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Thoughts and Progress

Development of an Electro-stethoscope System and Design of an Optimum Filter Based on Tissue Sound Transmission for Noninvasive Early Diagnosis of Malfunction of an Implanted Mechanical Total Artificial Heart

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Abstract: Early diagnosis of the malfunction of a mechanical artificial heart implanted in a patient who has been discharged from hospital is very important. We have developed an electro-stethoscope system that enables the malfunction of an artificial heart to be detected from the analysis of sound signals from the artificial heart. The sound data can be transmitted to a hospital via a mobile telephone or the Internet, so that doctors can examine the condition of the artificial heart. The optimum frequency characteristics of a low-pass filter for the elimination of ambient sound through the electro-stethoscope casing were obtained by simulating sound transmission through tissue. We evaluated the usefulness of the electro-stethoscope system using a goat in which an undulation pump total artificial heart had been implanted. A frequency analysis of the sound signal provided information on the degree of degradation of each mechanical component of the artificial heart. The results of this study showed that the electro-stethoscope system is useful for the early detection of the malfunction of an artificial heart at home, and that the use of the system contributes to improvement in the quality of life of patients. **Key Words:** Artificial heart—Electro-stethoscope—Telemedicine—Stethoscope—Tissue characteristics.

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While a reliable method for the early detection of the malfunction of an implanted artificial heart is needed to ensure the safety of patients with implanted artificial hearts, an early diagnosis system of the implanted artificial heart has hardly been researched.

Sound signals generated by an artificial heart may be an index of the degree of mechanical deterioration, because characteristics of sound signals generated by an artificial heart are related to the condition of each mechanical component of the artificial heart (1,2). For detecting the malfunction of an artificial heart, we have developed an electro-stethoscope system, which enables sound from the artificial heart to be detected on the surface of the patient's body.

The object of this study was to demonstrate the feasibility of the newly-developed electro-stethoscope system by means of in vivo experiments using a total artificial heart (TAH).

METHOD

The electro-stethoscope system consists of a pick-up microphone built into a commercially available stethoscope, an amplifier and a mobile computer. Sound data, which are detected on the surface of the body of a patient in whom a TAH has been implanted, are fed into the mobile computer via an A/D converter.

While tissue has the same frequency characteristics of sound transmission as that of a low-pass filter, the frequency components of the measured sound signal involve higher frequency components, which come from ambient sound around a patient.

In this study, we designed an optimum low-pass filter that has the same frequency characteristics as those of sound transmission through tissue, in order to eliminate the high-frequency noise signal. The theoretical relationship between the pressure of the sound source P_0 and the sound pressure P_m measured by the microphone is given by

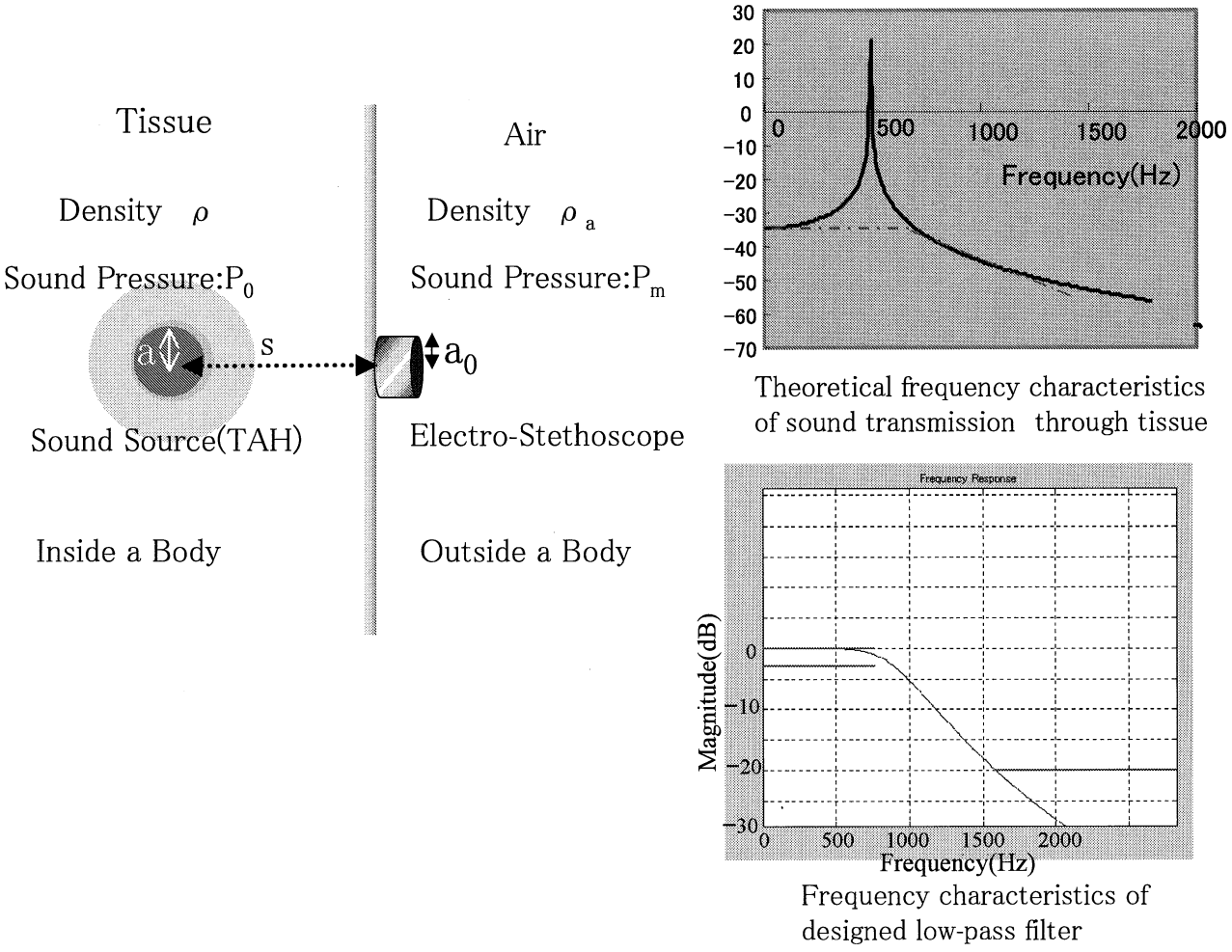

FIG. 1. Calculation results of the tissue characteristics of sound transmission, and a designed low-pass filter for the elimination of ambient sound.

$$\frac{P_m}{P_0} = \frac{0.8488 \cdot a}{s^2} \cdot \frac{1}{1 - \left(f / f_c\right)^2} \quad (1)$$

$$f_c = \frac{1}{2\pi} \sqrt{\frac{\rho_a \cdot c_a^{\,2}}{0.4244 \cdot \rho \cdot a_0 \cdot d}}, \quad (2)$$

where the density of air is $\rho_a = 1.18$ kg/m^3, the density of tissue is $\rho = 1000$ kg/m^3, the sound velocity in air is $c_a = 340$ m/s, and the radius and heights of an air cavity of the microphone are $a_0 = 17$ mm and $d = 2$ mm (Fig. 1) (3). An undulation pump TAH (UPTAH) with a radius of 38 mm was used as the sound source a (4). The depth of the position of the UPTAH implantation was measured as $d = 170$ mm using a goat (body weight of 45 kg).

The frequency characteristics of a newly-designed low-pass filter using results of tissue frequency characteristics are also shown in Fig. 1. The cut-off frequency of the low-pass filter was designed to be 850 Hz, according to the frequency characteristics of sound transmission through tissue.

RESULTS

The performance of the newly-developed electrostethoscope system for the detection of the malfunction of an artificial heart was evaluated in in vivo experiments using an UPTAH. An UPTAH was implanted into a goat weighing 45 kg and was driven by an external controller through a percutaneous lead. Sound data were fed into a personal computer via an A/D converter (sampling frequency of 44 kHz, data length of 16 bits). Frequency components in the sound data were analyzed on a personal computer using signal processing software (Matlab, Cybernet System, Tokyo, Japan).

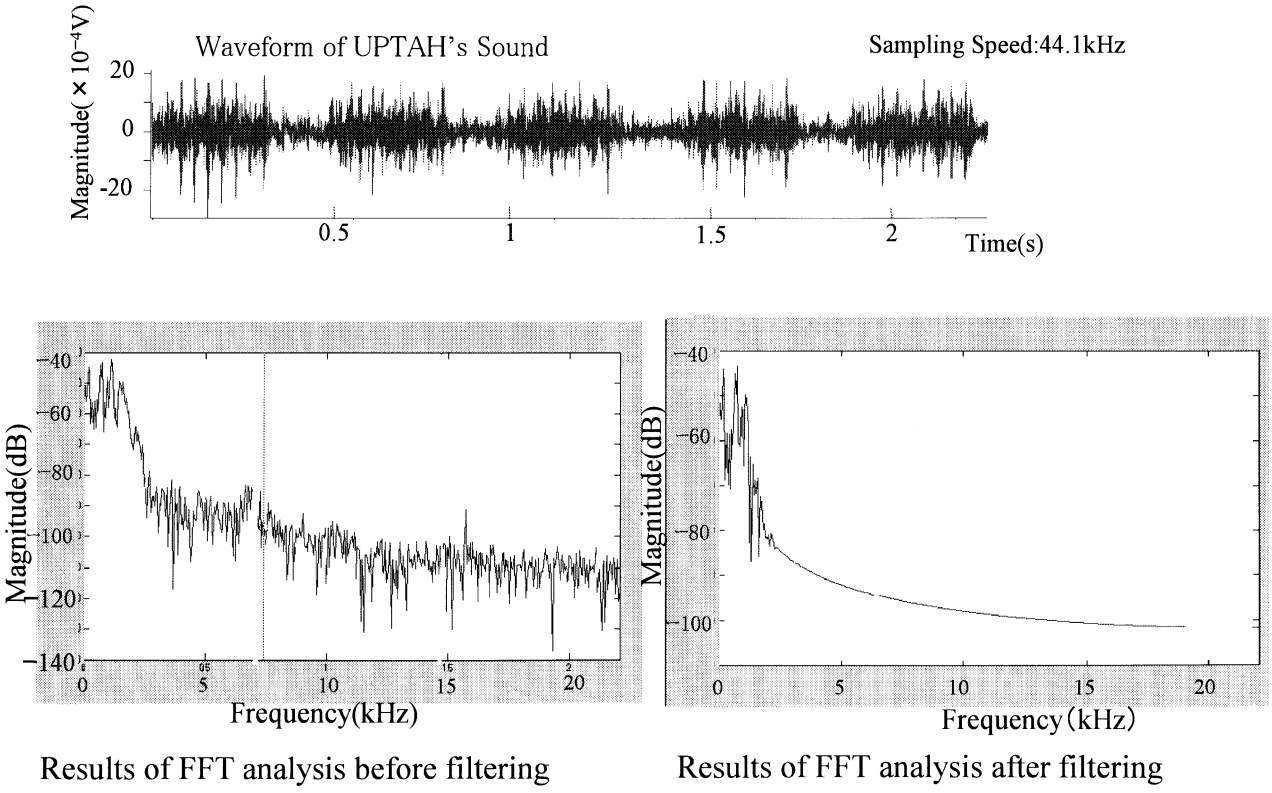

FIG. 2. UPTAH's sound signal and fast Fourier transform (FFT) analysis in animal experiments.

Figure 2 shows the measured waveform of the UPTAH's sound signal detected on the goat's body and the result of a frequency analysis during the higher motor velocity phase. While characteristics of sound transmission through the skin had that of a low-pass filter, as can been seen in Fig. 1, the measured sound signal included high-frequency components.

Figure 2 also shows frequency components of the sound data after eliminating high-frequency components by using a low-pass filter. The elimination of the high-frequency components of the signal enabled the sound of the UPTAH to be heard clearly, and it contributed to an improvement in the early diagnosis of mechanical malfunction in the UPTAH.

DISCUSSION

Early detection of the malfunction of a mechanical artificial heart implanted in a patient who has been discharged from hospital is important because the malfunction of the implanted artificial heart may be fatal for the patient. However, an early diagnosis system for malfunctions has not been developed.

We have developed an electro-stethoscope system that enables the early detection of the malfunction of an implanted mechanical artificial heart. The motor current may also be a useful index for early detection of a malfunction, but motor current data only provide information on the overall degree of degradation of the artificial heart. Sound data provide information on the degree of degradation of each mechanical component of the artificial heart by means of investigating the change in each frequency component, because the frequency of sound and the vibration of each mechanical part depend on the rotational velocity and structure of each mechanical component (1,2).

Results of a fast Fourier transform (FFT) analysis showed that sound from the UPTAH includes a frequency component of 8.33 Hz and its harmonics, corresponding to a left motor speed of 500 rpm during the high-velocity phase. The sound also includes a frequency component of 13.3 Hz and its harmonics, corresponding to a right motor velocity of 800 rpm. Similar results were obtained during the low-motor-speed phase. The time series change of amplitude in each frequency component provides information on the degree of degradation of

each mechanical component of the artificial heart's actuator.

In this article, we have proposed a new design method for eliminating high-frequency noise in the sound from an artificial heart. Optimum characteristics of a low-pass filter can be obtained by simulating sound transmission through tissue. The newly-designed low-pass filter, whose cut-off frequency is 850 Hz, showed an improvement in the quality of the measured artificial heart sound, allowing doctors to detect a change in the condition of the artificial heart.

CONCLUSION

We have developed an electro-stethoscope system for detecting the malfunction of an implantable artificial heart by means of measuring sound from the artificial heart on the surface of the patient's body. The results of this study showed that this electro-stethoscope system contributes to the early detection of a malfunction in the artificial heart of a patient, and also use of this system promises an improvement in the quality of life of a discharged artificial heart patient.

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Symptomatic Hypocalcemia Due to the Inadvertent Use of a Calcium-free Hemodialysate

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Abstract: Hemodialysis using a bath with less-than-normal calcium level can cause hypocalcemia. The development of symptomatic hypocalcemia with resultant hypotension after the inadvertent use of a calcium-free dialysate in a maintenance hemodialysis patient is described. It is suggested that the occurrence of similar mishaps in the future can be reduced by close checking of dialysate concentrate labels. **Key Words:** Hemodialysis—Calcium-free dialysate—Hypocalcemia—Medical errors.

Dialysate calcium concentrations can be customized depending on the current and target serum calcium levels as well as the desire to maintain hemodynamic stability during dialysis (1,2). The dialysate calcium concentration commonly used for maintenance hemodialysis ranges from 1.25 to 1.75 mM (5–7 mg/dL) (3). Dialysis with a dialysate having a calcium level less than the prevailing serum value can be associated with a negative calcium balance and hypocalcemia (4) with resultant hypotension. Herein we describe the development of symptomatic intradialytic hypocalcemia after the inadvertent use of a calcium-free dialysate in a chronic hemodialysis patient.

CASE REPORT

A 55-year-old maintenance hemodialysis patient had a history of diabetes, hypertension, coronary artery disease, and frequent arterio-venous graft infections in the past. His medications included aspirin, calcium acetate, metoprolol, diltiazem, hydralazine, terazosin, insulin, and acetaminophen/hydroxycodone. Dialytic therapy consisted of thrice-weekly dialysis treatments, each of 4 h duration. The patient's predialysis weight was 79 kg and his interdialytic weight gains were in the realm of 2 kg. Two days prior to the dialysis session described in the present article, his serum inorganic phosphorus level

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was found to be 1.2 mM (3.7 mg/dL), his serum total calcium value was 2.28 mM (9.1 mg/dL), and his serum parathyroid hormone level was 112 ng/L (the normal being 10–65 ng/L).

On the day of the adverse event, he was inadvertently dialyzed with a calcium-free dialysate. The dialysate was prepared by mixing 1.72 parts of a "bicarbonate concentrate," 1 part of a "calcium-free acid concentrate" and 42.28 parts of product water. The dialysate had the following concentrations (in mM): sodium 137, potassium 2, calcium 0, magnesium 0.375, bicarbonate 33, chloride 103, acetate 4, and glucose monohydrate 11. A high-flux polyamide dialyzer (Polyflux 21 S, Gambro Dialysatoren GmbH & Co., Stockholm, Sweden) was used. The dialyzer urea clearance as measured in vitro at a dialysate flow rate of 500 mL/min and a blood flow rate of 300 mL/min, was 267 mL/min. During dialysis, the dialysate flow rate amounted to 600 mL/min and the blood flow rate was 400 mL/min.

Thirty minutes into the dialysis session and after only 600 mL of ultrafiltrate had been removed, he was found to be hypotensive. He complained of generalized weakness, nausea and a tingling sensation in both hands. His blood pressure was noted to have fallen to 100/65 mm Hg from a predialysis value of 160/80 mm Hg. The blood pressure came up to 110/65 mm Hg with the intravenous administration of 500 mL of physiological saline, and dialysis was continued without ultrafiltration. He continued to feel weak. Instead of discontinuing dialytic treatment, the dialysis was inadvertently continued until the end of the fourth hour. Shortly after stopping dialysis, the results of his serum electrolyte concentrations measured at 2 h into dialysis became known. The serum total calcium was less than 1.25 mM (5 mg/dL) (our laboratory was not geared to measure serum calcium levels accurately if the values were less than 1.25 mM), while inorganic phosphorus was 0.42 mM (1.3 mg/dL), albumin 38 g/L, sodium 138 mM, potassium 3.3 mM, chloride 102 mM, CO_2 23 mM, and glucose 8.6 mM. The postdialysis electrocardiogram showed a normal sinus rhythm, an incomplete right bundle branch block and a prolonged QT interval of 0.48 s. The QT interval corrected for the prevailing heart rate was 0.54 s (the upper limit of the normal rate being 0.46 s). On discovering that the serum total calcium concentration was low, 4 g of calcium gluconate (9.6 mM of elemental calcium) were administered intravenously and his weakness improved. One hour after the calcium administration, the serum total calcium value was found to be 1.6 mM (6.5 mg/dL). He was discharged home with instructions to take oral calcium carbonate at a dosage of 3 g (30 mM of elemental calcium), three times a day for two days. Serum total calcium level two days after the incident was 2.7 mM (10.8 mg/dL).

DISCUSSION

Although the extracellular fluid calcium content represents only a small fraction of the total body calcium stores, the serum ionized calcium concentration does influence various physiological functions in a major manner and must be maintained within a narrow range (5). Acute hypocalcemia can have a myriad of untoward manifestations including nausea, vomiting, muscle weakness, paresthesias, neuromuscular irritability and tetany. In addition, hypotension, myocardial dysfunction, QT interval prolongation, and cardiac arrhythmias are common cardiovascular manifestations of hypocalcemia (5).

The use of calcium-free dialysates in normocalcemic individuals has previously been practiced in both animals and humans (4,6,7). Hemodialysis with calcium-free dialysates has induced hypocalcemia in goats (4) and dogs (6). In one of the human studies, five out of eighteen chronic hemodialysis patients developed symptomatic hypocalcemia during the first 60 min of dialysis with calcium-free dialysates (7). In view of this, calcium-free dialysates are often used only in hypercalcemic patients (2). We believe that our patient is the first reported case of symptomatic hypocalcemia caused by the inadvertent use of a calcium-free dialysate.

Medical errors are important causes of morbidity and mortality. The delivery of health care is a complicated undertaking and therefore prone to accidents. Human errors are major contributors to accidents in the setting of modern health care (8,9). In our instance, the "calcium-free acid dialysate concentrate" used for our patient happened to be stored alongside other "calcium-containing acid dialysate concentrates." Instead of using a "calcium-containing acid concentrate" as originally planned for our patient, a "calcium-free acid concentrate" was inadvertently employed. This mishap underscores the importance of meticulous efforts to label dialysate concentrates distinctly and to store different concentrates in separate areas, so as to prevent similar accidents from occurring in the future. Checking the label on the canister of a dialysate concentrate being used in the face of unusual intradialytic symptoms can lead to more prompt recognition of similar, inadvertent errors. Furthermore, the clinical course of our patient lends support to the notion that iatrogenic hypocalcemia is an important and evitable cause of intradialytic hypotension.

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Biological Reactions Resulting from Endotoxin Adsorbed on Dialysis Membrane: An In Vitro Study

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Abstract: Some types of dialysis membrane are known to adsorb endotoxin (ET). It is suggested that the biocompatibility of dialysis membrane is enhanced by adsorption and inhibition of ET. This study attempts to clarify the membrane-mediated biological reaction of the ET that is adsorbed to a dialysis membrane. After a dialysis circuit was prepared, contaminated dialysate was introduced on the dialysate side of a polyether polymer alloy (PEPA) membrane that adsorbs ET while saline solution or blood were introduced on the blood side, and the difference in ET adsorption between the two set-ups was measured. Further, the side filled with blood was left standing for 2 h, after which the changes in the amount of interleukin 1 receptor antagonist (IL-1Ra) produced from the whole blood were also assayed. Significantly more ET was adsorbed to the dialysis membrane when blood rather than saline was on the other side. In addition, the IL-1Ra production from the dialysis membrane that adsorbed ET was significantly higher. The ET adsorbed to the dialysis membrane may influence a living body even if it does not pass through the membrane. Accordingly, it is difficult to assume that the adsorption of ET to the membrane enhances its biocompatibility.

Key Words: Endotoxin—Dialysate—Adsorption—Dialysis membrane—Biological reaction.

Hemodialysis (HD) is performed as a substitute for normal kidney function in about 95% of 200,000 or more chronic renal failure patients in Japan (1). Since the prevalence of renal transplantation as an alternative treatment is low in this country, the duration of HD treatment is long in many patients so that the quality of dialysis has a substantial influence on the quality of life. Above all, the fluid quality of the dialysate is crucial.

Naturally occurring water bacteria such as *Pseudomonas* species are commonly found in dialysate. When microbial contamination, especially endotoxin (ET), reaches an excessively high concentration, serious health risks can result to HD patients, including pyrogenic reactions. It was reported that purification of dialysate alone led to clinical improvement of renal anemia (2) and a decrease in chronic inflammatory reaction (3). These reports suggest that the improved fluid quality is effective in the prevention and inhibition of the progress of dialysis related amyloidosis (DRA) which is the most serious complication in long-term hemodialysis (4).

Among the recently used synthetic membranes, especially the high flux membranes, it is suggested that there is a flow of ET in the dialysate into the blood due to back filtration and back diffusion (5). However, direct inflow of ET is not evident because it is difficult to accurately assay a trace of ET in the blood at present (6). In perfusion experiments, there are some reports of inflow (7) and some reports that deny this (8).

Some synthetic membranes adsorb ET (9) because hydrophilic polyvinylpyrrolidone (PVP) is a component of the membrane. It is possible that the adsorption of ET by the membrane may reduce the influence on a living body. However, ET may still have influence if its adsorption influences the inside of the dialysis membrane.

In this regard, we investigated the biological reaction of membranes with adsorbed ET in vitro.

MATERIALS AND METHODS

Influence of blood factor on ET adsorption

Polyether polymer alloy (PEPA, Nikkiso Co., Ltd, Tokyo, Japan) membrane with an area of

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0.15 m^2 was used. After the blood side, inside, and dialysate side of the membrane were washed with saline solution, the dialysate side was filled with 20 ml of contaminated dialysate (mean ET concentration: 4.29 ± 0.64 EU/ml). The ET in the dialysate side was assayed in a group whose blood side was filled with saline solution (saline group; $n = 4$) and a group whose blood side was filled with blood (blood group; $n = 4$) at 0, 1, and 2 h after filling with each fluid. Endotoxin Single Test Wako (Wako Pure Chemical Industries, Ltd, Osaka, Japan) was used for determination of ET and the changes in the adsorption volume were investigated.

Biological reaction of ET-adsorbed membrane

After informed consent was obtained, 20 ml of blood was collected from four healthy subjects in sterile conditions. The blood was added to 20 ml saline solution to make a 2-fold dilution. To prevent coagulation, 5 ml of heparin was also mixed with this. After the PEPA membrane was primed with saline solution as described above, the blood side of the dialysis membrane was filled with blood (10 ml) while either 10 ml of dialysate whose ET concentration was below the detection limit (purified dialysate) or contaminated (4.29 ± 0.64 EU/ml) dialysate (contaminated dialysate) was placed in the dialysate side.

After 2 h, 2 ml of blood was collected from these two groups, and interleukin 1 receptor antagonist (IL-1Ra) contained in the supernatant was measured by the ELISA method (R & D Systems, Minneapolis, MN, U.S.A.).

The statistically significant difference was calculated by Mann–Whitney *U*-test and the differences with a probability of 5% or less were regarded as statistically significant ($P < 0.05$).

RESULTS

Influence of blood factors on ET adsorption

When the blood side was filled with saline solution, the ET concentrations on the dialysate side at 0, 1, and 2 h afterwards were 4.70 ± 0.44, 0.88 ± 0.15, and 0.38 ± 0.14 EU/ml, respectively. However, when the blood side was filled with whole blood the concentrations at the respective time points were 1.41 ± 0.41, 0.53 ± 0.18, 0.39 ± 0.14 EU/ml. This indicates a significant adsorption increase at 0 and 1 h in comparison with the saline solution version (Fig. 1). However in neither of the cases was ET detected on the blood side.

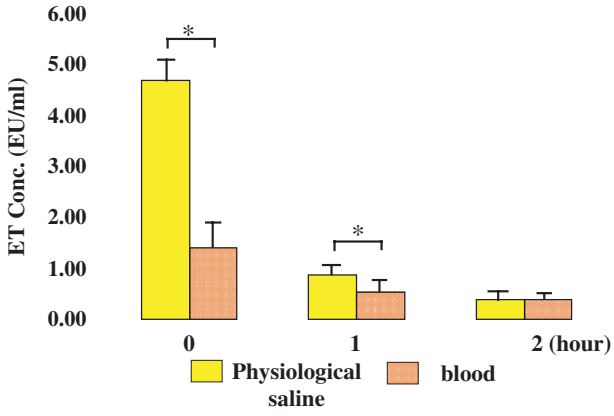

FIG. 1. Influence of blood factor on the ET adsorption to the dialysis membrane. (*$P < 0.05$).

Biological reaction of ET-adsorbed membrane

When purified dialysate was used, the IL-1Ra on the blood side after 2 h was 140.6 ± 46.7 pg/ml. However, the value went up to 177.6 ± 103.3 pg/ml when the contaminated dialysate was used, indicating a significant ($P < 0.05$) adsorption increase of ET with whole blood.

DISCUSSION

Sodium bicarbonate dialysate has recently become the main choice of fluid for use in HD. It is feared that the ET in the dialysate becomes fragmented and passes through the dialysis membrane to enter the blood (10). Furthermore, as dialysis membranes with larger pore diameters are used for low molecular weight protein removal, it is suggested that ET will pass through the membrane by back filtration and back diffusion and that it can affect a living body (5). If pyrogen activity is the strongest effect in the ET, it will cause various clinical conditions in the living body, such as generation of heat from production of inflammation cytokines. However, it has not been clinically demonstrated how much dialysate enters and influences a living body (8). Some dialysis membranes are manufactured to adsorb ET (9) because the adsorption to the membrane is considered to prevent ET in the dialysate from entering an organism. However, in which part of the dialysis membrane it is adsorbed (the inside of the membrane or the outside of the membrane) is unknown. A reaction may occur in a living body when ET has reached the inner side of a dialysis membrane. Accordingly, we investigated an ET-adsorbing membrane and its reaction to a living body in vitro.

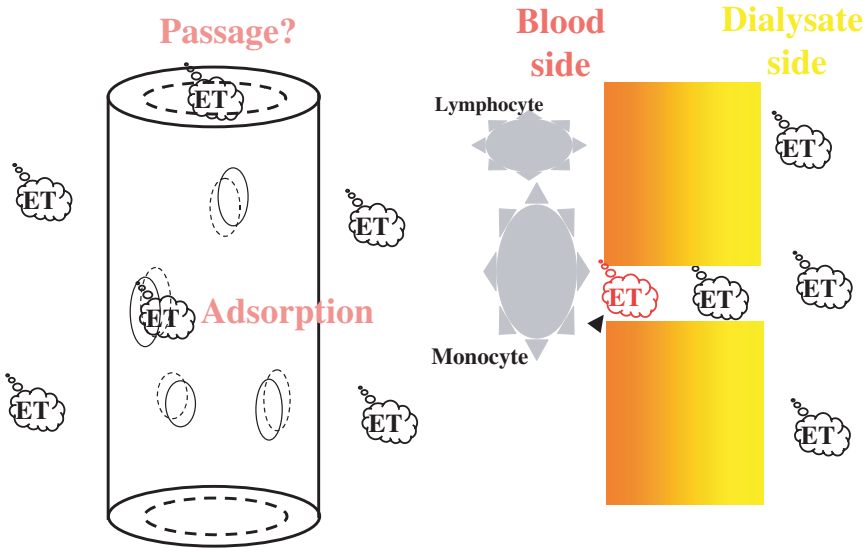

FIG. 2. Scheme indicating the passage through and adsorption on the dialysis membrane of ET. Although it is not clear whether ET passes through a dialysis membrane, adsorption is certainly present. Even if only adsorption occurs, it is possible to reach the other side, i.e., blood side. It is thought that lymphocytes and monocytes in the blood act even if the ET does not pass completely through the membrane.

PEPA was chosen as a typical dialysis membrane used to adsorb ET (11). It is a dialysis membrane which consists of hydrophobic synthetic polyallylate-polyethersulfone fiber as a material. Because PVP, which is a hydrophilic agent, is not used at all, the whole membrane has hydrophobic characteristics. ET is basically amphiphilic but its adsorption is greater on a hydrophobic surface (12). In the case of PEPA, the dialysis membrane itself is made hydrophobic based on an idea that the more hydrophobic a membrane is, the more adsorption of ET will be increased. As shown in Fig. 1, the membrane demonstrates a substantial ET adsorption effect, indicating the excellent function of PEPA membranes in adsorbing ET. The ET diffusing from dialysate side to the blood compartment can be 4 times higher when the blood compartment contains saline than when it contains blood at time 0. It is possible that a mechanism of adsorption can be adsorbed onto a dialysis membrane in a short time, because the membranous adsorption characteristic has been potentially changed by the protein, mainly albumin, and lipids contained in the blood. Yamamoto et al. reported that ET was removed by ultrafiltration through high-flux, hollow fiber filters and especially complete removal of ET molecules could be achieved by ultrafiltration through hydrophobic membranes having a high adsorptive capacity in addition to an appropriate sieving property for ET molecules (13). However in our study, there was a marked adsorption of ET to the membrane both from the dialysate and the blood side. The adsorption tendency showed a significant increase when the blood faced the dialysis membrane. It is conceivable that the adsorption of ET on the dialysis membrane is influenced not only by the hydrophobic or hydrophilic features of membrane itself but also by the corpuscles, proteins, and lipids contained in the blood.

When the membrane was in contact with the contaminated dialysate (rather than purified dialysate), IL-1Ra production in the blood increased. This result suggests that the ET adsorbed on the dialysis membrane had penetrated inside the membrane, possibly influencing the living body. It was reported that the production of IL-1Ra is earlier than other inflammatory cytokine. Therefore, ET adsorbed to the membrane results in the synthesis and release of IL-1Ra via the activation of monocytes/macrophages in this early time. That is, the ET adsorbed on the membrane penetrated into the membrane. As a result, it is likely that smaller molecular weight ET reached further into the inside of the membrane by diffusion. As a result, it is possible that ET that is present near the lumen influences lymphocytes and monocytes in the organism so that a biological reaction is induced (Fig. 2).

It is difficult to conclude that ET in the dialysate influences a living body through the membrane because accurate determination of the trace amounts of ET in the blood is not possible at present (6). However, even if the penetration of ET does not take place, there is still a possibility that a reaction in a living body is induced. Accordingly, the purification of the dialysate is considered to be very important when a high flux membrane is used. Because the dialysate and blood in this experiment were not perfused but were left undisturbed, it is too early to conclude that the same result is clinically observed

in the course of treatment. However, the result obtained in this study indicates the need for further detailed investigation of the behavior of ET in dialysate in the future.

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Ozonated Autohemotherapy in Patients on Maintenance Hemodialysis: Influence on Lipid Profile and Endothelium

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Abstract: Ozonated autohemotherapy (O3-AHT) is used in the treatment of atherosclerotic ischemia of lower limbs (AILL). The impact of ozone on serum lipids and endothelium injury is of particular interest since these factors are important in the development of atherosclerotic lesions. To evaluate this issue, a prospective, placebo-controlled study was designed. Twelve hemodialyzed subjects with AILL received autohemotherapy with oxygen as a control followed by O3-AHT with ozone concentration of 50 μg/ml. Serum lipids and plasma activity of von Willebrand factor (vWF) were measured. After O3-AHT, total cholesterol significantly decreased compared to the baseline (–8.34%) [$P < 0.01$]. LDL cholesterol was also significantly lower than the initial value (–17.71%) [$P < 0.001$]. No significant changes in the activity of vWF were found after the first session of O3-AHT and after all nine sessions of O3-AHT. The study demonstrated that O3-AHT did not affect deleteriously the endothelium in patients with chronic renal failure on maintenance hemodialysis. It may stimulate beneficial changes in serum lipid profile manifesting as a decrease in the total- and LDL-cholesterol levels. **Key words:** Ozone—Autohemotherapy—Lipids—Endothelium—Hemodialysis—Renal failure.

Ozonated autohemotherapy (O3-AHT) has been used in the treatment of atherosclerotic ischemia of lower limbs (AILL) for many years (1). In our previous report, the beneficial impact of O3-AHT on the clinical signs of AILL was shown in dialyzed subjects (2). Quite recently, comparatively favorable effects with regard to prolongation of intermittent claudication distance were observed in our placebo-controlled study (3). Exposure to ozone has also been suggested as inducing beneficial alterations in serum lipid profile (4,5). Furthermore, ozone given intra-arterially has been shown to inhibit the progres-

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sion of atherosclerotic lesions in a rabbit model of atherosclerosis (6). This issue is, however, controversial since the deleterious effects of ozone exposure are also known. Ozone may induce oxidative stress (7), which in turn can cause endothelial injury (8), the first and principal step in the development of atherosclerosis. Oxidized forms of lipids are considered another important risk factor of this process. Their generation after O3-AHT was noted in some studies (9) but not in all (5). In view of these conflicting results, it is difficult to conclude whether ozonotherapy promotes atherosclerosis or rather attenuates its development. This problem is of particular interest with regard to patients with end-stage renal disease (ESRD) on maintenance dialysis who manifest complications of atherosclerosis very often. To gain a better insight into this issue, a prospective oxygen controlled study was performed to evaluate the influence of O3-AHT on the endothelium and serum lipid profile.

MATERIALS AND METHODS

Subjects

Twelve chronic hemodialysis (HD) subjects (eight male, four female), aged 64.8 ± 7.6 (range 50–75) years manifesting symptomatic AILL were recruited. They underwent regular bicarbonate HD treatment, three times per week for more than one year (average 4.5 ± 3.1 years). The HD prescription, namely, dialyzer type, HD session length, rate of dialysis solution and blood flows remained unchanged during the study. No pharmacological treatment was either changed or newly administered.

Study design

Subjects received 9 sessions of autohemotherapy connected with the blood exposure to medical oxygen (AHT) as a control, followed by 9 sessions of autohemotherapy along with exposure to an oxygen-ozone mixture with ozone concentration of 50 μg/ml (O3-AHT). The details of O3-AHT were described previously (2). The sessions were performed three times a week just before the hemodialysis session in a single blind manner. The plasma activity of von Willebrand factor (vWF) and the serum lipid levels were measured. Blood samples were collected before the first AHT, after the ninth AHT and after the ninth O3-AHT. To evaluate the effects of the first exposure to ozone blood was also withdrawn before and 20 min after the first O3-AHT.

Biochemical analysis

Serum levels of total cholesterol (TCH), HDL cholesterol (HDL-CH), and triglycerides (TG) were measured calorimetrically using standard methods. LDL cholesterol (LDL-CH) was calculated according to Friedewald's formula (none of the patients had triglyceride levels higher than 400 mg/dl). vWF plasma activity was analyzed using an ELISA kit (vWF200, Axis-Shield Diagnostic, Dundee, UK) according to the kit-provider's recommendations.

Statistics

Data are expressed as mean ± SD. Differences of variables measured more than twice were assessed by the analysis of variance for repeated measurements or Friedman's test; otherwise by Student's *t*-test for paired comparison or the Wilcoxon test. Data were evaluated using STATISTICA (version 5.1, StatSoft Inc.).

RESULTS

The effects of O3-AHT on serum lipid profile are shown in Table 1. After O3-AHT, the TCH level was significantly lower compared both to baseline (–8.34%) [$P < 0.01$], and to the AHT (control) [$P < 0.01$]. LDL-CH level was also significantly lower than the initial value (–17.71%) [$P < 0.001$], and lower than the level after AHT (control) [$P < 0.001$]. Table 1 also presents the effects of O3-AHT and AHT on the plasma activity of vWF. No significant changes were found after the first and the ninth O3-AHT.

TABLE 1. *Lipid profile and activity of vWF at the baseline, after control AHT and after O3-AHT*

	Baseline	After AHT(control)	After 1st O3-AHT	After 9th O3-AHT
TCH (mg/dl)	211.0 ± 46.1	215.0 ± 49.5		193.4 ± 53.4[a]
LDL-CH mg/dl)	130.3 ± 42.6	129.0 ± 46.7		107.2 ± 44.4[b]
HDL-CH (mg/dl)	44.6 ± 9.1	45.3 ± 9.3		45.9 ± 8.7 NS
TG (mg/dl)	171.5 ± 96.2	167.4 ± 92.8		164.4 ± 92.8 NS
vWF (%)	72.6 ± 55.7	61.3 ± 53.8	78.7 ± 50.4	83.4 ± 65.9 NS

[a] Significant difference ($P < 0.01$): 9th O3-AHT vs. baseline, and 9th O3-AHT vs. AHT.
[b] Significant difference ($P < 0.001$): 9th O3-AHT vs. baseline, and 9th O3-AHT vs. AHT.
TCH, total cholesterol; LDL-CH, low-density lipoprotein cholesterol; HDL-CH, high-density lipoprotein cholesterol; TG, triglycerides; vWF, von Willebrand factor; NS, nonsignificant difference.

DISCUSSION

Abnormalities in lipid metabolism are common in patients with ESRD. In HD subjects further acceleration of lipid abnormalities is observed (10). It is suggested that ozonotherapy ameliorates these alterations. We demonstrated that O3-AHT caused a significant decrease in LDL-CH and TCH levels. The fall of LDL-CH was particularly evident. Our results are in close agreement with findings on subjects with normal renal function treated with O3-AHT (5). Ozone given intra-arterially has also been found to induce comparable results (4). None of the above-mentioned studies revealed any changes in TG and HDL-CH levels. Parallel conclusions were drawn from another study, where rabbits with dietary induced atherosclerosis displayed a decrease in TCH and LDL-CH after intravenous administration of ozone (11). The method of LDL-CH level evaluation used in the present study (Friedewald equation) limits the significance of our findings on this point. Direct measurement would be a better analytical tool in this regard.

The mechanism by which ozone causes a decrease in blood cholesterol is not clear. The theory involving oxysterols (OSTs) as a critical regulatory factor responsible for such ozone activity was proposed originally by Hernandez (5). OSTs, oxygenated derivatives of cholesterol, were shown to induce a suppressive effect on the synthesis of lipids both at transcriptional and posttranscriptional levels (12). It was suggested that ozone, when in contact with the blood during O3-AHT, reacts with polyunsaturated fatty acids producing organic radicals and hydrogen peroxide. These products induce oxidation of cholesterol when the blood is reinfused to a patient after ozonation. The oxidation of cholesterol may lead to its excretion with bile or further oxidation to water-soluble bile acids. It is likely that OSTs created in these processes are able to decrease the rate of cholesterol synthesis (5).

The possibility that ozone mediates the creation of oxidized LDL, resulting in increased uptake by macrophages and a subsequent decrease in LDL-CH level in plasma, can not be excluded. This could be, of course, harmful. Oxidized LDL was not measured in the present study. Conflicting data on this point exist in the literature (5,9). To clarify this issue in vitro studies and animal experiments are necessary.

HD patients are exposed to endothelial stimulation or even injury (13). Since ozone can induce the generation of free radicals, ozonotherapy may be supposed to be additionally harmful for these patients as far as endothelial injury is concerned. To verify this hypothesis, the influence of O3-AHT on the plasma activity of vWF was examined. vWF is believed to serve as an index of endothelial injury, or more likely, of altered endothelial function (14). There was no change in vWF activity after the first and the ninth session of O3-AHT when compared to the baseline level. These results are in line with findings on subjects with normal renal function treated with an ozonated isotonic solution of NaCl given intravenously (15). In view of these reports, the deleterious effect of ozone in therapeutic dosages on endothelium appears unlikely. Since a clinical study to look at endothelial function/scarring has a limited scientific value, in vitro experiments should also be performed to confirm these findings. Considering that theoretically possible endothelial injury may reflect oxidative cell modification, the precise control of ozone dosage seems to play a crucial role. Oxidative properties of ozone have been shown to be dose-dependent and the therapeutic concentrations window was found to range between 20 and 80 μg/ml (1). On the basis of the results of another study performed in our center, it seems likely that O3-AHT with ozone dosage of 50 μg/ml does not cause oxidative injuries in HD patients (16). The antioxidant defense system is able to neutralize the oxidative properties of ozone and protect against oxidative cell damage. This study confirmed this thesis with respect to endothelial cells.

As was mentioned above, ozone has been shown to ameliorate atherosclerosis in an animal study (6). Unfortunately, there are no human studies evaluating this controversial issue. Only the impact of OSTs on the development of atherosclerosis was evaluated. Of 13 studies, six indicated the proatherogenic effect of OSTs, four indicated an antiatherogenic effect, whereas three showed no clear-cut activity (17). With the current knowledge it is not possible to draw firm conclusions as to whether ozonotherapy enhances atherosclerosis or rather protects against this process. Summarizing, the study demonstrated that O3-AHT with ozone concentration of 50 μg/ml, applied three times a week, did not affect deleteriously the endothelium in HD patients. It may stimulate potentially beneficial changes in serum lipid profile manifesting in a decrease of TCH and LDL-CH levels.

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