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V. 研究成果の刊行物・別刷

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Modulation of Smooth Muscle Cell Migration by Members of the Low-Density Lipoprotein Receptor Family

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Modulation of Smooth Muscle Cell Migration by Members of the Low-Density Lipoprotein Receptor Family

Hideaki Bujo, Yasushi Saito

Abstract—Low-density lipoprotein receptor family members (LRs) play a key role in the catabolism of many membrane-associated proteins, such as complexes between proteinases and their receptors, in addition to being involved in lipoprotein metabolism as suspected by the hitherto well-established functions of low-density lipoprotein receptor, in a variety of tissues. Recent studies using receptor-deficient or -overexpressing animals and cells have suggested that certain LRs are important regulators of the migration (and proliferation) of vascular smooth muscle cells (SMCs). LR expression is markedly induced in intimal or medial SMCs during the formation of atherosclerotic lesions. Because LRs can modulate the activity of the urokinase-type plasminogen activator (uPA) receptor and possibly of the platelet-derived growth factor (PDGF) receptor, LRs may influence the migration of SMCs through functional modulation of these membrane receptors. Therefore, SMC migration may be regulated by time-restricted expression of LRs. In agreement with the concept of functional interaction between LRs and membrane signaling receptors, a negative regulator of uPA receptor protein catabolism, LR11, has been identified. Statins modulate the PDGF-induced migration of intimal SMCs via the LR11/uPA receptor cascade. Selective modification of the LRs/uPA receptor/PDGF receptor systems in SMCs may be important for suppression of atherosclerotic plaque formation as well as for preventing intimal thickening after angioplasty. (***Arterioscler Thromb Vasc Biol.* 2006;26:1246-1252.**)

Key Words: LDL receptor family ■ smooth muscle cells ■ migration ■ LR11 ■ urokinase-type plasminogen activator receptor ■ PDGF receptor

The members of the low-density lipoprotein receptor family (LRs) are characterized by distinct functional domains present in characteristic numbers and arrangements (Figure 1). The common structural domains in most LRs are the so-called low-density lipoprotein (LDL) receptor ligand binding repeats (type A), epidermal growth factor precursor homology repeats (type B1 and B2), epidermal growth factor precursor homology repeats with a consensus tetrapeptide, Tyr-Trp-Thr-Asp, and in the cytoplasmic region, signals for receptor internalization via coated pits. These LRs discovered to date are the LDL receptor, LDL receptor-related protein-1 (LRP-1), megalin, the very low-density lipoprotein (VLDL) receptor/LR8, apolipoprotein E receptor 2/LR8B, LR11, and, most recently, LRPs 3 through 7.[1–3] LRP-1 and megalin are giant LRs in which the amino acid sequence contains multiple repeats of each functional component of the LDL receptor.[4,5] The domain structures of VLDL receptor/LR8 and apolipoprotein E receptor 2/LR8B are most similar to that of the LDL receptor.[6–8] LRs indeed show considerable sequence identity (70% to 100%) between molecules harboring common structures and among a wide range of species. Such sequence conservation is thought to indicate evolution from an ancestral gene by duplication or exon shuffling. The avian VLDL receptor/LR8 is essential for reproduction as a receptor for the yolk accumulation.[8,9]

LRs play a key role in lipoprotein metabolism, as demonstrated by the well-established actions of the LDL receptor in a variety of tissues.[1] Extensive functional analyses have also revealed that LRs play an important role in the catabolism of many membrane-associated proteins such as complexes between proteinases and their receptors.[1–3] Recent studies using receptor-deficient or -overexpressing animals and cells have suggested that certain LRs are also important as regulators of the migration (and proliferation) of various cells such as fibroblasts, neurons, and vascular smooth muscle cells (SMCs).[10–17]

Histochemical studies have revealed that the expression of LRs, as well as scavenger receptors, is markedly induced during the development of atherosclerotic lesions.[1,18] For instance, the VLDL receptor/LR8 is highly expressed by SMCs, macrophages, and endothelial cells in rabbit atherosclerotic lesions, whereas the LDL receptor is not abundant in arterial walls.[18,19] LRP-1 expression is also induced in atheromatous plaques.[18–20] We identified strong LR11 expression inside plaques, particularly by intimal SMCs located at the interface between intima and media.[21,22] In addition, LRP-1B

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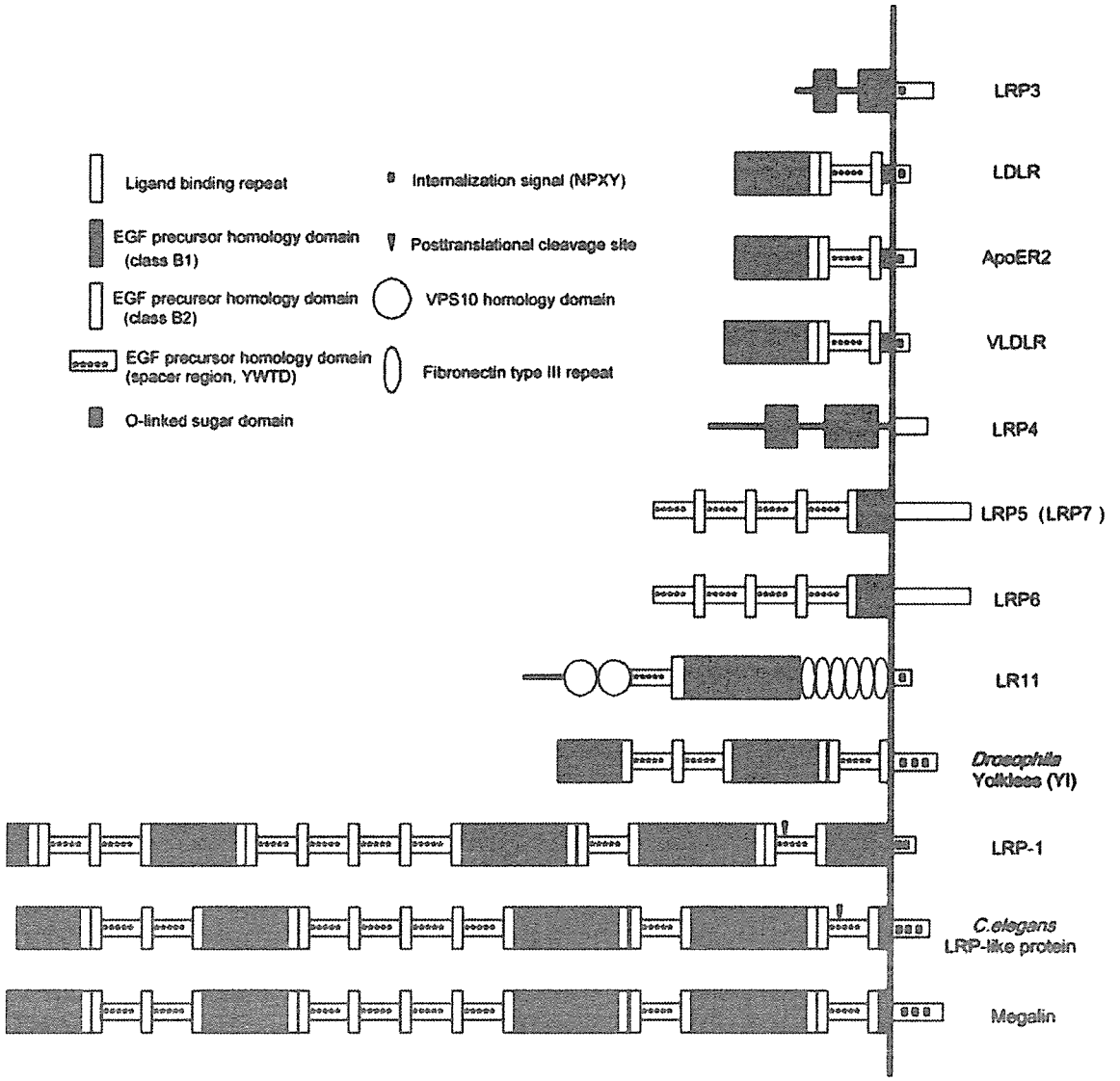

Figure 1. Schematic presentation of the LRs. The common structural modules in most of LRs are: (1) the so-called "LDL receptor ligand binding repeats (type A)," complement-type domains consisting of ≈40 residues displaying a triple-disulfide-bond-stabilized negatively charged surface; (2) epidermal growth factor (EGF) precursor homology repeats (type B1 and B2), also containing 6 cysteins each; (3) EGF precursor homology repeats consisting of ≈50 residues each, most often in groups of 5, with a consensus tetrapeptide, Tyr-Trp-Thr-Asp (YWTD); and (4) in the cytoplasmic region, signals for receptor internalization via coated pits, containing the consensus tetrapeptide Asn-Pro-Xaa-Tyr (NPXY).

is expressed by SMCs of the medial layer and in thickened intimal regions.[23] Thus, changes in the expression of LRs by vascular cells, particularly SMCs, may play a role in the development of atherosclerosis.

The migration and proliferation of SMCs, as well as extracellular matrix (ECM) production and catabolism by these cells, are important events in the development of atherosclerosis and intimal thickening after coronary angioplasty.[24] When thickening of the intima occurs, SMCs migrate from the media into the intima. During migration, SMCs acquire or lose various functions to perform the above-mentioned activities in the intima.[25,26] However, the mechanisms that control the migration of intimal SMCs have not been clarified because of the complex intracellular machinery and the interactions of numerous internal or external factors and signaling pathways. There is conclusive evidence that migration of SMCs from the media into the intima contributes to the formation of stable plaque.[27,28] Here, we focus on the role of LRs in regulating membrane receptor functions related to the migration of SMCs associated with atherosclerosis.

Platelet-Derived Growth Factor–Mediated Migratory Activity of Intimal SMCs

There is a distinct difference in migratory activity between cultured SMCs isolated from the intimal and medial layers of atherosclerotic aortas.[29] Cultured intimal SMCs differ from medial SMCs in many ways, including their morphology, proliferative potential, and gene expression.[29-31] The phenotypic modifications of SMCs that migrate to the intimal layer seem to contribute to an enhanced synthetic capacity, representing a mechanism that influences plaque stability. In fact,

cultured intimal SMCs exhibit a phenotype resembling that of fetal or dedifferentiated SMCs.[25,26] Among the many genes involved in the process of phenotypic modification that occurs in the intima,[32,33] the expression of myosin heavy chain isoforms, such as SM1, SM2, and SMemb/nonmuscle myosic heavy chain-B (NMHC-B), has been well characterized.[25,26,34]

Many factors may contribute to altering the migratory potential of SMCs in the intima, including changes of contact with the ECM and exposure to growth factors. Cultured SMCs tend to mimic these changes because primary cultured cells rapidly lose their differentiation markers and develop a synthetic phenotype. Conversely, SMCs grown in 3D cultures, such as a honeycomb structure, are able to retain the contractile phenotype.[35] Thus, various cell culture models have provided information about factors that influence the migration of intimal SMCs. Among them, sensitivity to growth factors (including platelet-derived growth factor [PDGF]) is known to be important for inducing SMC migration.[25] PDGF-BB–mediated intracellular signals induce migration, which is commonly observed using a migration assay system such as Boyden's chamber. The influence of PDGF-BB on the migration of SMCs is mediated by a specific membrane receptor: PDGF β-receptor.[36] During the process of migration of SMCs from the media into the intima, one of the strongly expressed genes is PDGF β-receptor,[37] which contributes to the migratory capacity of intimal SMCs.[38,39] The PDGF β-receptor is highly expressed even in the media of diabetic models, which show accelerated plaque formation.[40,41] PDGF-BB negatively regulates the transcription of multiple genes in SMCs and thus modulates differentiation.[42] Accordingly, the switch that induces PDGF β-receptor gene expression seems to be closely related to increasing the migratory capacity of intimal SMCs.

Urokinase and Its Receptor System Are Activated During SMC Migration

In addition to chemoattractants, several proteases and their inhibitors are involved in the migration of SMCs through the process of matrix degradation.[24] Local protease activation is important for enhancing the mobility of migrating cells, particularly for SMCs to migrate through the ECM to target sites in plaque or thickened intima. Thus, matrix metalloproteinases (MMPs) are integral for SMC migration into the intima.[24] Conversion of pro-MMPs to active MMPs, as well as MMP-9 expression, is mediated by urokinase-type plasminogen activator (uPA)–generated plasmin.[43,44] The resulting matrix degradation releases growth factors such as fibroblast growth factor-2 and latent transforming growth factor-β, and these chemoattractants further promote the migration of SMCs. Thus, urokinase appears to be necessary for migration of SMCs through the surrounding ECM.

Both tissue-type plasminogen activator and uPA cleave plasminogen to release plasmin. Expression of tissue-type plasminogen activator and uPA is increased in atherosclerotic plaque,[45–47] and a study using knockout mice has revealed a role of uPA in the development of intimal hyperplasia.[48] Accordingly, uPA is thought to play an important role in the target-oriented movement of SMCs because its activation can be localized via binding to its receptor (the uPA receptor) on the cell surface. The receptor-mediated potentiation of protease activity for plasminogen also causes an increase of plasmin activation around cell surface receptors. Subsequent production of plasmin leads to the degradation of ECM components and also has the potential to activate some MMPs. The essential role of this process in enhancing cell mobility has been intensively studied with regard to tumor invasion and neuronal migration.[49,50]

Expression of uPA by medial SMCs increases rapidly and significantly after balloon catheter injury to a vessel, corresponding with the time course of SMC migration.[51] Virally mediated overexpression of uPA by the endothelial cells of the carotid arteries promotes lesion growth in cholesterol-fed rabbits.[52] After arterial injury, intimal thickening is significantly reduced in uPA-deficient mice.[48,53] Thus, uPA itself seems to promote intimal thickening after vascular injury. However, despite the ability of uPA to influence the migration of cultured SMCs,[54–56] intimal formation is unaffected in uPA receptor knockout mice.[57] The specific proteolytic activity of uPA plays a role in the processes of arterial repair after injury, although the details of the mechanism regulating association with its receptor have not been clarified in the setting of atherosclerosis.

In addition to the proteolytic cascade initiated by binding of uPA to its cell surface receptor, uPA possibly facilitates cell migration by inducing intracellular signaling pathways.[58] The uPA receptor is a glycosylphosphatidylinositol-anchored protein, and therefore signaling activity is mediated by its interaction with other membrane molecules. Binding of uPA to its receptor on the cell surface influences the migratory activity through the formation of a complex involving the uPA receptor, vitronectin, and integrin.[50,58] These interactions at the cell membrane stimulate intracellular signaling cascades, as well as uPA receptor–mediated activation of extracellular proteolysis.[50,58] uPA stimulates the migration of SMCs via its receptor signaling cascade containing the Janus kinase, Tyk2, and phosphatidylinositol 3-kinase. Active GTP-bound forms of small GTPases (RhoA and Rac1) are the downstream targets for Tyk2 and phosphatidylinositol 3-kinase activation. Phosphorylation of myosin light chain is one of the end points of the uPA receptor–mediated signaling pathways. Observations suggesting a possible role of uPA (independent of ECM degradation) in cell migration have been reported so the uPA receptor may also modulate migration/invasion in a protease-independent manner. These findings, together with the results obtained in uPA receptor knockout mice,[57] have led to the conception that the uPA receptor modulates SMC migration through cooperation between extracellular proteolysis and intracellular signaling. Proteolysis of the ECM accelerates migration and is coordinated with adhesive and structural changes that promote cell motility, with both processes leading the cells to their targets in the plaques.

LRs Are Novel Modulators of uPA Receptor Function During PDGF-Mediated Migration of SMCs

Functional modulation of the uPA receptor through the pathways with participation of LRs has been established.[59]

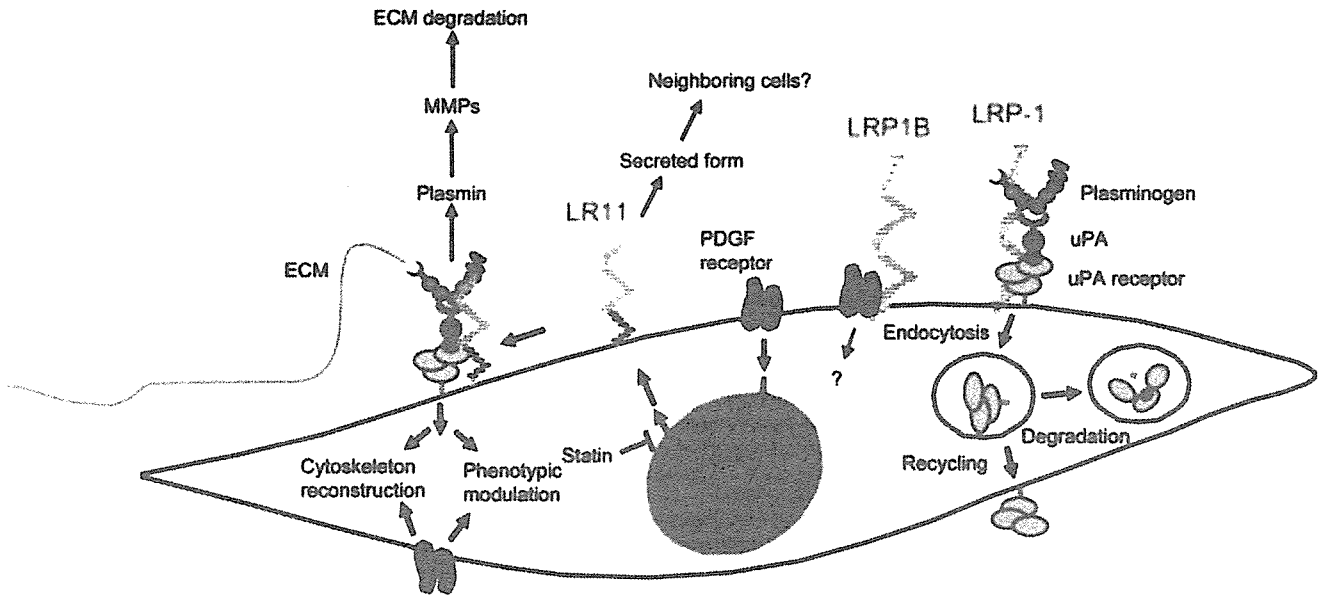

Figure 2. Proposed model for the regulation of SMC migration by LRs through the uPA/uPA receptor system. The uPA/uPA receptor system induces cell migration through both increased degradation of the ECM and receptor-mediated intracellular signaling that promotes motility. uPA receptor expression is regulated by LRs such as LRP-1, VLDL receptor/LR8, and LRP-1B. SMCs in plaques produce LR11, which is localized on the cell surface and also secreted by the cells. LR11 binds to and interacts with the uPA receptor on the cell surface or on neighboring cells. Formation of this complex inhibits internalization of the uPA receptor via other LRs (LRP-1, LRP-1B, etc.) and thereby prevents its degradation and relocation, resulting in the enhanced uPA receptor expression on the cell surface. Finally, SMCs expressing LR11 gain an increased migratory capacity that is mediated by activation of the uPA/uPA receptor system. LR11 gene transcription is induced by PDGF-BB and mediated by the PDGF β-receptor. LRP-1 (and LRP-1B) interacts with the PDGF β-receptor and modulates receptor-mediated intracellular signaling by PDGF-BB, which promotes migratory activity. Thus, LRs possibly regulate the migration of intimal SMCs in atherosclerotic plaques via modulation of PDGF receptor-meditated signaling, which is also linked with the uPA/uPA receptor system. Statins inhibit the migration of intimal SMCs by decreasing uPA receptor expression via the downregulation of LR11 gene expression.

LRs are known to play an integral role in the catabolism of lipoproteins and of complexes between proteinases and their receptors.[2,3] A large member of the family, LRP-1, is involved in the intake of uPA receptors and uPA/uPA receptor complexes by cells for subsequent degradation or recycling.[60] Extensive studies have revealed that other LRs, such as VLDL receptor/LR8[12] and LRP-1B,[61] also have the capacity to catabolize uPA/uPA receptor complexes.

LRP-1 is involved in the internalization of the uPA/uPA receptor complex, in which formation is induced by plasminogen activator inhibitor-1, and this process is dependent on LRP-1.[10,11,62,63] LRP-1 is a large molecule composed of 2 subunits. Two NPXY motifs exist in the intracellular domain of LRP-1, and these motifs are not only important for endocytosis but also for intracellular signaling through molecules such as Shc.[64-66] Inhibition of uPA receptor internalization increases cell surface uPA receptor expression and enhances cell motility.[10,16,63,67]

Deficiency of LRP-1 in SMCs causes atherosclerosis, which is mediated by the modulation of intracellular PDGF signaling.[17] This is attributable to the influence of LRP-1 on PDGF β-receptor signaling or metabolism, possibly because of a molecular interaction at the cell surface.[17,68-70] LRP-1B is the giant family member that is most similar to LRP-1; it also binds to the PDGF β-receptor and modulates receptor-mediated signaling in SMCs.[23] These findings suggest that SMC migration might be regulated by the time-restricted expression of LRs, which determines the outcome of PDGF β-receptor– and uPA receptor–mediated signaling. In accordance with the concept of functional interaction between LRs and membrane signaling receptors, LR11 has been identified by us and others as a negative regulator of protein catabolism for uPA receptor.[71,72] Previous histochemical studies have revealed that LRs are markedly induced during the development of atherosclerotic lesions.[1,18] Altered expression of LRP-1 and the uPA receptor possibly reflects the vascular response to injury. Upregulation of LRP-1 mRNA has been detected in the aortas of rabbits fed a high-cholesterol diet.[1,18] Both LRP-1 mRNA and protein are expressed in normal and atherosclerotic human arteries.[19,20] Increased vascular expression of the uPA receptor is observed in cholesterol-fed rabbits and human atherosclerotic arteries.[73] Because LRs are able to modulate uPA receptor activity and possibly PDGF receptor activity, LRs are expected to regulate the migration of SMCs through the functional modulation of these membrane receptors (Figure 2).

Involvement of LRs in Regulating SMC Migration in the Intima

Recent functional studies using genetically altered animals or cells revealed that LRs are important regulators of the migration of various cells via modulation of cytokine signaling or protease activation.[13,16] SMC-specific inactivation of LRP-1 in mice has revealed a novel role of LRP-1, which forms a complex with the PDGF receptor.[17] LRP-1 ablation results in a decrease of vascular wall integrity and causes marked susceptibility to cholesterol-induced atherosclerosis in mice.[17] In murine embryonic fibroblasts and fibrosarcoma

cells, loss of LRP-1 expression is associated with increased cell surface expression of the uPA receptor and is correlated with increased cell migration in vitro.[10] Similar changes were reported to occur when VLDL receptor/LR8 activity was neutralized in cultured breast cancer cells.[12] LR-mediated regulation of cell migration appears to depend partly on modulation of the uPA/uPA receptor system involved in the degradation of the ECM or modulation of uPA receptor-mediated intracellular signaling through activation of extracellular signal-regulated kinase and Rac1.

A negative regulator of receptor catabolism, LR11, controls uPA receptor localization on the plasma membrane because both the membrane-spanning and secreted forms of LR11 bind to and colocalize with the uPA receptor on the cell surface.[21,74] Expression of LR11 is induced by stimulation of PDGF-BB in SMCs and is observed in intimal SMCs localized at the intima/media border in the atherosclerotic plaques of experimental animals.[21] Overexpression of LR11 by SMCs enhances their migration by elevating uPA receptor expression.[21] Contrarily, neutralization of LR11 reduces the intimal thickening after cuff injury in mice.[21]

Modulation of the LR11/uPAR Pathway for Prevention of Atherosclerosis

Statins are potent inhibitors of the 3-hydroxy-3-methylglutaryl–coenzyme A reductase that are known to be effective for preventing atherosclerosis. Statins have recently been shown to perform a multitude of activities that are involved in the functional modulation of vascular cells such as influences on cell proliferation and secretion.[75,76] One of the major effects of statins on SMCs is modulation of migration. However, the mechanism involved and clinical significance of such inhibition of migration, which has been observed in vitro, have not been elucidated. PDGF-induced migration of SMCs is suppressed by statins in vitro.[77,78] Statins reduce protease expression in atheromatous plaques, and hydrophilic statins decrease SMC numbers and collagen gene expression in vivo.[79] However, phenotypic modulation of intimal SMCs by statins has not yet been investigated. LR11 plays an important role in the induction of migration after enhancement by PDGF-BB in vitro. A potent 3-hydroxy-3-methylglutaryl–coenzyme A reductase inhibitor, pitavastatin, reduced the expression of both LR11 and SMemb/NMHC-B in atherosclerotic plaques (unpublished data, 2006). In fact, the enhanced expression of LR11, uPA receptor, and SMemb/NMHC-B by cultured intimal SMCs is reduced by pitavastatin to the levels seen in cells from the media. When expression of the uPA receptor, SMemb/NMHC-B, and endogenous LR11 is increased by PDGF-BB, the enhanced migratory activity of SMCs is blocked by pitavastatin via suppression of endogenous LR11 production. Thus, modulation of the LR11/uPA receptor system plays a role in PDGF-induced migration of intimal SMCs (Figure 2).

It has not yet been clarified whether inhibition of the migration of intimal SMCs leads to the regression of atherosclerotic plaque or prevents restenosis after coronary angioplasty. Activation of pathways mediated by the uPA receptor and the PDGF receptor that increase the migration of intimal SMCs is thought to be essential for the formation of mature plaque after endothelial injury leads to the initiation of atherosclerosis. Unregulated expression of these membrane receptors may reduce the stability of plaque because the programmed migration of SMCs from the media to target regions in the intima would be disturbed. LRs are a possible candidate for modulating SMC migration to control the process of atherosclerosis. Selective modification of the LRs/uPA receptor/PDGF receptor system in SMCs, associated with the change to a dedifferentiated phenotype, appears to be important for the occurrence of intimal thickening after angioplasty as well as plaque formation in atherosclerosis.

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Effect of a Newly Developed Charging Chamber for the Treatment of Hypotension during Hemodialysis

Tadayoshi Ikegami, Zenzo Fujii, Masae Minami, Machiko Matsumoto, and Masunori Matsuzaki

We developed a new method of hemodialysis using a charging chamber for treatment of hemodialysis patients with hypotension occurring during the latter half of hemodialysis (collapse). The purpose of this method was to recover systolic blood pressure (BP) by returning a part of blood within the chamber into the body circulation when hemodialysis collapse occurred. Using this method, systolic BP recovery (ΔBP) in ten hemodialysis patients (4 males, 6 females, mean age 66.0 years old) was compared to a control group treated with intravenous administration of 20 ml of 10% NaCl. When hemodialysis collapse occurred, 60 ml of blood within the chamber in this method and 20 ml of 10% NaCl intravenously in the control group were administered and systolic BP was measured 20 minutes later. The results showed that ΔBP using this method was 26.0 mm Hg (ANOVA: $p = 0.0072$), while in the control group it was 30.2 mm Hg (ANOVA: $p = 0.0003$), and there was no significant difference between the systolic BP recovery of both groups (paired t test: $p = 0.4196$). *ASAIO Journal* 2006; 52:80–85.

The number of patients who start hemodialysis because of chronic renal failure continues to increase, and with this increase the various complications associated with hemodialysis become more and more important as factors that determine a patient's prognosis. The most frequently seen complication during hemodialysis is hypotension (hemodialysis collapse), the causes of which are reported to relate to: 1) a decrease of blood volume (BV) by water deletion during hemodialysis,[1] 2) insufficient water refilling from extravascular space for BV decrease, 3) decrease of peripheral vascular resistance due to autonomic nervous system dysfunction,[1,2] 4) cardiac dysfunction, 5) nitric oxide production,[3] and 6) adenosine production.[4]

Hemodialysis collapse causes the patient to feel ill, with symptoms of nausea and shock, making it mentally and physically difficult to continue the hemodialysis session. As a result, there is a decreased efficiency in the hemodialysis and insufficient water deletion, causing a vicious cycle of increased load for the next session. To prevent these consequences, the patient's optimal dry weight should be established and any weight gain should be brought to the patient's attention. In addition, there should be an evaluation of the hemodialysis conditions (extracorporeal ultrafiltration method,[1] low-temperature dialysate,[5,6] high-Na dialysate,[7] biofeedback system,[8] and profiled dialysis[9]) and of the water deletion plan (the change of the water deletion rate based on an evaluation of the change of effective arterial blood volume using crit-line, or thoracic electrical impedance[10]). When hemodialysis collapse is still inevitable, then water deletion stoppage, infusion (isotonic saline, hypertonic saline,[11] human albumin,[12] hydroxyethyl starch[13]), and administration of vasoconstrictor (amezinium,[14] midodrine[15,16]) are performed according to the need. However, when or if infusion is given, at the time of the restart of hemodialysis after BP recovery, the amount of infusion in addition to the originally planned amount of water deletion should be removed. Therefore, due to the potential danger of recurring hemodialysis collapse, it would be difficult to complete a predetermined water deletion plan.

It is known that the venous system *in vivo* in a state of suddenly decreasing BV, such as hemodialysis collapse, works to increase the venous return by decreasing the total venous capacity and increasing cardiac output attempting to raise BP.[17] When isotonic saline is infused at hemodialysis collapse, its volume will be distributed in the extracellular space, theoretically. So finally, about 20–25% of infusion volume remains in the intravascular space.[18] As compared with this, when a part of the extracorporeal BV is infused at hemodialysis collapse, all of its volume remains in intravascular space, and it is expected that this method is a very effective treatment for hemodialysis collapse. So we took notice of this action of the venous system and body fluid distribution, and made a charging chamber (C-C). This C-C functions as a venous bubble trap and as the charging volume that was not present previously in order to add the ability to change the volume for extracorporeal blood circulation. We studied the BP recovery effect by artificially increasing the BV in the body by returning the blood from C-C without infusion for hemodialysis collapse.

Materials and Methods

Patients

The study included 10 patients (4 males and 6 females) on hemodialysis because of end-stage renal disease, who maintained optimal dry weight without cardiac dysfunction and had hemodialysis collapse episodes (**Table 1**). Patients' profiles (hematocrit, albumin, ejection fraction, hemodialysis duration, dry weight, weight gain, and hemodialysis time) are shown in **Table 1**. All the laboratory data were collected immediately before dialysis was started. The mean age was 66.0 years old. Eight of the patients were diabetic. hemodialysis collapse is

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Table 1. Patient Profiles

No. of patients	10
No. of males/females	4/6
Age (y), mean ± SD	66.0 ± 9.2
DM/non-DM, n	8/2
Hematocrit (%), mean ± SD	31.0 ± 4.7
Albumin (mg/dl),	3.9 ± 0.3
EF (%), mean ± SD	68.4 ± 13.1
hemodialysis duration (mo)	50.9 ± 41.6
DW (kg)	51.0 ± 8.2
Alpha-stimulant (yes/no), n	5/5
Antihypertensive drug given just before hemodialysis (yes/no), n	4/6
QB (ml/min), mean ± SD	196.0 ± 8.2
Dialysate temperature (°C)	36.0
Residual glomerular filtration rate (ml/min), mean ± SD	5.7 ± 2.3

All laboratory data were collected immediately before dialysis was started. DM, diabetes mellitus; DW, dry weight; QB, blood flow rate.

defined as exhibiting the following during hemodialysis: 1) a decrease in systolic BP of 30 mm Hg or more compared to the starting BP or systolic BP less than or equal to 100 mm Hg and 2) having shock-like symptoms. Consent was obtained from all patients who were fully informed prior to the study.

Charging Chamber

The increased volume of the venous line chamber is used as the charging blood volume (**Figure 1a**). The number of chambers on the venous line was increased to two (connected with tubing), because it was suspected that a single chamber induces difficulty in the charging and discharging maneuvers. The priming volume was 172 ml. BV in the chambers can be increased or decreased by up to 60 ml by injecting or withdrawing air using a syringe (**Figure 1b**). When dialysis was performed at QB 200 ml/min with the chambers full, the fluid simulation showed that the values of QB flowing through the two tubes connecting the chambers were 124.7 ml/min and 73.6 ml/min (**Figure 1c**), and there was no fluid stagnation (**Figure 1d**). All measurements were computed by a Dell dimension 8100 OS WindowsNT computer using CFX TASC flow software. The flowfield was analyzed with general-purpose heat fluid analysis software (CFX TASC flow ver2.10, available commercially). It was assumed that blood was incompressible Newtonian fluid, with specific gravity 1.05, and the viscosity was assumed to be 3 cp. The laminar flow model was used.

Method

Hemodialysis sessions were conducted two or three times per week (bicarbonate dialysate, dialysate flow 500 ml/min, dialysate temperature 36.0°C, dialysate composition Na 140 mEq/l, K 2.0 mEq/l, Ca 3.0 mEq/l, HCO3 25 mEq/l).

Using an extracorporeal circulation circuit with charging chamber (C-C), we performed priming with the C-C empty, connected the patient and circuit under the usual dialysis conditions, and started dialysis. Ten minutes later, blood pressure was checked for stability. Any air was removed from the chamber using a syringe and the chamber was filled with blood in 5 minutes.

Ten minutes after the chamber was filled with blood, blood pressure and heart rate were measured to confirm that hypotension due to an increase in extracorporeal BV was not induced, and dialysis was continued. When hemodialysis collapse occurred during dialysis, we first elevated the lower extremities, decreased QB (60-80 ml/min), and stopped the hemodialysis. Although it may not be necessary to slow the blood flow rate before discharging the chamber, we were concerned about transmembrane pressure and venous pressure elevation by pushing a portion of the blood volume from C-C, so we slowed the blood flow rate before blood was discharged from C-C. Then we put 60 ml BV into the body from the C-C. Twenty minutes after increasing the intracorporeal BV artificially, we measured the systolic BP to see if there was an increase of 20 mm Hg or more or recovery of systolic BP to greater than or equal to 110 mm Hg. In the control group, 20 ml of 10% NaCl was administered intravenously when hemodialysis collapse occurred.

Statistical Analysis

Analysis of variance and paired *t* tests were performed for statistical evaluation. Values of p less than or equal to 0.05 were considered to be significant.

Results

Hypotension Induction by Starting Hd

We measured the systolic BP and heart rate (HR) at the following points and evaluated the hemodynamic changes: 1) immediately before inserting the needle into the shunt, 2) immediately before C-C filling (10 minutes after connection of the circulation circuit), and 3) 10 minutes after C-C filling. There was no significant decrease in BP during these processes (**Figures 2a and 2b**).

Effect of the Charging Chamber on Blood Pressure Recovery from Hemodialysis Collapse

We compared the systolic BP and HR (for C-C and control) at the following points and evaluated the effect of BP recovery: 1) before the start of hemodialysis, 2) 30 minutes before hemodialysis collapse occurred, 3) When hemodialysis collapse occurred, and 4) 5,10,15 and 20 minutes after hemodialysis collapse occurred (5,10,15 and 20 minutes after blood administration from C-C), 5) immediately before the end of hemodialysis, 6) 20 minutes after the end of hemodialysis. At hemodialysis collapse, systolic BP decreased by 38.6 mm Hg ($p = 0.0002$) and HR increased by 12.0 bpm ($p = 0.0226$); however 5, 10, 15, and 20 minutes later, systolic BP recovered by 18.0, 22.1, 24.2, and 26.0 mm Hg ($p = 0.0054, 0.0052, 0.0085, 0.0072$), and it was possible to maintain a definite BP elevation immediately before ending hemodialysis and 20 minutes after ending hemodialysis, compared with the time of hemodialysis collapse (**Figure 3a,b,c**).

Recovery Effect from Hemodialysis Collapse by Intravenous Administration of 10% NaCl

For the C-C study, systolic BP and HR at points 1–6 were compared and the effect of BP recovery was evaluated. The

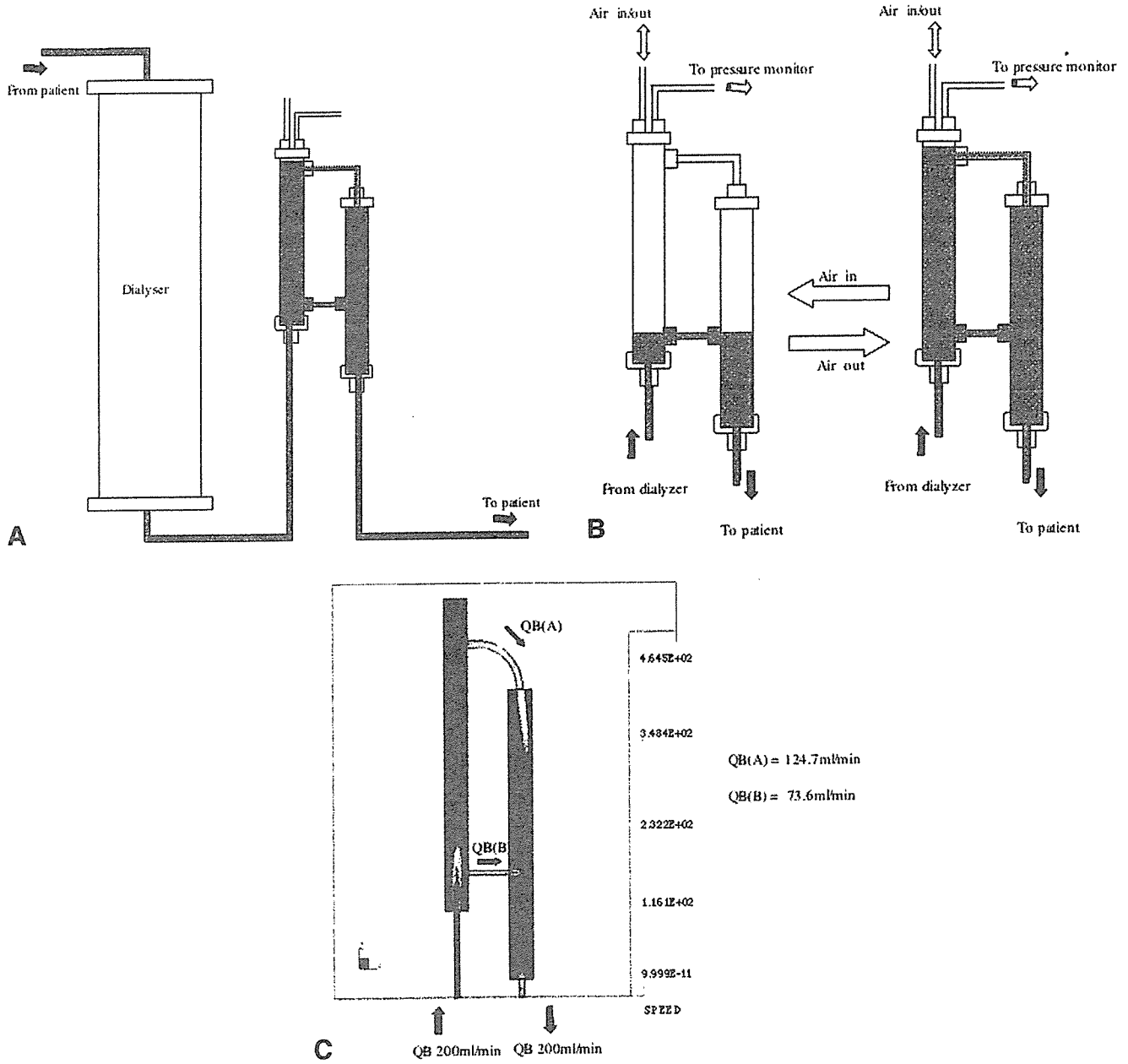

Figure 1. a: The charging-chamber device. Arrows indicate the direction of blood flow. **b:** The black area in the charging chamber is space filled by blood; white areas are empty. BV in the chambers can be increased or decreased up to 60 ml by injecting or withdrawing air using a syringe. **c.** Fluid simulation showed that the values of QB flowing through the connecting tube A and B were 124.7 ml/min and 73.6 ml/min. **d.** There was no fluid stagnation in the charging chamber and connecting tube.

results were that, at hemodialysis collapse, systolic BP decreased by 40.6 mm Hg ($p < 0.0001$) and HR increased by 10.0 bpm ($p = 0.0057$); however, 5, 10, 15, and 20 minutes later, systolic BP recovered by 20.5, 24.2, 28.7, and 30.2 mm Hg ($p = 0.0025, 0.0056, 0.0004, 0.0003$), respectively **(Figure 3c)**.

Comparison of the Bp Recovery Effect

Hemodialysis was performed under the condition of water deletion shown in **Table 2** (by the C-C and 10% NaCl methods) and degree of BP recovery (ΔBP) was evaluated by using paired *t* tests. Total amount of fluid removed with the C-C and 10% NaCl methods was 2.14 (SD; 0.62) kg and 2.11 (SD; 0.62) kg, respectively. Total hemodialysis time with the C-C and 10% NaCl methods was 228.5 (SD; 39.7) minutes and 226.0 (SD; 39.3) minutes, respectively. There were no significant differences in total amount of fluid, total hemodialysis time, and BP recovery effect at 5, 10, 15, and 20 minutes ($p = 0.5346, 0.8315, 0.6342, 0.4196$, respectively; **Figure 4**).

Blood Clotting in the Chamber

Blood clotting occurred in the venous chamber in 1 of 10 patients. However, in that patient, even during usual dialysis,