Dysregulated gene expression of extracellular matrix and adhesion molecules in saphenous vein conduits of hemodialysis patients

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Objective: The incidence of cardiovascular disease was approximately 10 times higher in hemodialysis patients with end-stage renal disease than in the general population. The saphenous vein is the most commonly used conduit for coronary artery bypass grafting. However, the extracellular matrix and adhesion molecule characteristics of saphenous vein in hemodialysis patients remain unclear. The aim of the present study was to survey the extracellular matrix gene expression profile of the saphenous vein in hemodialysis patients undergoing coronary artery bypass grafting.

Methods: A total of 34 patients undergoing elective coronary artery bypass grafting were enrolled. Of the 34 patients, 15 with end-stage renal disease required maintenance hemodialysis. The control group consisted of the other 19 patients without preoperative renal disease. Samples of the saphenous vein were obtained at coronary artery bypass grafting. The expression profile of the extracellular matrix genes was analyzed by microarray. The tissue matrix metalloproteinase/tissue inhibitor of metalloproteinase protein activities in the saphenous vein were evaluated by immunocytochemistry and Western blotting.

Results: Nineteen extracellular matrix and adhesion molecule-focused genes demonstrated at least a threefold difference in expression between the 2 groups. Upregulation was observed in 16 genes, and 3 genes appeared to be downregulated. Notable imbalanced matrix metalloproteinase/tissue inhibitor of metalloproteinase protein activities of saphenous vein exposed to end-stage renal disease conditions was found.

Conclusions: The results from present study suggest that the native extracellular matrix gene expression profile of the saphenous vein conduits in hemodialysis patients show signs of the vein graft disease process before coronary surgery. Furthermore, some preoperative profiles of hemodialysis patients undergoing coronary artery bypass grafting might provide some useful clues regarding vein graft quality and prompt adjustment in surgical strategy. (J Thorac Cardiovasc Surg 2012;144:684-9)

The incidence of cardiovascular disease is approximately 10 times greater in hemodialysis (HD) patients with end-stage renal disease (ESRD) than in the general population.¹ Moreover, the cardiovascular death rates in HD patients are also greatly increased compared with those in the general population.²⁻⁴ Although percutaneous coronary intervention has made significant inroads in the revascularization of patients with coronary artery disease, some HD patients still do significantly better in terms of survival and a reduced need for additional intervention with coronary artery bypass grafting (CABG).⁵ The saphenous vein (SV) is the most commonly used CABG conduit. However, within

10 years, vein graft occlusive disease occurs in nearly one half of the conduits.^{6,7} Vein graft failure occurs secondary to the deposition of extracellular matrix (ECM).⁸⁻¹⁰ However, to date, no studies have been published on the identification and understanding of the ECM and adhesion molecule characteristics of SV conduits in HD patients. The aims of the present study were, therefore, to survey the gene expression profile of the ECM and adhesion molecules in SV conduits of HD patients undergoing CABG using microarray technology.

METHODS

Study Subjects

For the present study, 34 patients with coronary artery disease undergoing elective CABG at our institute were enrolled. All these subjects fulfilled the inclusion criteria and agreed to participate. The local research ethics committee approved study, and each patient gave written informed consent. Of the 34 subjects, 15 with ESRD were receiving maintenance HD. The non-HD (control) group included the other 19 patients who had no preoperative history of renal disease. All patients were receiving conventional anti-angina therapy. The HD sessions were performed 3 times for 4 hr/wk using polysulphone membrane dialysers. The exclusion criteria were any inflammatory, infective disease, overt clinical heart failure, neoplastic disease, or acute coronary event during the previous 2 months. The patients previously diagnosed with diabetes mellitus (fasting glucose >5.5 mmol/L), hyperlipidemia (total cholesterol level >5.2 mmol/L), or hypertension (systolic blood pressure >140 mm Hg, diastolic blood

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Supported by the Shanghai Medical Development Research Fund (Grant 20001-2D002).

Disclosures: Authors have nothing to disclose with regard to commercial support. Yongxin Sun and Wenjun Ding contributed equally to this study.

Received for publication Sept 11, 2011; revisions received Nov 1, 2011; accepted for publication Dec 14, 2011; available ahead of print Jan 24, 2012.

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0022-5223/\$36.00

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doi:10.1016/j.jtcvs.2011.12.037

Abbreviations and Acronyms	
ECM	= extracellular matrix
ESRD	= end-stage renal disease
CABG	= coronary artery bypass grafting
HD	= hemodialysis
SV	= saphenous vein

pressure >90 mm Hg) were receiving appropriate glucose, lipid-lowering, or hypertension treatment. The individual characteristics of the patients are listed in Table 1.

Tissue Samples and RNA Extraction

Samples of the SV were obtained at CABG. Segments of a few millimeters from the SV were isolated and immediately put into RNeasyTM (Ambion, Austin, Tex) for storage at -80°C. Total RNA extraction was performed using the RNeasy Mini kit (Qiagen, Valencia, Calif) according to the manufacturer’s instructions. Next, 1 to 2.0 μg of total RNA was reverse transcribed into single strand cDNA using MuLV reverse transcriptase (Applied Biosystems, Foster City, Calif).

cDNA Gene Expression Array Analysis

The expression profile of extracellular matrix and adhesion molecules genes was analyzed using the RT² ProfilerTM PCR Array Human Extracellular Matrix and Adhesion Molecules (PAHS-013A; SuperArray Bioscience, Frederick, Md). The results were analyzed using ScanAlyze and GEArray Analyzer (SuperArray Bioscience).

Immunocytochemistry Evaluation of MMP-2, MMP-9, TIMP-2, and TIMP-3 Activity in SV of HD and Control Groups

The optimal dilutions for each primary antibody (Santa Cruz Biotechnology, Santa Cruz, Calif) were found. The sections were incubated with goat anti-mouse biotinylated second antibody IgGs (Beijing Zhongshan, Beijing, China). Semiquantitative evaluation was performed by computer-assisted image analysis (Chengdu Taimeng, Sichuan, China).

Western Blot Analysis of Tissue MMP-2, MMP-9, TIMP-2, and TIMP-3 Levels

Total protein was fractionated and transferred to polyvinylidene difluoride membranes (Millipore, Bedford, Mass). Each membrane was incubated with specific antibodies (Calbiochem, San Diego, Calif) with secondary antibody (Jackson Immunolab, West Grove, Pa). Immune complexes were visualized with the enhanced chemiluminescence detection system (Amersham, Piscataway, NJ).

Statistical Analysis

All values are expressed as the mean ± standard deviation. Quantitative reverse transcriptase-polymerase chain reaction results are reported as the relative gene expression, and the x-fold change in target genes was determined using the 2-ΔΔC_t method, as previously described.¹¹ To evaluate the gene expression profile in the HD group, the x-fold change was determined as (C_t HD - C_t Actin) - (C_t Control - C_t Actin). The group comparisons were performed using the Student *t* test. Univariate and multivariate linear regression analyses (Cox regression model) were performed to analyze the extent of matrix metalloproteinase-2/tissue inhibitor of metalloproteinase-2 (MMP-2/TIMP-2) imbalance in relation to the preoperative variables.

TABLE 1. Demographics of studied population

Demographic	HD group (n = 15)	Control group (n = 19)	P value
Gender (male)	11 (73.3%)	15 (78.9%)	.39
Age (yr)	62.6 ± 10.3	60.1 ± 15.5	.44
Body weight (kg)	66.2 ± 10.6	68.5 ± 13.7	.41
History of smoking (n)	6 (40.0%)	8 (42.1%)	.38
History of MI (n)	4 (26.7%)	4 (21.1%)	.23
Previous PCI (n)	9 (60.0%)	11 (57.9%)	.29
Hypertension (n)	8 (53.3%)	8 (42.1%)	.12
Diabetic mellitus (n)	5 (33.3%)	6 (31.5%)	.34
Cerebral vessel disease (n)	5 (33.3%)	4 (21.1%)	.07
Peripheral vascular disease (n)	5 (33.3%)	4 (21.1%)	.09
Duration of HD (yr)	2.2 ± 0.9		
Total cholesterol level (mmol/L)	5.80 ± 2.25	5.04 ± 1.46	.14
Lipoprotein(a) (mg/L)	721.0 ± 253.6	267.3 ± 119.8	<.01
Apolipoprotein A (g/L)	2.48 ± 1.13	1.39 ± 0.84	.04
Apolipoprotein B (g/L)	3.09 ± 1.54	1.67 ± 0.92	<.01
Calcium concentration (mmol/L)	2.64 ± 1.31	2.25 ± 0.77	.20
Phosphorus concentration (mmol/L)	2.77 ± 0.86	1.05 ± 0.24	<.01
Calcium × phosphorus product	55.74 ± 13.08	35.16 ± 4.31	<.01
PTH (ng/L)	509.6 ± 313.6	80.5 ± 44.7	<.01
Homocysteine (mmol/L)	37.6 ± 10.9	18.8 ± 6.5	.02
IMT in carotid arteries (mm)	1.46 ± 0.32	0.90 ± 0.14	<.01
Coronary artery calcification score	695.0 ± 126.8	507.3 ± 88.2	.03

HD, Hemodialysis; MI, myocardial infarction; PCI, percutaneous coronary intervention; PTH, parathyroid hormone; IMT, intima-media thickness.

RESULTS

Demographic Patient Characteristics

The demographics of the population studied did not differ between the HD and control groups, except for the parameters related to ESRD. The 2 groups were well matched for age, gender, and presentation profile (Table 1).

Dysregulated Expression of Gene Profiles

Using the RT² ProfilerTM PCR Array Human Extracellular Matrix and Adhesion Molecules (SuperArray Bioscience), we examined the gene expression profile and compared the relative expression of ECM and adhesion molecule genes in SV between the HD and control groups. Of the 84 ECM and adhesion molecule-focused genes in this microarray, 19 genes demonstrated at least a threefold difference in expression between the HD and control groups. Upregulation was observed in 16 genes, and 3 genes appeared to be downregulated (Table 2). The extent of MMP-2/TIMP-2 imbalance was calculated with the x-fold changes of the genes. The extent of MMP-2/TIMP-2 imbalance of the SV in the HD group appeared significantly increased compared with the normal control veins (4.57 ± 1.24 vs 0.84 ± 0.29, *P* < .05). Univariate and multivariate

TABLE 2. ECM-related genes regulated in saphenous vein of hemodialysis patients compared with control group*

Symbol	Description	x-Fold difference	P value
CDH1	Cadherin 1, type 1, E-cadherin	23.83 ± 11.09	<.001
COL12A1	Collagen, type XII, alpha 1	6.74 ± 2.25	.003
COL16A1	Collagen, type XVI, alpha 1	5.61 ± 2.06	.005
COL1A1	Collagen, type I, alpha 1	3.29 ± 1.44	.011
ITGA3	Integrin, alpha 3	4.15 ± 1.63	.008
MMP1	Matrix metalloproteinase 1	8.15 ± 3.80	.001
MMP2	Matrix metalloproteinase 2	19.93 ± 11.75	<.001
MMP12	Matrix metalloproteinase 12	13.00 ± 13.55	<.001
MMP13	Matrix metalloproteinase 13	6.76 ± 2.59	.003
MMP3	Matrix metalloproteinase 3	10.85 ± 9.06	<.001
MMP7	Matrix metalloproteinase 7	15.51 ± 38.62	<.001
MMP8	Matrix metalloproteinase 8	17.91 ± 30.58	<.001
MMP9	Matrix metalloproteinase 9	19.36 ± 5.50	<.001
SGCE	Sarcoglycan, epsilon	28.91 ± 13.40	<.001
THBS1	Thrombospondin 1	15.30 ± 6.93	<.001
TIMP1	Tissue metalloproteinase inhibitor 1	-4.60 ± 0.58	.007
TIMP2	Tissue metalloproteinase inhibitor 2	-3.58 ± 0.96	.009
TIMP3	Tissue metalloproteinase inhibitor 3	-11.30 ± 4.38	<.001
TNC	Tenascin C	7.81 ± 2.01	.002

x-Fold change for each gene from HD group to control group calculated as $2^{-\Delta\Delta Ct}$. Among 84 genes analyzed, 16 genes were upregulated >3.0-fold, and 3 were down-regulated in SV conduits of HD patients. *ECM*, Extracellular membrane; *HD*, hemodialysis; *SV*, saphenous vein. *Gene expression changes ≥ 3.0 -fold versus control in RNA level.

linear regression analyses showed that the extent of MMP-2/TIMP-2 imbalance of the SV was directly related to some preoperative ESRD-related parameters (Tables 3 and 4).

Changed Activities of MMP-2, MMP-9, TIMP-2 and TIMP-3 in SV of HD Group

A semiquantitative analysis showed that the MMP-2 and MMP-9 levels in the SV of the HD group (grade, 2.6 ± 0.5 and 1.8 ± 0.4 , respectively) were significantly greater than those in the normal SV (grade, 0.7 ± 0.16 and 0.6 ± 0.15 ; $P < .01$ and $P < .02$, respectively). TIMP-2 and TIMP-3 immunostaining were notably reduced in the SV of the HD group compared with those in the control group (grade, 0.6 ± 0.19 vs 2.2 ± 0.3 and 0.9 ± 0.28 vs 2.4 ± 0.7 ; $P < .05$ and $P < .05$, respectively). Figure 1 shows the results of the immunocytochemistry evaluation.

Alteration of Tissue MMP-2, MMP-9, TIMP-2 and TIMP-3 Protein Levels Induced by ESRD

The SV exposed to ESRD conditions demonstrated a notable increase in MMP-2 and MMP-9 protein levels compared with the normal SV (1.48 ± 0.33 vs 0.92 ± 0.16 densitometry unit and 1.72 ± 0.49 vs 0.98 ± 0.22 densitometry unit, $P < .05$ and $P < .05$, respectively). In contrast, the TIMP-2 and TIMP-3 protein levels in the SV of the HD group were significantly decreased compared with that in the normal control veins (66.3 ± 15.8 vs 119.4 ± 20.1

TABLE 3. Univariate linear regression analysis of ESRD-related parameters in relation to MMP-2/TIMP-2 imbalance of SV conduits

Variable	Odds ratio	95% Confidence interval	P value
Gender (male vs female)	1.247	0.48–2.6	.93
Age	1.004	0.97–1.09	.81
Body weight	1.005	0.96–1.04	.08
Hypertension	0.69	0.073–2.88	.26
Hyperlipidemia	0.55	0.084–2.51	.89
Cerebral vessel disease	1.83	0.042–2.05	.10
Periphery vessel disease	1.66	0.105–2.79	.14
Diabetic mellitus	1.902	0.73–3.68	.09
Total cholesterol level	1.57	0.048–2.39	.10
Lipoprotein(a)	3.19	1.92–4.08	.03
Apolipoprotein A	1.17	0.54–1.85	.15
Apolipoprotein B	4.48	1.77–15.49	.02
Calcium concentration	0.66	0.19–1.31	.17
Phosphorus concentration	0.42	0.051–1.65	.11
Calcium $\times$ phosphorus product	0.93	0.063–1.82	.14
PTH	0.55	0.041–1.07	.19
Homocysteine	1.038	0.96–1.54	.08
IMT in carotid arteries	7.05	2.041–8.07	.01
Coronary artery calcification score	6.49	1.56–7.13	.04

ESRD, End-stage renal disease; *MMP*, matrix metalloproteinase; *TIMP*, tissue metalloproteinase inhibitor; *SV*, saphenous vein; *PTH*, parathyroid hormone; *IMT*, intima-media thickness.

densitometry unit and 73.7 ± 11.6 vs 98.5 ± 18.2 densitometry unit, $P < .05$ and $P < .05$, respectively). Figure 2 displays the results from the Western blotting analysis.

DISCUSSION

It is well known that the arterial alterations induced by ESRD include changes in arterial wall thickness due to rearrangements of the cellular elements and ECM of the vessel wall.^{12,13} Although arterial conduit use has become the standard procedure for CABG, with the concern of the limited life expectancy of the HD patient with severe coronary artery disease, the complications of dialysis, including osteoporosis and coagulation dysfunction, flow steal during hemodialysis with upper extremity arteriovenous fistula, and the patients' poor native coronary artery conditions, we did not use the left internal mammary artery conduits as much as we expected during clinical practice.¹⁴ SV conduits can be the more reliable and available conduits in these cases. The cardiovascular

TABLE 4. Multivariate linear regression analysis of ESRD-related parameters in relation to MMP-2/TIMP-2 imbalance of SV conduits

Variable	Odds ratio	95% Confidence interval	P value
IMT in carotid arteries	16.43	2.02–18.37	<.01
Coronary artery calcification score	5.57	1.32–7.59	.01

ESRD, End-stage renal disease; *MMP*, matrix metalloproteinase; *SV*, saphenous vein; *IMT*, intima-media thickness.

FIGURE 1. Immunohistochemical evaluation of matrix metalloproteinase (MMP)-2, MMP-9, tissue inhibitor of metalloproteinase (TIMP)-2, and TIMP-3 activity in saphenous vein (SV) of hemodialysis (HD) and control groups (A–H, DAB staining, original magnification $\times 100$). SV of HD group ($n = 15$) showed high expression of MMP-2 and MMP-9 (A and C, respectively). In contrast, in control group ($n = 19$), their expression was low (B and D); TIMP-2 and TIMP-3 immunostaining were notably reduced in SV of HD group (E and G, respectively) compared with those in control group (F and H, respectively).

damage in HD patients includes a variety of changes in the vessels.^{15,16} The vascular changes involve both cellular and extracellular components. The ECM is a dynamic structure that requires constant synthesis and degradation by MMPs. This is tightly controlled by the TIMPs. Therefore, increased ECM protein synthesis, increased MMP activity, and decreased TIMP activity might contribute to vascular ECM deposition and fibrosis. ECM displays a very dynamic equilibrium in which there is constant synthesis, degradation, and reorganization.¹⁷ Our results revealed the ECM and adhesion molecule characteristics in the SV conduits of HD patients. Before being exposed to the increased blood flow and pressure in the arterial system, the pathologic remodeling of the SV, characterized by ECM deposition, in HD patients has begun. Furthermore, the Cox linear regression analysis showed the relationship between the extent of MMP-2/TIMP-2 imbalance and some important preoperative variables. Therefore, if the SV quality revealed by the preoperative profile was poor, the surgical strategy should be adjusted, and more arterial conduits

should be used, although some limitations and concerns exist about the use of arterial conduits for CABG in HD patients.

To determine how the activity of the ECM and adhesion molecules is altered in SV of HD patients, the gene expression profile was investigated using microarray analysis. Our data indicate that some risk factors induced by ESRD in HD patients deeply influence the biologic properties of ECM activity in SV conduits. The ECM and adhesion molecule gene expression profile of the SV conduits in HD patients showed signs of the vein graft disease process before coronary surgery. Some important ECM and adhesion molecule genes, including the MMP/TIMP system and collagen, showed significantly different activity.

In addition to the increased traditional risk factors, HD patients could also have a number of nontraditional cardiovascular risk factors that might play an important role in the pathogenesis of vein graft disease.^{18,19} Also, the pathogenesis of cardiovascular damage in HD patients is far more complex than in the general population. Moreover, this increase in

FIGURE 2. Alteration of saphenous vein (SV) tissue matrix metalloproteinase (MMP)-2, MMP-9, metalloproteinase (TIMP)-2 and TIMP-3 protein levels induced by end-stage renal disease (ESRD). A, Representative Western blot showing tissue MMP-2, MMP-9, TIMP-2, and TIMP-3 expression in SV samples of hemodialysis (HD) and control groups. β -Actin was the loading control. B, Densitometric evaluation of MMP-2 expression; C, MMP-9; D, TIMP-2; and E, TIMP-3.

the risk of coronary heart disease is not entirely explained by the traditional risk factors for cardiovascular disease. The complex effect of some nontraditional risk factors, including lipoprotein(a), apolipoprotein A, apolipoprotein B, homocysteine, calcium, phosphorus, and parathyroid hormone, on SV conduits of HD patients is not well known. Our findings also suggest that the extent of MMP-2/TIMP-2 imbalance of SV was directly related to some preoperative ESRD-related parameters.

The atherosclerotic changes in the carotid arteries measured by ultrasonography mirror the atherosclerosis of the coronary arteries in the general population and in HD patients.^{20,21} In some studies, a significant increase was found in the intima-media thickness and plaque occurrence in the carotid arteries in the HD patients compared with the age- and gender-matched healthy control subjects.²² In the present study, a positive correlation between intima-media thickness and plaque occurrence plaques in the carotid arteries with the extent of MMP-2/TIMP-2 imbalance in the SV conduits in HD patients was also observed. Therefore, intima-media thickness and plaque occurrence plaques in

carotid arteries could be considered a mirror of the SV condition in HD patients. Because coronary artery calcification score is a sensitive marker of coronary artery atherosclerosis, it would be of interest to determine whether it has a direct relationship with the MMP-2/TIMP-2 imbalance of the SV in HD patients.²³ Our data have shown that coronary artery calcification score can be viewed as a sign of SV remodeling in HD patients. It was observed that increased lipoprotein(a) and apolipoprotein B levels had significant correlation with carotid atherosclerosis in HD patients. Such a significant correlation was also found when SV remodeling was considered. Although high apolipoprotein A, homocysteine, calcium, phosphorus, and parathyroid hormone levels have been independently associated with greater rates of cardiovascular disease in patients with ESRD, the data did not show a direct correlation between them and SV conduit damage in the HD patients in our study. The explanation for such unexpected results might be related to the limited number of patients. Nevertheless, these findings have demonstrated the importance of careful monitoring and management for some nontraditional risk

factors in patients with ESRD, particularly if they have undergone CABG. Therefore, the assessment of some nontraditional risk factors in HD patients scheduled to undergo CABG might offer useful information on conditions of grafting conduits for coronary surgery.

In conclusion, the results from our study suggest that the ECM and adhesion molecule gene expression profile of the SV conduits in HD patients has shown signs of the vein graft disease process before coronary surgery. Some important ECM and adhesion molecule genes showed significantly different activity that was induced by ESRD. Moreover, these observations raise the question of whether a local genetic anticoagulant treatment should be considered for HD patients with vein bypass grafts. Novel therapeutic approaches could develop that transform the SV conduit into a vessel with increased long-term patency.²⁴ This question, however, remains to be answered by appropriately designed clinical trials.

Study Limitations

Several limitations of the present study have to be addressed. First, the study focused on the native pathologic remodeling of the SV in HD patients. However, surgical manipulation could affect SV graft integrity and might promote neointima characterized by ECM deposition, which need to be investigated. Second, although there were significantly different ECM and adhesion molecule gene activities between the HD and normal SV conduits, the numbers of specimens was still too small to reach the definitive conclusion. Third, although the microarray demonstrated the expression files of 84 ECM and adhesion molecule-focused genes, techniques with greater sensitivity and more detailed profiles to investigate the pathologic remodeling of SV characterized by the ECM deposition still need to be found.

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