Study of Effectiveness of Prevention by Double Filtration Plasmapheresis (DFPP) Against Arteriosclerotic Disease: Removal of Oxidized LDL-cholesterol and Pentosidine by DFPP

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Abstract

Purpose: Among the wide range of diseases for which apheresis is indicated, the clinical effectiveness of double filtration plasmapheresis (DFPP) for arteriosclerotic disease, typified by familial hyperlipidemia and arteriosclerosis obliterans, is well known. In the present study, we focused on the effects of removing oxidized LDL and pentosidine with the purpose of investigating further preventive effects against arteriosclerotic disease.

Methods: Subjects were 41 patients (31 men, 10 women; mean age, 56.5±9.7 years), 29 with hyper-LDL-cholesterolemia and 12 with borderline hyper-LDL-cholesterolemia, who began DFPP at our clinic from July 2011. Of these, 15 had already been started on medication for dyslipidemia and 15 were under observation with lifestyle guidance. Complications other than dyslipidemia were type 2 diabetes in 18, hypertension in 19, chronic kidney disease in 3, and a history of smoking in 13. A full explanation of the study was provided to the subjects in advance, and written consent was obtained. DFPP was conducted in accordance with the following procedure. Blood was drained from the body via the median cubital vein, and was separated into blood cell and plasma components using a Plasmaflo OP-05W (Asahi Kasei Medical Co., Ltd.) membrane-type plasma separator. Pathogenic substances were then removed from the plasma using a Cascadello EC-50W (Asahi Kasei Medical Co., Ltd.) plasma component separator. Heparin was used as an anticoagulant. Blood flow was 30-60 mL/min, plasma flow was 30-33%, and the target plasma treatment volume was taken to be 1,500-2,000 ml.

Results: There were significant decreases after DFPP in both LDL cholesterol (129.4±26.6 mg/dl → 81.7±21.9 mg/dl) and triglycerides (263.9±113.1 mg/dl → 97.6±59.7 mg/dl) (p<0.001). The decrease rate was 36.7±10.39% for LDL cholesterol and 63.8±16.9% for triglycerides. Of the 25 patients with oxidized LDL, improvements were seen in 21 (85.5±26.3 mg/dl → 65.4±26.0 mg/dl, decrease rate 21.8±24.2%, p<0.009). Pentosidine decreased from 129.4±29.7 pg/ml to 100.7±24.1 pg/ml (decrease rate 21.3±12.9%, p<0.001). Decreases in high-sensitivity CRP were seen in all patients (0.188±0.312 mg/dl → 0.129±0.228 mg/dl, decrease rate 36.7±10.39%, p<0.001). The only adverse event immediately after DFPP was a complaint of mild malaise in one patient.

Discussion: Oxidative and glycation stress was reduced by DFPP, resulting in decreases in inflammation and risk factors for arteriosclerosis, which suggests the possibility of a preventive effect against arteriosclerosis.

KEY WORDS: Oxidative stress, glycation stress, double filtration plasmapheresis (DFPP), LDL cholesterol, pentosidine

Introduction

Medical institutions that practice Anti-Aging Medicine usually evaluate functional age based on muscular age, vascular age, neurological age, hormonal age and bone age 1). The main objectives of Anti-Aging Medicine are to prevent aging and promote rejuvenation in terms of functional age. To maintain a young vascular age, it is important to prevent the onset of arteriosclerosis. The four major risk factors for arteriosclerosis are smoking 2) (which causes oxidative stress), hypertension, diabetes 3,4) (which causes glycation stress) and dyslipidemia. From the perspective of prevention and treatment of arteriosclerosis, it is important to eliminate the risk factors for arteriosclerosis, as well as to decrease low density lipoprotein-cholesterol (LDL-C). Among the different modes of apheresis (methods to remove blood components), double filtration plasmapheresis (DFPP) 3) most potently eliminates LDL-C 6-11). The clinical effectiveness of DFPP for arteriosclerotic disease, typified by arteriosclerosis obliterans, is well known.

Glycation stress has attracted attention for its association with physical aging and onset of age-related diseases via accumulation of advanced glycation end products (AGEs), diminished physiological activities of enzymes and hormones, and the induction of inflammation through receptors for AGEs 1,4).
In the present study, we investigated the effects of removing high-sensitivity C-reactive protein (CRP), which is an inflammatory marker, oxidized LDL, which is an oxidative stress marker, and pentosidine, which is a glycation stress marker, with the purpose of studying their possible preventive effects against arteriosclerotic disease. We herein report our findings.

Subjects and methods

Subjects

Methods: Subjects were 41 patients (31 men, 10 women; mean age, 56.5±9.7 years); 29 with intractable hyper-LDL-cholesterolemia and 12 with borderline hyper-LDL-cholesterolemia, who have undergone DFPP since July 2011 at our clinic. Fifteen of these patients were treated with oral medication, and had some of the following complications. Eighteen had type 2 diabetes, 19 had hypertension, 13 had a history of smoking, 2 had anamnesis or cerebrovascular event (cerebral infarction, myocardial infarction) and 3 had stage III chronic kidney disease (CKD) (eGFR 50.2/47.3/42.4%). There were no malignant tumors or autoimmune diseases. Of the 18 patients with diabetes, serum pentosidine was measured in 9 (8 men, 1 woman; age, 62.4±7.1 years). Prior to enrollment, written consent was obtained from each subject.

Double-filtration plasmapheresis (DFPP)

DFPP was conducted as described previously. Blood was drained from the body via the median cubital vein, and separated into blood cell and plasma components using a Plasmaflo OP-05W (Asahi Kasei Medical Co., Ltd., Chiyoda-ku, Tokyo, Japan) membrane-type plasma separator. Pathogenic substances were removed from the isolated plasma using a Cascadeflo EC-50W (Asahi Kasei Medical Co., Ltd.) plasma component separator, and plasma and blood cells were returned to the subject. An ACH Σ (Asahi Kasei Medical Co., Ltd.) blood purification device equipped with an extracorporeal circulation pump was used for blood and plasma circulation.

Evaluation method

Subjective and objective symptoms, physical findings and hematology/urinalysis were evaluated before and after DFPP. Subjective symptoms were classified as either “physical symptoms” or “mental symptoms,” and evaluated on a 5-point scale using the Anti-Aging QOL Common Questionnaire (AAQol) as described in previous reports. Subject body weight was measured using a scale. Blood biochemistry was analyzed at BML Inc. (Shibuya-ku, Tokyo, Japan).

Statistical analysis

Data are expressed as means ± standard deviation. Changes in test values before and after treatment were compared by statistical analysis using paired t-test. The statistical analysis software SPSS II (IBM Japan, Ltd., Minato-ku, Tokyo, Japan) was used for statistical analysis. The significance level was set at less than 5% risk percentage on two-sided test.

Ethical standards

The present study was conducted at the authors’ clinic in compliance with ethical principles based on the Helsinki Declaration and the Personal Information Protection Act, with reference to the “Ministerial Ordinance on Good Clinical
Practice” (Ordinance No. 28 of the Ministry of Health and Welfare (MHW), March 27, 1997). A meeting of our hospital’s internal ethics committee was held to discuss the ethics and validity of this study. The present study was conducted under the approval of the ethics committee of our clinic, and in accordance with the approved study protocol.

**Results**

Subjective symptoms based on the Anti-Aging QOL Common Questionnaire

There were no items that improved significantly in the group after DFPP among the physical symptoms on the common questionnaire (Table 1). Similarly, no significant changes were observed for mental symptoms or lifestyle.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Change of physical symptoms (Anti-Aging QOL Common Questionnaire) (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
</tr>
<tr>
<td>Tired eyes</td>
<td>3.4 ± 1.0</td>
</tr>
<tr>
<td>Cough and sputum</td>
<td>2.3 ± 1.0</td>
</tr>
<tr>
<td>Bleediness</td>
<td>2.4 ± 1.0</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>2.0 ± 1.2</td>
</tr>
<tr>
<td>Eye pain</td>
<td>2.3 ± 1.1</td>
</tr>
<tr>
<td>Constipation</td>
<td>1.7 ± 1.0</td>
</tr>
<tr>
<td>Stiff shoulders</td>
<td>3.6 ± 1.3</td>
</tr>
<tr>
<td>Gray hair</td>
<td>2.9 ± 1.2</td>
</tr>
<tr>
<td>Arthralgia/stiffness</td>
<td>3.0 ± 1.4</td>
</tr>
<tr>
<td>Hair loss</td>
<td>2.7 ± 1.1</td>
</tr>
<tr>
<td>Palpitation</td>
<td>1.7 ± 0.8</td>
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<tr>
<td>Headache</td>
<td>2.4 ± 1.1</td>
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<tr>
<td>Shortness of breath</td>
<td>1.9 ± 1.1</td>
</tr>
<tr>
<td>Dizziness</td>
<td>1.9 ± 1.5</td>
</tr>
<tr>
<td>Tendency to gain weight</td>
<td>3.6 ± 0.8</td>
</tr>
<tr>
<td>Tinnitus</td>
<td>2.1 ± 1.5</td>
</tr>
<tr>
<td>Weight reduction</td>
<td>1.1 ± 0.4</td>
</tr>
<tr>
<td>Lumbar pain</td>
<td>2.7 ± 1.5</td>
</tr>
<tr>
<td>Languor</td>
<td>2.7 ± 1.1</td>
</tr>
<tr>
<td>Arthralgia</td>
<td>2.1 ± 1.1</td>
</tr>
<tr>
<td>No feeling of good health</td>
<td>2.7 ± 0.8</td>
</tr>
<tr>
<td>Swelling</td>
<td>2.1 ± 1.1</td>
</tr>
</tbody>
</table>

Physical findings and blood vessel function test

No significant differences were seen before and after DFPP in body weight, body mass index (BMI) and blood pressure (Table 2).

On peripheral blood tests, there was a significant elevation in white blood cell count (WBC) after DFPP (p<0.001). On biochemical tests, there were significant changes in total protein (TP) (p<0.001), albumin (Alb) (p<0.001), CPK (p=0.014) and amylase (AMY) (p<0.001), all of which decreased with DFPP. Among the lipids, LDL-C decreased from 129.4±26.6 mg/dl before DFPP to 81.7±21.9 mg/dl afterward (p<0.001), with a mean clearance rate of 36.7±10.3% (Fig. 1). Triglycerides decreased from 263.9±113.1 mg/dl before DFPP to 97.6±59.7 mg/dl afterward (p<0.001), with a mean clearance rate of 63.8±16.9% (Fig. 2). HDL-C decreased from 48.8±14.0 mg/dl to 39.8±11.1 mg/dl. Oxidized LDL-C decreased from 85.5±26.3 mg/dl to 65.4±26.0 mg/dl (p=0.009), with a mean clearance rate of 21.8±24.2% (Fig. 3). For subclass analysis, subjects were divided into 2 groups based on oral anti-dyslipidemia drug administration. However, there were no significant differences in the clearance rate of LDL-C between subjects taking anti-dyslipidemia drugs (35.8±9.0%, n=26) and those not taking anti-dyslipidemia drugs (35.8±9.4%, n=26) and those not taking anti-dyslipidemia drugs (37.8±10.3%, n=15). In addition, there were no significant differences in the clearance rate of LDL-C between male (35.8±9.4%, n=26) and female subjects (38.6±11.8%, n=10).

In the tests of the nine type 2 diabetes patients, pentosidine decreased from 129.4±29.7 pmol/ml before DFPP to 100.7±24.1 pmol/ml afterward, with a mean clearance rate of 21.3±12.9% (Fig. 1). The HbA1c clearance rate in diabetic subjects was not significantly different from that without diabetes, although the number of diabetic subjects was fewer.

In the inflammatory response test, CRP decreased from 0.9±1.0%, n=4) was not significantly different from that without diabetes, although the number of diabetic subjects was fewer.

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Fig. 1. Change in LDL-C

***p<0.001, paired t test, n=41.

Fig. 2. Change in TG

***p<0.001, paired t test, n=41.
**Removal of Oxidized LDL-cholesterol and Pentosidine by DFPP**

**Fig. 3.** Change in oxidized LDL

*\( **p=0.009, \) paired \( t \) test, \( n=41 \).

**Fig. 4.** Change in pentosidine

*\( **p<0.001, \) paired \( t \) test, \( n=9 \) ([diabetes as complication]).
Removal of Oxidized LDL-cholesterol and Pentosidine by DFPP

Discussion

In the present study, we investigated the effects of DFPP on reducing LDL-C, oxidative stress and glycation stress, which are risk factors for arteriosclerosis, for the purpose of evaluating the possibility that DFPP has a preventive effect on arteriosclerotic diseases. DFPP was performed in a total of 41 patients; 29 with hyper-LDL-cholesterolemia and 12 with borderline hyper-LDL-cholesterolemia. Reduction of LDL-C, triglycerides, oxidized LDL and pentosidine was observed, and high-sensitivity CRP was reduced in all patients. As a result of lowering oxidative and glycation stress with DFPP, inflammation was reduced and a decrease in risk factors for arteriosclerosis was observed. Based on the above, DFPP is expected to have preventive effects against arteriosclerosis.

DFPP has previously been reported to decrease serum LDL-C 22-26) and oxidized LDL 16-27), but this is the first report on its effectiveness in removing AGEs such as pentosidine.

Involvement of glycation stress in arteriosclerosis

Degenerative changes occur in the blood vessels with aging, leading to arteriosclerosis. Arteriosclerosis is a state of deterioration in which arteries lose elasticity, and it is pathologically divided into three major types: atherosclerosis, Mönckeberg’s medial sclerosis, and fibrosis of the vascular walls (Fig. 6) 29).

Atherosclerosis is produced by deposition of a mixture of cholesterol, calcium (Ca) and inflammatory cells called atheromas in the intima 29). Atheromas consist of macrophages, smooth muscle cells, extracellular matrix and elastic fibers. Macrophages contribute to the progression of these lesions, when LDL-C is converted to modified LDL-C from the effects of oxidative stress or glycation stress, followed by phagocytosis via macrophage scavenger receptors 3). Macrophages that contain large volumes of lipid droplets are called foam cells, and accumulate in the lesions. Ca deposits and fibrosis also progress, narrowing the vessel lumen. Dyslipidemia (high LDL-cholesterol), smoking (oxidative stress) and diabetes (glycation stress) are major risk factors for arteriosclerosis.

Oxidized LDL is the generic term for LDL that has undergone oxidative degradation, and LDL in which lipid peroxidation products modify the major LDL protein apo B is oxidized LDL 16). Malondialdehyde (MDA) has been identified as a typical lipid peroxidation product, and LDL in which apo B is modified by MDA is known as malondialdehyde-modified LDL (MDA-LDL). In a study of diabetes mellitus (DM) patients treated with percutaneous coronary intervention (PCI), an investigation into the relationship between MDA-LDL value before PCI and restenosis after PCI showed that MDA-LDL was higher in the restenosis group than in the no restenosis group, and that the risk of restenosis was higher, with a relative risk of 5.3, when MDA-LDL was ≥110 U/l than when MDA-LDL was <110 U/l 18). MDA-LDL and other oxidized LDL are thought to be related to the progression of arteriosclerosis and the onset of coronary artery disease 17,18,30,31).

Wnt signaling is an important intracellular signaling pathway involved in fibrosis of the vascular wall 32). Myoblasts and fibroblasts are differentiated from a common progenitor cell, and when Wnt signaling is activated, differentiation into fibroblasts is accelerated and fibrosis progresses. The Klotho
gene and translated Klotho protein are involved in various aging phenomena. It has been reported that muscle fibrosis is inhibited when Wnt signal inhibitor DKK1 is administered to senescent mouse muscles, and that Klotho binds directly to Wnt and inhibits Wnt activity. As inflammatory cytokines and oxidative stress are known to be factors that activate Wnt signaling, it is possible that glycation stress stimulates Wnt signaling via the mechanism of AGEs leading to RAGE activation, which in turn leads to an increase in inflammatory cytokines.

Calcification is an age-related change in vascular walls. When the product of serum Ca × phosphorus rises as a result of kidney dialysis or excess phosphorus intake, the Ca metabolism in blood vessels changes due to the occurrence of secondary hyperparathyroidism with elevated phosphorus and low bone turnover with elevated Ca. Klotho protein functions as an inhibitory factor of Ca metabolism. It responds to decreased Ca, inducing Na+, K4+-ATPase recruitment to cell surfaces, Ca reabsorption in renal tubules, and PTH secretion from the parathyroid. When Klotho is impaired at the gene or protein synthesis level, Ca mobilization from bone, serum phosphorus and Ca elevation, and calcification of kidney and vascular wall becomes pronounced. AGEs promote calcification of cultured vascular smooth muscle cells and medial calcification via RAGE (receptor for AGEs).

Significance of DFPP

Removal by DFPP

There are a number of risk factors for cardiovascular events, such as LDL-C, oxidative stress, and glycation stress, and the risk is multiplied with increases in the number of factors. DFPP, in addition to its effect of removing LDL-C, can reduce multiple risk factors through the reduction of oxidative stress. Excess accumulation of LDL-C produces inflammation via macrophage system cells, and may accelerate development of atherosclerosis or progression to non-alcoholic steatohepatitis (NASH) from simple fatty liver. It has been reported that removal of cholesterol with DFPP may be able to break this negative cycle. Among AGEs, pentosidine strongly induces NF-κB, an inflammatory cytokine transcription factor. Consequently, the pentosidine reduction by DFPP in this study inhibited the production of inflammatory cytokines and reduced CRP. The number of cardiovascular event risk factors can be further reduced by the relief of glycation stress and suppressing inflammatory responses, and DFPP may thus be expected to contribute greatly to inhibiting cardiovascular events. In addition, the present finding of significant reductions in serum pentosidine, a potential risk factor of osteoporosis, indicates that DFPP may contribute to preventing deterioration of bone quality such as osteoporosis induced by diabetes.

Adverse events

Apheresis therapies such as DFPP have been mostly performed for life-saving purposes in cases of serious liver disease, such as fulminant hepatitis and acute liver failure, serious pancreatitis and renal diseases. In that respect, they have been established as a safe therapy. Adverse events reportedly include nausea, vomiting, decreased blood pressure, yawning, sweating, cold sweat, tachycardia, fever and Ca loss, but these symptoms are mostly transient. We have seen no critical adverse events in our clinic.

Our previous report showed, with regard to the
clinical features of cases receiving DHPP, that serum LDL-C decreases just after DFPP, and within 1–2 weeks, returns back to or exceeds baseline levels. This is known as the rebound phenomenon. However, during the period with unchanged or increased serum LDL-C values, as LDL-C is undoubtedly removed from the body with improvement of inflammation, fatty liver and visceral fat status improves. It therefore remains necessary to increase the number of cases and extend the surveillance term in order to better understand these phenomena.

**Conclusion**

The reductions in inflammatory markers and oxidized LDL by DFPP suggest that DFPP decreases the risk factors of arteriosclerosis and may have preventive effects against arteriosclerosis. A significant reduction in pentosidine, a glycation stress marker, was observed, which may suggest a secondary improvement mechanism via anti-inflammatory effects in addition to the one of direct removal.

**Conflict of interest statement**

In conducting the present study, the Anti-Aging Research Center, Graduate School of Life and Medical Sciences, Doshisha University received a grant from Asahi Kasei Medical Co., Ltd.

A summary of this study was presented at the 12th Scientific Meeting of the Japanese Society of Anti-Aging Medicine (2012).

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