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Intraoperative and Histochemical Comparison of the Skeletonized and Pedicled Internal Thoracic Artery

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ABSTRACT

Background. Skeletonization of the internal thoracic artery (ITA) has advantages, but the variation of ITA preparation may be traumatic for the arterial wall. We sought to compare intraoperative results and endothelial nitric oxide synthase (e-NOS) expression on the vessel wall after left ITA harvesting with skeletonization and the conventional technique.

Methods. A prospective evaluation of 84 consecutive patients undergoing coronary artery bypass grafting was performed: 40 patients with skeletonized and 44 patients with pedicled left ITA. The lengths of ITA and free ITA blood flow were measured. Distal ITA segments were analyzed histopathologically and stained by antibodies against e-NOS.

Results. In the skeletonized group, the length of the ITA were significantly longer than in the pedicled group (15.7 ± 0.4 cm versus 19.0 ± 0.6 cm; P = .001). Also, the free-flow capacity of the ITA was significantly higher than in the pedicled group (62.4 ± 4.8 mL/min versus 88.6 ± 6.9 mL/min; P = .001). e-NOS expressions on endothelial cells were similar between the groups. Dense e-NOS immunostaining was observed in vaso vasorum of the adventitia in the pedicled group. However, there was not any e-NOS immunostaining in vaso vasorum of the adventitia in the skeletonized group.

Conclusions. Although skeletonization of the ITA is a more technically demanding procedure, it provides some advantages such as increased available graft length and reduced sternal devascularization. This technique did not have any detrimental effects on the endothelial cell lining and e-NOS expressions on the endothelial layer. To reach a definitive judgment for using skeletonized ITA, we need information about the long-term angiographic patency rates.

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INTRODUCTION

The internal thoracic artery (ITA) is used extensively as a superior arterial conduit in coronary artery bypass grafting (CABG). Studies confirm that the ITA provides better long-term patency and survival rates because of its high resistance to atherosclerosis [Barner 1985; Loop 1985]. Recently, harvesting of the ITA as a skeletonized graft is preferred because this technique reduces sternal devascularization, development of postoperative respiratory complications, and wound infections and enables sequential anastomosis by increasing the length of the ITA [Calafiore 1999; Athanasiou 2004].

Nitric oxide (NO) is involved in several intracellular and intercellular signaling functions such as smooth muscle relaxation and inhibition of smooth muscle cell proliferation, reducing the chance of vascular intimal thickening [Broeders 2001; Gaudino 2003]. Endogenous NO is produced by the enzyme NO synthase (NOS) from the conversion of L-arginin to L-citrullin [Mayer 1997]. Three forms of NOS have been demonstrated. NOS I (neuron NOS) and NOS III (endothelial NOS [e-NOS]) are regulated by Ca^{+2} and expressed in neurons and endothelial cells. NOS II (inducible NOS) is an inducible form, Ca^{+2} independent, whose expression is induced by cytokines and other inflammatory agents. It is usually thought that e-NOS is expressed only by the endothelial layer and affects the long-term graft patency [Pollock 1993].

Although studies showed that skeletonization of the ITA was clinically acceptable, few reports demonstrated the effect of endothelial integrity and NO release of the vessel wall after skeletonization [Gaudino 1999; Ueda 2003]. We sought to compare the clinical results and e-NOS expression on the vessel wall after ITA harvesting between skeletonization and the conventional technique.

MATERIALS AND METHODS

Eighty-four consecutive patients underwent elective CABG at our institution from September 1 through October 12, 2006. Patients were randomly assigned into 2 groups, one consisting of 44 patients receiving pedicled left ITA grafts and the other consisting of 40 patients receiving skeletonized left

Table 1. Preoperative Patient Characteristics

	Skeletonized	Pedicled
Age, y	65.3 ± 6.5	63.1 ± 5.5
Male sex, n	34 (85.0%)	36 (81.8%)
Hypertension, n	18 (45.0%)	20 (45.5%)
Diabetes mellitus, n	14 (35.0%)	16 (36.4%)
Family history, n	10 (25.0%)	14 (31.8%)

ITA grafts. Preoperative patient characteristics are shown in Table 1. Approval of the local ethical committee and informed consent from the patients were obtained.

Operative Technique

All of the patients were operated on by the same surgeon (O.K.). The left pleura was opened in all of the patients. In the pedicled group, the internal thoracic fascia was incised by electrocautery, and the flap of fascia, muscle, and fat tissue containing the left ITA and concomitant veins was dissected.

In the skeletonized group, a longitudinal incision of the fascia was performed using low-setting electrocautery. The skeletonization of the left ITA was performed from the first intercostal space, leaving distally both of the concomitant veins and the other surrounding structure on the chest wall. The branches of the ITA were clipped both proximally and distally and transected using scissors. After heparin administration, distal parts of the ITA were transected and clipped at the bifurcation point in both of the groups. The ITA was sprayed with diluted papaverine solution (50 mg/20 mL). Before ITA anastomosis, the length and flow rates of the ITA were measured. The redundant 1 cm of the ITA was collected for histological and immunohistochemical analysis.

Histopathological Procedure

Harvested ITA segments were fixed in 10% neutral formaline solution and embedded in paraffin. Five micrometers of serial transverse sections were then cut and processed for histochemical (hematoxylin and eosin) and immunohistochemical studies. These sections were assessed for vessel-wall integrity and endothelial continuity.

Immunohistochemical Analysis

For immunohistochemical studies, immunostaining was performed according to the avidin-biotin-peroxidase complex technique. Paraffin sections were collected on slides, deparaffinized, and dehydrated. Endogenous peroxidase activity was blocked using a 3% hydrogen peroxide solution for 10 minutes. The sections were incubated with primary antisera including e-NOS, endothelial (e-NOS) antibody (Rabbit polyclonal, RR-1711-R7, Neomarkers; Lab Vision, Fremont, CA, USA) for 1 hour at room temperature. After washing in phosphate-buffered saline, the tissues were incubated with biotin-conjugated secondary antibody and then a streptavidin-biotin system for 30 minutes at room temperature. The reactions were visualized by diaminobenzidine tetrahydrochloride. The sections were counterstained using hematoxylin stain, then cleared and mounted. The intensity of immunostaining was graded semiquantitatively on a scale of 0 to 4, with 0 representing the absence of staining, and 4 the maximum intensity of staining.

Statistical Analysis

Statistical analysis was carried out using SPSS for Windows (version 11.0; Chicago, IL, USA). Variables are presented as mean ± standard deviation. The normal distributions of variances among groups for continuous variables were evaluated by Levene's test. Chi-square testing was used for comparing dichotomous variables, and the Student *t* test for continuous variables.

RESULTS

Intraoperative Assessment

Intraoperative comparisons of the skeletonized and pedicled groups are summarized in Table 2. In the skeletonized group, the length of the ITA was significantly longer than in the pedicled group (15.7 ± 0.4 cm versus 19.0 ± 0.6 cm; $P = .001$). Also, the free-flow capacity of the ITA was significantly higher than in the pedicled group (62.4 ± 4.8 mL/min versus 88.6 ± 6.9 mL/min; $P = .001$). During these measurements, the mean arterial pressure on cardiopulmonary bypass was similar (56.0 ± 4.2 mmHg versus 57.9 ± 3.9 mmHg; $P = .1$).

Histopathological Results

Microscopic findings showed that the wall structures of both skeletonized and pedicled ITA were almost intact without any hematomas or dissections in the media. Endothelial cells lining the luminal surface were well preserved in both groups.

Immunohistochemical Results

Semiquantitative evaluation of e-NOS expression is shown in Table 3. e-NOS expressions were clearly observed (Figure). There were no significant differences between the groups for e-NOS expression in the luminal endothelium (3.7 ± 0.4 skeletonized versus 3.6 ± 0.4 pedicled; $P > .05$). The e-NOS expression was significantly denser in the endothelium of the adventitial vaso vasorum of the pedicled group (0.5 ± 0.4 skeletonized versus 3.3 ± 0.6 pedicled; $P = .001$).

Table 2. Intraoperative Assessment of Skeletonized and Pedicled Internal Thoracic Arteries

	Skeletonized	Pedicled	*P*
Length, cm	19.0 ± 0.6	15.7 ± 0.4	<.001
Free-flow capacity, mL/min	88.6 ± 6.9	62.4 ± 4.8	<.001
Mean arterial pressure, mmHg	56.0 ± 4.2	57.9 ± 3.9	Not significant

Table 3. Endothelial Nitric Oxide Synthase (e-NOS) Expressions in Different Layers of Skeletonized and Pedicled Internal Thoracic Arteries

e-NOS Grade	Skeletonized	Pedicled
Luminal endothelium*		
0+	–	–
1+	–	–
2+	–	–
3+	10/40	14/44
4+	30/40	30/44
Endothelium of the adventitial vaso vasorum†		
0+	24/40	–
1+	16/40	–
2+	–	4/44
3+	–	22/44
4+	–	18/44

*Not significant.
†P = .001.

DISCUSSION

Keeley and coworkers described the operative technique of skeletonized dissection of the ITA in 1987 [Keeley 1987]. Nevertheless, the pedicled surgical variant has developed into widespread, clinical practice because of the apparently reduced risk of trauma to the ITA. Cunningham and colleagues in 1992 published their experience with the standardized operative technique with the skeletonized ITA [Cunningham 1992]. Excellent clinical long-term results with this technique in 560 consecutive patients have been reported by Bical and coauthors [1996].

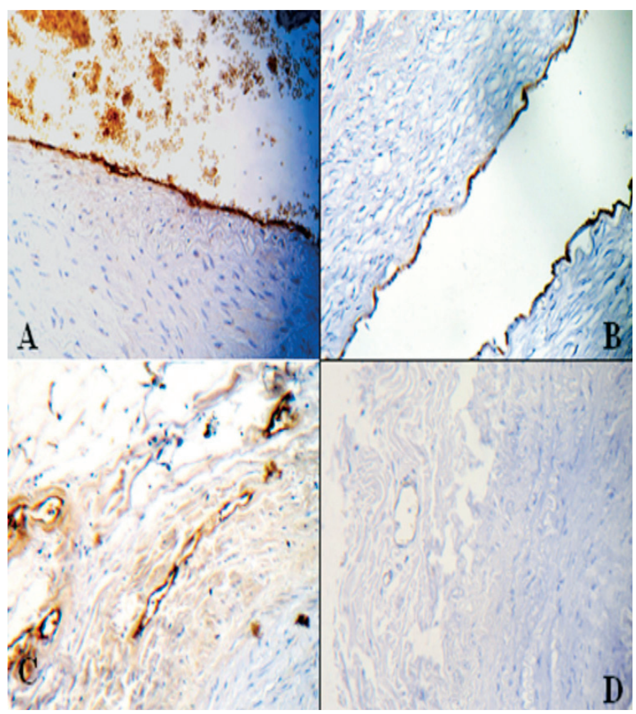
Skeletonization of the ITA has the advantages of increased available graft length, reduced sternal devascularization, and reduced postoperative respiratory complications when compared with pedicled grafts [Calafiore 1999; Campo 2003; Athanasiou 2004]. But skeletonization of the ITA preparation is more technically demanding and may be traumatic for the arterial wall. There are few reports explaining the effects of skeletonization on vessel-wall integrity [Deja 1999; Gaudino 1999; Ueda 2003; Yoshikai 2004].

Skeletonization offers the advantage of extra length, permitting complete arterial revascularization [Raja 2005]. Combining the T graft or sequential grafting makes it possible for most patients to undergo bypass with both left and right skeletonized ITA grafts [Wendler 1999]. In the present study, skeletonization provided a significantly longer graft length than pedicled ITA.

Wendler and coauthors concluded that preparation of the ITA by skeletonization resulted in a significantly higher free-flow capacity than for pedicled grafts [Wendler 1999]. Also, Deja and coworkers showed that free flow from the skeletonized ITA was twice as much as that of the pedicled ITA [Deja 1999]. On the contrary, in a large retrospective study by Huddlestone and coauthors, there were not any significant differences in free-flow capacities between skeletonized and pedicled ITA [Huddlestone 1986]. We found that before distal anastomosis, skeletonized ITA had a higher free-flow capacity than pedicled ITA. Local sympathectomy, which probably occurs with skeletonization, or diluted papaverine solution on the naked arterial wall may cause this difference. Higher free-flow capacity may increase the safety of arterial revascularization.

Sternal devascularization after pedicled ITA harvesting is well documented [Seyfer 1988]. There is a higher risk of damage to the collateral blood supply after pedicled ITA harvesting. Cohen and coworkers compared single photon emission computed tomography of 2 sides of the sternum of patients pre- and post-CABG. Results demonstrated significantly reduced postoperative sternal vascularity after pedicled ITA harvesting [Cohen 1999]. In our study, there were not any significant differences concerning wound infections due to the inadequate blood supply between skeletonized and pedicled groups.

This variation of ITA preparation is more technically demanding and may cause mechanical and physical damage to the vessel wall. Loss of vaso vasorum may cause ischemia in the media, and loss of the draining vein may induce stasis



Immunohistochemical identification of endothelial nitric oxide synthase (e-NOS). A, e-NOS immunostaining is seen in the luminal endothelium in the pedicled group. B, e-NOS is associated in the luminal endothelium in the skeletonized group. C, Dense staining of the adventitial vaso vasorum endothelium in the pedicled group. D, Much of the adventitia was removed and e-NOS immunostaining had not been observed in the skeletonized internal thoracic artery.

and edema in the vessel wall. There are few reports explaining the effects of skeletonization on vessel-wall integrity [Deja 1999; Gaudino 1999; Ueda 2003; Yoshikai 2004]. Gaudino and colleagues used immunohistochemistry with light and electron microscopy to compare skeletonized and pedicled groups. They have not demonstrated any cases of dissection, vessel disruption, or macroscopic thrombus formation. However, they showed 2 cases of microthrombi adherent to the endothelial layer [Gaudino 1999]. Noera and coworkers have not found any evidence of superior damage in the skeletonized group compared with a pedicled group [Noera 1993]. In our study, we have not found any damage to the endothelial layer such as intimal dissection, irregularity, or thrombus formation. Also, e-NOS expressions in the endothelial layer were similar between the 2 groups. Dense e-NOS immunostaining was observed in vaso vasorum of adventitia in the pedicled group. However, in the skeletonized group, no e-NOS immunostaining was observed. It has been thought that the thinner walled arteries could be nourished by luminal diffusion, which can reach a range of 350 to 600 μm into the superficial part of the arterial wall [Goff 1988]. Sasajima and colleagues showed that the thickest part of the ITA was approximately 152 μm, so the ITA could be nourished by luminal diffusion even after total disruption of the vaso vasorum [Sasajima 1998].

In conclusion, although skeletonization of the ITA is a more technically demanding procedure as it is time consuming and requires experience, it provides some advantages such as increased available graft length and reduced sternal devascularization. This technique did not have any detrimental effects on the e-NOS expressions in the endothelial layer. To reach to a definitive judgment concerning the use of skeletonized ITA, we need information about the long-term angiographic patency rates.

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