Heavy Smoking Before Coronary Surgical Procedures Affects the Native Matrix Metalloproteinase-2 and Matrix Metalloproteinase-9 Gene Expression in Saphenous Vein Conduits

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Background. **Smoking has numerous effects that may promote atherosclerosis, but the pathogenesis of smoking-related vein graft disease after coronary artery bypass grafting (CABG) remains incompletely understood. Matrix metalloproteinase (MMP) subtypes MMP-2 and MMP-9 have been identified as the key components in vascular remodeling processes. However little is known about the native *MMP2* and *MMP9* gene expression in saphenous vein (SV) conduits of heavy smokers undergoing CABG.**

Methods. **Two hundred eight patients were divided into 6 groups: nonsmokers, heavy smokers, 3-month quitters, 6-month quitters, 12-month quitters, and long-term quitters. mRNA and protein levels of MMP-2, MMP-9, and tissue inhibitors of metalloproteinases (TIMPs) 1 and TIMP-2 were analyzed. In a clinical study, SV graft patency after surgical procedures was followed up.**

Results. **Compared with the nonsmoker group, *MMP2* and *MMP9* gene expression was significantly increased in the other 5 groups ($p < 0.05$). In contrast to *MMP* response, *TIMP1* and *TIMP2* gene expression was significantly decreased ($p < 0.05$). An association of increased *MMP2* and *MMP9* gene expression with poor SV graft patency could be found in the clinical data from follow-up.**

Conclusions. **Heavy smoking noticeably increases native *MMP2* and *MMP9* gene expression in the SV before CABG. Even after long-term cessation of smoking, the dysregulated *MMP2* and *MMP9* gene expression cannot recover to normal levels. With the elevated native *MMP2* and *MMP9* gene expression in the SV induced by heavy smoking, more vein graft disease can be found on long-term follow-up.**

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The long-term results of coronary artery bypass grafting (CABG) are limited by stenosis and subsequent occlusion of saphenous vein (SV) grafts [1]. SV grafts generally exhibit unfavorable vascular remodeling properties after CABG [2, 3]. The native matrix metalloproteinases (MMPs), a family of endopeptidases that can degrade extracellular matrix (ECM) components in both physiologic and pathophysiologic states, play an important role in the tendency of pathologic vessel remodeling after coronary operations [4–7]. MMP subtypes MMP-2 and MMP-9 have been identified as the key components in these vascular remodeling processes [8, 9]. Some studies suggest that patients who smoke are at greater risk for vein graft disease than are nonsmokers [10–12]. Smoking has numerous effects that may promote atherosclerosis through vascular inflammation and oxidative stress, but the pathogenesis of smoking-related vein graft disease remains incompletely understood [13, 14]. Thus far little is known about the native *MMP2* and *MMP9* gene expression in SVs of heavy smokers who undergo CABG. As we know, smoking cessation can dramatically reduce the likelihood of revascularization after CABG [15]. Therefore it will be valuable to investigate to determine the ideal length of time before coronary surgical procedures that the patient should quit smoking so that the native *MMP2* and *MMP9* gene expression can recover to normal levels in SV conduits of heavy smokers.

Material and Methods

Patients

For this study, 208 patients undergoing elective CABG in our institution were enrolled. All these patients fulfilled the inclusion criteria and agreed to participate. The study was approved by the local research ethics committee, and each patient gave written informed consent. Exclusion criteria included any inflammatory infective disease, overt clinical heart failure, malignancy, or an acute coronary event during the previous month. Patients with a

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Abbreviations and Acronyms

CABG	= coronary artery bypass grafting
CAG	= coronary angiography
CCABG	= conventional coronary artery bypass graft
CPD	= cigarettes per day
CVD	= cerebral vessel disease
DVD	= double vessel disease
ECM	= extracellular matrix
LIMA	= left internal mammary artery
LM	= left main
MI	= myocardial infarction
MMP-2	= matrix metallopeptidase 2
MMP-9	= matrix metallopeptidase 9
MSCT	= multidetector spiral computed tomography
OPCAB	= off-pump coronary artery bypass
PCI	= percutaneous coronary intervention
PVD	= peripheral vascular disease
RA	= radial artery
Real-time RT-PCR	= real-time reverse transcriptase polymerase chain reaction
RNA	= ribonucleic acid
SMCs	= smooth muscle cells
SV	= saphenous vein
TIMP-1	= tissue inhibitor of metalloproteinase 2
TIMP-2	= tissue inhibitor of metalloproteinase 3
TVD	= triple vessel disease

history of psychiatric illness requiring current treatment with psychoactive medications, those with a history of dependence on alcohol or a non-nicotine substance in the past year, or those currently using tobacco products other than cigarettes were also excluded. Smoking status was assessed on the basis of information obtained from the hospital medical records and by direct interviews with the patient or family members. The patients were divided into 6 groups: nonsmokers (36 patients, control group), defined as patients who had never smoked cigarettes regularly; heavy smokers (45 patients), defined as those who had smoked a minimum of 20 cigarettes per day for a minimum of 20 years until hospitalization for CABG; 3-month quitters (33 patients), defined as those who were also heavy smokers but stopped smoking within 3 months before hospitalization for coronary operation; 6-month quitters (27 patients), defined as those who had stopped smoking for more than 3 months but no more than 6 months; 12-month quitters (38 patients), defined as those who had stopped smoking for more than 6 months but less than 12 months; long-term quitters (29 patients), defined as those who had stopped smoking for at least 12 months.

Tissue Samples and RNA Extraction

Samples of SV were obtained at the time of CABG. Total RNA extraction was carried out using the RNeasy Mini kit (Qiagen Inc, Valencia, CA). One to 2.5 μg of total RNA was reverse transcribed into single-strand complementary DNA (cDNA) using MuLV Reverse Transcriptase (Applied Biosystems, Foster City, CA).

Real-Time Reverse Transcriptase Polymerase Chain Reaction

Real-time reverse transcriptase polymerase chain reaction (real-time RT-PCR) was performed with an Applied Biosystems 7300 Real-Time PCR System with TaqMan Universal PCR Master Mix and TaqMan Gene Expression Assays (Applied Biosystems). The amplification cycle consisted of 2 minutes at 50°C, 10 minutes at 95°C, 15 seconds at 95°C, and 1 minute at 60°C. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) served as a control for PCR. Relative gene expression levels were quantified using the $2^{(-\Delta\Delta Ct)}$ formula.

Immunocytochemical Evaluation

The optimal dilutions for each primary antibody (mouse monoclonal anti–MMP-2, anti–MMP-9, anti–tissue inhibitors of MMP-1 [TIMP-1], anti-TIMP-2; Santa Cruz Biotechnology Inc, Santa Cruz, CA) were found. The sections were then incubated with goat antimouse biotinylated second antibody immunoglobulins (Beijing Zhongshan Co, Ltd, Beijing, China). Semiquantitative evaluation was performed by computer-assisted image analysis (Chengdu Taimeng Co, Ltd, Chengdu, China). The mean optical density was measured at 100 times original magnification in 10 random fields of coded sections.

Western Blot Analysis

Total protein was fractionated and transferred to polyvinylidene difluoride membranes (Millipore, Bedford, MA). Each membrane was incubated with specific primary antibodies (Calbiochem, San Diego, CA) and subsequently with secondary antibody (Jackson Immunolab, West Grove, PA). Immune complexes were visualized with the enhanced chemiluminescence detection system (GE Healthcare, Piscataway, NJ).

SV Graft Failure Assessment by Multidetector Spiral Computed Tomography

Patients returned to our institution for follow-up multidetector spiral computed tomography (MSCT) coronary angiographic assessment. SV failure was defined as stenosis of at least 75% of the diameter of the graft. The SV graft patency was assessed not only at the anastomotic site but also along the main body of the SV graft as well. The duration of SV graft life was calculated from the time of operation to the last time the graft showed patency or when the SV graft was found to be occluded. According to the schedule, all patients underwent MSCT coronary angiography at 1 month, 1 year, and 2 years after operation. MSCT data were analyzed by 2 radiologists who