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Effects of Atorvastatin on Serum Lipids, Lipoproteins, and Hemostasis

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Serum levels of lipids and lipoproteins were examined in individuals with hyperlipidemia treated with atorvastatin or colestimide and in healthy volunteers. Modified low-density lipoprotein (LDL) was measured by its faster electrophoretic mobility and expressed as charge modification frequency (CMF). Serum levels of total cholesterol (t-chol), triglyceride (TG), very low-density lipoprotein (VLDL)-chol, low-density lipoprotein (LDL)-chol, and CMF were significantly higher in hyperlipidemia, but there was no significant difference in serum high-density lipoprotein (HDL)-chol levels between hyperlipidemic and healthy subjects. Treatment with atorvastatin resulted in significant decreases of serum t-chol, TG, and LDL-chol levels but not serum HDL-chol and VLDL-chol. Treatment with colestimide significantly reduced serum t-chol, HDL-chol, and LDL-chol levels but not those of TG and VLDL-chol. CMF was significantly reduced by treatment with atorvastatin but not by colestimide. Atorvastatin significantly reduced plasma levels of thrombomodulin, thrombin antithrombin complex (TAT) and tissue type plasminogen activator–plasminogen activator inhibitor-I complex. Colestimide moderately prolonged activated partial thromboplastin time and reduction of TAT. Based on its actions of lowering modified LDL and improving hemostatic abnormalities, we postulate that atorvastatin might inhibit the onset of ischemic diseases. Am. J. Hematol. 78:1–6, 2005. © 2004 Wiley-Liss, Inc.

Key words: lipids; lipoproteins; hyperlipidemia; atherosclerosis; colestimide; atorvastatin; hypercoagulability; atherogenesis

INTRODUCTION

Clinical trials [1–3] have demonstrated that total cholesterol (t-chol)-lowering therapy reduces the incidence of major cardiac events in patients with hypercholesterolemia. Colestimide, a bile acid sequestering resin, was reported to reduce low-density lipoprotein (LDL)-chol [4]. On the other hand, atorvastatin, a powerful inhibitor of 3-hydroxyl-3-methylglutaryl coenzyme A (HMG CoA), prevents the progression of atherosclerosis in familial hypercholesterolemia [5], coronary and stroke events in hypertensive patients [6], and recurrent ischemic events in acute coronary syndrome [7]. However, the results of previous clinical trials have also shown that a substantial number of individuals who achieved the target LDL-chol level still developed a clinical event. The reason for this finding may be explained by the prevalence of other metabolic abnormalities that are linked to atherosclerosis [8]. Over the past several years, the clinical importance of other

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dyslipidemias, such as hypertriglyceridemia and the predominance of small dense LDL particles, has attracted much attention [8]. Oxidative LDL is thought to be a major contributor to the development of atherosclerosis [9]. Several studies showed that atherosclerotic plaques contain oxidized lipids [10–12]. Studies using antibodies against protein–aldehyde adducts have demonstrated that these adducts are present in atherosclerotic plaques [13]. Other studies [14] identified antibodies to various protein–aldehyde adducts in plasma.

The association between thrombus formation and atherosclerosis was first suggested by Rokitansky et al. [15] and by Duguid [16], who postulated that atherosclerotic lesions resulted principally from the organization of intravascular fibrin deposits. Ross and Glomset [17] and Ross [18] proposed the "response to injury" hypothesis of atherogenesis, based on the theory that the initiation of atherogenesis and thrombosis is related to endothelial cell compromise or injury. Some attention has been focused on the role of hypercoagulability in atherogenesis [19,20].

The aim of the present study was to determine the relationships between the effects of atorvastatin and colestimide and hemostatic abnormalities. For this purpose, we measured serum levels of lipids and lipoproteins in patients with hyperlipidemia treated with the drugs and in healthy volunteers. The results showed that atorvastatin reduced modified LDL and improved hemostatic abnormalities.

MATERIALS AND METHODS

Serum levels of lipids and lipoproteins were measured in 124 healthy subjects (females, 64; males, 60; age, 37.5 ± 12.3 years, mean ± SD) and 60 individuals with hyperlipidemia (females, 44; males, 16; age, 61.3 ± 9.7 years). Of the 60 patients, the first 30 (females, 22; males, 8; age, 59.8 ± 8.3 years) were treated with colestimide (Mitsubishi, Tokyo, Japan) at 3 g/day, while the remaining 30 (females, 22; males, 8; age, 62.7 ± 10.8 years) were treated with atorvastatin (Yamanouchi, Tokyo, Japan) at 10 mg/day. The study protocol was approved by the Human Ethics Review Committee of Mie University School of Medicine, and a signed consent form was obtained from each subject.

Serum levels of t-chol and triglyceride (TG) were measured using Datamina-L TC II (Kyowamedix, Tokyo, Japan) and Datamina-L TG II (Kyowamedix), respectively. Each lipoprotein fraction was measured by agarose gel electrophoresis.

AGAROSE GEL ELECTROPHORESIS

Sample application (1 μL), electrophoresis (400 V, 15 min), staining, drying, and densitometric scanning (570 nm) were performed using the rapid electrophoresis system (Helena Laboratories, Beaumont, TX). A Rep Lipo-30 plate (Helena Kenkyusho, Saitama, Japan) was used as the agarose gel. To avoid any influence of gel variations on migration distance, the gel was divided into two halves: one half for chol and the other for TG staining. Samples were loaded into wells on one side of the plate, a space (3 wells) was left in the center, and the same samples were loaded, in a symmetrical fashion, into wells on the opposite side. Lateral inversion of samples enabled more precise superimposition of bands that developed after enzymatic staining and scanning. Chol and TG levels of lipoprotein fractions were visualized after treatment with enzymatic staining reagents (Helena Kenkyusho) [21,22], which contained cholesterol dehydrogenase and glycerol-3-phosphate dehydrogenase, respectively. The visualized gel plate was scanned on a densitometer after fixation (5% acetic acid, 30 min), washing (distilled water, 30 min) and drying. The scanning patterns of chol and TG staining were superimposed to delimit each lipoprotein fraction using analytical software (Electrophoresis Data Bank, Helena Kenkyusho). The chol and TG levels of each fraction were estimated from the area percentages and total concentrations (Fig. 1A).

Modified LDL

Modified LDL showing faster electrophoretic mobility was easily distinguished from native LDL. The modification frequency indicates sample variation (cathodic or anodic) of LDL compared to a LDL standard. The modification frequency of standard "0" was determined by the mean migration distance from the origin to the peak of LDL in 10 samples from healthy donors of age 35 years and under. Accordingly, the migration distance of normal LDL from the origin was calculated using controlled LDL modification frequency. In general, A is the distance between normal LDL and origin, and B is the distance between the patients' LDL and the origin. Modified LDL is expressed as $[(B - A)/A] \times 100$ and designated as charge modification frequency (CMF) (Fig. 1B).

For the assays of hemostatic parameters, whole blood was anticoagulated by the addition of 9 volumes of blood to 1 volume of 3.8% trisodium citrate solution. Prothrombin time (PT) and activated partial thromboplastin time (APTT) were measured by Thromborel S (Behringwerke, Marburg, Germany) and Patromtin (Behringwerke). The activities of plasma antithrombin (AT) and protein C (PC) were measured by amidolytic assays using Berichrom AT III and Berichrom Protein C, respectively (Behringwerke). The plasma levels of thrombin–antithrombin complex (TAT), plasmin–plasmin

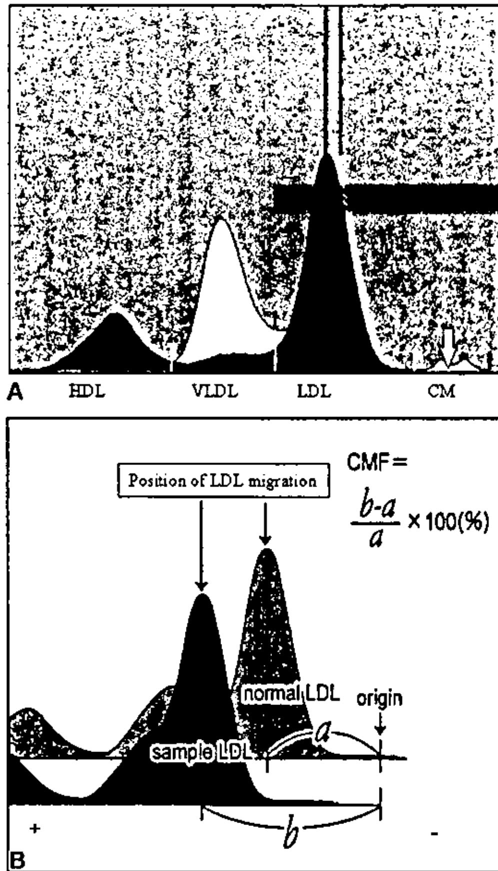

Fig. 1. Agarose gel electrophoresis of t-chol and TG. (A) Electrophoretic pattern of t-chol and TG: black area, t-chol; white area, TG; gray area, both t-chol and TG. (B) Calculation of modified LDL.

inhibitor complex (PPIC), FDP-D-dimer, soluble fibrin (SF) [22], thrombomodulin (TM), and tissue plasminogen activator (tPA)–plasminogen activator inhibitor-I (PAI-I) complex were determined with TAT-test Kokusai-F (Kokusai-Shiyaku, Koube, Japan), PIC-test Kokusai-F (Kokusai-Shiyaku), D-dimer test Kokusai-F (Kokusai-Shiyaku), Enzymum test (Boehringer Mannheim, Mannheim, Germany), TM-test Kokusai (Kokusai-Shiyaku), and tPA-PAI-I complex Kokusai (Kokusai-Shiyaku), respectively. The coagulant activities of factors VIII, IX, X, XI, and XII were measured using Patromtin and FVIII-, FIX-, FX-, FXI-, and FXII-deficient plasma (Behringwerke), respectively.

Statistical Analysis

Data shown in tables are expressed as mean ± standard deviation. Differences between groups were examined for statistical significance using the Mann–Whitney U-test (two-sided). A P value less than 0.05 denoted the presence of a statistically significant difference.

RESULTS

Serum levels of t-chol, TG, very low-density lipoprotein (VLDL)-chol, LDL-chol, HDL-TG, VLDL-TG, LDL-TG, and CMF were significantly higher in individuals with hyperlipidemia than in healthy subjects (P ± 0.001, each), but there was no significant difference in serum HDL-chol levels between hyperlipidemia and healthy volunteers (Table I). There were no significant differences in serum t-chol, TG, VLDL-chol, LDL-chol, HDL-TG, VLDL-TG, and CMF between patients treated with atorvastatin and those with colestimide. Treatment with atorvastatin resulted in significant reductions of serum levels of t-chol, TG, LDL-chol, VLDL-TG, LDL-TG ($P < 0.001$, each), but no changes were noted in those of HDL-chol, VLDL-chol, and HDL-TG, compared with before treatment (Table II). In contrast, treatment with colestimide resulted in significant reductions of serum concentrations of t-chol, HDL-chol, and LDL-chol ($P < 0.001$, each) but not those of TG, VLDL-chol, HDL-TG, VLDL-TG, and LDL-TG (Table III). CMF was significantly reduced by treatment with atorvastatin (8.63 ± 5.45 vs. 2.23 ± 5.67, $P < 0.001$), but not by colestimide (8.63 ± 5.45 vs. 6.57 ± 7.20, NS) (Fig. 2).

Treatment of hyperlipidemia by atorvastatin or colestimide resulted in moderate prolongation of APTT and significant reduction of plasma levels of TAT, TM, and tPA–PAI-I complex (Table IV). Atorvastatin produced no significant changes in APTT, international normalized ratio (INR), or plasma levels of PPIC, D-dimer, PC, and AT but significantly

TABLE I. Serum Levels of Lipid of Lipoprotein in Hyperlipidemia and Healthy Volunteers*

	Hyperlipidemia	Healthy subjects	Significance
Total cholesterol (mg/dL)	255.4 ± 38.0	190.6 ± 32.6	$P < 0.001$
Triglycerides (mg/dL)	186.7 ± 78.0	83.9 ± 43.1	$P < 0.001$
HDL cholesterol (mg/dL)	58.7 ± 20.1	60.0 ± 15.7	NS
VLDL cholesterol (mg/dL)	19.2 ± 11.0	9.4 ± 6.0	$P < 0.001$
LDL cholesterol (mg/dL)	173.1 ± 37.3	119.8 ± 30.4	$P < 0.001$
HDL triglycerides (mg/dL)	16.5 ± 8.8	10.2 ± 4.3	$P < 0.001$
VLDL triglycerides (mg/dL)	102.6 ± 61.9	41.8 ± 36.7	$P < 0.001$
LDL triglycerides (mg/dL)	57.6 ± 25.5	27.9 ± 10.4	$P < 0.001$
Charge modification frequency	7.60 ± 6.36	−1.98 ± 4.35	$P < 0.001$

*Data are mean ± SD; NS, not significant.

TABLE II. Effects of Atorvastatin on Lipid Levels*

	Baseline	Post-treatment	Significance
Total cholesterol (mg/dL)	261.2 ± 34.5	183.7 ± 27.5	$P < 0.001$
Triglycerides (mg/dL)	214.2 ± 79.0	154.3 ± 58.4	$P < 0.001$
HDL cholesterol (mg/dL)	55.8 ± 21.9	54.9 ± 18.0	NS
VLDL cholesterol (mg/dL)	22.7 ± 13.6	18.1 ± 8.9	NS
LDL cholesterol (mg/dL)	177.7 ± 37.7	101.2 ± 29.4	$P < 0.001$
HDL triglycerides (mg/dL)	18.2 ± 11.1	18.3 ± 7.5	NS
VLDL triglycerides (mg/dL)	116.4 ± 65.7	83.6 ± 49.5	$P = 0.001$
LDL triglycerides (mg/dL)	67.8 ± 31.1	42.1 ± 15.2	$P < 0.001$

*Data are mean ± SD; NS, not significant.

TABLE III. Effects of Colestimide on Lipid Levels

	Baseline	Post-treatment	Significance
Total cholesterol (mg/dL)	248.8 ± 42.1	200.4 ± 34.2	$P < 0.001$
Triglycerides (mg/dL)	159.2 ± 70.3	140.7 ± 59.0	NS
HDL cholesterol (mg/dL)	61.6 ± 18.8	54.6 ± 16.4	$P < 0.001$
VLDL cholesterol (mg/dL)	15.7 ± 9.2	15.3 ± 8.8	NS
LDL cholesterol (mg/dL)	168.3 ± 37.4	127.7 ± 30.9	$P < 0.001$
HDL triglycerides (mg/dL)	14.9 ± 5.7	15.1 ± 6.7	NS
VLDL triglycerides (mg/dL)	88.9 ± 56.8	78.4 ± 51.6	NS
LDL triglycerides (mg/dL)	47.4 ± 13.7	44.6 ± 13.3	NS

*Data are mean ± SD; NS, not significant.

increased plasma levels of fibrinogen ($P < 0.05$) and decreased TAT ($P < 0.05$), TM ($P < 0.01$), and tPA–PAI-I complex levels, relative to the baseline (pretreatment) levels (Table V). In contrast, colestimide resulted in a moderate prolongation of APTT ($P < 0.01$) and significant decrease of TAT ($P < 0.05$) but no significant changes in INR, plasma levels of fibrinogen, PPIC, D-dimer, PC, AT, TM, or tPA–PAI-I complex levels, compared with baseline levels (Table VI). Treatment with colestimide resulted in slight and insignificant reductions of plasma levels of factors VIII (98.0% ± 37.9% vs. 92.2% ± 39.6%, NS), IX (106% ± 12.6% vs. 97.3% ± 12.4%, NS), X (116% ± 21.2% vs. 98.9% ± 10.8%, NS), and XI (109% ± 21.2% vs. 99.5% ± 17.5%, NS), and slight

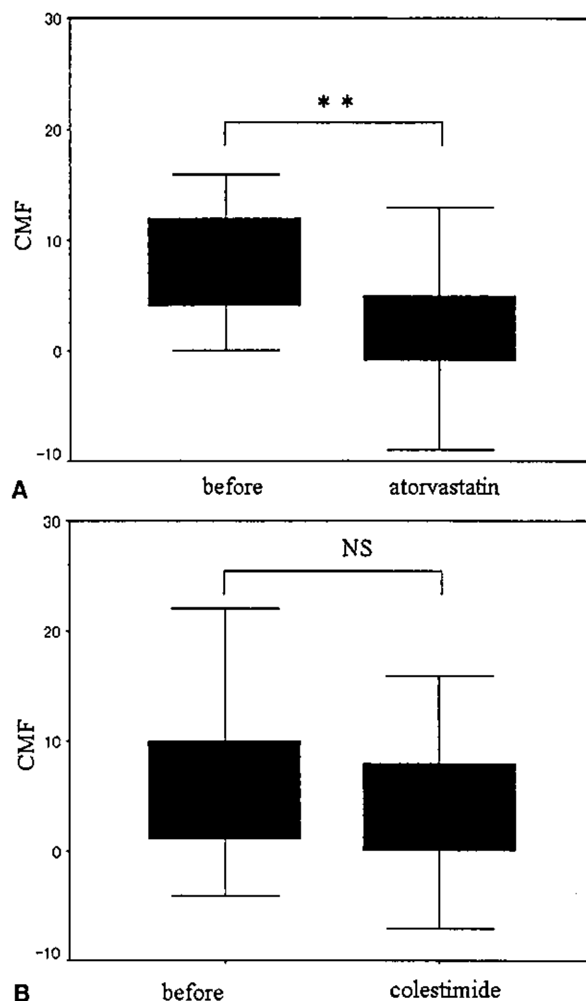

Fig. 2. Box plots of CMF values before and after treatment with atorvastatin (A) and colestimide (B). **$P < 0.01$.

TABLE IV. Effects of Hypolipidemic Agents on Hemostatic Parameters in the Entire Group of 60 Patients*

	Before	Post-treatment	Significance
APTT (sec)	33.8 ± 4.9	38.1 ± 9.2	$P < 0.01$
International normalized ratio	1.00 ± 0.07	1.00 ± 0.08	NS
Fibrinogen (mg/dL)	288 ± 53.5	303 ± 73.5	$P = 0.05$
TAT (ng/mL)	2.66 ± 3.96	1.12 ± 1.12	$P < 0.01$
PPIC (μg/mL)	0.72 ± 0.29	0.71 ± 0.27	NS
D-dimer (μg/mL)	0.36 ± 0.26	0.34 ± 0.29	NS
Protein C (%)	126 ± 19.9	123 ± 18.9	NS
Antithrombin (%)	96.9 ± 10.1	96.7 ± 10.2	NS
Thrombomodulin (ng/mL)	12.2 ± 2.67	10.6 ± 2.40	$P < 0.01$
PA-PAI-I complex (ng/mL)	15.4 ± 6.1	12.7 ± 5.4	$P < 0.01$

*Data are mean ± SD; NS, not significant.

TABLE V. Effects of Atorvastatin on Hemostatic Parameters in 30 Patients Treated With the Drug*

	Before	Post-treatment	Significance
APTT (sec)	32.8 ± 3.6	32.3 ± 2.8	NS
International normalized ratio	1.01 ± 0.08	1.02 ± 0.09	NS
Fibrinogen (mg/dL)	304 ± 53	334 ± 83	$P < 0.05$
TAT (ng/mL)	3.29 ± 5.15	1.23 ± 1.36	$P < 0.05$
PPIC (μg/mL)	0.65 ± 0.23	0.65 ± 0.19	NS
D-dimer (μg/mL)	0.40 ± 0.21	0.39 ± 0.29	NS
Protein C (%)	128 ± 22	125 ± 20	NS
Antithrombin (%)	99.7 ± 9.6	101.5 ± 8.8	NS
Thrombomodulin (ng/mL)	12.7 ± 2.70	10.5 ± 2.0	$P < 0.01$
PA–PAI-I complex (ng/mL)	16.3 ± 6.9	12.1 ± 3.9	$P < 0.01$

*Data are mean ± SD; NS, not significant.

TABLE VI. Effects of Colestimide on Hemostatic Parameters in 30 Patients Treated With the Drug*

	Baseline	Post-treatment	Significance
APTT (sec)	34.8 ± 5.7	44.0 ± 9.7	$P < 0.01$
International normalized ratio	0.99 ± 0.07	0.98 ± 0.06	NS
Fibrinogen (mg/dL)	273 ± 50	273 ± 47	NS
TAT (ng/mL)	2.03 ± 2.13	1.00 ± 0.50	$P < 0.05$
PPIC (μg/mL)	0.79 ± 0.34	0.77 ± 0.33	NS
D-dimer (μg/mL)	0.31 ± 0.30	0.29 ± 0.18	NS
Protein C (%)	125 ± 19	122 ± 19	NS
Antithrombin (%)	95.5 ± 10.5	92.4 ± 10.5	NS
Thrombomodulin (ng/mL)	11.8 ± 5.6	10.7 ± 4.8	NS
PA–PAI-I complex (ng/mL)	14.5 ± 5.1	13.3 ± 6.5	NS

*Data are mean ± SD; NS, not significant.

reduction in plasma levels of factor XII (94.4% ± 12.2% vs. 72.0% ± 11.0%, $P < 0.01$) compared to baseline levels.

DISCUSSION

Serum levels of t-chol, TG, VLDL-chol, LDL-chol, and CMF were significantly high in individuals with hyperlipidemia, but serum levels of HDL-chol were high in some and low in others, relative to the normal control. The aim of most clinicians is to at least reduce t-chol levels. Among the many independent risk factors for coronary heart disease (CHD) identified by epidemiological studies, a low level of plasma HDL concentration is one of the strongest [23]. The Framingham Heart Study demonstrated that, for any given LDL concentration, the HDL cholesterol concentration is inversely correlated with the risk of CHD [24].

Treatment with atorvastatin and colestimide significantly reduced serum levels of both t-chol and LDL-chol. In addition, atorvastatin reduced serum TG levels, while colestimide reduced HDL-chol levels. These results show that atorvastatin reduced small-size LDL-chol by decreasing the TG level. CMF was significantly reduced by treatment with atorvastatin but not by colestimide. CMF is related to small-size LDL-chol and reflects modified LDL or oxidative LDL. Myeloperoxidase, chlorinated lipids, chlorinated proteins, and hydroxyphenylacetaldehyde have been isolated from atherosclerotic plaques, suggesting the presence of oxidatively active phagocytic cells such as neutrophils or macrophages in these plaques [25–27]. These results suggest that CMF, modified LDL, is an important risk factor for atherosclerosis. Considered together, our findings suggest that atorvastatin seems be a more potent anti-atherosclerotic agent than colestimide.

Treatment of hyperlipidemia by atorvastatin and colestimide resulted in moderate prolongation of APTT and significant reduction of plasma levels of TAT, TM, and tPA–PAI-I complex, suggesting that reduction of serum lipid levels improved hypercoagulability and vascular endothelial cell injury. Atorvastatin treatment increased plasma fibrinogen levels and decreased TM and tPA–PAI-I complex levels. Statins are known to improve endothelial function [28], decrease platelet aggregability and thrombus deposition [29], and reduce vascular inflammation [30]. The increased level of fibrinogen and decreased level of TAT observed in our study might have resulted from improvement of hypercoagulability, while the decreased TM and t-PA–PAI-I complex levels may have been due to improved endothelial function. Furthermore, the decreased levels of tPA–PAI-I complex may have been secondary to improvement of hypofibrinolysis. A reduction in modified LDL causes reduced activation of monocytes and vascular endothelial cells, with subsequent inhibition of activation of the coagulation system. Atorvastatin may therefore improve hemostatic abnormalities by reducing modified LDL (shown as CMF). Treatment with colestimide resulted in a moderate prolongation of APTT and reduction of TAT level, suggesting improvement of the hypercoagulable state. Since colestimide prevents the absorption of bile acid, the production of clotting factors might be reduced. Improvement of abnormalities of lipid metabolism by atorvastatin or colestimide might therefore prevent the progression of atherosclerosis and onset of thrombosis.

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Adenovirus-Mediated Transfer of Human Placental Ectonucleoside Triphosphate Diphosphohydrolase to Vascular Smooth Muscle Cells Suppresses Platelet Aggregation In Vitro and Arterial Thrombus Formation In Vivo

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Background—Platelet-rich thrombus formation is a critical event in the onset of cardiovascular disease. Because ADP plays a significant role in platelet aggregation, its metabolism is important in the regulation of platelet activation and recruitment. Ectonucleoside triphosphate diphosphohydrolase (E-NTPDase) is a key enzyme involved in vascular ADP metabolism. We recently isolated 2 isoforms of E-NTPDase from the human placenta. The present study examined whether these isoforms suppress platelet aggregation and thrombus formation after adenovirus-mediated gene transfer to vascular smooth muscle cells (SMCs).

Methods and Results—We constructed adenovirus vectors expressing human placental E-NTPDase isoforms I (AdPlac I) and II (AdPlac II) or bacterial β-galactosidase (AdLacZ). Vascular SMCs infected with AdPlac I expressed significant NTPDase activity and inhibited the platelet aggregation induced by ADP and collagen in vitro. In contrast, SMCs infected with AdPlac II and AdLacZ did not exert antiplatelet effects. To investigate the antithrombotic and antiproliferative effects of placental E-NTPDase isoform I in vivo, we generated thrombosis in rat carotid arteries by systemically administered rose Bengal and transluminal green light 5 days after gene transfer and examined neointimal growth 3 weeks after thrombus formation. Blood flow in AdLacZ-infected arteries rapidly deteriorated and vanished within 96±18 seconds of occlusive thrombus formation. In contrast, blood flow in AdPlac I–infected arteries was preserved for at least 10 minutes during irradiation. In addition, thrombus formation and subsequent neointimal growth were obviously suppressed.

Conclusions—The local expression of placental E-NTPDase in injured arteries might prevent arterial thrombosis and subsequent neointimal growth. (***Circulation*. 2005;111:808-815.**)

Key Words: platelets ■ thrombosis ■ genetics ■ muscle, smooth ■ arteries

Thrombus formation is a critical event in the onset of cardiovascular disease. Thrombotic occlusion still occasionally arises after vascular interventions, such as angioplasty and stent implantation. Platelets play a pivotal role in the development of arterial thrombosis. Therefore, antiplatelet agents, including aspirin, ADP receptor blockers, and platelet glycoprotein (GP) IIb/IIIa antagonists, are systemically administered to prevent cardiovascular events.[1] Although some of these agents effectively reduce cardiovascular events,[1] they can also produce systemic hemorrhagic side effects.[2]

Platelet adhesion to injured vascular walls leads to platelet activation and the release of additional agonists such as ADP, serotonin, and thromboxane A_2, which cause further platelet recruitment to injured sites. Because ADP plays a key role in platelet aggregation,[3] its metabolism in the blood is important in the regulation of platelet activation and recruitment. Ectonucleoside triphosphate diphosphohydrolase (E-NTPDase; EC 3.6.1.5, or CD39) is a major metabolic enzyme of ADP in the vasculature.[4] Originally identified in B lymphocytes,[5] E-NTPDase is a membrane-bound enzyme that rapidly hydrolyzes both ATP and ADP to AMP and thereby inhibits platelet aggregation.[6–8] Makita et al[9] purified human placental E-NTPDase (Plac I) as an isoform of CD39 that alternatively differed at the *N*-terminus from that of CD39. Matsumoto et al[10] recently isolated another truncated variant

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of placental E-NTPDase (Plac II). Although these structural differences might affect their enzymatic activities,[11,12] the properties of the 2 isoforms remain unknown.

The present study used adenovirus-mediated gene transfer into vascular smooth muscle cells (SMCs) in vitro and in vivo to investigate whether the 2 isoforms of placental E-NTPDase inhibit platelet aggregation, thrombus formation, and neointimal growth.

Methods

Preparation of Recombinant Adenovirus With Placental E-NTPDase

Replication-defective $E1^-$ and $E3^-$ adenoviral vectors encoding Plac I (AdPlac I) and Plac II (AdPlac II) were generated with the Adeno-X expression system (Clontech) according to the manufacturer's instructions.[13] In brief, cDNA clones of Plac I (1554 bp) and Plac II (921 bp) were isolated at the Department of Blood Transfusion Medicine of Nara Medical University[10] and subcloned into the mammalian expression cassette pShuttle. Recombinant pShuttle was digested and inserted into the Adeno-X viral DNA. The recombinant viruses were propagated in HEK293 cells. Viral titers were determined by limiting dilution as plaque-forming units (PFU).[14] We also constructed control recombinant adenovirus encoding bacterial β-galactosidase (AdLacZ).

Cell Culture

Arterial SMCs were isolated from explanted thoracic aortas of Sprague-Dawley rats. Cells were cultured in SmGM2 growth medium (Sanko Junyaku) containing 5% fetal bovine serum and antibiotics. Confluent cells were immunopositive to anti-smooth muscle actin antibody (Dako Japan).

In Vitro Gene Transfer

Cells from passages 3 to 6 were incubated with the adenovirus vectors in the serum-free SmGM2 medium at the indicated multiplicity of infection (MOI). After incubation for 24 hours at 37°C, cells were washed twice with sterile phosphate-buffered saline and incubated in complete medium until assay.

Western Blots

Four days after infection, cells were lysed in Tris-buffered saline (pH 8.0) containing 150 mmol/L NaCl, 1% Nonidet P-40, 1% Triton X-100, and 1 mmol/L phenylmethylsulfonyl fluoride. After centrifugation at 12 000g for 5 minutes, the supernatant was resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions on 4% to 12% gradient gels, and then separated proteins were electrophoretically transferred to an Immobilon membrane (Millipore). Nonspecific binding was blocked with 5% skimmed milk, and then the membrane was incubated overnight at 4°C with a monoclonal anti-human placental E-NTPDase antibody (YH34). This novel antibody was produced in mice by a standard procedure at the Department of Blood Transfusion Medicine of Nara Medical University. Purified placental E-NTPDase used as an immunogen was prepared on an immunoadsorbent column as described.[9] Western blotting showed that YH34 bound with high affinity to the purified enzyme (≈80 kDa) under both nonreducing and reducing conditions (data not shown). We determined the immunoglobulin subclass and idiotype of YH34 (IgG_1-κ) by using an isotyping kit (Serotec). We purified YH34 IgG from mice ascites on a protein A-Sepharose CL-4B column (Amersham Bioscience Corp). The antibody recognizes the external domain of both Plac I and Plac II. Specific binding was visualized with a horseradish peroxidase-conjugated goat anti-mouse IgG (Nacalai Tesque) and an enhanced ChemiLuminescence reagent (DuPont NEN).

Analysis of NTPDase Activity

The activity of NTPDase in transfected SMCs was determined by measuring extracellular ATP and ADP concentrations with luciferin-luciferase.[15] In brief, cells (5×10^3) were seeded in 96-well plates and cultured in serum-free SmGM2 medium with 0.1% bovine serum albumin for 2 days. After 2 washes with phosphate-buffered saline, 100 μL of firefly luciferase ATP assay mixture (ATP determination kit, Molecular Probes) was added to each well. Luminescence was measured with a microplate luminometer (Fluoroskan Ascent FL, Labsystems). After 10 minutes of equilibration, 100 nmol/L exogenous ATP was added, and luminescence was measured at 1-minute intervals for up to 15 minutes. ATPase activity, calculated by measuring the degradation of exogenous ATP, was expressed as picomoles of inorganic phosphate per minute per milligram.

We estimated the ADP concentration by converting ADP to ATP.[16] After the cells were washed and the firefly luciferase ATP assay mixture was added, 100 nmol/L exogenous ADP was added to each well. After a 5-minute incubation, 1 U pyruvate kinase and 1 mmol/L phosphoenolpyruvate were added and luminescence was measured. ADPase activity, calculated by measuring the degradation of exogenous ADP, was expressed as picomoles of inorganic phosphate per minute per milligram.

Platelet Aggregation

We evaluated platelet aggregation in the presence of SMCs by using a modification of a described method.[17] Uninfected or infected SMCs detached by EDTA-collagenase were washed 3 times. Blood samples collected in 3.8% sodium citrate (9:1, vol/vol) were centrifuged at 900 or 3000 rpm for 10 minutes to prepare platelet-rich plasma (PRP) or platelet-poor plasma (PPP), respectively. PRP (5×10^8 platelets) and SMCs (5×10^5 cells) were incubated in siliconized cuvettes at 37°C in a PA-20 aggregometer (Kowa) that had been calibrated with PRP and PPP for 0% and 100% transmission, respectively. Thereafter, either ADP (10 μmol/L final concentration) or collagen (10 μg/mL, final concentration) was added to the cuvettes, and platelet aggregation was measured.

Animal Care

The Animal Care Committee of Miyazaki Medical College (No. 1998-025-6) approved the study protocols. We used 59 male Sprague-Dawley rats weighing 400 to 500 g. The animals received humane care according to the *Guide for the Care and Use of Laboratory Animals* prepared by the Institute of Laboratory Animal Resources and published by the National (Bethesda, Md) Institutes of Health (NIH publication No. 86-23, revised 1985). Aseptic surgery proceeded under general anesthesia induced by an intraperitoneal injection of pentobarbital (50 mg/kg body weight).

In Vivo Gene Transfer Into Injured Arteries

The common carotid arteries of the rat were exposed and isolated by temporary ligation at a distance of 1.5 cm. A 31-gauge needle was inserted into the proximal side of the segment. Thereafter saline was flushed into the segment, and then air was infused at a rate of 50 mL/min for 3.5 minutes to denude endothelial cells.[18] After the segment was filled with 0.1 mL of saline containing AdPlac I or AdLacZ (final titer, 5×10^8 PFU) or saline alone (n=6 each) for 30 minutes, the mixture was aspirated and blood flow was restored.[19] The vessels harvested 2 to 5 days after gene transfer were perfusion-fixed in 4% paraformaldehyde and embedded in paraffin by standard procedures. We detected the expression of human placental E-NTPDase protein by immunohistochemistry (EnVision+ kits) by using the primary monoclonal antibody YH34. The negative control included nonimmune mouse IgG_1 instead of YH34.

NTPDase Activity in Vessels

Carotid arteries without perfusion-fixation were excised and homogenized with a Polytron PT3000 (Kinematica) in Tris-buffered saline (pH 7.4) containing aprotinin and phenylmethylsulfonyl fluoride.[20,21] The homogenates were then incubated with the firefly luciferase ATP assay mixture. Extracellular ATP and ADP concentrations were

determined by measuring luminescence as described earlier. Protein concentrations of homogenates were determined by the bicinchoninic acid assay with bovine serum albumin as the standard.

Arterial Thrombus Formation and Neointimal Growth

Thrombus formation was produced in rat common carotid arteries 5 days after gene transfer by photochemical exposure under anesthesia.[22] Blood flow in the distal side of the arteries was recorded with a transit-time blood flowmeter (T106, Transonic Systems Inc) with a PowerLab system (AD Instruments Pty Ltd). After baseline blood flow was established, rose Bengal (20 mg/kg; Wako) was slowly injected into the jugular vein, and the carotid arteries were irradiated with green light (wavelength, 540 nm) with a xenon lamp (Hamamatsu Photonics) equipped with heat-absorbing and green filters. Blood flow was measured during irradiation for 10 minutes, and then the arteries were perfusion-fixed and stained with hematoxylin and eosin/Victoria blue for histological examination. To evaluate the neointimal growth 3 weeks after thrombus formation, the areas (μm^2) of neointima and media were measured with an image analyzing system (Axio Vision 2.05, Carl Zeiss) by 2 investigators (K.M. and K.H.) who were blinded to the treatment assignment.

Ex Vivo Platelet Aggregation, Prothrombin Time, and Activated Partial Thromboplastin Time

To confirm a systemic antithrombotic effect by local gene transfer, we evaluated platelet aggregation in response to either ADP or collagen ex vivo as described earlier. Prothrombin time and activated partial thromboplastin time were measured with a coagulation timer (Behring Fibrintimer, Behring Diagnostics) before and 5 days after gene transfer.

Statistical Analysis

All data are presented as mean±SE. An unpaired Student t test and ANOVA with Bonferroni multiple comparisons were used for comparisons between groups. A value of $P<0.05$ was considered significant.

Results

Expression of Human Placental E-NTPDases and NTPDase Activity of Infected SMCs In Vitro

We examined whether SMCs infected with AdPlac I, AdPlac II, or AdLacZ can produce biologically active E-NTPDases. Figure 1 shows 82- and 50-kDa immunoreactive bands that were detected from 2 days after gene transfer in lysates of cells infected with AdPlac I and AdPlac II, respectively.[9] These proteins persisted for at least 8 days thereafter (data not shown) but were undetectable in lysates of parental SMCs or of those infected with AdLacZ.

The ATPase activities of uninfected SMCs and of those infected with AdLacZ were 77.5±5.5 and 79.3±8.2 pmol $Pi \cdot min^{-1} \cdot mg^{-1}$, respectively (Figure 2A). In contrast, the ATPase activity of SMCs infected with AdPlac I was significantly high at an MOI of 100. We found that the ADPase activity of SMCs infected with AdPlac I was also high in an MOI-dependent manner (Figure 2B); however, the activities of ATPase and ADPase in AdPlac II–infected SMCs were similar to those of parental and AdLacZ-infected SMCs.

Platelet Aggregation With Infected SMCs In Vitro

We investigated whether the expression of Plac I and Plac II in SMCs inhibits platelet aggregation in vitro. Platelet aggregation induced by ADP was significantly and MOI-dependently suppressed in the presence of SMCs infected with AdPlac I, but not when the cells were infected with AdPlac II or AdLacZ (Figure 3A). Collagen-induced platelet aggregation was significantly suppressed in the presence of SMCs infected with AdPlac I at an MOI of 100 but not at that of 10 (Figure 3B). AdPlac I–infected SMCs dose-dependently suppressed platelet aggregation (data not shown), whereas SMCs infected with either AdPlac II or AdLacZ did not affect ADP/collagen-induced aggregation.

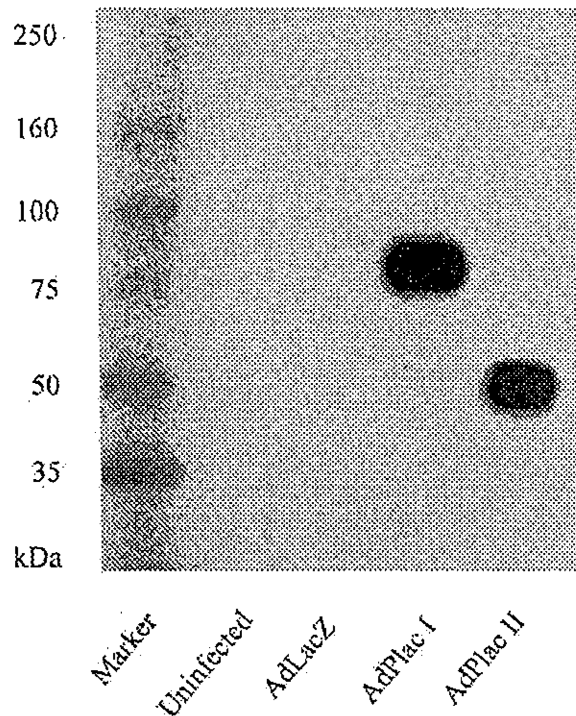

Figure 1. Expression of human placental E-NTPDases in infected SMCs 4 days after gene transfer. Proteins of whole-cell extracts from SMCs infected or not with AdLacZ, AdPlac I, or AdPlac II were separated by SDS-PAGE and immunoblotted against monoclonal anti-human placental E-NTPDase antibody YH34.

Inhibition of Arterial Thrombus Formation and Neointimal Growth in Arteries Infected With AdPlacI In Vivo

Five days after gene transfer, Plac I protein was broadly expressed in the medial SMCs of arteries infected with AdPlac I (Figure 4A) but was undetectable in control arteries infected with AdLacZ (Figure 4C). Arteries infected with AdPlac I were immunonegative for Plac I protein when the antibody was replaced with nonimmune mouse IgG_1 (Figure 4B). Figure 5 shows NTPDase activities in infected arteries (Figure 5). Two days after endothelial denudation with a saline infusion and AdPlac I infection, ATPase and ADPase activities were significantly reduced as compared with those of normal carotid arteries (control) by endothelial denudation. Five days after infection with AdPlac I, however, ATPase and ADPase activities were 2.0- and 1.7-fold higher, respectively.

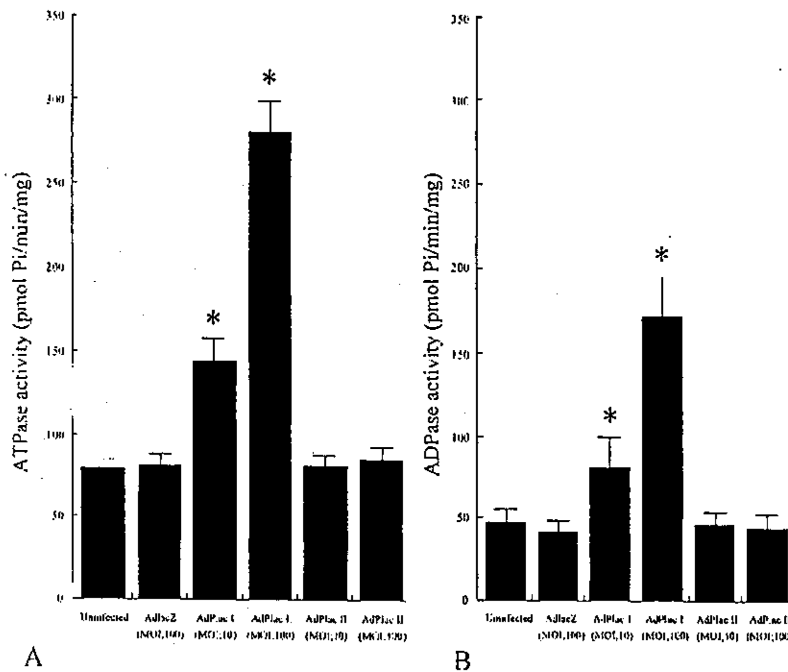

Figure 2. NTPDase activity of infected SMCs. Activities of NTPDase in transfected SMCs were determined by measuring extracellular ATP and ADP concentrations. Activities of ATPase (A) and ADPase (B) were determined in SMCs infected or not with AdLacZ at MOI 100, AdPlac I at MOI of 10 or 100, or AdPlac II at MOI of 10 or 100 (n=5 each; *$P<0.05$ vs AdLacZ).

than those after AdLacZ infection. The numbers of mRNA copies of Plac I per 10^6 copies of the housekeeping gene, glyceraldehyde 2-phosphate dehydrogenase, 2 and 5 days after gene transfer were 15.2±3.5 and 1456±65, respectively. These results indicated that Plac I protein expressed in carotid arteries had biological NTPDase activity in the vascular wall.

Blood flow in arteries infected with AdLacZ rapidly deteriorated after irradiation and vanished within 96±18 seconds (n=6, Figure 6A). In contrast, blood flow in arteries infected with AdPlac I was preserved for at least 10 minutes during green light irradiation (n=6, Figure 6B). Histological analyses revealed that occlusive thrombi, mainly consisting of aggregated platelets, had blocked arteries infected with AdLacZ (Figure 6C), whereas only small mural thrombi had developed in arteries infected with AdPlac I (Figure 6D). The neointimal growth 3 weeks after thrombus formation was significantly reduced by AdPlac I infection. Area of neointima and neointima/media ratio in arteries infected with AdLacZ versus AdPlac I were 97 500±14 000 μm^2 versus 45 300±5900 μm^2 (n=8 each, $P<0.01$) and 1.03±0.13 versus 0.41±0.05 (n=8 each, $P<0.001$), respectively.

Platelet Function and Blood Coagulation in Rats Infected With AdPlac I

Platelet aggregation induced by ADP and collagen, prothrombin time, and activated partial thromboplastin time did not significantly differ between rats infected with AdPlac I and AdLacZ (see Table).

Discussion

We have demonstrated that human placental E-NTPDase isoform I expressed in vascular SMCs hydrolyzed ATP/ADP, prevented platelet aggregation in vitro, and significantly suppressed photochemically induced arterial thrombus formation, as well as subsequent neointimal growth in vivo. Placental E-NTPDase isoform II expressed in SMCs did not exert antiplatelet effects.

Fresh platelet-rich thrombi frequently develop in association with cardiovascular events, including unstable angina and acute myocardial infarction, as well as immediately after interventions such as angioplasty, stent implantation, and atherectomy.[23,24] Platelet activation induced by ADP plays a pivotal role in arterial thrombus formation.[3] In normal vessels, ADP is rapidly metabolized to AMP by E-NTPDase on the endothelial cell surface, which is subsequently converted to adenosine by the 5′-nucleotidase, also localized on the endothelial cell membrane. Recent in vivo and in vitro gene transfer studies of NTPDase have shown that increased NTPDase activity in the vasculature confers vascular protective effects and also survival benefits on cardiac grafts by blocking thrombotic sequelae.[25,26] Therefore, retaining high NTPDase activity in vascular SMCs should reduce the incidence of thrombus formation after vascular injury.

Vascular E-NTPDase was identified along with CD39, which has 2 putative transmembrane domains and an extracellular domain containing an enzymatically active region.[11,12] The extracellular domain contains 5 apyrase conserved regions (ACRs), of which ACR-1, -4, and -5 are important for maintaining enzymatic activity.[12] Plac I also has 2 putative transmembrane domains and an extracellular domain like CD39, whereas Plac II lacks ACR-5 and a putative transmembrane sequence in the C-terminal region.[10] Our results indicate that ACR-5 is essential for NTPDase activity.

Thrombus formation was almost completely suppressed in arteries infected with AdPlac I. Makita et al[9] showed that placental E-NTPDase blocks platelet aggregation under low shear stress (12 dyne/cm^2) but did not significantly inhibit

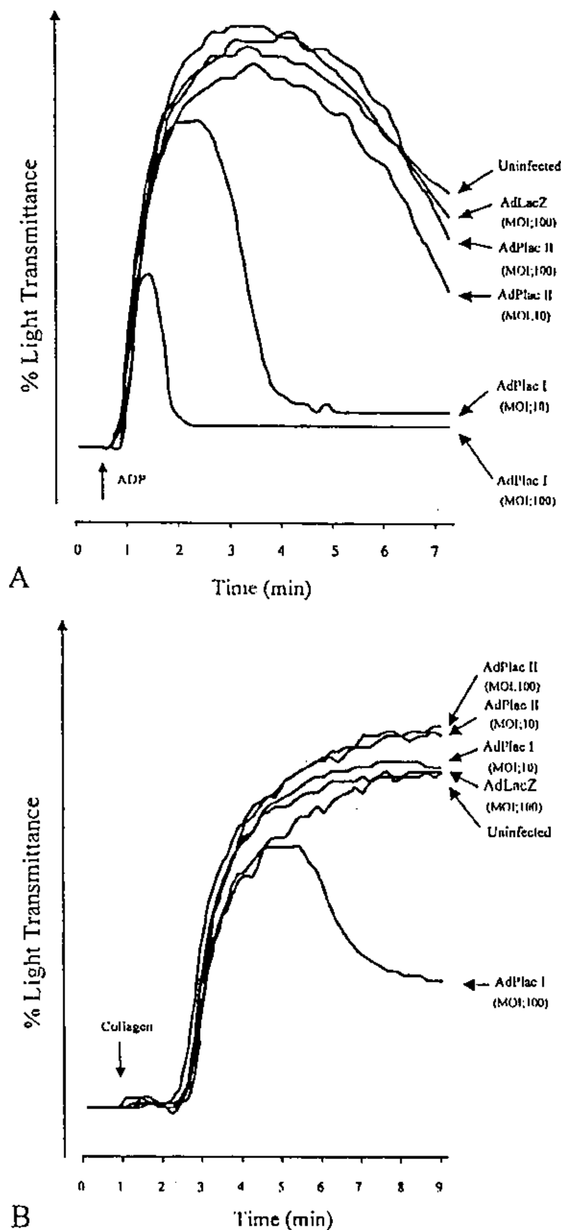

Figure 3. Platelet aggregation in presence of SMCs induced by ADP and collagen. ADP (A) or collagen (B) was added to PRP in presence of uninfected SMCs or SMCs infected with AdLacZ (MOI=100), AdPlac I (MOI=10 and 100), and AdPlac II (MOI=10 and 100), and platelet aggregation was measured.

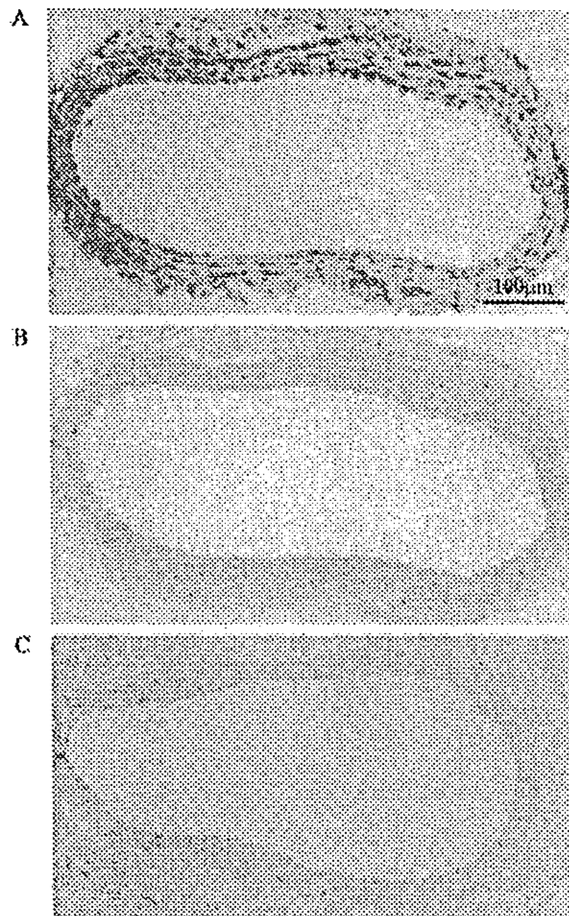

Figure 4. Immunohistochemical staining for human placental E-NTPDase in infected carotid arteries. Representative immunohistochemical microphotographs of rat carotid arteries infected with AdPlac I (A and B) or AdLacZ (C). SMCs in whole wall are immunopositive for human placental E-NTPDase in artery infected with AdPlac I (A) but not with AdLacZ (C). Replacing antibody with nonimmune mouse IgG_1 generated immunonegative reactions (B).

initial aggregation under high shear stress (108 dyne/cm^2), although platelets disaggregated during the later phase in vitro. These results imply that E-NTPDase plays an antithrombotic role under relatively low flow conditions. The finding that this enzyme localizes on syncytiotrophoblasts and in endothelial cells of the umbilical vein, rather than the artery,[8] might be consistent with the results in vitro; however, the present study showed that arterial thrombus formation under high flow conditions was obviously suppressed in vessels infected with AdPlac I. Interactions between von Willebrand factor and the platelet membrane receptors GP Ib and IIb/IIIa are crucial for platelet aggregation when blood flow is high,[27] and ADP and its receptors are key mediators of such interactions.[28,29] Thrombus formation was photochemically induced in the present study.[22] Photochemical exposure produces highly reactive oxygen species that react with cell membrane lipids to cause endothelial cell damage and platelet activation. In this animal model therefore, ADP released from damaged tissue and activated platelets played a critical role in thrombus formation. These lines of evidence indicate that placental E-NTPDase isoform I overexpression in injured vessels is highly antithrombotic, even under high blood flow, which is characteristic of stenosed atherosclerotic arteries.

A key advantage of the local expression of E-NTPDase in the rat model was the absence of systemic side effects.

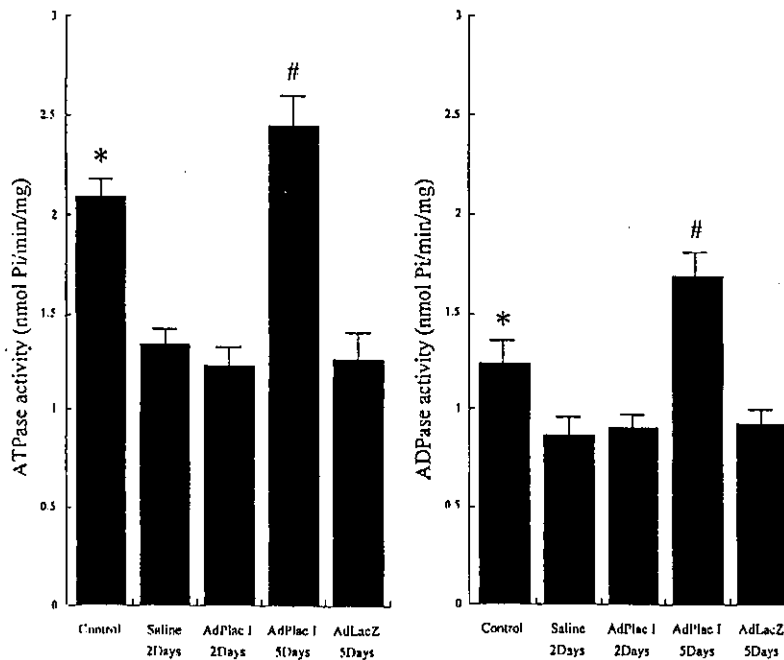

Figure 5. Activities of NTPDase in infected carotid arteries. Homogenized arteries were incubated for 5 minutes with exogenous ATP or ADP, and then ATPase and ADPase activities were determined. Control indicates rat normal carotid artery; saline 2 days, 2 days after endothelial denudation with saline infusion (n=5 each; *$P<0.05$, control vs saline 2 days and AdPlac I 2 days; #$P<0.05$, AdPlac I 5 days vs AdLacZ 5 days).

Animal studies have shown that a new soluble form of the extracellular region of CD39 (solCD39) has systemic antithrombotic effects[30,31]; however, antiplatelet therapy also tends to induce systemic bleeding.[2] This is an important limitation to clinical applicability and indicates a potential advantage of local gene transfer into injured vessels. Our study demonstrated that E-NTPDase expressed on SMCs inhibited platelet aggregation induced not only by ADP but also by collagen, although a high viral titer was required. Thus, local expression of E-NTPDase in diseased arteries should reduce the incidence of cardiovascular events without side effects.

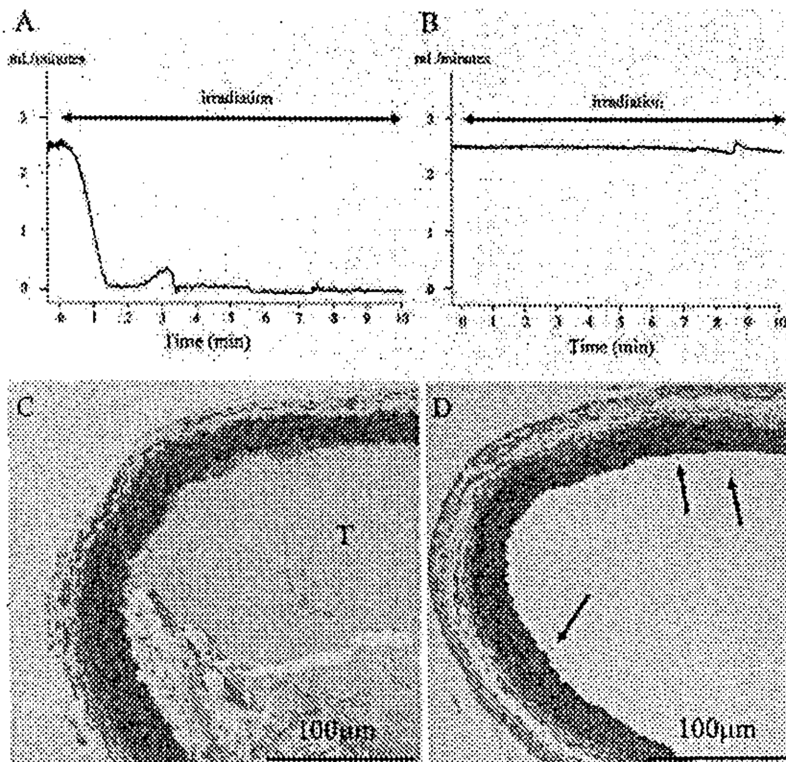

Figure 6. Arterial thrombus formation in arteries injured by air. Thrombus formation induced by photochemical exposure in rat common carotid arteries 5 days after gene transfer. Blood flow of carotid arteries infected with AdLacZ (A) or AdPlac I (B) was recorded during irradiation for 10 minutes. Histologically occlusive thrombus (T) formed in arteries infected with AdLacZ (C), whereas small mural thrombi (arrows) developed in those infected with AdPlac I (D). Results were similar in 6 rats.

Platelet Aggregation and Coagulation Parameters

	Before Virus Infection	AdLacZ	AdPlac I
% Maximum platelet aggregation			
ADP (10 μmol/L)	83.3±3.8	82.0±3.5	84.0±3.5
Collagen (10 μg/mL)	91.3±5.3	90.8±3.3	89.8±4.3
Coagulation parameters			
Prothrombin time, s	8.6±0.5	8.9±0.2	8.6±0.5
Activated partial thromboplastin time, s	14.6±0.5	14.5±0.5	14.8±0.6

Abbreviations are as defined in text.
n=4 each, P>0.1.

The endothelium predominantly expresses E-NTPDase and, to a lesser extent, so do other vascular cells.[32] The enzyme in vascular SMCs would modulate vascular tone via P2 purinoceptors.[33] The present study detected placental E-NTPDase isoform I expression by immunohistochemistry in whole walls of arteries infected with AdPlac I. Antiplatelet ability is thought to depend mainly on the NTPDase activity of luminal SMCs. Gangadharan et al[21] reported that adenovirus-mediated CD39 gene transfer augments NTPDase activity in rabbit iliac arteries injured with balloons but does not significantly reduce platelet deposition on the injured luminal surface. They detected CD39 expression only in luminal SMCs. Although the methods of gene transfer and/or species differences might be relevant factors in explaining this discrepancy, the powerful inhibitory effect against occlusive thrombus formation in AdPlac I–infected arteries might depend not only on antiplatelet function but also on reduced vasoconstriction in injured vessels. We evaluated total NTPDase activities of the vessel wall, which includes potential sources of the enzyme. We could not evaluate native E-NTPDase of rat vascular SMCs. Occlusive thrombi were photochemically induced in all arteries infected with AdLacZ, and the level of E-NTPDase/CD39 mRNA expression in rat carotid arteries was very low (data not shown). These results suggest that native E-NTPDase in SMCs would not have putative antithrombotic roles in vivo.

In addition to thrombus formation, subsequent neointimal growth was suppressed in arteries infected with AdPlac I. Other studies in vitro have revealed that purinergic signaling modulates the proliferation and death of SMCs and endothelial cells.[34] Extracellular ATP and ADP released from platelets, as well as injured or activated SMCs and endothelial cells, is mitogenic for intimal SMCs via P2Y receptors[35] and synergistically acts with growth factors such as platelet-derived growth factor and basic fibroblast growth factor.[35,36] The neointima in the present study was exclusively composed of SMCs and extracellular matrix, suggesting that increased NTPDase activity on SMCs suppresses neointimal growth via the inhibition of SMC proliferation. In addition, thrombus itself contributes to neointimal formation and plaque progression.[37] Taken together, increased NTPDase activity on SMCs directly and/or indirectly might contribute to neointimal growth after thrombosis.

In summary, the present study showed that transfer of the adenovirus-mediated human placental E-NTPDase gene into vascular SMCs eliminates platelet aggregation induced by ADP and collagen as well as occlusive thrombus formation in injured arteries. In the clinical setting of cardiovascular events, not only the development of platelet-rich thrombi but also the rapid activation of the blood coagulation system plays a significant role in thrombus formation. The antithrombotic efficacy of AdPlac I infection should be evaluated in arteries with atherosclerosis.

Acknowledgments

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その他

平成１６年度厚生労働科学研究費補助金
難治性疾患克服研究事業
血液凝固異常症に関する調査研究班

第２回班会議
日時：平成１７年１月15日（土）午前１０時〜４時３０分終了予定
場所：慶應義塾大学医学部北里記念図書館２Ｆ　北里講堂

プログラム

抄録集

主任研究者　池田　康夫

平成16年度厚生労働科学研究費補助金　難治性疾患克服研究事業

血液凝固異常症に関する調査研究班

第2回班会議

日時：平成17年1月15日（土）午前10時〜4時30分終了予定

場所：慶應義塾大学医学部北里記念図書館2F　北里講堂

プログラム

（サブグループ研究報告:30分　各個研究:10分　討論含む）

10：00〜　主任研究者　挨拶　池田康夫

10：10〜　厚生労働省（疾病対策課）挨拶

10：20〜　ITP研究班　…　統括報告　藤村欣吾

サブグループリーダー：藤村欣吾　広島大学医学部
班　　員：桑名正隆　慶應義塾大学医学部
　　　　　倉田義之　大阪大学医学部
研究協力者：藤沢康司　慈恵会医科大学
　　　　　降旗謙一　エスアールエル
　　　　　野村昌作　岸和田市民病院
特別協力者：杉野稔・伊津野孝　東邦大学医学部（疫学班）

10：50〜　TTP研究班　…　統括報告　藤村吉博

サブグループリーダー：藤村吉博　奈良県立医科大学
班　　員：宮田敏行　国立循環器病センター研究所
　　　　　村田　満　慶應義塾大学医学部
　　　　　和田英夫　三重大学医学部
特別協力者：杉野稔・伊津野孝　東邦大学医学部（疫学班）

11：20〜　特発性血栓症研究班　…　統括報告　宮田敏行

サブグループリーダー：宮田敏行　国立循環器病センター研究所
班　　員：小嶋哲人　名古屋大学医学部
　　　　　坂田洋一　自治医科大学
　　　　　辻　　肇　京都府立医科大学
　　　　　村田　満　慶應義塾大学医学部
　　　　　川崎富夫　大阪大学医学部
研究協力者：猪子英俊　東海大学医学部
特別協力者：杉野稔・伊津野孝　東邦大学医学部（疫学班）

11：50〜12：30　昼休み

１２：３０～１３：３０

TTP 班員報告：

松本雅則（藤村吉博）「TMA 患者における血漿 ADAMTS13 抗原解析」

小亀浩市（宮田敏行）「ADAMTS13 の消光性蛍光基質の開発と血栓症の解析への応用」

村田　満　「ADAMTS13 と血栓症」

和田英夫　「TTP の二次アンケート調査の解析途中経過」

１３：３０～１４：３０

特発性血栓症班員報告：

川崎富夫「日本の現状に即した新しい発想の肺塞栓症予防戦略－大阪大学病院の試み」

窓岩清治（坂田洋一）「敗血症 DIC の病態と線溶系因子」

辻　肇　「先天性アンチトロンビン欠損症の遺伝的背景」

小嶋哲人「血栓性素因プロテインＳ欠損所の遺伝子解析」

１４：３０～１５：００　休　憩

１５：００～１６：００

ITP 班員報告：

桑名正隆「ITP の診断基準改定をめざして」

倉田義之「血小板数著減検体における正確な血小板数測定法の確立
－血小板測定への破砕赤血球の影響についての検討－」

降旗謙一「検査センターにおける ITP 診断検査の準備状況について」

終　了

平成 16 年度厚生労働科学研究費補助金　難治疾患克服研究事業
血液凝固異常症に関する調査研究班
主任研究者：池田康夫
事務局：慶應義塾大学医学部内科池田教授室　Tel: 03-3353-1211 内線 62421

ITP研究グループ　総括報告

広島大学大学院病態薬物治療学講座　血液・腫瘍科　藤村　欣吾

成人特発性血小板減少性紫斑病（ITP）に対する治療ガイドラインの提案

標準的ITP治療は１９８２年に厚生省特発性造血器障害治療研究班からITP治療指針として発表され今日まで定着し汎用されている。
本指針を用いたプロスペクティブな治療研究結果は完全寛解率は約４０％、無反応例は約３０％である。また副腎皮質ホルモン単独による完全寛解は３３％に認められたが、その後の施設間での寛解率は１０～３０％と必ずしも良好ではない。また長期の副腎皮質ホルモン服薬による副作用対策が必要となり、医療情報の普及、意識の向上など従来の指針が医師、患者に受け入れにくい場合も生じている。
さらに免疫学の進歩により発症機序からより的確なITP診断法が提案され、新たな免疫抑制療法の有用性が示唆されている。
また本研究班におけるレトロスペクティブ研究において、ヘリコバクターピロリ菌（HP菌）感染ITP症例に対する除菌療法は未治療例、既治療例を問わず、治療抵抗性を超えて除菌成功例では約６５％に血小板数の増加が認められ、殆どの症例はITPの治療から脱却可能となっている。除菌療法は１週間で、副作用が軽微でありHP菌陽性ITP症例においては有用な治療と考えられた。この除菌効果は米国、北欧などでは認められず本療法を治療に組み入れるガイドラインは本邦で独自に作成される必要がある。
これらの理由によりITP治療ガイドライン（２００４年試案）を作成した。
本ガイドラインの特徴は①ピロリ菌の検査、除菌療法を組み込み、②除菌無効例、ピロリ菌陰性例に対して First line, Second line 治療を示し、③それぞれに治療目標を設定し、④自覚症状、臨床症状、など臨床実態に即したもので、⑤副腎皮質ステロイドの使用期間を極力短くして副作用の発現を少なくする、⑥緊急時、重篤な出血時にはガンマーグロブリン療法を積極的に用いる、などである。
本ガイドラインを履行することによりQOLの改善、全体的なITP治療費の削減が可能になると期待される。