo^{1,2*} , Hiroshi Tsukao^{1,2}, Kozue Kobayashi^{1,2}, Minoru Hirose^{1,2} and Hirosuke Kobayashi^{1,2}

Abstract

Background: Dialysis membranes that release nitric oxide (NO) from their surface, mimicking one of the functions of endothelial cells, may suppress platelet reactivity during hemodialysis treatment. The aim of the present study was to examine whether the addition of a NO donor to the dialysis fluid can suppress platelet reactivity during dialysis.

Methods: Porcine whole blood was circulated for 4 h through a polysulfone (PS) dialyzer or polymethylmethacrylate (PMMA) dialyzer. After the blood was circulated through the blood circuit and dialyzer, sodium nitroprusside was added to the dialysis fluid as a NO donor. The changes in the platelet reactivity, measured by the platelet aggregation activity by the addition of adenosine diphosphate or collagen in the blood sample, were evaluated during ex vivo dialysis experiments in the presence of a dialysis fluid containing or not containing a NO donor.

Results: The platelet aggregation activity was significantly decreased at 30 min after the start of the experiment in the case where nitroprusside was added to the dialysis fluid (the NO (+) condition) as compared to the case where no nitroprusside was added to the dialysis fluid (the NO (–) condition), for both the PS and PMMA membranes. The suppression of the platelet reactivity in the NO (+) condition was sustained until the end of the experimental period (240 min). The platelet cyclic guanosine monophosphate level was also significantly increased in the NO (+) condition as compared to the NO (–) condition.

Conclusions: NO in the dialysis fluid appears to be capable of suppressing the increase of the platelet reactivity observed during dialysis.

Keywords: Hemodialysis, NO-containing dialysis fluid, Platelet reactivity, platelet aggregation, cyclic guanosine monophosphate

* Correspondence: kokubo@kitasato-u.ac.jp

¹Kitasato University Graduate School of Medical Sciences, Kanagawa, Japan

²Department of Medical Engineering and Technology, Kitasato University School of Allied Health Sciences, 1-15-1 Kitasato, Sagami-hara Minami-ku, Kanagawa 252-0373, Japan



© The Author(s). 2020 **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated in a credit line to the data.

Introduction

Hemodialysis is a widely adopted renal replacement therapy for patients with end-stage chronic kidney disease. However, hemodialysis itself is well-known to induce oxidative stress [1] and platelet activation [2, 3], and to be associated with an increased risk of vascular disease, including arteriosclerosis and coronary artery disease [4, 5]. Platelet reactivity, which is also known to be increased in patients undergoing hemodialysis [6], has been reported to be positively correlated with the incidence of coronary artery disease [4, 7, 8]. Even with the currently used dialysis membranes, which show improved biocompatibility owing to improved smoothness/use of improved surface materials [9], it is difficult to suppress all the biological reactions caused by contact of blood with foreign materials. In the blood vessels, nitric oxide (NO), generated by the nitric oxide synthase expressed in the endothelial cells, suppresses platelet aggregation and activation [10–12]. NO suppresses platelet aggregation by stimulating guanylate cyclase and increasing the cellular concentration of cyclic guanosine monophosphate (cGMP) [13] or via other pathways [14]. Therefore, we considered that if the surface of the dialysis membrane could also release NO like endothelial cells, the platelet reactivity during hemodialysis might possibly be suppressed. The present study was aimed at examining whether NO released from a NO donor added to the dialysis fluid can suppress the platelet reactivity that is observed during ex vivo dialysis experiment.

Materials and methods

Ex vivo dialysis experiment

The blood side of the experimental system consisted of the blood circuit, dialysis membrane, and a soft bag (FCB-3, Asahi Kasei Kuraray Medical, Tokyo, Japan). The arterial side and venous side of the blood circuit were connected to the soft bag, so that the blood circulated through the blood circuit and the dialyzer (Fig. 1).

A polysulfone (PS) dialyzer (TS-1.6UL, Toray Medical, Japan) or polymethylmethacrylate (PMMA) dialyzer (BQ-1.6PQ, Toray Medical, Japan) was used (Table 1). The dialysate side of the circuit was a closed loop, and the dialysis fluid was circulated using a pump (MASTER FLEXR L/S, Cole-Parmer Instrument Company, IL, USA). The blood-side circuit was primed with saline and the dialysate side with dialysis fluid (CARBOSTAR® L, Yosindo Inc., Toyama, Japan). After priming, the saline filling the blood circuit and dialyzer was replaced with porcine blood or platelet-rich plasma (PRP), and the remaining porcine blood or PRP was filled into the soft bag. The porcine blood or PRP was circulated for 4 h at a flow rate of 200 mL/min. The dialysis fluid was circulated co-current with the blood at a flow rate of 200 mL/min. As the NO donor, 0.1 mM sodium nitroprusside (Wako Pure Chemical Industries, Ltd., Osaka, Japan) was continuously injected into the dialysis fluid at a flow rate of 100 μ L/min using a syringe pump (1235 N, Atom Medical Corporation, Tokyo, Japan). If it were assumed that all the added nitroprusside would completely liberate and release NO, then 10 nmol/min of NO would be delivered to the blood in the steady state under the above condition. The soft bag was placed in a constant-temperature (37 °C) bath.

NO donor

Sodium nitroprusside was used as the NO donor. Its molecular weight is 298 Da, and its half-life is as short as 3 to 4 min. One mole of NO is liberated from 1 mol of sodium nitroprusside.

Measurement of platelet aggregation activity

The platelet aggregation activity was measured using WBA-Carna (IMI CO. LTD., Saitama, Japan). To evaluate the platelet aggregation activity, a platelet aggregation-inducing substance was added to the whole-blood sample,

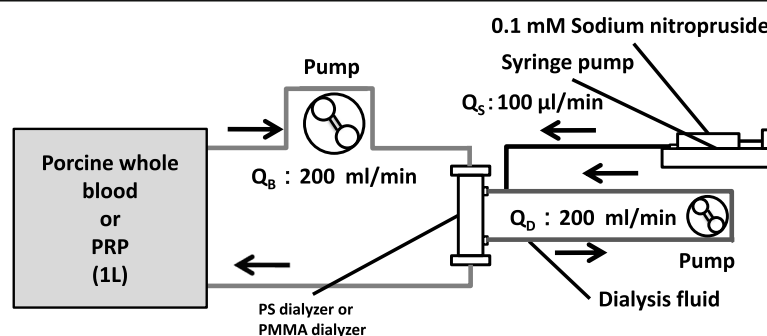


Fig. 1 Schematic diagram of the ex vivo dialysis experiment. The blood side of the circuit in this experiment consisted of the blood circuit, dialyzer, and a soft bag. The dialysate side of the circuit was a closed loop, and the dialysis fluid was circulated using a pump. Porcine blood or PRP was circulated for 4 h at a flow rate of 200 mL/min. The dialysis fluid was circulated co-current with blood at a flow rate of 200 mL/min. As the NO donor, 0.1 mM sodium nitroprusside was continuously injected into the dialysis fluid at the rate of 100 μ L/min using a syringe pump. The soft bag was placed in a constant-temperature (37 °C) bath

Table 1 Technical data on the dialyzer

| Dialyzer | TS-1.6UL | BG-1.6PQ |
|--|-------------|------------------------|
| Hollow-fiber material | Polysulfone | Polymethylmethacrylate |
| Inner diameter of the hollow fiber (μm) | 200 | 200 |
| Membrane thickness (μm) | 40 | 30 |
| Effective length of hollow fiber (mm) | 257 | 195 |

which was then agitated for a certain period of time, and sucked through a micromesh filter. More specifically, the blood sample was dispensed in 200- μL aliquots into 4 tubes containing a stirrer, placed in a device containing a constant-temperature (37°C) bath, and stirred for 1 min. Thereafter, a platelet aggregation-inducing substance, namely, adenosine diphosphate (ADP) or collagen, was added to each of the four tubes at different concentrations. After allowing for a reaction time of 5 min, the blood was aspirated into a tube through a micromesh filter ($20 \times 20 \mu\text{m}$). The platelet aggregation activity was determined by measuring and analyzing the suction pressure during the suction operation.

Measurement of the changes in the platelet count

The platelet count was measured using a cell counter for animal blood (Celltac α MEK-6450, Nihon Kohden Corp., Tokyo, Japan), and the percent change of the platelet count was determined. The measurement was conducted in blood samples obtained at the start of dialysis (prior to the addition of sodium nitroprusside to the dialysis fluid), and at 30, 60, 90, 120, 180, and 240 min after the start of dialysis.

Preparation of platelet-rich plasma

PRP was prepared in accordance with the procedure described elsewhere [15]. Briefly, porcine blood containing 10 mM of sodium citrate was purchased on the morning of the day of the experiment, delivered carefully to the lab. The plasma sample obtained by centrifugation of the porcine blood at $140\times g$ for 20 min was used. PRP obtained from the same animal was divided into two portions, one used for the NO (+) condition and the other for the NO (–) condition of the experiment in this study.

Measurement of the cGMP level in the platelets

The cGMP levels in the platelets were measured by an enzyme-linked immunosorbent assay. First, 1 mL of the sampled PRP was dispensed into a microtube. Then, 1 mL of 0.1 M hydrochloric acid solution was added to destroy the platelets, followed by centrifugation at 15,000 rpm for 2 min. The supernatant of the centrifuged sample was used for the measurement. The sample was treated in accordance with the manufacturer's instructions (cyclic GMP Complete ELISA kit, Assay Designs Inc., Michigan, USA), and the absorbance at a

wavelength of 405 nm was measured with an absorbance meter (model 680, Bio-Rad Laboratories, Inc., CA, USA).

Statistical analysis

Statistical analyses were performed using the FreeJSTAT software. The data are presented as the means \pm standard deviations. Two-way analysis of variance (ANOVA) was applied to compare the platelet aggregation activity and changes in the platelet count between the two experimental conditions (the NO (+) and NO (–) conditions). The cGMP level in the platelets was compared between the two experimental conditions using a paired *t* test. A probability (*P*) value of < 0.05 was set as denoting statistical significance.

Results

Then the platelet aggregation activity during circulation of the blood through the dialysis circuit was measured using WBA-Carna (Figs. 2 and 3). At the start of dialysis, there was no difference in the platelet aggregation activity between the two experimental conditions, namely, the NO (–) and NO (+) conditions. At 30 min after the start of dialysis, the platelet aggregation activity in the NO (+) condition was significantly decreased as compared to that in the NO (–) condition. The same result was obtained, regardless of whether the dialyzer used was a PS dialyzer or a PMMA dialyzer, and also irrespective of whether the platelet aggregation-inducing substance used was ADP or collagen. However, while the platelet aggregation activity in the NO (+) condition remained significantly decreased as compared to that in the NO (–) even at 240 min after the start of dialysis when the PS dialyzer was used, irrespective of whether the platelet aggregation-inducing substance used was ADP or collagen, the difference in the platelet-aggregation activity observed at 30 min between the NO (–) and NO (+) conditions was only seen at 240 min after the start of dialysis when the PMMA dialyzer was used and aggregation-inducing substance used was ADP.

There was no significant difference in the platelet counts between the two experimental conditions (Fig. 4), irrespective of whether the PS or PMMA dialyzer was used.

The platelet cGMP level during circulation of PRP through the dialysis membrane was measured to confirm that NO was delivered into the blood, and that the suppression of platelet activation occurred via a NO-

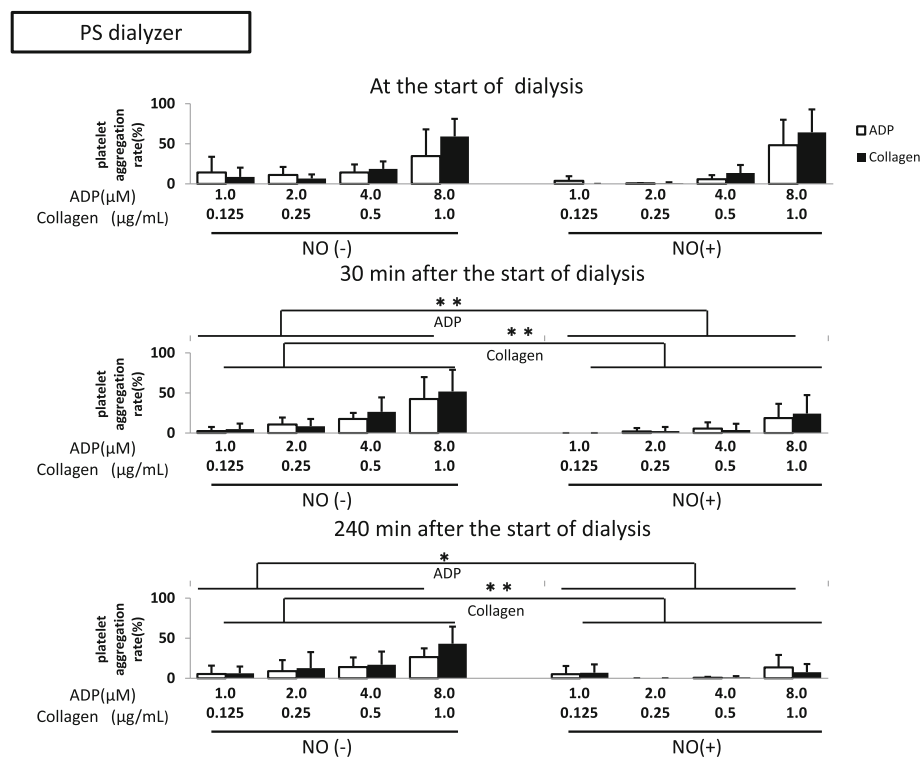


Fig. 2 Platelet aggregation activity in the PS dialyzer group ($n = 4$). The white bars show the increase in the platelet aggregation activity in response to the addition of ADP, and the black bars show the increase in the platelet aggregation activity in response to the addition of collagen. At the start of dialysis, there was no difference in the platelet aggregation activity between the NO (-) and NO (+) conditions. However, at 30 min after the start of dialysis, the platelet aggregation activity in the NO (+) condition was significantly decreased as compared to that in the NO (-) condition ($P < 0.01$). The platelet aggregation activity in the NO (+) condition was still significantly decreased as compared with that in the NO (-) condition even at 240 min after the start of dialysis ($P < 0.05$)

dependent mechanism (Fig. 5). At the start of dialysis, there was no difference in the platelet cGMP level between the NO (+) and NO (-) conditions. However, at 30 min after the start of dialysis, the platelet cGMP level significantly increased in the NO (+) condition as compared to the NO (-) condition. This difference was sustained until the end of the experiment, at 240 min after the start of dialysis.

Discussion

While currently used dialysis membranes show improved biocompatibility owing to the use of improved materials or their improved smoothness [9], it remains difficult to suppress the increased platelet reactivity induced by contact of blood with foreign materials. In the present study, we verified the hypothesis that during dialysis, addition of a NO donor, sodium nitroprusside, to the dialysis fluid can suppress the platelet reactivity.

The main findings of the present study were that the addition of sodium nitroprusside to the dialysis fluid increased the concentration of cGMP in the platelets and suppressed the platelet aggregation activity observed when the blood comes in contact with the dialysis

membrane. This finding indicates that the NO released from the sodium nitroprusside into the dialysis fluid can suppress the platelet reactivity, such as platelet aggregation activity and platelet adhesion, during dialysis. Although the presence of NO in the dialysis fluid suppressed the platelet reactivity, it had no significant effect on the blood platelet count. The decrease in the platelet count was slight and not sufficient to attribute it to the presence of NO because the activation of platelets and coagulation was well-controlled in this experiment, even under the NO (-) condition. Therefore, the main effect of NO observed in this study was suppression of the platelet aggregation activity, along with increase of the platelet cGMP level. NO is known to stimulate soluble guanylate cyclase (sGC) and induce the production of cGMP, which promotes the upregulation of protein kinase G (PKG) [16]. PKG reduces platelet reactivity by phosphorylating inositol-1,4,5-triphosphate and its receptor, preventing an increase of the intracellular calcium concentration [17]. In platelets exposed to high concentrations of NO, sGC-independent mechanisms are also known, namely, enhanced sarco/endoplasmic reticulum calcium-ATPase activity with sequestration of

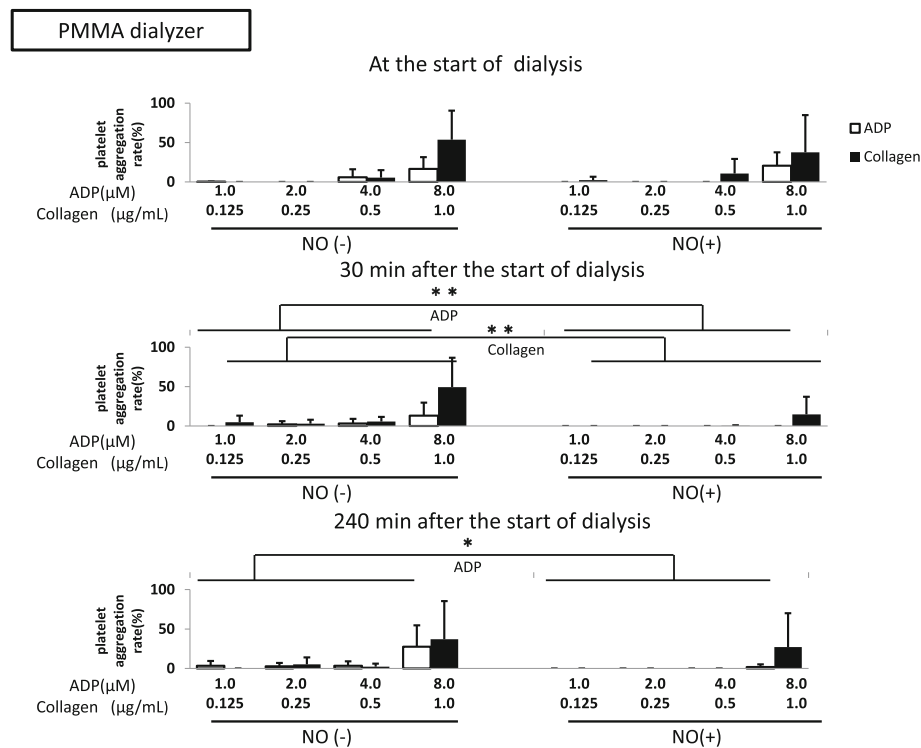


Fig. 3 Platelet aggregation activity in the PMMA dialyzer group ($n = 3$). The white bars show the increase in the platelet aggregation activity in response to the addition of ADP, and the black bars show the increase in the platelet aggregation activity in response to the addition of collagen. At the start of dialysis, there was no difference in the platelet aggregation activity between the NO (-) and NO (+) conditions. However, at 30 min after the start of dialysis, the platelet aggregation activity in the NO (+) condition was significantly decreased as compared with that in the NO (-) condition ($P < 0.05$)

calcium into the intracellular stores [14] and lowering of the intracellular calcium concentrations, which prevents the platelet activation-like cytoskeleton rearrangement and integrin activation. In the present study, the suppression of the platelet reactivity by NO was considered to have been mediated by an sGC-

dependent mechanism because increase of the platelet cGMP level was observed.

Attempts have been made previously, with successful reduction of the platelet adhesiveness, to use NO gas in membrane oxygenators, by mixing NO with the oxygenator sweep gas [18, 19] or use of a NO-releasing material

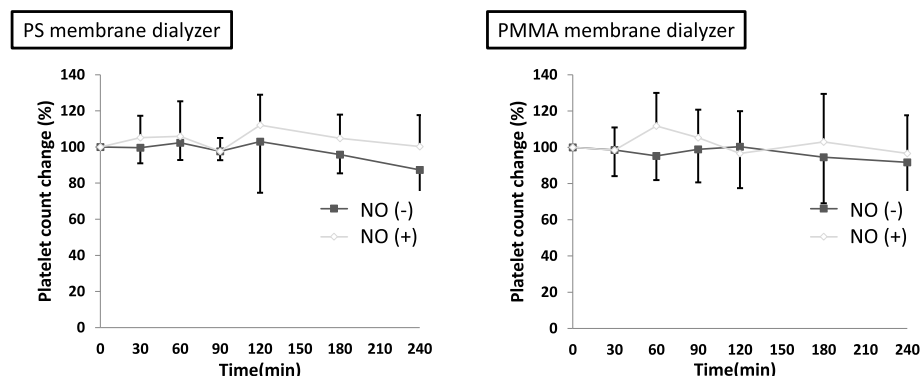


Fig. 4 Platelet count change in the PS dialyzer group ($n = 4$) and PMMA dialyzer ($n = 3$) group. The gray dot denotes the NO (-) condition, and the white dot denotes the NO (+) condition. There was no difference in the degree of change of the platelet count between the NO (-) and NO (+) conditions, in either the PS dialyzer group or the PMMA dialyzer group

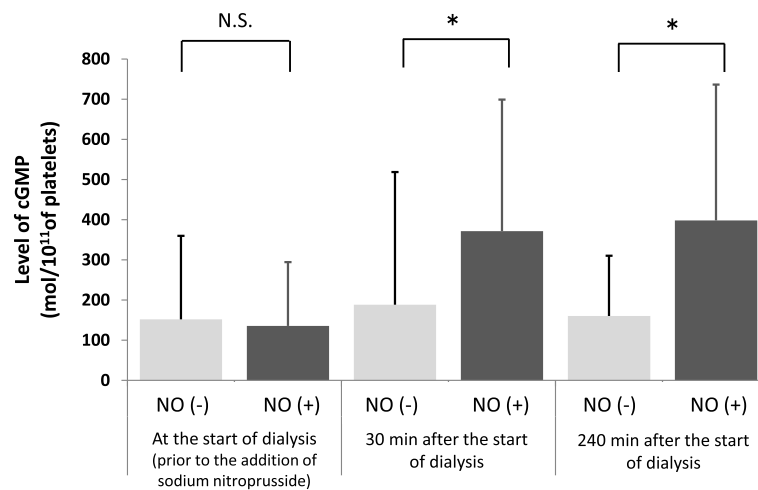


Fig. 5 The level of cGMP in the platelets. The platelet cGMP levels were measured when the PS membrane dialyzer was used ($n = 6$). The gray bar denotes the NO (-) condition, and the black bar denotes the NO (+) condition. There was no significant difference in the platelet cGMP levels between the two conditions at the start of dialysis. However, 30 min after the start of dialysis, significant increase of the level of cGMP in the platelets was observed in the NO (+) condition as compared to the NO (-) condition ($P < 0.05$). This difference between the two groups was sustained even until 240 min after the start of dialysis ($P < 0.05$)

[20, 21]. Modification of the surface of a dialysis membrane, like in the case of a membrane oxygenator [20, 21], by the addition of a NO-releasing substance, however, causes alterations of the surface texture and chemical properties of the membrane, resulting in deterioration of the biocompatibility of the dialysis membrane itself. Therefore, supplying NO from the dialysate side while using the currently available improved dialysis membranes is considered as the most reliable way of supplying NO without interfering with the biocompatibility of the dialysis membrane.

There are limitations to using the NO delivery system adopted in this study in clinical settings. We used sodium nitroprusside as the NO donor in this experiment. The molecular weight of sodium nitroprusside is 298 Da, so that it can diffuse from the dialysis fluid into the blood. When used clinically, sodium nitroprusside has the potential side effect of hypotension, caused by the vasodilatory action of NO and cyanide intoxication. Even though sodium nitroprusside has a short half-life of only 3 to 4 min, the vasodilatory effect of NO [22, 23] released from sodium nitroprusside in the peripheral blood vessels and the potential fall of blood pressure is a cause for concern when this substance is used in clinical settings. Therefore, sodium nitroprusside is considered as not being entirely suitable for clinical use. From the results of the present study, however, we can confirm that NO contained in the dialysis fluid has strong potential to suppress the platelet reactivity during dialysis treatment. Therefore, NO gas, a NO donor with a shorter half-life, or a NO donor that cannot permeate

through the dialysis membrane would be a good candidate to realize this concept in clinical settings.

We used the co-current setting in this study to ensure sufficient delivery of NO from the dialysis fluid to the blood, especially to the blood at the inlet side. But this setting reduces the solute removal efficiency as compared to the counter-current setting used in daily clinical practice. Therefore, this system may be potentially applicable to indications for prolonged dialysis treatments where a high solute removal efficiency is not critical, such as continuous hemodialysis for acute-phase patients and nocturnal hemodialysis. On the other hand, to apply this system for routine maintenance hemodialysis, other options for adding NO to the blood available in the counter-current setting should be developed; immobilizing the NO donor on the outer aspect of the hollow fiber or on the dialyzer housing could be an effective solution.

We used the PS membrane dialyzer or the PMMA membrane dialyzer. The PS membranes are currently the most frequently used material in hemodialysis treatment, and the PMMA membranes have unique properties of adsorption. We did not focus much on the membrane material in the present study because the main purpose was to clarify the potential of NO contained in the dialysis fluid. However, the effects of NO on the platelets during hemodialysis treatment may differ by the type of membrane material used. Further experiments are required to address this point.

In conclusion, addition of sodium nitroprusside to the dialysis fluid increases the platelet concentration of cGMP and suppresses the platelet reactivity during dialysis. This finding indicates the potential of NO supplied from the dialysate side through the dialysis membrane to inhibit platelet aggregation or adhesion while allowing the good biocompatibility of the inner surface of the dialysis membrane to be maintained.

Abbreviations

NO: Nitric oxide; cGMP: Cyclic guanosine monophosphate; PS: Polysulfone; PMMA: Polymethylmethacrylate; PRP: Platelet-rich plasma; ADP: Adenosine diphosphate; ANOVA: Analysis of variance; *P* value: Probability value

Acknowledgments

Not applicable.

Authors' contributions

SU provided the research design and carried out the experiment and the data analysis, and also wrote the manuscript. KeK provided the working hypothesis, participated in the research design, and wrote the manuscript. HT, MH, and KoK participated in the research design and substantially contributed to the study concept. HK provided the working hypothesis, participated in the research design, and substantially contributed to the study concept. The authors read and approved the final manuscript.

Funding

This work was supported in part by JSPS KAKENHI grant numbers JP21591060 and JP16K09653.

Availability of data and materials

The datasets analyzed during this study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Received: 31 May 2020 Accepted: 28 July 2020

Published online: 14 August 2020

References

1. Campean V, Neureiter D, Varga I, Runk F, Reiman A, Garlachs C, et al. Atherosclerosis and vascular calcification in chronic renal failure. *Kidney Blood Press Res.* 2005;28:280–9.
2. Lam JY, Latour JG, Lesperance J, Waters D. Platelet aggregation, coronary artery disease progression and future coronary events. *Am J Cardiol.* 1994; 73:333–8.
3. Daub K, Lindemann S, Langer H, Seizer P, Stellos K, Siegel-Axel D, et al. The evil in atherosclerosis: adherent platelets induce foam cell formation. *Semin Thromb Hemost.* 2007;33:173–8.
4. Lindner A, Charra B, Sherrard DJ, Scribner BH. Accelerated atherosclerosis in prolonged maintenance hemodialysis. *N Engl J Med.* 1974;290:697–701.
5. O'Hare AM, Hsu CY, Bacchetti P, Johansen KL. Peripheral vascular disease risk factors among patients undergoing hemodialysis. *J Am Soc Nephrol.* 2002; 13:497–503.
6. Aggarwal A, Kabbani SS, Rimmer JM, Gennari FJ, Taatjes DJ, Sobel BE, et al. Biphasic effects of hemodialysis on platelet reactivity in patients with end-stage renal disease: a potential contributor to cardiovascular risk. *Am J Kidney Dis.* 2002;40:315–22.
7. Thaulow E, Erikssen J, Sandvik L, Stormorken H, Cohn PF. Blood platelet count and function are related to total and cardiovascular death in apparently healthy men. *Circulation.* 1991;84:613–7.
8. Trip MD, Cats VM, van Capelle FJ, Vreken J. Platelet hyperreactivity and prognosis in survivors of myocardial infarction. *N Engl J Med.* 1990;322: 1549–54.
9. Kokubo K, Kurihara Y, Kobayashi K, Tsukao H, Kobayashi H. Evaluation of the biocompatibility of dialysis membranes. *Blood Purif.* 2015;40:293–7.
10. de Graff JC, Banga JD, Moncada S, Palmer RMJ, de Groot PG, Sixma JJ. Nitric oxide functions as an inhibitor of platelet adhesion under flow conditions. *Circulation.* 1992;85:2284–90.
11. Radomski MW, Vallance P, Whitley G, Foxwell N, Moncada S. Platelet adhesion to human vascular endothelium is modulated by constitutive and cytokine induced nitric oxide. *Cardiovasc Res.* 1993;27:1380–2.
12. Wilcox JN, Subramanian RR, Sundell CL, Tracey WR, Pollock JS, Harrison DG, et al. Expression of multiple isoforms of nitric oxide synthase in normal and atherosclerotic vessels. *Arterioscler Thromb Vasc Biol.* 1997;17:2479–88.
13. Radomski MW, Palmer RM, Moncada S. An L-arginine/nitric oxide pathway present in human platelets regulates aggregation. *Proc Natl Acad Sci U S A.* 1990;87:5193–7.
14. Sylman JL, Lantvit SM, Reynolds MM, Neeves KB. The relative role of soluble guanylyl cyclase dependent and independent pathways in nitric oxide inhibition of platelet aggregation under flow. *Cell Mol Bioeng.* 2014;7:421–31.
15. Tsukao H, Kokubo K, Takahashi H, Nagasato M, Endo T, Iizuka N, et al. Activation of platelets upon contact with a vitamin E-coated/non-coated surface. *J Artif Organs.* 2013;16:193–205.
16. Naseem KM, Roberts W. Nitric oxide at a glance. *Platelets.* 2011;22:148–52.
17. Antl M, et al. IRAG mediates NO/cGMP-dependent inhibition of platelet aggregation and thrombus formation. *Blood.* 2007;109:552–9.
18. Mellgren K, Friberg LG, Mellgren G, Hedner T, Wennmalm Å, Wadenvik H. Nitric oxide in the oxygenator sweep gas reduces platelet activation during experimental perfusion. *Ann Thorac Surg.* 1996;61:1194–8.
19. Mellgren K, Mellgren G, Lundin S, Wennmalm A, Wadenvik H. Effect of nitric oxide gas on platelets during open heart operations. *Ann Thorac Surg.* 1998;65:1335–41.
20. Amoko KA, Montoya PJ, Major TC, Suhaib AB, Handa H, Brant DO, et al. Fabrication and in vivo thrombogenicity testing of nitric oxide generating artificial lungs. *J Biomed Mater Res A.* 2013;101:3511–9.
21. El-Ferzli GT, Andukuri A, Alexander G, Scopel M, Ambalavanan N, Patel RP, et al. A nitric oxide-releasing self-assembled peptide amphiphile nanomatrix for improving the biocompatibility of microporous hollow fibers. *ASAIO J.* 2015;61:589–95.
22. Moncada S, Higgs A. The L-arginine-nitric oxide pathway. *N Engl J Med.* 1993;329:2002–12.
23. Ignarro LJ, Napoli C, Loscalzo J. Nitric oxide donors and cardiovascular agents modulating the bioactivity of nitric oxide: an overview. *Circ Res.* 2002;90:21–8.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Ready to submit your research? Choose BMC and benefit from:

- fast, convenient online submission
- thorough peer review by experienced researchers in your field
- rapid publication on acceptance
- support for research data, including large and complex data types
- gold Open Access which fosters wider collaboration and increased citations
- maximum visibility for your research: over 100M website views per year

At BMC, research is always in progress.

Learn more biomedcentral.com/submissions

