A combination of omental flap and growth factor therapy induces arteriogenesis and increases myocardial perfusion in chronic myocardial ischemia: Evolving concept of biologic coronary artery bypass grafting

Kiyoaki Takaba, MD,[a] Chunli Jiang, MD,[a] Shintaro Nemoto, MD, PhD,[a] Yoshiaki Saji, MD,[a] Tadashi Ikeda, MD, PhD,[a] Shinichi Urayama, MS,[b] Takashi Azuma, BS,[c] Akishige Hokugo, DDS, PhD,[c] Sadami Tsutsumi, PhD,[c] Yasuhiko Tabata, PhD, DMedSc, DPharm,[c] and Masashi Komeda, MD, PhD[a]

Supplemental material is available online.

Objective: The purpose of this study was to evaluate the therapeutic efficacy of the combined growth factor therapy with an omental flap in a rabbit model of chronic myocardial ischemia.

Methods: Chronic ischemia was created in rabbits by placing a constrictor on the left circumflex artery. Four weeks later the animals were divided into 3 groups: group FG, in which a gelatin hydrogel sheet incorporating 100 μg of basic fibroblast growth factor was placed over the left circumflex region followed by covering with the omental flap including the intact gastroepiploic artery; group F, in which only the basic fibroblast growth factor sheet was placed; and group N, in which no treatment was done.

Results: Cine magnetic resonance imaging analysis showed a greater percentage wall thickening in the left circumflex region in group FG than in other groups (group FG, 49.2% $\pm$ 4.5%; group F, 41.2% $\pm$ 3.8%; group N, 32.1% $\pm$ 2.5%, $P = .035$, group FG vs group F). A colored microsphere assay showed higher perfusion in the left circumflex region in group FG than in group F. Perfusion in the left circumflex region was decreased after clamping the gastroepiploic artery pedicle in group FG (before clamping, 2.83 $\pm$ 0.72 mL $\cdot$ min^{-1} $\cdot$ g^{-1}; after clamping, 1.93 $\pm$ 0.59 mL $\cdot$ min^{-1} $\cdot$ g^{-1}; $P < .01$). In vivo angiography via gastroepiploic artery showed direct "to-and-fro" visible collaterals between the gastroepiploic and occluded left circumflex coronary arteries in group FG.

Conclusion: The combined growth factor therapy with an omental flap induced arteriogenesis and provided additional perfusion via the gastroepiploic artery to ameliorate regional dysfunction in the chronically ischemic myocardium.

From the Department of Cardiovascular Surgery,[a] Human Brain Research Center,[b] Kyoto University, Graduate School of Medicine, Kyoto, Japan, and the Institute for Frontier Medical Sciences,[c] Kyoto University, Kyoto, Japan.

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Address for reprints: Masashi Komeda, MD, PhD, Professor and Chairman, Department of Cardiovascular Surgery, Graduate School of Medicine, Kyoto University, 54 Shogoin Kawahara-machi, Sakyo-ku, Kyoto 606-8507, Japan (E-mail: komelab@kuhp.kyoto-u.ac.jp).

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Despite advances in the treatment for ischemic heart disease, there exist patients who are not eligible for current revascularization procedures because of chronic, diffuse, and poorly graftable coronary lesions.[1]

As progress has been made in the basic studies on growth factors in the normal angiogenic process, the concept of therapeutic angiogenesis was developed as an alternative treatment for these patients over the past 2 decades.[2] Preclinical animal studies with various growth factor delivery strategies have shown promising data.[3,4] However, recent randomized double-blind clinical trials showed disappointing results with respect to therapeutic efficacy.[5,6]

Historically, before the advancement of cardiopulmonary bypass, the concept of employing an omental flap to provide revascularization for the ischemic myocardium was attempted in patients with ischemic heart disease. However, the thera-

Abbreviations and Acronyms

bFGF = basic fibroblast growth factor
FS = fractional shortening
GEA = gastroepiploic artery
LCx = left circumflex coronary artery
LV = left ventricular
LVEDD = left ventricular end-diastolic dimension
LVEF = left ventricular ejection fraction
LVESD = left ventricular end-systolic dimension
MRI = magnetic resonance imaging

peutic efficacy of omentopexy was not so efficient for rapid recovery. There exist the clinical limitations with the therapeutic efficacy of ometopexy as well as with that of growth factor therapy.[7]

To offer a more effective therapeutic option for these patients, we developed a combined method involving an omental flap and growth factor as a new alternative surgical method. We called this strategy "biologic coronary artery bypass grafting." Our previous study demonstrated that the method augmented the therapeutic efficacy of growth factor therapy using sustained-release basic fibroblast growth factor (bFGF) by applying a pedicled omental flap in a rabbit model of acute myocardial infarction, whereas omentopexy alone was not so effective as growth factor therapy.[8] Regarding the clinical application, the purpose of the present study was to evaluate its therapeutic efficacy and to verify its superior therapeutic effect over growth factor therapy in the chronically ischemic myocardium.

Materials and Methods

Experimental Animals and Study Protocol

Forty adult male white Japanese rabbits (weighing 3.5-4.0 kg) (Shizuoka Laboratory Animal Center, Shizuoka, Japan) were used in this study. All the animal experiments in this study were performed according to the institutional guidelines on animal experimentation of Kyoto University, which conform to the "Guidance for the Care and Use of Laboratory Animals" law in Japan.

Each animal received two consecutive operations during the study. In the first operation, we created chronic myocardial ischemia by placing an ameroid constrictor. Four weeks after the first operation, animals were assigned into 3 groups as follows: group N (n = 8) received no additional treatment after creation of the myocardial ischemia; in group F (n = 8) a gelatin hydrogel sheet incorporating 100 μg of bFGF was placed over the epicardium of the ischemic area; and in group FG (n = 8) a gelatin hydrogel sheet incorporating 100 μg of bFGF was placed over the ischemic area, followed by covering with an omental flap. Four weeks after the second operation, animals were put to death with an overdose of pentobarbital to harvest the cardiac tissue for further assessments.

Anesthetic protocol for the surgical procedures and functional measurements. All surgical procedures in this study were performed with the animals under general anesthesia as described below. The rabbits were sedated with an intravenous injection of sodium pentobarbital (30 mg/kg) and then intubated with an endtracheal tube (4.0-mm inner diameter, neonatal end-tracheal tube; Mallinckrodt Medical, St Louis, Mo) for mechanical ventilation (tidal volume of 10-15 mL and a minute ventilation rate of 40-60 breaths/min) (7025 Rodent Ventilator; Ugo Basile, Rome, Italy). General anesthesia was maintained with 1.0% to 2.0% of isoflurane mixed with room air. Rectal temperature was maintained at 38°C-39°C with a heat pad during the surgical procedures.

Creation of Chronic Myocardial Ischemia With an Ameroid Constrictor: The First Operation

We used a rabbit model of chronic myocardial ischemia according to the method previously described by Operschall and coworkers.[9] In brief, a left thoracotomy was performed at the fourth intercostal space in a sterile manner. After the pericardium was opened, the main branch of the left circumflex (LCx) coronary artery was identified. We applied a commercially available hygroscopic ameroid constrictor specially designed for constricting rabbit coronary arteries (5-mm diameter, 1.5-mm height: Research Instruments SW, San Diego, Calif). We fixed it on the epicardial surface of the heart, surrounding the targeted coronary artery, with a 6-0 polypropylene suture placed around its circumference. The knot was softly tied to place the ameroid constrictor on the epicardial surface of the targeted artery preventing complete occlusion of the flow, as demonstrated by both electrocardiographic changes and visual blanching of the myocardium. Careful inspection was maintained for 10 minutes after placement of the constrictor. The thoracotomy was closed in layers, and residual air in the thoracic cavity was evacuated.

Echocardiographic measurements of time-course cardiac function. Left ventricular (LV) function was assessed by transthoracic echocardiography at each procedure (every 4 weeks) under general anesthesia as described above. A commercially available echocardiograph with a 7.5-MHz pediatric transducer (Vivid 7; GE Medical, Tokyo, Japan) was used for all studies to obtain serial images through a left parasternal approach. LV end-diastolic and end-systolic dimensions (LVEDD and LVESD, respectively) were measured with M-mode tracings from the short-axis view at the papillary muscle level. Fractional shorting (FS) was calculated from these data as follows: FS (%) = (LVEDD – LVESD)/(LVEDD) × 100. The data were averaged over 3 consecutive cardiac cycles. Another observer who was blinded to the treatment groups performed all measurements.

Preparation of the gelatin hydrogel sheet incorporating bFGF. Gelatin hydrogel sheets (Nitta Gelatin Co, Osaka, Japan) were prepared as described previously.[10] In brief, the sheets were freeze-dried and trimmed in 5 × 5–mm squares and 0.7-mm thick, then impregnated with an aqueous solution containing 100 μg of human recombinant bFGF (Kaken Pharmaceutical Co, Tokyo, Japan). All the processes were conducted under sterile conditions.

Treatment: The Second Operation

Four weeks after the first operation, each animal in groups FG and F received 2 different types of treatment as the second operation. We excluded rabbits if they showed an FS more than 30% or less than 15% in the echocardiograhic assessment before the second operation.