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ORIGINAL ARTICLE

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Abstract Oxygen radicals have recently been attracting close attention because of their involvement in tissue damage and their close relationship to various clinical conditions. It has been suggested that hemodialysis increases oxidative stress, triggering the development of complications such as atherosclerosis and dialysis-related amyloidosis. We recently developed a dialyzer containing a highly functional polysulfone membrane on which vitamin E had been bonded (PS-ViE). The present study was undertaken to evaluate the biocompatibility of this membrane and to conduct other experiments on the membrane in vitro. Human blood was dialyzed with minidialyzers (300–600 cm^2 membrane area) made of PS-ViE, cellulose, or untreated polusulfone (PS), and the effects of the dialyzers on complements (C3a, C4a, and C5a), cytokines (IL-1β and IL-8), and granulocyte elastase as well as their anti-oxidative activity were investigated (n = 6). The effect of PS-ViE on complement activation and its effects on cytokines were comparable to those of PS membrane, whereas granulocyte elastase following dialysis with the PS-ViE membrane tended to be lower than that seen with PS membrane. The effects of PS-ViE-induced methemoglobin, lipid peroxide, and oxygen radicals were significantly less than those of PS membrane, indicating the antioxidative activity of PS-ViE. Vitamin E-modified polysulfone membrane dialyzers were found to have favorable effects on the immune system and to express antithrombotic and antioxidative effects.

Key words Oxidative stress · Vitamin E-modified polysulfone membrane dialyzers · Biocompatibility · Antioxidative effects

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Introduction

Advances in dialysis

Hemodialysis systems account for a very major part of artificial kidney systems. The systems used for hemodialysis have been advancing both quantitatively and qualitatively.[1–5] At the same time, as the patients receiving hemodialysis become older and their duration of hemodialysis becomes longer, it is now required for hemodialysis to serve as a means of improving the quality of life (QOL) of patients, rather than simply serving as a life-saving treatment. The complications following prolonged use of hemodialysis have become a serious problem. In an attempt to deal with dialysis-related amyloidosis, which is one of the complications of hemodialysis, high-performance dialyzers[6–8] and internal filtration-enchanced hemodialysis (IFEHD)[9–11] have been introduced. However, current dialyzers do not provide an adequate solution to the problem of dialysis-related amyloidosis. Furthermore, other complications, especially atherosclerosis, still remain problematic. Thus, dialyzes are now required not only to suppress complement activation and possess antithrombotic activity but also to be more biocompatible than ever.

Hemodialysis and oxygen radicals

In recent years, about 40% of deaths of long-term hemodialysis patients have been attributable to cardiac diseases such as atherosclerosis. In the past, low-density lipoprotein (LDL)-cholesterol was considered to be responsible for atheroslerosis. In recent years, it has begun to be reported that oxidized LDL is really responsible for atherosclerosis. This is because oxidized LDL is more likely to injure the vascular endothelium and to induce monocytes to enter the vascular endothelium, leading to their transformation into macrophages. Furthermore, it has been reported that, compared to ordinary LDL cholesterol, oxidized LDL is several times more likely to be phagocytosed by macrophages, leading to the formation of foam cells and the narrowing of

blood vessels. These mechanisms for the development of atherosclerosis have been studied in more depth than ever, on the basis of extensive data collected in recent years. Now, the risk caused by oxidized LDL is being studied.

The formation of advanced glycation end products (AGEs) has been reported to serve as a factor inducing dialysis-related amyloidosis in patients on long-term hemodialysis. Among others, pentosidine and carboxymethyl lysine (CML) are reported to be closely related to the enhancement of oxidative stress, and these factors are now being studied actively.[12] Sanaka[13] proposed a hypothesis on oxygen radicals and reported that oxygen radicals are probably involved in atherosclerosis, hypotension, anemia, amyloidosis, susceptibility to carcinogenesis, susceptibility to infection, aging, and various other complications. Under such circumstances, oxidative stress during hemodialysis has been studied intensely, and a number of reports on this issue have been published in Japan, Europe, and other regions.[14–18]

Methods

Preliminary study

The level of oxidized LDL was monitored in 180 hemodialysis patients at 18 hemodialysis centers and compared with that of 30 healthy volunteers. End-stage renal disease patients (average age 60.2 ± 12.7 years) were treated three times a week with hemodialysis for 4–5 h a time. The hemodialysis membranes used were made of cellulose, cellulose acetate, polyethylene glycol-coated cellulose, polymethylmethacrylate, and polysulfone. A blood sample was drawn from the arterial line before and after hemodialysis and subjected to ultracentrifugation to separate an LDL fraction as follows. Plasma (1.5 ml) was placed into the tube for ultracentrifugation and 500 µl of 0.15 M NaCl containing 0.3 mM ethylenediaminetetraacetic acid (EDTA) was layered within the tube. Then ultracentrifugation at 185000 *g* (40000 rpm, X-80 Ultracentrifuge; Beckman, Fullerton, CA, USA) was performed for 2.5 h at 10°C. After ultracentrifugation, the upper white fraction [chylomicron and very low density lipoprotein (VLDL)] was discarded and the lower layer (1.5 ml) was collected and combined with 300 µl of aqueous solution of KBr (522 mg/ml). The mixture was then centrifuged at 234000 *g* and 10°C for 16 h. After centrifugation, the orange band (LDL layer) was carefully collected and dialyzed with phosphate-buffered saline (PBS) containing 0.25 mM EDTA for 2 h at 4°C (the 5 l of PBS was renewed twice at intervals of 30 min) using a dialysis tube Spectra/PorR 6 (molecular weight cut-off: 50000; Spectrum Medical, Bozeman, MT, USA). The LDL specimen so obtained was subjected to oxidized LDL measurement by enzyme-linked immunosorbent assay (ELISA) using a monoclonal antibody DLH3 that reacts with oxidized phosphatidylcholine. The LDL specimen was also subjected to quantitation of protein (Protein Assay; Biorad, Tokyo, Japan). The LDL-protein level was used for correction of the lipid peroxide measurement.

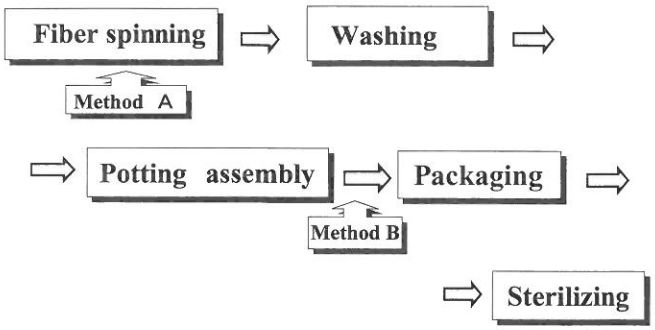


Fig. 1. Schematic of the manufacturing process of the vitamin E-modified polysulfone membrane dialyzers

Manufacture of vitamin E-modified dialyzers

Figure 1 shows how the modified dialyzers were manufactured. We manufactured vitamin E-modified polysulfone membrane dialyzers using two methods that we developed, i.e., method B, in which membranes in the assembled dialyzer are immersed in vitamin E solution and then dried to bond the vitamin E, and method A, in which vitamin E is added to the inner surface and bonded there (Fig. 2). The vitamin E used for the manufacture of dialyzers was α-tocophenol of a Japanese Pharmacopeia grade.

Hollow fibers were cut from commercial dialyzers and made into minimodules with a length of 14 cm and membrane areas of 300–600 cm^2. The cellulose membrane (CL-SS; Terumo, Tokyo, Japan) and PS membrane (PS-UW; Kawasumi, Tokyo, Japan) served as control membranes.

Evaluation

Vitamin E binding

Amount of bound vitamin E. The amount of bound vitamin E was determined as follows: ethanol (Wako, Osaka, Japan) was circulated (n = 6) through the vitamin E-modified polysulfone membrane dialyzers for 5 h. Extracted vitamin E was quantified by high-performance liquid chromatography (HPLC) (UV = 280 nm).

Vitamin E elution in bovine plasma. To test for exfoliation, bovine plasma (500 ml) was circulated (n = 6) through dialyzers with an effective membrane area of 1.2 m^2 at 37°C for 5 h with a blood flow rate (Q_B) of 200 ml/min and a dialysate flow rate (Q_D) of 0 ml/min. For analysis of vitamin E, the plasma was deproteinated with ethanol and vitamin E was extracted using hexane (Wako). Vitamin E was quantified by HPLC (UV = 280 nm). The yield of vitamin E from plasma was 92.2% (n = 6).

Biocompatibility evaluation in vitro

Complement activation. Minimodules with a membrane area of 300 cm^2 were filled (n = 6) with human serum, the

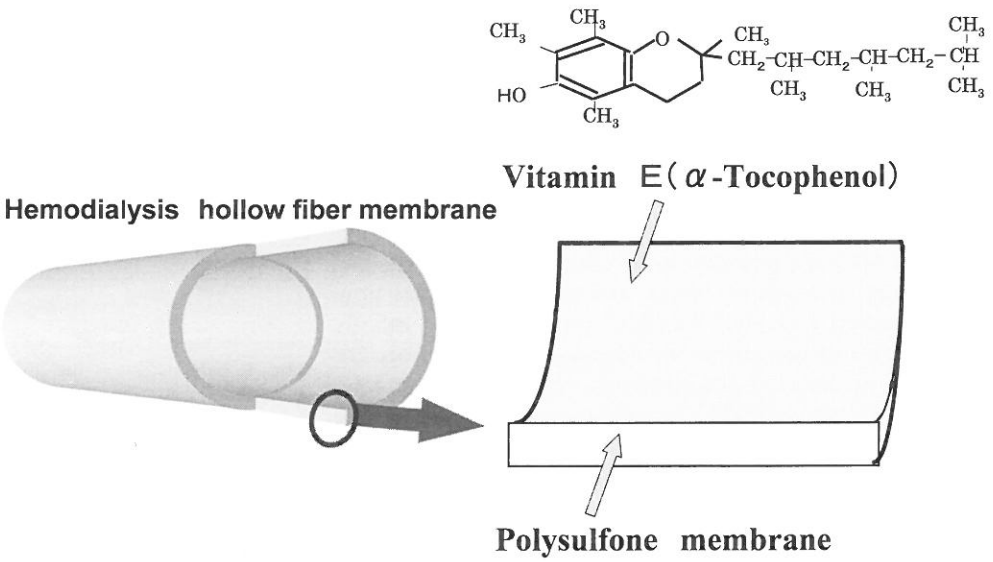


Fig. 2. Vitamin E-modified polysulfone membrane

contact area ratio of which was 190 cm^2/ml. After 1 h of incubation at 37°C, the mini-modules were placed in an ice-cooled bath to stop complement activation. The serum within the minimodules was collected in a glass tube and was pretreated, i.e., the serum (200 µl) was combined with 60 µl of 0.1 M EDTA and 140 µl of gelatin veronal buffer (GVB). The mixture was agitated and then left standing for 5 min. This was followed by centrifugation at 7000 rpm and 4°C for 15 min. The supernatant fluid was subjected to radioimmune assay (RIA), using an RIA kit (human complement C3a, C4a, C5a des Arg 125I assay system; GE Healthcare Bio-Sciences, Piscataway, NJ, USA).

Cytokines. Minimodules with a membrane area of 600 cm^2 were filled (n = 6) with human blood, the contact area ratio of which was 190 cm^2/ml. After 6 h of incubation at 37°C, the blood was collected from the minimodules. The collected blood (900 µl) was combined with 50 µl of 0.1 M EDTA (final 5 mM) and 50 µl of 20 U/ml aprotinin (final 1 U/ml). The mixture was centrifuged firstly at 400 g for 10 min, then at 1000 g for 10 min at 4°C. The supernatant fluid was collected and combined with twice its volume of chloroform. The mixture was centrifuged at 1000 g, for 10 min at 4°C and the supernatant fluid was then subjected to ELISA.

Granulocyte elastase. Minimodules with a membrane area of 300 cm^2 were filled with 10 U/ml heparinized human blood, the contact area ratio of which was 190 cm^2/ml, and incubated for 10 min at 37°C (n = 6). The blood collected from the minimodules (450 µl) was combined with 50 µl of EDTA (final 5 mM). The mixture was centrifuged at 1000 g for 10 min at 4°C. The plasma separated therefrom was subjected to measurement with an elastase test kit (Immunoassay PMN Elastase; Merck, Darmstadt, Germany).

Activation of the contact phase. The contact phase was activated by exposing each type of minimodule to human plasma for 4 h at 0°C (n = 6). Kallikrein, which was formed as a result of activation, was measured by the fluorescence-labeled synthetic substrate method. The intensity of fluorescence was measured at an excitation wavelength of 380 nm and an emission wavelength of 460 nm using a fluorescence spectrophotometer (F-4000; Hitachi, Tokyo, Japan).

Platelet spreadability. Human platelet-rich plasma (PRP), 10^5/µl, was placed in contact with each membrane for 30 min at room temperature (n = 6). After washing, the adherent platelets were observed under a scanning electron microscope (JSM-5300, JEOL, Tokyo, Japan). The degree of deformation in the adherent platelets was assessed on the following three-grade scale:

Type I: The platelet has changed form its normal disc-like form into a spherical form with three or four pseudopodia. This type of platelet seems to adhere relatively weakly to the surface of materials.

Type II: The platelet has developed several or more pseudopodia and is spread out to the extent that the length of pseudopodia has decreased to less than half that of the Type I platelet. Its adhesion to material surfaces seems strong.

Type III: The platelet has nearly flattened out to take a quasicircular form. Its adhesion to material surfaces seems extremely strong.

Oxygen radicals. Heparinized blood (20 U/ml) was gently mixed with 6% Dextran T-70 (Pharmacia) in physiological saline at a ratio of 9:1. The mixture was left standing upside down for about 2 h. The supernatant fluid was gently collected and used as a test solution. This test solution contained plasma, platelets, and leukocytes (monocytes/macrophages, lymphocytes, neutrophils, eosinophils, and basophils), and the phagocyte count (monocytes/macrophages, neutrophils, eosinophils, and basophils) was adjusted

with Hank's balanced salt solution to 1×10^6 cells/ml. The glass tube of the cuvette was coated with silicone (NCT-911; Toshiba, Tokyo, Japan) to avoid activation of leukocytes by the cuvette.

Chemiluminescence was measured as follows. First, each membrane with a membrane area of 40 cm^2 and the above-mentioned leukocyte-platelet-rich plasma (LPRP) solution (1.2 ml) were added to the cuvette ($n = 6$). Then, 10 µl of 0.1 mM 2-methyl-6(4-methoxyphenyl)-3,7-dihydroimidazol[1,2-a]pyrazin-3-one (MCLA) was added to the cuvette with a microsyringe, followed immediately by measurement of the intensity of luminescence using a luminescence reader (cumulative luminescence intensity for 15 min). At the same time, measurement of the cuvette alone (blank sample) was also performed as a control.

Formation of methemoglobin. Minimodules with a membrane area of 600 cm^2 were filled with 10 U/ml heparinized human blood, the contact area ratio of which was 190 cm^2/ml, and incubated at 37°C for 6 h ($n = 6$). The collected blood was subjected to measurement using the cyanomethemoglobin method.

Lipid peroxide in erythrocyte membranes. Minimodules with a membrane area of 600 cm^2 were filled with human heparinized blood (10 IU/ml) and incubated at 37°C for 6 h ($n = 6$). After incubation, blood was collected from the minimodules and hemolysis was induced in steps using the method of Burton et al.[19] The white erythrocyte ghost membranes thus obtained were subjected to malondialdehyde (MDA) measurement by the Yagi method. The value obtained was corrected with the erythrocyte count in the blood collected from the minimodules.

Oxidized LDL. Minimodules with a membrane area of 600 cm^2 were filled with human heparinized blood (10 IU/ml) and incubated at 37°C for 2 h ($n = 6$). After incubation, blood was collected from the minimodules and subjected to ultracentrifugation to separate an LDL fraction, as follows. Plasma (1.5 ml) was placed into the tube for ultracentrifugation and 500 µl of 0.15 M NaCl containing 0.3 mM EDTA was layered within the tube. Then ultracentrifugation at 185000 *g* (X-80 Ultracentrifuge; Beckman) was performed for 2.5 h at 10°C. After ultracentrifugation, the upper, white fraction (chylomicron and VLDL) was discarded and the lower layer (1.5 ml) was collected and combined with 300 µl aqueous solution of KBr (522 mg/ml). The mixture was then centrifuged at 234000 *g* and 10°C for 16 h. After centrifugation, the orange band (LDL layer) was carefully collected and dialyzed with PBS containing 0.25 mM EDTA for 2 h at 4°C (the 5 l of PBS was renewed twice at intervals of 30 min) using a Spectra/PorR 6 dialysis tube (molecular weight cut-off: 50000; Spectrum Medical). The LDL specimen so obtained was subjected to MDA measurement by the Yagi method (Lipid Peroxide Test; Wako). The LDL specimen was also subjected to quantitation of protein (Protein Assay; Biorad). The LDL-protein level was used for correction of the lipid peroxide measurement.

Statistical analysis

Data from pre- and post-dialysis values of oxidized LDL in the preliminary study were analyzed using a paired *t* test. The differences among various membranes were detected by Mann-Whitney *U* tests with Bonferroni correction. All values are expressd as mean ± standard error. *P* values of less than 0.05 were considered statistically significant.

Results and discussion

Preliminary study

We analyzed the oxidative status of patients on long-term hemodialysis using oxidized LDL as an indicator (Fig. 3). The level of oxidized LDL in hemodialysis patients was found to be about ten times that in healthy volunteers. More than 90% of hemodialysis patients showed elevated oxidized LDL levels. Our data thus endorsed the empirical knowledge that patients on hemodialysis are usually in a peroxidized state. Regarding the effects of extracorporal circulation, our results suggested that the oxidized LDL level after a session of hemodialysis was about twice that before the session.

Vitamin E-modified polysulfone membrane dialyzers

To cope with the problem that patients with a longer hemodialysis history require an increasing number of repeated extracorporeal circulations, dialyzers have evolved into more biofriendly devices, causing less damage to the living body. To further improve the biocompatibility of dialyzers, the author attempted to develop a new dialyzer on the basis of "positive thinking" instead of "negative thinking." Considering that extracorporeal circulation provides a good opportunity for blood to come into contact with drugs (the whole blood is exchanged 12.5 times during one session of dialysis for a 50-kg patient if the dialysis lasts for 4 h at a blood flow rate of 200 ml/min), the author attempted to bond an antioxidant to the membrane in the dialyzer. Vitamin E, which is abundant in organisms and exerts an antioxidative activity against various oxygen radicals such as hydroxyl radicals and superoxide radicals, was selected as the antioxidant. Of the eight isoforms of vitamin E, α-tocopherol with the highest physiological activity was used (Table 1). In addition to antioxidative activity, vitamin E has many other pharmacological actions such as suppression of platelet adhesion and aggregation and stabilization of membranes. To make full use of its activity, the author attempted to bind vitamin E to membranes.

We previously developed a vitamin E-modified cellulose membrane (CL-EE) by combining antioxidative vitamin E (tocopherol) with a composite membrane which was composed of a synthetic membrane layer and its covalently bonded cellulose membrane.[20–28] A number of clinical studies on CL-EE demonstrated the antioxidative effects of

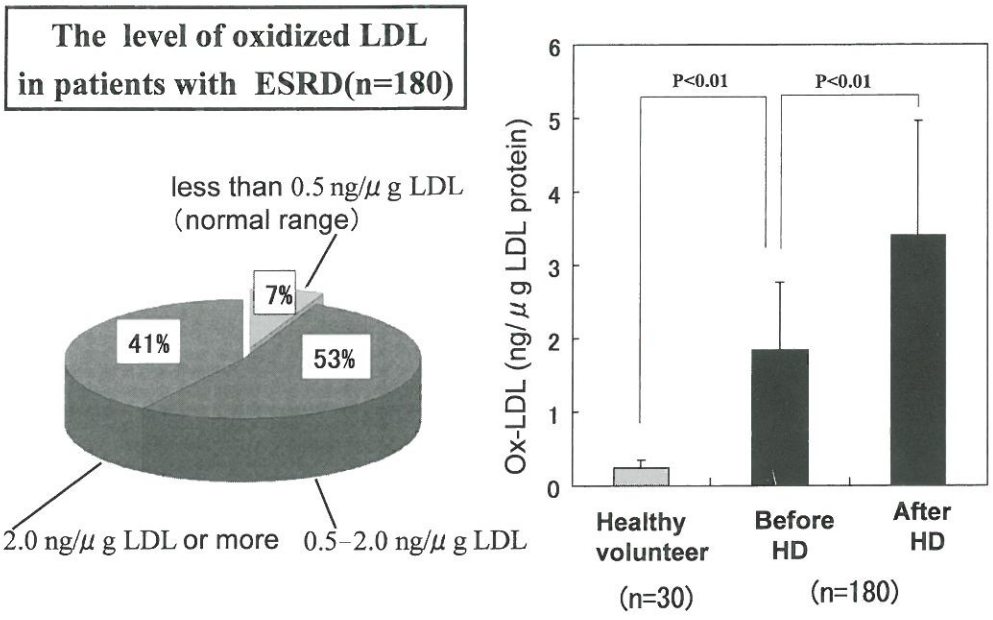


Fig. 3. Oxidation status in patients with end-stage renal disease (*ESRD*)

Table 1. Properties of vitamin E (α-tocophenol)

Main locations	Biological membranes, blood
Antioxidative activity against various oxygen radicals	$\cdot O_2^-$, ·OH, $^1O_2$, OCl^- RCOO·
Pharmacological actions	Antioxidative activity Stabilization of membranes Improvement of microcirculation dynamics Suppression of platelet adhesion and aggregation

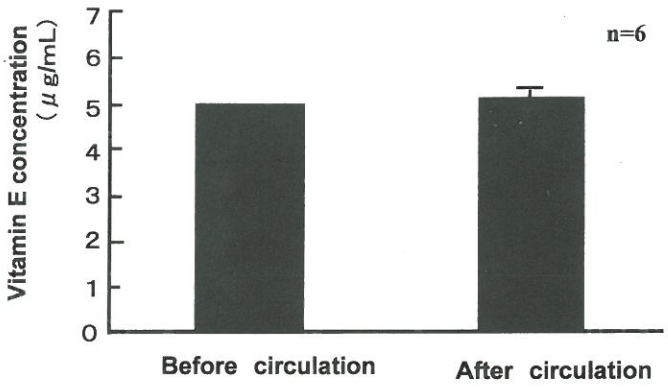


Fig. 4. Vitamin E elution in bovine plasma. Bovine plasma (500 ml) was circulated through vitamin E-modified polysulfone membrane dialyzers with an effective surface area of 1.2 m^2 at 37°C for 5 h with a blood flow rate (Q_B) of 200 ml/min and a dialysis flow rate (Q_D) of 0 ml/min

CL-EE and its favorable effects on oxidized LDL, AGE, and erythrocyte lifespan.[29–43]

The dialyzer prepared by the author was composed of polysulfone, which has such excellent properties as clearance, permeability, and is easy to process. The surface of the polysulfone membrane was modified before use. To preserve the pharmacological activity of vitamin E, a hydrophobic–hydrophobic bond was adopted.

Evaluation of the binding potential of vitamin E

The amount of vitamin E bonded onto the hemodialysis membrane was 63.5 ± 2.7 mg/m^2. In the experiment on elution of vitamin E by bovine plasma, the plasma vitamin E level was 4.84 μg/ml before circulation and it remained almost unchanged at 4.95 ± 0.22 μg/ml after circulation ($n = 6$) (Fig. 4). The difference in mean plasma vitamin E level was small (0.11 μg/ml). This amount corresponds to 0.15% of the amount of bonded vitamin E. The total amount of vitamin E was 94 μg/membrane area (1.2 m^2). Although vitamin E is abundantly found in cell membranes, it is also present in plasma in amounts between 0.7 and 1.6 mg/dl. Compared to these levels, the amount of vitamin E eluted into bovine plasma was very small in the present study. Vitamin E is extremely safe and it has been reported that no abnormalities were caused by ingestion of 800 mg vitamin E/kg body weight for 5 consecutive days.[44–46]

Biocompatibility evaluation in vitro

Because the number of samples (n = 6) was small, we adopted nonparametric statistical analysis and analyzed the data using the Mann-Whitney U test with Bonferroni correction. Although this might lead to some limitations in interpreting the results, the present study elucidated characteristics of vitamin E-modified polysulfone (PS-ViE) membrane thus leading to the following discussion on its biocompatibility.

Complement activation

Complement activation takes place as part of the host defense mechanism. Two pathways of complement activation

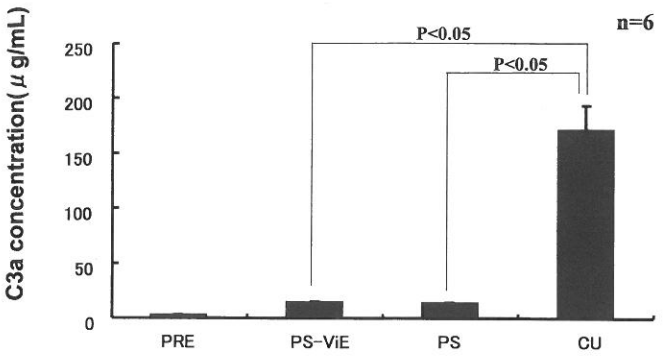


Fig. 5. Complement activation (*C3a*) concentrations for different membranes. Each minimodule with a membrane area of 300 cm^2 was filled with human serum with a contact area ratio of 190 cm^2/ml and incubated for 1 h at 37°C. *PRE*, initial value; *PS-ViE*, vitamin E-modified polysulfone membrane; *PS*, polysulfone membrane; *CU*, regenerated cellulose membrane

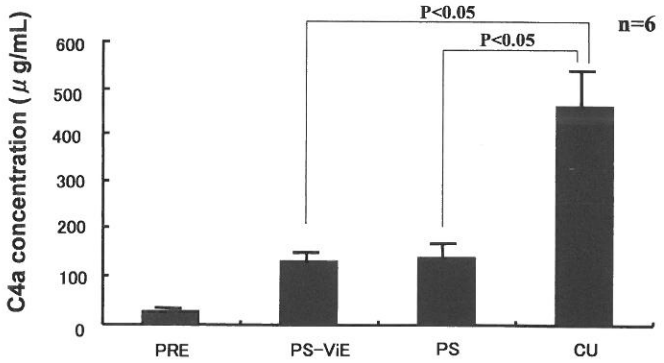


Fig. 6. Complement activation (*C4a*) concentrations with different membranes. Each minimodule with a membrane area of 300 cm^2 was filled with human serum with a contact area ratio of 190 cm^2/ml and incubated for 1 h at 37°C

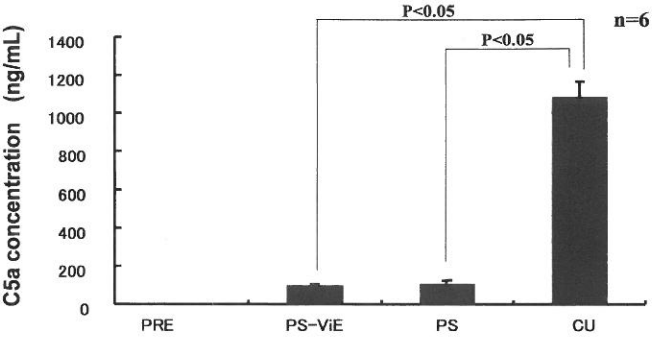


Fig. 7. Complement activation (*C5a*) concentrations with different membranes. Each minimodule with a membrane area of 300 cm^2 was filled with human serum with a contact area ratio of 190 cm^2/ml and incubated for 1 h at 37°C

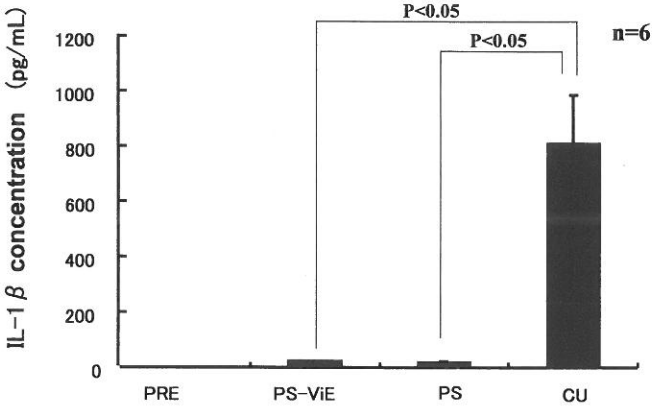


Fig. 8. Interleukin-1β (*IL-1β*) concentrations with different membranes. Each minimodule with a membrane area of 600 cm^2 was filled with human serum with a contact area ratio of 190 cm^2/ml and incubated for 6 h at 37°C

are known: (1) the classical pathway (antigen–antibody reaction) and (2) the secondary pathway (formation from polysaccharides on the surface of bacteria, etc.). It is known that ethylene-vinyl alcohol copolymers activate the classical pathway, whereas regenerated cellulose activates the secondary pathway. Unlike bacteria, regenerated cellulose is free of toxicity. However, since it has a framework of polysaccharides, it activates complement. Although the clinical effects of complement activation during hemodialysis have not been clarified, this phenomenon is often used for theoretical interpretation, such as in the monokine hypothesis.

Regarding complement activation, the present study analyzed C3a, C4a, and C5a, considering possible changes from C4a to C5a in the classical pathway and changes from C3a to C5a in the secondary pathway (Figs. 5–7). This study allowed us to confirm that PS-ViE membrane suppresses complement activation markedly to a level close to that seen when untreated PS membrane (known to have only weak complement activation) is used.

Cytokines

After Shaldon proposed his monokine hypothesis,[47] the number of reports on biocompatibility of hemodialysis membranes increased, ranging from earlier studies of temporary decrease in leukocytes to studies on complement activation and more recently to studies looking at cytokines. It has been revealed that acetate, endotoxins, C5a, and others are involved as inducers for interleukin-1β (IL-1β). These factors have been suggested to cause hyperthermia during hemodialysis; hypotension in the latter half of a dialysis session; and muscular atrophy, osteoporosis, and articular fibrosis in patients on long-term hemodialysis. Thus, these substances are attracting close attention as inflammation-associated factors that have systemic effects.[48,49] When the author studied IL-1β production under membrane stimulation (Fig. 8), it was found that the PS-ViE membrane suppresses the formation of cytokines, just as PS membrane does.

In the development of atherosclerosis, injury to the vascular endothelium is an important factor. The binding of leukocytes to the vascular endothelium involves rolling and subsequent induction of chemokine. Chemokine is a

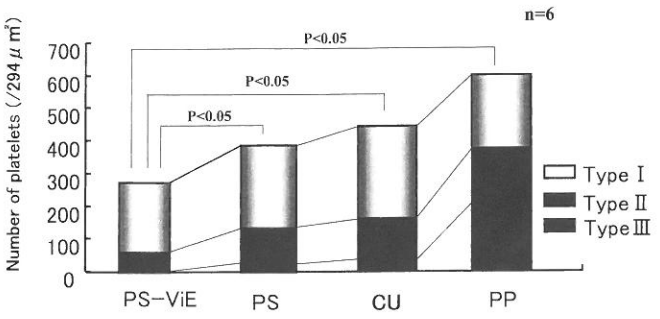


Fig. 12. The number of platelets within a given with different membranes. Types I–III represent a deformation scale for adhered platelets, with type III being the most deformed. *PP*, polypropylene

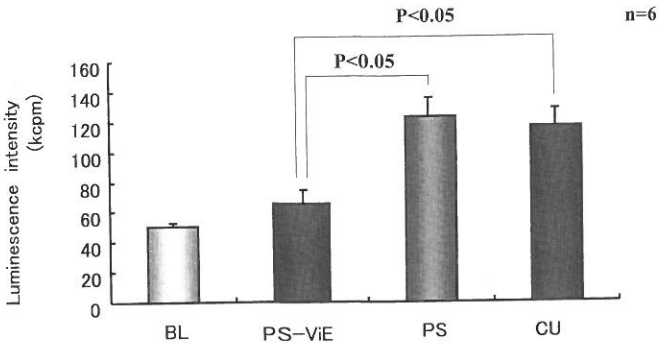


Fig. 13. Cumulative luminescence intensities as indices of active oxygen production for different membranes. The membrane area was 40 cm^2 and 1.2 ml leucocyte platelet rich plasma and 10 μl 0.1 mM 2-methyl-6(4-methoxyphenyl)-3,7-dihydroimidazol[1,2-a]pyrazin-3-one (MCLA) was used. *BL* (blank sample), control plasma

these factors acts on kininogen to induce the release of bradykinin, which stimulates the kinin system.

Figure 11 shows the results of contact phase activation on each hemodialysis membrane. In this experiment, human plasma was placed in contact with each hemodialysis membrane for 4 h at 0°C. When activation of the contact phase was measured, it was performed under nonphysiological conditions (incubation at 0°C). If incubated at 37°C, the activity reaches a peak within a few minutes, but the activity is depressesd rapidly by the inhibitory protein abundantly present in blood. At 0°C, this inhibitory reaction is negligible, thus providing optimal experimental conditions.[51]

It is said that activation of the contact phase (clotting system) is usually stimulated on intensely negatively charged surfaces [e.g., on polyacrylonitrile (PAN) membrane]. Clinically, it has been reported that the use of a membrane with a strong negative charge elevated the plasma bradykinin level. Cellulose membranes have weak negative charges, whereas PS membrane is almost electrically neutral. PS-ViE membrane was found to induce contact phase activation very slightly (Fig. 11).

Platelet spreadability

Another important factor in antithrombotic activity is the activation of platelets. Figure 12 shows the results of the platelet deforming experiment designed to examine the activation of platelets. Compared to PS membrane and cellulose membrane, PS-ViE membrane was found to cause significantly less deformation and attachment of platelets and thus to suppress the activation of platelets mildly.

These results indicate that PS-ViE membrane suppresses activation of both platelets and the contact phase (clotting system). This means that PS-ViE membrane has excellent antithrombotic activity, and this effect probably reflects the smoothing properties of the isoprenoid side-chain of vitamin E and the pharmacological activity (suppression of platelet adhesion and aggregation) of vitamin E.

Oxygen radicals

Patients on hemodialysis are usually in a peroxidized state. It has been reported that oxygen radicals are involved in various conditions associated with hemodialysis, such as stimulation of atherosclerosis and a high incidence of malignant tumors.[52,53]

Oxygen radical production when using the PS-ViE membrane (Fig. 13) was very small, the level of oxygen radicals being comparable to that of control plasma (BL). PS-ViE membrane significantly suppressed the release of oxygen radicals compared to PS and cellulose membranes. This probably represents the antioxidative activity of vitamin E in vivo. In addition to this activity, vitamin E has various physiological actions, including stabilization of membranes and suppression of platelet aggregation. Vitamin E-coated membrane is thus expected to suppress various complications in hemodialysis patients.

Methemoglobin formation

In vivo, hemoglobin undergoes two types of oxidation: (1) iron oxidation, by which an electron is removed from bivalent iron to yield trivalent iron, leading to the formation of methemoglobin, and (2) protein oxidation, by which the SH-group of globin is oxidized and undergoes disulfide binding, leading to denaturation and sedimentation. Methemoglobin, which is produced from trivalent heme iron by means of iron oxidation, no longer has the potential to bind to oxygen. It is thought that iron oxidation to form methemoglobin is induced by hazardous oxygen radicals produced in erythrocytes (hydrogen peroxide or the superoxide anion).[54]

Figure 14 shows the effects of erythrocyte oxidation (the percentage of methemoglobin) in each membrane dialyzer, as measured by the cyanomethemoglobin method. This indicates that oxidation of the erythrocyte membrane is suppressed significantly by PS-ViE membrane, compared to the other membranes.

Lipid peroxide in erythrocyte membranes

The most frequent complication seen in hemodialysis patients is renal anemia, which involves a marked reduction in erythrocyte count. In recent years, clinical introduction of

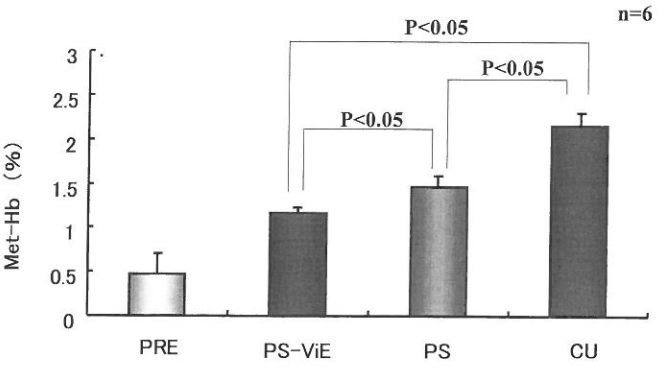


Fig. 14. The degrees of conversion to methemoglobin (*Met-Hb*) with different membranes. Each minimodule with a membrane area of 600 cm^2 was filled with human blood with a contact area ratio of 190 cm^2/ml and incubated for 6 h at 37°C

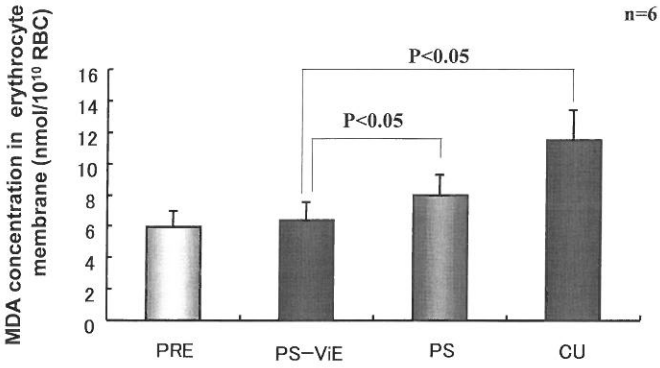


Fig. 15. Production of MDA in erythrocyte membrane with different dialyser membranes. Each minimodule with a membrane area of 600 cm^2 was filled with human blood with a contact area ratio of 190 cm^2/ml and incubated for 6 h at 37°C. *RBC*, red blood cells

recombinant erythropoietin preparations has made it possible to stimulate hemopoiesis in such cases. The turnover of erythrocytes is 120 days in healthy individuals, but it is much shorter (40–60 days) in hemodialysis patients. The involvement of oxidative stress in the short turnover of erythrocytes in hemodialysis patients has been reported. To examine oxidative stress in erythrocytes, the author analyzed the production of lipid peroxide in erythrocyte membrane (Fig. 15). This showed that the formation of lipid peroxide in the erythrocyte membrane was suppressed significantly by PS-ViE membrane, compared to cellulose and PS membranes. This probably reflects the antioxidative activity of vitamin E in vivo and the pharmacological action of vitamin E in stabilizing the membranes in vivo.

Oxidized LDL

As an indicator of atherosclerosis, the amount of LDL-fraction MDA under stimulation with each membrane dialyzer was examined (Fig. 16). The formation of oxidized LDL was suppressed significantly by PS-ViE membrane, compared to cellulose and PS membranes, probably reflecting the antioxidative activity of vitamin E.

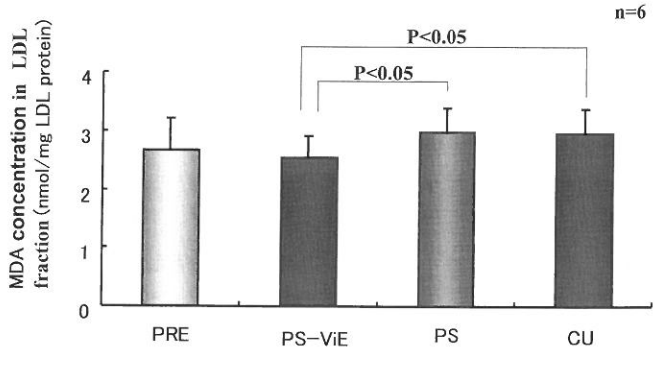


Fig. 16. Production of MDA in LDL fraction with different dialyser membranes. Minimodules with a membrane area of 600 cm^2 were filled with human blood with a contact area ratio of 190 cm^2/ml and incubated for 6 h at 37°C

Oxygen radicals are produced by direct contact of blood with dialyzer membranes, but PS-ViE membrane is thought to efficiently and effectively depress the production of oxygen radicals because the blood contacts the vitamin E layer, rather than directly contacting the membrane.

Conclusions

We attempted to bond vitamin E to a polysulfone membrane. Vitamin E is known to have antioxidative and various other pharmacological actions, and polysulfone membrane has excellent properties as a hemodialysis membrane. The physical characteristics and the in vitro biocompatibility of the vitamin E-modified polysulfone membrane were investigated. The following results were obtained:

1. When the binding potential of vitamin E on the dialysis membrane was examined, no significant release of vitamin E into bovine plasma was noted during circulation.
2. Regarding stimulation of the immune system by PS-ViE membrane, the potential for complement activation and cytokine formation was as favorable as that of PS membrane, and the granular elastase level tended to decrease in the presence of PS-ViE membrane compared to PS membrane.
3. In analysis of antithrombotic activity, the potential for activating the contact phase and inducing platelet deformation tended to be lower with PS-ViE membrane than with PS membrane. In analysis of the antioxidative activity, formation of methemoglobin, lipid peroxide, and oxygen radicals decreased significantly when PS-ViE membrane was used compared to PS membrane, thus indicating an antioxidative activity of the PS-ViE membrane.

The vitamin E-modified polysulfone membrane dialyzer was found to serve as a novel dialyzer that can make use of the advantages of vitamin E and exert antioxidative activity. This membrane is expected to protect hemodialysis patients through the physiological actions of vitamin E,

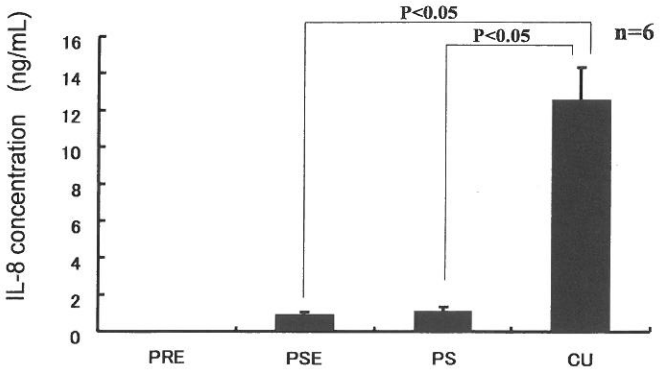


Fig. 9. Interleukin-8 (*IL-8*) concentrations with different membranes. Each minimodule with a membrane area of 600 cm^2 was filled with human serum with a contact area ratio of 190 cm^2/ml and incubated for 6 h at 37°C

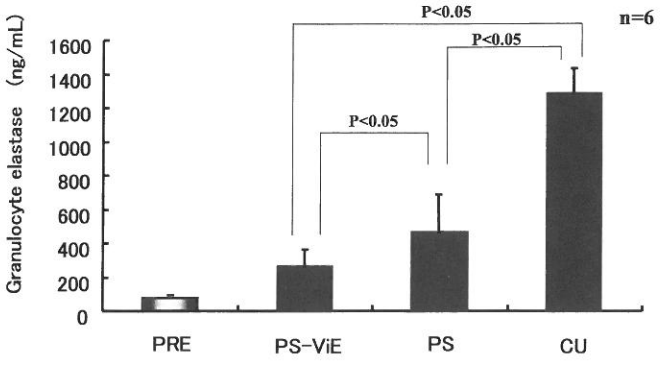
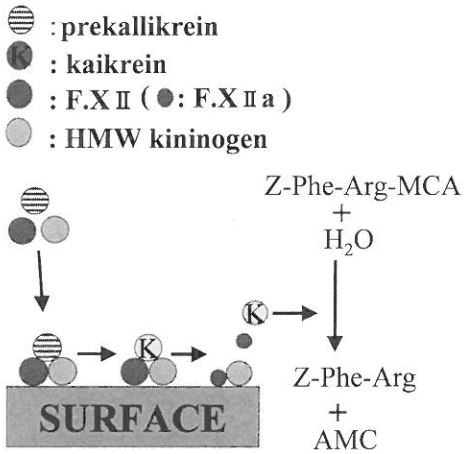
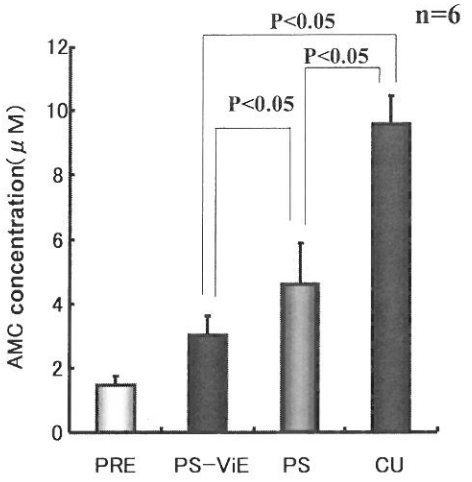


Fig. 10. Granulocyte elastase concentrations with different membranes. Each minimodule with a membrane area of 300 cm^2 was filled with human serum with a contact area ratio of 190 cm^2/ml and incubated for 10 min at 37°C

Fig. 11. Correction contact phase activation with different membranes. Each minimodule with a membrane area of 300 cm^2 was filled with human serum with a contact area ratio of 190 cm^2/ml and incubated for 4 h at 0°C. *AMC*, 7-amino-4-methylcoumarin; *F.XII*, factor XII; *F.XIIa*, factor XIIa; *HMW*, high-molecular-weight kininogen





cytokine and is composed of a group of physiologically active peptides with common structural features and chemotactic activity. More than 30 types of chemokines are known, including monocyte chemoattractant protein (MCP), macrophage inflammatory protein (MIP), and interleukin 8 (IL-8). In the present study, PS-ViE membrane suppressed the activity of IL-8 more markedly than did cellulose membrane and to a degree similar to that effected by PS membrane (Fig. 9).

It is well known from biocompatibility evalution that complement activation and the release of cytokines occur at a low level with PS membrane, and this membrane produces the least stimulus. In the present study, PS-ViE membrane did not suppress the activity of the immune system any more than untreated PS membrane did. Nevertheless, PS-ViE membrane was found to have favorable effects on the immune system, similar to those of PS membrane.

Granulocyte elastase

Granulocyte elastase is one of the neutral proteases possessed by granulocytes. Elastase is an enzyme that plays important physiological roles (e.g., digestion or degradation of bacteria and foreign matter). If released from cells, elastase degrades plasma proteins and stromal components and produces various physiologically active substances, probably injuring the living body.[50] Elastase degrades not only elastin but also degrades components of the stroma (collagen, proteoglycan, fibronectin, and others), converts complement C3 into C3a or C5 into C5a, degrades C3a and C5a, and deprives each granulocyte of its chemotacic activity. It also destroys the clotting and fibrinolytic factors; the activity of this enzyme is amplified by PF4, etc. Elastase also degrades amyloid A protein and is probably involved in the mechanism of the onset of amyloidosis.

Figure 10 shows the amount of granulocyte elastase released in each membrane dialyzer. The release was smaller with the PS-ViE membrane than the PS membrane. When compared to the cellulose membrane, the PS-ViE membrane significantly suppressed the release of granulocyte elastase.

Activation of contact phase

Activation of the contact phase serves as an important factor in antithrombotic activity. When blood contacts the surface of foreign matter, three factors (XII, prekallikrein, and high-molecular-weight kininogen) form a complex and induce coagulation (Fig. 11). At the same time, activation of

contributing to suppression of complications associated with hemodialysis.

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