Secondhand Smoking and Matrix Metalloproteinase-2 and -9 Gene Expression in Saphenous Veins of Women Nonsmokers

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Background. **Active smoking upregulated matrix metalloproteinase-2 (MMP-2) and MMP-9 gene expression in saphenous veins (SVs) before coronary operation. However, little is known about the effects of secondhand smoke (SHS), a smoking status that is more widely harmful to the general population, on MMP gene expression in SVs. Health effects of SHS were investigated mainly in nonsmokers married to smokers. Because the vast majority of women are nonsmokers in China, we had an opportunity to evaluate the relations between SHS and MMP gene expression in SVs of women patients before operation.**

Methods. **A total of 258 woman patients were divided into three groups: control group, nonsmokers; SHS group, patients exposed to SHS until operation; active smoking group, patients who smoked a minimum of 1 package of cigarettes every day for more than 20 years. Messenger RNA and protein levels of MMP-2 and MMP-9 were analyzed. Saphenous vein graft patency after coronary operation was evaluated.**

Results. **The clinical backgrounds in the three groups were comparable. Compared with the control group, MMP-2 and MMP-9 gene expression was significantly increased in the SHS and active smoking groups ($p < 0.05$). The degree of MMP gene expression changed by SHS or active smoking was comparable. A significant difference of SV graft patency was found among the three groups. An association of increased MMP gene expression with lowered SV graft patency was found in follow-up.**

Conclusions. **Our data revealed that SHS had similar power as active smoking to affect MMP gene expression in SVs before operation and SV graft patency after coronary operation in women nonsmokers.**

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Secondhand smoke (SHS) is the combination of smoke given off by the burning end of a tobacco product and the smoke exhaled by the smoker. Secondhand smoke exposure is associated with elevated risks of coronary artery disease [1–3] and stroke [4]. Our previous study had found that active smoking (AS) noticeably increased matrix metalloproteinases (MMPs) MMP-2 and MMP-9 gene expression in saphenous veins (SVs) before coronary artery bypass graft (CABG) [5]. However, the impact from SHS, a smoking status that is more widely harmful to the general population, on MMP-2, MMP-9, tissue inhibitors of metalloproteinases-1 (TIMP-1), and TIMP-2 gene expression in SVs still remains unknown. Furthermore, the relation between SHS exposure and the loss of SV graft patency after CABG procedure has not also been fully illustrated.

The health effects of SHS have been investigated mainly in nonsmokers married to smokers. In 2010, the prevalence of smoking among adults in China was 28.1%: 52.9% for men and only 2.4% for women [6]. Because the vast majority of women are nonsmokers in China, we have a unique opportunity to evaluate the impact of SHS on the native MMP/TIMP gene expression in SV conduits before coronary operation and SV graft patency after CABG in woman patients who were lifelong nonsmokers.

Material and Methods

Study Design and Patients

This study protocol followed the Declaration of Helsinki and was approved by the Ethics and Research Committee of Zhongshan Hospital of Fudan University, and each patient provided written informed consent. From February 2007 to January 2010, 258 patients were enrolled. The inclusion criteria were that all patients were female, retired (>60 years), postmenopausal, and admitted for elective CABG surgery in our hospital. All the patients had different preoperative smoking status, such as nonsmoking, SHS, and AS. Exclusion criteria were any inflammatory, infective disease, overt clinical heart failure, malignancy, or acute coronary event during the last 1 month. Patients were also excluded if they required emergency or concomitant major surgery, unstable angina requiring emergency revascularization, ventricular aneurysm requiring repair, and left ventricular

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Abbreviations and Acronyms

AS = active smoking
MMP = matrix metalloproteinase
SHS = secondhand smoke
SV = saphenous vein
TIMP = tissue inhibitors of metalloproteinases

ejection fraction less than 0.40. Patients with a history of psychiatric illness requiring current treatment with psychoactive medications, a history of dependence on alcohol or a nonnicotine substance in the past year, or current use of 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 interview with the patient, family members. According to preoperative smoking status, the patients were divided into three groups: control group, nonsmokers (105 patients), defined as patients who had never smoked cigarettes regularly and no SHS exposure; SHS group (87 patients), those who were exposed to SHS from family members or coworkers until CABG operation; and AS group (66 patients), those who had smoked a minimum of one package (20 cigarettes) per day for a minimum of 20 years until hospitalization for CABG operation.

Definition of Secondhand Smoke Exposure

Secondhand smoke exposure was defined as exposure to another person's tobacco smoke at home or in the workplace for at least 15 minutes daily for more than 1 day every week for at least 2 years during the past 10 years. Each participant was asked about personal lifetime exposure to SHS in the home and workplace.

Tissue Samples and RNA Extraction

All SV segments were harvested from the legs using the open method. Saphenous vein samples represented the remaining distal segments after each procedure. The specimens were placed in cold Dulbecco modified Eagle medium and kept on ice. The specimens were then cut into 5-mm segments, and immediately snap frozen. Total RNA was extracted 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 murine leukemia virus (MuLV) reverse transcriptase (Applied Biosystems, Foster City, CA).

Real-Time Reverse Transcriptase–Polymerase Chain Reaction

Real-time reverse transcriptase–polymerase chain reaction was performed with an Applied Biosystems 7300 Real-Time PCR System with TaqMan Universal PCR Master Mix and TaqMan Gene Expression Assays (Applied Biosystems). Glyceraldehyde-3-phosphate dehydrogenase served as controls for PCR. Relative gene expression levels were quantified using the $2^{(-\triangle\triangle Ct)}$ formula [7].

Immunocytochemical Staining

Histologic assessment was carried out on formalin-fixed specimens processed for light microscopy. The optimal dilutions for each primary antibody (mouse monoclonal anti-MMP-2, anti-MMP-9, anti-TIMP-1, and anti-TIMP-2; Santa Cruz Biotechnology Inc, Santa Cruz, CA) were determined. The sections were then incubated with goat anti-mouse biotinylated second antibody immunoglobulins (Beijing Zhongshan Co, Ltd, Beijing, China). Semiquantitative evaluation was performed by computer-assisted image analysis system (Chengdu Taimeng Co, Ltd, Chengdu, China). Immunohistochemical staining was evaluated using a semiquantitative immunoreactive score from 0 to 3 based on their staining intensity [8]. The mean optical density was measured at 100 times original magnification in 10 random fields of coded sections.

Western Blot Analysis

Total protein extracted from frozen samples of SV 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).

Saphenous Vein Graft Failure Assessment by Multidetector Spiral Computed Tomography

Patients returned to our institution for follow-up of multidetector spiral computed tomography coronary angiographic assessment. Saphenous vein graft 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. 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 multidetector spiral computed tomography coronary angiography at 1 month, 1 year, and 2 years after operation. The multidetector spiral computed tomography data were analyzed by 2 radiologists who were unaware of the pathologic characteristics of the SV conduits studied.

Data Analysis

All values were expressed as mean ± standard deviation. Continuous variables were estimated as mean ± standard deviation and compared among the three groups by one-way analysis of variance, followed by the multiple comparisons test. The χ^2 and Fisher's exact tests were used to compare qualitative variables among the groups. Tests were two-tailed, and probability values of less than 0.05 were considered statistically significant. Statistical analysis was performed with SAS 6.12 software (SAS Institute Inc, Chicago, IL).