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Therapeutic Potential of Low-Density Lipoprotein Apheresis in the Management of Peripheral Artery Disease in Patients With Chronic Kidney Disease

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Abstract: Cardiovascular disease (CVD) is a major cause of death in patients with chronic kidney disease (CKD). Patients with CKD are reported to have a significant greater risk of CVD-associated mortality than that of the general population after stratification for age, gender, race, and the presence or absence of diabetes. CKD itself is also an independent risk factor for the development of atherosclerosis, and in particular, patients undergoing dialysis typically bear many of the risk factors for atherosclerosis, such as hypertension, dyslipidemia and disturbed calcium-phosphate metabolism, and commonly suffer from severe atherosclerosis, including peripheral arterial disease (PAD). Low-density lipoprotein (LDL) apheresis is a potentially valuable treatment applied to conventional therapy-resistant hypercholesterolemic patients with coronary artery disease and PAD. Although previous and recent studies have suggested that LDL apheresis exerts beneficial effects on the peripheral circulation in dialysis patients suffering from PAD, probably through a reduction of not only serum lipids but also of inflammatory or coagulatory factors and oxidative stress, the precise molecular mechanisms underlying the long-term effects of LDL apheresis on the improvement of the peripheral circulation remains unclear and warrants further investigation. **Key Words:** Chronic kidney disease, Hemodialysis patients, Low-density lipoprotein apheresis, Oxidative stress, Peripheral artery disease.

Cardiovascular disease (CVD) has a major effect on the prognosis of patients with chronic kidney disease (CKD), particularly for those patients on dialysis, and atherosclerotic vascular changes play a critical role. Renal deterioration in CKD promotes hypertension, dyslipidemia, insulin resistance, disturbed calcium-phosphate metabolism and renal anemia, and these are all risk factors for atherosclerosis. In addition, chronic inflammation, oxidative stress and variability in blood pressure and circulating blood volume also promote atherosclerotic vascular changes in dialysis CKD patients. Among the systemic atherosclerotic vascular diseases, peripheral arterial disease (PAD) is prevalent in dialysis CKD patients. In dialysis CKD patients, the PAD lesions are prone to being distributed in the arteries of the lower limbs and exhibit a severely stenotic lumen

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with vascular calcification without the development of any collateral circulation, and patients with CKD and PAD have higher mortality rates than those with either of these conditions alone (1).

Percutaneous transluminal angioplasty (PTA) for the treatment of PAD is reported to suffer from the problem of an increased rate of restenosis in dialysis CKD patients (2). PAD in HD patients is often therapy resistant and closely associated with an increased risk of cardiovascular mortality, morbidity, and hospitalization as well as a reduced Health-Related Quality of Life (HRQOL), even after bypass surgery and leg amputation (3,4). In certain countries, including most of the countries in Europe, LDL apheresis is the treatment of choice in patients with homozygous familial hypercholesterolemia (FH), particularly those refractory to statins, as described in a recent review (5). LDL apheresis has been shown to exert beneficial effects on aortic and coronary atherosclerosis and to reduce the risk of coronary artery disease in patients with homozygous FH (6,7). Recently LDL apheresis has become the adjuvant treatment of choice for dialysis CKD patients with PAD, particularly those refractory to statins and on whom it is difficult to perform PTA or bypass surgery (8,9). In this review we briefly summarize the clinical applications of LDL apheresis in Japan and mechanistic basis for its benefit in PAD in dialysis patients.

PRESENT INDICATION OF LDL APHERESIS FOR THERAPY OF PAD IN JAPAN

In Japan, indicated usage of LDL apheresis for the treatment of PAD under the government system of insurance coverage, includes the following. (i) clinical signs of poor peripheral circulation, such as cold, decolored or ulcerated extremities, or intermittent claudication consistent with a Fontaine classification of Grade II or more; (ii) refractoriness to conventional medical or surgical treatment; (iii) an excessively high LDL cholesterol (LDL-C) or total cholesterol (TC) levels (LDL-C >140 mg/dL or TC >220 mg/dL) in spite of drug treatment (10). Japanese government insurance coverage permits 10 sessions of LDL apheresis for each patient to be carried out during a 3-month period.

THE PATHOGENESIS OF LIPOPROTEIN ABNORMALITIES IN CKD

Although hypercholesterolemia is a major requisite for the governmental insurance-covered application of LDL apheresis for the treatment of PAD in Japan, the regulatory system of lipid metabolism is reported to be highly disturbed in CKD patients, owing to alterations in apolipoproteins, lipid transfer proteins, lipolytic enzymes and lipoprotein receptors (11,12). Previous studies demonstrated that LDL particles are heterogeneous with respect to their size, density and lipid composition (13,14). Among the LDL particles, the smaller and denser LDL particles (small dense low-density LDL particles) are more atherogenic (13), and the small dense low-density LDL phenotype is strongly associated with the development of coronary heart disease (15).

In CKD patients, triglyceride concentrations increase while HDL cholesterol (HDL-C) concentrations decline, and there is a progressive accumulation of the more atherogenic, small dense low-density LDL particles, in spite of low-to-normal TC and LDL-C levels (11). In addition, in dialysis CKD patients, dyslipidemia is typified by a marked increase in triglyceride-rich apo B-containing particles, a decreased HDL concentration and a predominance of small dense LDL particles, with a normal LDL-C level but an increased lipoprotein(a) (Lp[a]) concentration. Also, there is reportedly a persistent disturbance in the apolipoprotein profile, with reduced apo AI and apo AII concentrations and significant increases in the apoB, apoCIII and apoE concentrations (11,16).

LDL APHERESIS FOR THE TREATMENT OF PAD

There are presently several systems of LDL apheresis in use, including cascade and lipid filtration, immunoadsorption, heparin-induced LDL precipitation, dextran sulfate LDL adsorption, and the LDL hemoperfusion (17,18). In Japan, LDL apheresis therapy using the strategy of LDL absorption with dextran sulfate (Liposorber LA-15, Kaneka, Japan) is most commonly performed (19,20). Low-molecular dextran sulfate (MW 4500) selectively absorbs all substances containing apoB. The binding mechanism is the direct interaction between the dextran sulfate and the positively charged surface of apoB-containing lipoproteins (LDL-C, very low density lipoprotein-cholesterol [VLDL-C], and Lp[a]). Dextran sulfate has a structure similar to that of the LDL receptor and seems to act as a type of pseudoreceptor. Approximately 2.5 g LDL-C can be bound per column. After primary separation, the plasma is perfundated through the columns, where all material containing apoB such as cholesterol, LDL-C, VLDL-C, and triglycerides is absorbed, but without any absorption of HDL-C, which does not contain apoB (18).

In our hospital, LDL apheresis is performed using hollow polysulfone fibers (Sulflux, Kaneka, Osaka, Japan) as the plasma separator and a dextran sulfate cellulose column (Liposorber LA-15, Kaneka) as the LDL absorber. Blood flow from the A-V fistula access in the case of dialysis CKD patients is typically in the range of 80–100 mL/min, the plasma flow is 25–30 mL/min, and 3000–4000 mL of the plasma volume is treated per session. Heparin or nafamostat mesilate is given as an anticoagulant for extracorporeal circulation. To maintain good adsorption efficiency with an increasing quantity of plasma treated, two columns arranged in a row are used in turn automatically, so that one column can be washed with the specific liquid and regenerated after the treatment of the plasma while the other is in operation (MA-03 system, Kaneka) (21,22). LDL apheresis is carried out once or twice a week on non-HD days in the case of dialysis CKD patients, and 10 sessions of apheresis are performed in each patient.

THERAPEUTIC EFFECTS OF LDL APHERESIS IN DIALYSIS CKD PATIENTS WITH PAD

Although dialysis CKD patients with PAD often exhibit low-to-normal TC and LDL-C levels along with increased triglycerides and reduced HDL-C levels, previous studies showed that LDL apheresis by the dextran sulfate cellulose column (Liposorber LA-15, Kaneka) is clinically effective even in PAD patients undergoing HD (23–28). Since PAD patients undergoing HD tend to be resistant to any treatment and are at high risk for lower-extremity amputation, LDL apheresis is suggested to be a useful strategy in the multidisciplinary approach for therapy of PAD (Fig. 1).

In a recent clinical study we conducted, 25 dialysis CKD patients with PAD were enrolled, and the therapeutic effects of LDL apheresis in 19 patients were ultimately analyzed (22). Blood samples were collected before and after the first session, at the start of the 10th session, and at 3 months after the end of treatment (before regular HD). The absolute walking distance and ankle-brachial pressure index (ABI) were principally estimated on non-HD days prior to the 1st and 10th sessions and at 3 months after the end of treatment, and the long-term periods in this study were defined as the time from the 1st session to the 10th session and the time from the 1st session up to the third month after the end of the 10th sessions.

Because most of these patients were unable to perform treadmill exercise because of conditions such as a previous heart attack or paralysis, the absolute walking distance was evaluated by medical staff on a flat floor in the hospital. Of the 25 patients enrolled, five patients could not complete the study because of death ($N = 3$), amputation ($N = 1$) or PTA ($N = 1$) during the study period, and they were excluded from the analysis. On the whole, the absolute walking distance improved significantly by the 10th session of LDL apheresis compared with baseline and was still improved even at 3 months after the end of the treatment (Table 1) (22). Similarly, the ABI was improved by the 10th session compared with baseline (Table 1). Subsequently, the patients were classified into two groups according to the changes in the ABI at 3 months after the end of

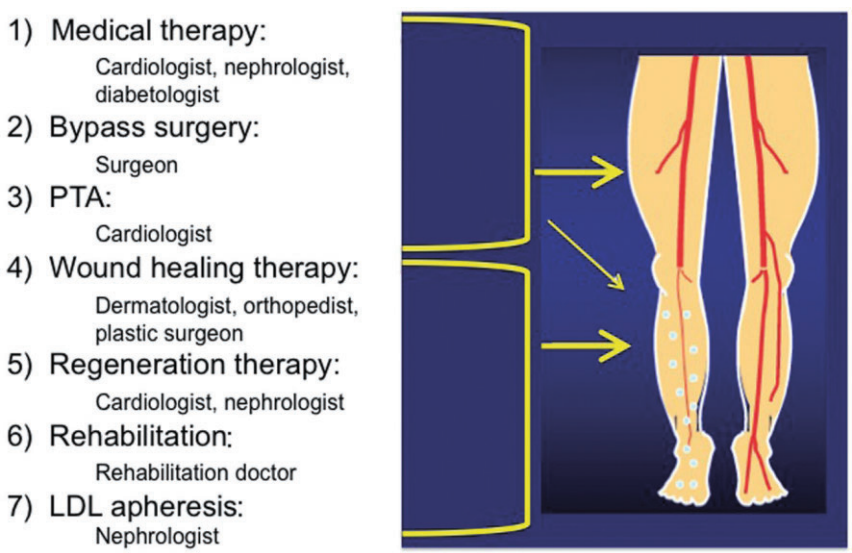

FIG. 1. Multidisciplinary therapeutic approach to peripheral arterial disease (PAD) in Japan.

TABLE 1. *Therapeutic effects of low density lipoprotein (LDL) apheresis in dialysis chronic kidney disease (CKD) patients with peripheral arterial disease (PAD)*

Clinical parameters	Baseline	After 1st apheresis	At 10th apheresis	3 months after 10th apheresis	*P1*	*P2*	*P3*
Total patients ($N = 19$):							
Walking distance (m)	171 ± 33	N/A	294 ± 34	270 ± 42	N/A	<0.05	<0.05
ABI	0.59 ± 0.04	N/A	0.67 ± 0.04	0.64 ± 0.04	N/A	<0.05	NS
ABI responders ($N = 10$):							
Walking distance (m)	118 ± 26	N/A	333 ± 45	297 ± 63	N/A	<0.05	<0.05
ABI	0.53 ± 0.06	N/A	0.69 ± 0.06	0.69 ± 0.05	N/A	<0.005	<0.005
LDL cholesterol (mg/dL)	88 ± 7	32 ± 3	78 ± 9	98 ± 11	<0.01	NS	NS
Oxidized LDL (U/L)	38 ± 3	20 ± 2	32 ± 3	38 ± 4	<0.01	<0.05	NS
Fibrinogen (mg/dL)	400 ± 14	308 ± 18	337 ± 32	394 ± 35	<0.01	0.07	NS
CRP (mg/dL)	0.87 ± 0.40	0.49 ± 0.20	0.39 ± 0.23	0.75 ± 0.47	<0.05	0.07	NS
ABI non-responders ($N = 9$):							
Walking distance (m)	232 ± 58	N/A	254 ± 49	238 ± 59	N/A	NS	NS
ABI	0.65 ± 0.05	N/A	0.63 ± 0.05	0.59 ± 0.06	N/A	NS	NS
LDL cholesterol (mg/dL)	83 ± 8	26 ± 2	75 ± 10	104 ± 16	<0.05	NS	NS
Oxidized LDL (U/L)	38 ± 5	18 ± 2	32 ± 4	46 ± 6	<0.05	NS	<0.05
Fibrinogen (mg/dL)	388 ± 38	264 ± 28	340 ± 45	427 ± 35	<0.05	NS	NS
CRP (mg/dL)	0.81 ± 0.47	0.34 ± 0.21	1.12 ± 1.02	0.61 ± 0.35	<0.05	NS	NS

Parameters are shown as the mean ± standard error. *P1* indicates the baseline vs. after the 1st apheresis; *P2*, the baseline vs. at the 10th apheresis; *P3*, the baseline vs. 3 months after the 10th apheresis (Modified from Tsurumi-Ikeya Y et al. *Arterioscler Thromb Vasc Biol* 2010;30:1058–1065). ABI, ankle-brachial pressure index; CRP, C-reactive protein; N/A, not applicable; NS, not significant.

treatment. The two groups were patients with an improved ABI (ABI responders, $N = 10$) and patients with a worsened ABI (ABI non-responders, $N = 9$). The serum levels of LDL-C and oxidized LDL, along with the C-reactive protein (CRP) and fibrinogen concentrations were significantly reduced after a single session in both groups. However, in the responders, LDL apheresis showed a trend toward a long-term reduction of the circulating levels of oxidized LDL, CRP, and fibrinogen (Table 1), in addition to a short-term dramatic decrease in the TC and LDL-C levels after each LDL apheresis session (Table 1).

When the baseline parameters of the ABI responders and non-responders were compared in order to analyze factors involved in the therapeutic effects of LDL apheresis, the walking distance as well as ABI tended to be lower in the ABI responders than in the ABI non-responders (walking distance, 118 ± 26 vs. 232 ± 58 m, $P = 0.08$; ABI, 0.53 ± 0.06 vs. 0.65 ± 0.05, $P = 0.12$), thereby suggesting that dialysis CKD patients with severe symptoms of PAD may be afforded a long-term therapeutic benefit by LDL apheresis. However, although the absolute walking distance and ABI still remained significantly improved in the responders 3 months after the tenth apheresis compared to these parameters at baseline, the LDL apheresis-mediated decrease in the oxidized LDL, CRP and fibrinogen concentrations lasted until just after the tenth apheresis, but not at 3 months after the tenth apheresis. Thus, there is a discrepancy between the long-term therapeutic effects of LDL apheresis on the clinical parameters of the walking distance and ABI, and the improvements in the laboratory parameters, including oxidized LDL, CRP and fibrinogen.

Thus, in order to examine the mechanism by which the absolute walking distance and ABI improved, even though the LDL apheresis did not result in a decrease in either oxidized LDL or inflammation at 3 months after the tenth apheresis, additional statistical correlation analyses were performed (Fig. 2) (22). As a result, there were statistically significant correlations between the walking distance and the plasma oxidized LDL ($R = –0.448$, $P < 0.05$, Fig. 2A) and fibrinogen ($R = –0.779$, $P < 0.05$, Fig. 2B) levels in the responders. In the non-responders, there was only a marginal correlation between the walking distance and the plasma oxidized LDL ($R = –0.329$, $P = 0.07$, Fig. 2C). Therefore, the therapeutic effects of LDL apheresis appear to be related to a chronic reduction of oxidized LDL and fibrinogen, as revealed by the significant negative relationships between the walking distance and the laboratory parameters.

Nevertheless, one of the obvious limitations of this study is the small number of enrolled patients. In addition, most of the patients were unable to perform treadmill exercise because of conditions such as previous heart attack or paralysis, but these are two distinctly different pathologies. Thus, although there should be at least four groups, that is, responders and non-responders in patients with heart attack and those with paralysis, respectively, to be strictly correct for the purposes of analysis, this was not possible due

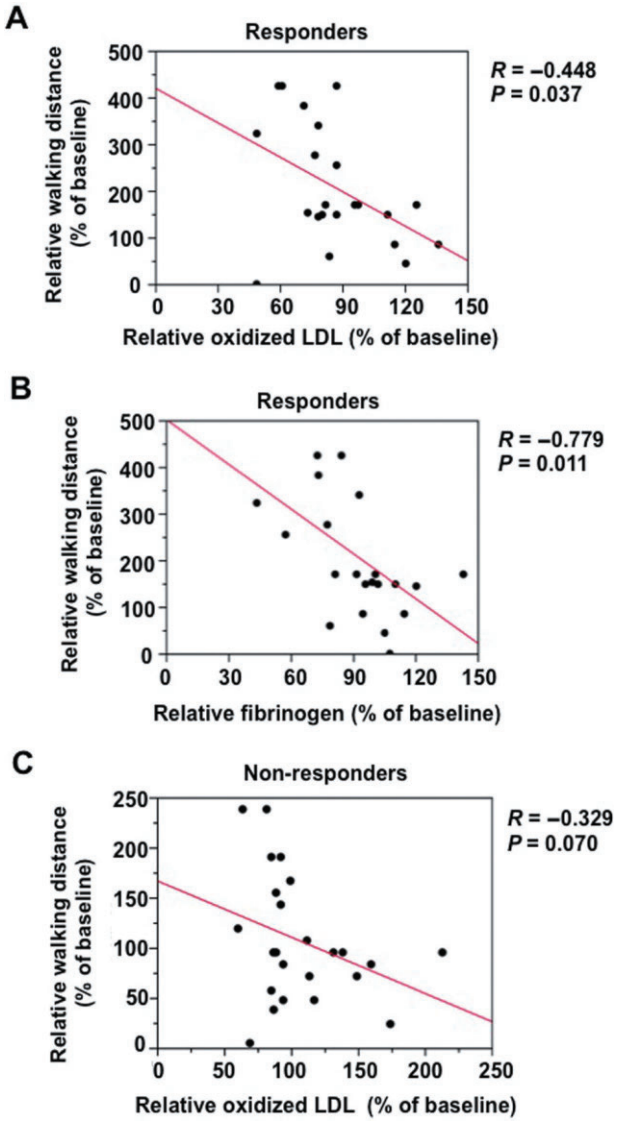

FIG. 2. Relationships between the walking distance (relative walking distance) and plasma oxidized low density lipoprotein (LDL) (relative oxidized LDL) (A), fibrinogen (relative fibrinogen) levels (B) in the responders, and between the walking distance (relative walking distance) and plasma oxidized LDL (relative oxidized LDL) (C) in the non-responders. The respective values were calculated relative to those achieved at baseline in either the responder group or the non-responder group (Modified from Tsurumi-Ikeya Y et al. *Arterioscler Thromb Vasc Biol* 2010; 30: 1058–65).

to the limited number and type of patients available. Furthermore, there is a possibility that other factor(s), which have not been identified in these studies, play a critical role in mediating the long-term therapeutic effects of LDL apheresis in CKD patients with PAD. Also, the components of the non-responders' serum responsible for the insufficient clinical improvement remain to be determined. Therefore, further studies, such as investigations using mass spectrometry, are needed (29).

MECHANISMS INVOLVED IN THE THERAPEUTIC EFFECTS OF LDL APHERESIS IN DIALYSIS CKD PATIENTS WITH PAD

Low density lipoprotein apheresis not only improves clinical symptoms rapidly, but also results in sustained improvement, although the mechanism has not been fully elucidated. Recently, LDL-C crystals in the phagosome of vascular macrophages were shown to directly activate Nod-like receptor family, pyrin domain–containing 3 (NLRP3) inflammasomes and thus to trigger atherogenesis in the early phase (30,31), and previous studies demonstrated that a single LDL apheresis decreased not only the TC and LDL-C concentrations, but also the oxidized LDL-C, CRP and fibrinogen concentrations in the short term (18). Dialysis CKD patients are reportedly characterized by higher levels of oxidative and inflammation than healthy subjects (11,32–34). Oxidative stress and inflammation are correlated strongly with tryglycerides, VLDL-C, apoC-III and apoC-III bound to apoB-containing lipoproteins, but not with either TC or LDL-C (35).

With respect to the long-term effects of LDL apheresis on lipid-related oxidative stress, a previous study has shown both acute and chronic effects of LDL apheresis in lowering the susceptibility of LDL to oxidation in non-CKD patients with severe, genetically determined hypercholesterolemia (36). In our previous study, the therapeutic effects of LDL apheresis were related to the relatively sustained decrease in oxidized LDL and fibrinogen, which are markers of lipid peroxidation and blood coagulation, respectively (Table 1, Fig. 2) (22). Other studies also showed LDL apheresis-mediated reduction of thiobarbituric acid-reactive substances, thiobarbituric acid being a marker of lipid peroxidation, and also a production of reactive oxygen species via the suppression of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase expression in leukocytes in HD patients (28). Therefore, these results suggest that LDL apheresis-mediated suppression of lipid peroxidation is one of the contributing factors to its therapeutic effect on the peripheral circulation in end-stage renal disease patients with PAD. Other potential mechanisms have also been proposed to play a role in the therapeutic effects of LDL apheresis on atherosclerotic vascular lesions (Table 2).

The therapeutic effects of LDL apheresis on the inflammatory profile have also been reported. Stefanutti et al. showed that LDL apheresis resulted in an anti-inflammatory and anti-atherogenic cytokine profile in the plasma of non-CKD patients with

TABLE 2. *Proposed effects of low density lipoprotein (LDL) apheresis*

- Reduction of whole blood and plasma viscosity
- Improvement of red blood cells (deformability)
- Increase of vasodilation factors (bradykinin, NO, PGI_2)
- Reduction of coagulation factors (fibrinogen etc.)
- Reduction of cell adhesion factors (ICAM-1, ELAM-1etc.)
- Reduction of CRP and MMP-9
- Inhibition of platelet activation
- Increase of HGF and endothelial progenitor cells

CRP, C-reactive protein; ELAM-1, endothelial leukocyte adhesion molecule-1 (E-selectin); HGF, hepatocyte growth factor; ICAM-1, intercellular adhesion molecule-1; MMP-9, matrix metalloproteinase-9; NO, nitric oxide; PGI_2, prostacyclin.

severe dyslipidemia and pre-existing angiographically demonstrated atherosclerotic lesions, that is, those patients at the highest level of individual cardiovascular risk (37). In another previous study, several cytokines and complement activation products, which are important for the progression of vascular atherosclerosis as well as plaque instability, were differently affected by the three apheresis columns DL-75 (whole blood adsorption), LA-15 (plasma adsorption), and EC-50W (plasma filtration) (38). This was true even in cases in which the LDL-C was reduced equally by all of them, and the adsorption columns displayed an apparently more beneficial inflammatory profile than the filtration device (38).

Vascular endothelial cells play important preventive roles against the development of atherosclerotic vascular disease (39). Previous studies showed that a single LDL apheresis session enhanced the peripheral microcirculation, probably by increasing the production of nitric oxide (NO) and bradykinin (40), reducing blood viscosity and adhesion molecules (41), and inducing endothelium-dependent vasodilatation (42). Another study demonstrated that endothelium-dependent vasodilation was significantly increased even 4 weeks after the final LDL apheresis in dialysis CKD patients with PAD (26). Thus, to investigate the molecular mechanism involved in the long-term therapeutic effects of LDL apheresis on endothelial cells, we examined the effects of LDL apheresis on vascular endothelial cell functions in vitro by analyzing the expression of the activated form of endothelial nitric oxide synthase (eNOS), which is phosphorylated at Ser-1177 (43), and cellular proliferative activity (22). The expression of the activated eNOS protein in human umbilical vein endothelial cells (HUVECs) was significantly increased by incubation with the serum from the responders at the 10th session compared with the serum collected after the first apheresis (22). Furthermore, the proliferative activity of HUVECs was increased by the serum collected from the responders at 3 months after the end of treatment (22). Collectively, these results suggest that the therapeutic effects of LDL apheresis on CKD patients with PAD are at least partly dependent on the sustained reduction of oxidized LDL-C and fibrinogen, along with the activated eNOS-mediated improvement of endothelial cell function (Fig. 3). Because the

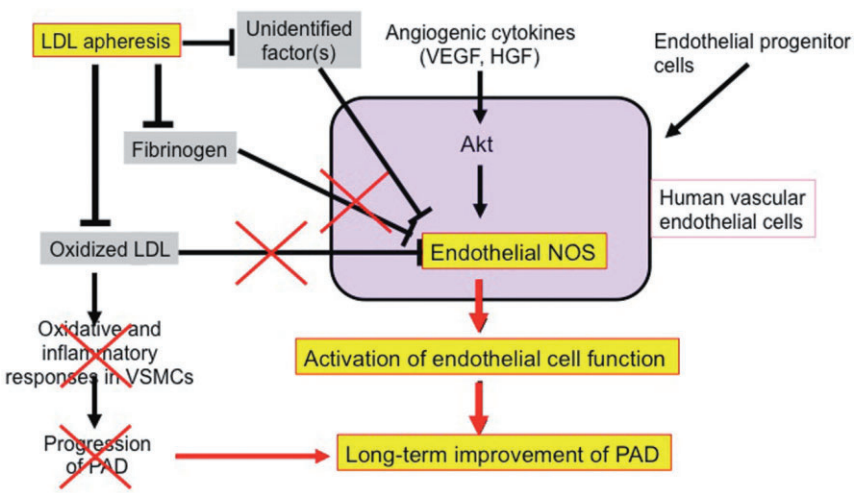

FIG. 3. Low density lipoprotein (LDL) apheresis exerts long-term therapeutic effects on peripheral arterial disease (PAD) in chronic kidney disease (CKD) patients, at least partly via a sustained reduction of oxidized LDL and fibrinogen, along with an activated endothelial nitric oxide synthase (eNOS)-mediated improvement of endothelial cell function. There is a possibility that other factor(s), which have not been identified yet, may play a critical role in mediating the therapeutic effects of LDL apheresis on endothelial cellular function. Further efforts, such as microarray analysis, are needed to identify the precise molecular mechanism of the LDL apheresis-mediated effects on endothelial cells and to improve the therapeutic efficacy of LDL apheresis. HGF, hepatocyte growth factor; PAD, peripheral arterial disease; VEGF, vascular endothelial growth factor; VSMCs, vascular smooth muscle cells.

activation of endothelial cells is an important strategy for the amelioration of the atherosclerotic vascular process (39) and there is a possibility that other factor(s), which have not been identified yet, play a critical role in mediating the therapeutic effects of LDL apheresis on endothelial cellular function, further investigative efforts, such as microarray analysis, should be used to identify the precise molecular mechanism of the LDL apheresis-mediated effects on endothelial cells and to improve the therapeutic efficacy of LDL apheresis (44).

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