Denervation of gastroepiploic artery graft can reduce vasospasm

Yasutaka Yokoyama, MD,[a] Satoshi Matsushita, MD, PhD,[a] Takafumi Iesaki, MD, PhD,[b] Taira Yamamoto, MD, PhD,[a] Hirotaka Inaba, MD, PhD,[a] Takao Okada, MD, PhD,[b] and Atsushi Amano, MD, PhD[a]

Background: The right gastroepiploic artery is useful as an in situ arterial graft for coronary artery bypass grafting. However, the gastroepiploic artery is more likely to cause vasospasms compared with the internal thoracic artery. We hypothesized that the cause of the spasms is the stimulation of the periarterial sympathetic nerve, because the gastroepiploic artery is classified as a muscular artery. In this study, we examined whether the spasm is reduced by removing the periarterial sympathetic nerve.

Methods: Unused parts of the gastroepiploic artery were obtained from patients who underwent coronary artery bypass grafting. The vessel was cut into 2 segments, and they were assigned to control (N+) and denervation (N−) groups. The periarterial nerve was microscopically removed from the vessels of the N− group. The vessels in both groups were investigated by hematoxylin–eosin or immunohistochemical staining, and they were stimulated by electrical field stimulation with serial frequency for isometric tension measurement.

Results: Histologic analyses revealed that periarterial connective tissues including neuropeptide Y were removed to expose the external elastic membrane in the N− vessel, whereas they were preserved in N+. The mean contraction by electrical field stimulation with serial frequency was consistently lower in N− than in N+ ($P < .05$ at 20 and 50 Hz; n = 8 each). Endothelium-dependent relaxation and contractile function of the smooth muscle were similar in both groups.

Conclusions: The removal of the periarterial sympathetic nerve from the human gastroepiploic artery reduced vascular contraction, elicited by peripheral nerve stimulation, without disturbing endothelial and smooth muscle contractile functions. This reduction may contribute to the prevention of vasospasms. (J Thorac Cardiovasc Surg 2014;147:951-5)

The use of the right gastroepiploic artery (GEA) in coronary artery bypass grafting (CABG) was first reported in 1987.[1,2] The GEA has the ability to supply blood to the posterolateral wall of the heart. Because it is an in situ arterial graft that obtains direct blood flow from systemic circulation, the use of the GEA prevents cerebral infarctions caused by operating on the ascending aorta.[3] In case of a multivessel disease, complete revascularization has been shown to improve long-term survival.[4] According to a recent report, CABG using multiple arterial grafts shows better late survival than CABG with left internal thoracic artery (ITA) to left anterior descending artery bypass and additional saphenous vein grafting.[5] This indicates the advantage of multiple arterial conduits for complete revascularization. With increased prevalence of arteriosclerosis-causing diseases such as diabetes, the number of patients with multivessel coronary artery disease is increasing. Thus, there will be a growing demand for GEA.

However, the GEA grafts develop vasospasms more frequently than the ITA grafts. A vasospasm can result in postoperative shock and decreased long-term patency,[6-8] and is known to be triggered by endothelial and autonomic nervous dysfunction.[9,10] According to a study of canine femoral arteries, which is classified as an elastic artery, vascular tonus effect of the sympathetic nerve is superior than the effect of an endothelial function.[11] Because the GEA is a muscular artery that adjusts blood flow by the periarterial sympathetic nerve (PSN), there should be a greater effect of the periarterial nerve to its vascular contraction. This corresponds with the reports that vasospasm occurs more frequently in the GEA than in the ITA, which is classified as an elastic artery.[12] In this study, we investigate whether removal of the PSN can reduce vasospasm using immunohistochemical and physiologic analyses.

MATERIALS AND METHODS

Human Sample Collections

Patients who underwent CABG using GEA in Juntendo University Hospital were included in this study. Handling of human samples followed the Declaration of Helsinki. This study was approved by the Human Ethics Committee of Juntendo University Hospital, and written informed consent was obtained from each patient.

From the Department of Cardiovascular Surgery,[a] Juntendo University School of Medicine, Tokyo, Japan; and Department of Physiology,[b] Juntendo University Faculty of Medicine, Tokyo, Japan.

Disclosures: Authors have nothing to disclose with regard to commercial support.

Received for publication Nov 13, 2012; revisions received Jan 6, 2013; accepted for publication Feb 12, 2013; available ahead of print March 13, 2013.

Address for reprints: Satoshi Matsushita, MD, PhD, Juntendo University, School of Medicine, Cardiovascular Surgery, 2-1-1, Hongo, Bunkyo-ku, Tokyo, N/A 113-8421 Japan (E-mail: saty-m@juntendo.ac.jp).

0022-5223/$36.00

http://dx.doi.org/10.1016/j.jtcvs.2013.02.022

Abbreviations and Acronyms

CABG = coronary artery bypass grafting
EFS = electrical field stimulation
GEA = gastroepiploic artery
H&E = hematoxylin–eosin
ITA = internal thoracic artery
NPY = neuropeptide Y
PSN = periarterial sympathetic nerve
TTX = tetrodotoxin

Experimental Protocols

Tissue preparation. Immediately after dissection, the GEA sample was transferred to the laboratory in a cold (4°C) HEPES buffered (25 mmol/L of HEPES, pH 7.4) Krebs bicarbonate solution containing the following: 118 NaCl mmol/L, 4.7 KCl mmol/L, 1.5 $CaCl_2$ mmol/L, 25 $NaHCO_3$ mmol/L, 1.1 $MgSO_4$ mmol/L, 1.2 KH_2PO_4 mmol/L, and 5.6 glucose mmol/L. All samples for physiologic study were investigated on the day of the collection. For the experiments, we used 2 cm from the proximal side of each vessel. Surrounding connective tissue and adipose tissue were removed using a pair of tweezers and scissors under the microscope, and the vessel was cut into 2 segments. One segment was used as control group (N+), and the other segment was used as the denervation group (N−). Connective tissue around the vessel of the denervation group was subsequently trimmed to the layer of external elastic membrane to remove periarterial nerves. After trimming, vessels in both groups were cut into 3 segments, with each segment measuring approximately 3 mm. Two segments of the three in each group were used for isometric tension measurement, and the remaining segment was used for histologic study. Histologic study consisted of hematoxylin–eosin (H&E) staining and immunohistochemical staining, and they were performed using frozen-section and whole mount of the vessel rings.

Hematoxylin–eosin staining. The frozen section was prepared by immersing the vessel rings in 4% paraformaldehyde for 30 minutes and then dehydrated by sucrose solutions of increasing concentration of 10%, 15%, and 20%. These rings were embedded in optimal cutting temperature compound (Sakura, Tokyo, Japan) and stored at −80°C. The frozen block was sectioned at 20 μm using a cryomicrotome (Leica, Wetzlar, Germany) for H&E staining. H&E staining was applied to the sections for 15 minutes. After washing with water, the sections were immersed in 95% alcohol for 30 seconds and in eosin (Sigma-Aldrich, St Louis, Mo) for 60 seconds.

Immunohistochemical staining. Two axes (short and long axes) of immunostaining were performed. The short-axis slices were prepared using the frozen block as described earlier. The frozen tissue was sectioned at 5 μm using a cryomicrotome and immersed in 0.5% Triton X-100 overnight. After blocking with 2% of goat serum (Sigma-Aldrich) for 30 minutes, the section was incubated with anti-neuropeptide Y (NPY) antibody (Enzo Life Sciences, Inc, Tokyo, Japan) at a dilution of 1:50 for 72 hours at 4°C. F-actin was costained using rhodamine-phalloidin (Sigma-Aldrich) at a dilution of 1:200 for 1 hour at room temperature. After incubating with secondary antibodies (Alexa Fluor 488; Invitrogen, Tokyo, Japan) for 60 minutes, the section was observed under a fluorescence microscope (Keyence, Osaka, Japan).

In long-axis immunostaining, the whole mount vessel was used. The vessel was immersed in Zamboni solution (Sigma-Aldrich) for 48 hours and treated with 0.5% Triton X-100 overnight. After blocking with 1% of goat serum for 60 minutes, the vessel was incubated with anti-NPY antibody at a dilution of 1:50 for 72 hours at 4°C. After incubating with secondary antibodies (Alexa Fluor 488) for 60 minutes, the vessel was observed with a confocal laser-scanning microscope (Leica).

Isometric tension measurement. Two vessel rings were prepared from each group. A total of 4 vessel rings were mounted on Magnus electrode wire hooks (Unique Medical, Tokyo, Japan) that were connected to force transducers (Nihon Kohden, Tokyo, Japan). Electrical field stimulation (EFS) was provided by an electrical stimulator (Dia Medical System, Tokyo, Japan), and the changes in isometric force were recorded by a polygraphic recording system (Rikadenki, Tokyo, Japan). The vessel rings were incubated in individually thermostated (37°C) 10-mL Magnus baths for 60 minutes at an optimal passive tension of 4 g in Krebs bicarbonate buffer gassed with 95% O_2-5% CO_2. Vascular contraction of each vessel caused by high-KCL (high K^+) Krebs in which NaCl was replaced by KCl (KCl: 122.7 mmol/L) was defined as maximum contraction (100%).

Optimal voltage of EFS for each vessel was defined at the volt when the vascular contraction of N+ occurred by increasing the voltage from 1 to 20 volts (V) with square-wave pulses (2.0 ms of duration) and 1 Hz frequency. After optimal voltage was obtained, vascular contraction was recorded with stimulate frequency of 1, 2, 5, 10, 20, and 50 Hz. The stimulation was applied for 30 seconds, and the vessels were stabilized until vascular contraction returned to baseline. The vessel rings that were not contracted by high K^+ Krebs or that were in vasospasm more than 6 hours were excluded from the study.

Statistical Analysis

The EFS data were evaluated using LabChart 7 software (AD Instruments, Nagoya, Japan), and the results were expressed as the means ± standard error of the mean. The Student *t* test was used to compare the data.

RESULTS

Histologic Analyses

H&E staining revealed that the periarterial tissues outside the external elastic membrane were maintained in N+ vessel, whereas they were removed in N−. Moreover, smooth muscle and endothelial cells were observed inside the external elastic membrane in both groups. These results showed that even after successful denervation, smooth muscle and endothelial cells were not injured by trimming (Figure 1, *A* and *B*).

Double immunostaining of short-axis vessels was performed using NPY as a marker for sympathetic nerve and F-actin as a marker for smooth muscle. The NPY signals were detected around the external smooth muscle of N+, and the signals were substantially reduced around the external smooth muscle of N− (Figure 1, *C* and *D*). Immunostaining of long-axis vessels with NPY as a marker for sympathetic nerve was performed. The NPY signals that formed a network around the vessel of N+ had nearly disappeared in the vessel of N− (Figure 1, *E* and *F*).

Isometric Tension Measurement

GEA contraction data from 8 cases are analyzed in Figure 2. Optimal volts for EFS in each vessel were 8 V in 4 cases, 10 V in 3 cases, and 20 V in 1 case. The mean contraction by EFS was consistently lower in N− than in N+: 2.5% ± 1.5%, 4.7% ± 2.8%, 9.9% ± 4.6%, 19.5% ± 7.3%, 21.3% ± 7.2%, and 23.1% ± 5.1% versus 7.0% ± 3.9%, 12.6% ± 7.3%, 22.5% ± 9.5%, 32.7% ± 8.3%, 43.5% ± 4.9%, and 44.9% ± 5.2% at 1, 2, 5, 10, 20, and 50 Hz, respectively ($P < .05$ at 20 and 50 Hz).