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Vascular Remodeling in the Internal Mammary Artery Graft and Association With In Situ Endothelin-1 and Receptor Expression

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Background—The vasoconstricting peptide endothelin-1 (ET-1) has been associated with atherosclerotic cardiovascular disease, vascular smooth muscle cell (VSMC) growth stimulation, and intimal thickening. ET-1 binds 2 receptor subtypes, endothelin A and B, and the ET_A receptor mediates vasoconstriction and VSMC growth. This study aims to quantitatively assess arterial remodeling variables and compare them with changes in ET-1, ET_A, and ET_B expression in the internal mammary artery (IMA).

Methods and Results—Specimens from 55 coronary artery disease (CAD) patients (45 men, 10 women; mean age 65 years) and 14 control IMA specimens (from 7 men and 7 women; mean age 45 years) were collected. IMA cross sections were assessed by histochemical and immunohistochemical staining methods to quantify the levels of medionecrosis, fibrosis, VSMC growth, ET-1, ET_A, ET_B, and macrophage infiltration. The percentage area of medionecrosis in the patients was almost double that in the controls ($31.85 \pm 14.52\%$ versus $17.10 \pm 9.96\%$, $P=0.0006$). Total and type 1 collagen was significantly increased compared with controls ($65.8 \pm 18.3\%$ versus $33.7 \pm 13.7\%$, $P=0.07$, and $14.2 \pm 10.0\%$ versus $4.8 \pm 2.8\%$, $P=0.01$, respectively). Despite ACE and/or statin therapy, ET-1 expression and cell cycling were significantly elevated in the patient IMAs relative to the controls (46.27 ± 18.46 versus 8.56 ± 8.42 , $P=0.0001$, and 37.29 ± 12.88 versus 11.06 ± 8.18 , $P=0.0001$, respectively). ET_A and ET_B staining was elevated in the patient vessels ($46.88 \pm 11.52\%$ versus $18.58 \pm 7.65\%$, $P=0.0001$, and $42.98 \pm 7.08\%$ versus $34.73 \pm 5.20\%$, $P=0.0067$, respectively). A mild presence of macrophages was noted in all sections.

Conclusions—Elevated distribution of collagen indicative of fibrosis coupled with increased cell cycling and high levels of ET-1 and ET_A expression in the absence of chronic inflammation suggests altered IMA VSMC regulation is fundamental to the remodeling process. (*Circulation*. 2006;113:1180-1188.)

Key Words: endothelin ■ receptors ■ remodeling ■ coronary disease

The internal mammary artery (IMA) is a small vessel located in the thoracic cavity that branches off the subclavian artery. It is unusual with respect to its histological character in that it has the features both of an elastic and of a muscular artery and has therefore been referred to as a transition artery. Previous histological reports on IMA structure indicated that the media consists of a network of circularly and longitudinally interlacing elastic lamellae, in which vascular smooth muscle cells (VSMCs) are dispersed and circumferentially arranged.¹ Because of these attributes, the IMA is considered to be less prone to intimal hyperplasia than muscular arteries and veins and is currently the most preferable conduit for coronary revascularization surgery. It functions well as a

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conduit, with patency rates between 85% and 95%² at least 10 years postoperatively.

Despite the fact that this vessel has a much higher patency than other vascular grafts used for CABG surgery,³ it is not considered a passive conduit and is sometimes limited in function owing to poor flow and the fact that it is susceptible to perioperative spasm⁴ mediated by vasoconstrictors such as endothelin (ET)-1. Investigating the effects of endothelin on IMA graft specimens, Verma and colleagues⁵ demonstrated that the endothelin receptor antagonist bosentan improved the vasodilatory function of the IMA, albeit in an experimental

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group that appeared to have an unusually low response to acetylcholine compared with other studies of similar design.⁶ In a histological study, Göbel et al⁷ observed medial ET-1 immunoreactivity in 89% of IMA biopsy samples from CABG patients. This group observed that the amount of ET-1 staining was significantly higher in patients with hypercholesterolemia and non-insulin-dependent diabetes mellitus than in those without these risk factors. Furthermore, Rossi et al⁸ demonstrated that individuals with hypertension or coronary artery disease (CAD) had increased levels of preproET-1, endothelin-converting enzyme-1 mRNA, and ET-1 immunoreactivity in the IMA. In particular, they noted significant correlations between ET-1 immunoreactivity in the tunica media and mean blood pressure, cholesterol, and the number of atherosclerotic sites. It was also suggested that in contrast to endothelial ET-1, which is subject to short-term regulation, the presence of ET-1 in the media contributes a more important and stable source.

ET-1 binds 2 G-protein-coupled receptors: endothelin A (ET_A) and B (ET_B). In both atherosclerotic and nondiseased coronary artery, ET_A receptors are far more dominant in VSMCs of the tunica media than the ET_B subtype.⁹ In experimental vein grafts, Dashwood et al,¹⁰ using autoradiography, observed greater proportions of ET_A relative to ET_B present in the tunica media and neointima. Dagassan and colleagues¹¹ compared the densities of both endothelin receptor subtypes in cardiomyopathic hearts and observed that ET_A was significantly more prevalent in the vessel media (83% versus 17%, respectively); however, in atherosclerotic coronary arteries, levels of ET_A and ET_B receptor binding were comparable (51% versus 49%, respectively).

In addition to its role in advanced atherosclerotic disease, ET-1 is known to participate in preatherogenic changes within the vasculature at a cellular and molecular level. ET-1-stimulated collagen deposition has been documented in the heart, intracardiac arteries,¹² and aorta¹³ and has been reversed with a specific ET_A receptor antagonist. Tharaux et al¹⁴ observed that in renal microvessels, when nitric oxide production was suppressed, ET-1 synthesis was increased and was associated with accumulation of collagen type 1. Moreover, ET-1 has also been shown to stimulate DNA synthesis in various quiescent VSMC lines,^{15,16} in which peptide expression is especially strong during the S phase, when cells produce new DNA. Azuma et al¹⁷ noted increased ET-1 immunoreactivity 24 and 72 hours after balloon angioplasty in the rabbit carotid artery, in which an increase in cells labeled with proliferating cell nuclear antigen (PCNA, a diagnostic S-phase growth marker) was also apparent.

This investigation aimed to assess the IMA histological profile and quantitatively measure vascular wall remodeling, including changes in extracellular matrix composition and VSMC proliferation in conjunction with the presence of in situ ET-1, ET_A, and ET_B expression in CABG patients and control IMA specimens. Although the IMA is a sound graft, this study focuses on the pathological role of ET-1 and receptors in abnormal vessel wall remodeling, and as such, it essentially used the IMA as an in situ model. Giannoukas and colleagues¹⁸ have demonstrated that ultrasound evaluation and histology of the long saphenous vein do not correlate and

have shown that ultrasound was only effective in detecting ≈10% of moderate/severe fibrosis in this vessel. This study is novel in that it additionally evaluates structural and cellular abnormalities in association with ET-1, a pathological and potential preclinical marker of atherogenesis that is well known for its association with endothelial dysfunction.

Methods

Tissue Source

Fifty-five ungrafted IMA (UIMA) segments were collected from patients undergoing CABG surgery (45 men, 10 women; mean age 65 years). Fourteen IMA specimens, collected from organ donors (7 men and 7 women; mean age 45 years) were used as controls (CIMA). None of the donors had coronary atherosclerotic disease, hypertension, hyperlipidemia, or diabetes mellitus at the time of death. Cause of death for the organ donors included acute head trauma, subarachnoid hemorrhage, cerebral infarct, and subdural hemorrhage. All CABG subjects gave written informed consent before study participation, and likewise, the next of kin gave permission for Queenslanders Donate to collect an artery specimen for a cardiovascular research study. Approval for this project had been conferred from the medical research ethics committees of The Prince Charles Hospital (EC2126) and The University of Queensland (clearance No. 2003000113) before commencement.

Histology and Immunohistochemistry

A section of the distal left UIMA, which was not required for left anterior descending coronary artery grafting, was collected from all CABG participants. In an additional 2 cases, a section from the mid-distal left UIMA and proximal right UIMA was also discarded during the surgical procedure and collected for research. A small segment of the left distal IMA was harvested from the organ donors. All specimens were fixed in 10% phosphate-buffered formalin and were processed within 48 hours of fixation. Specimens were paraffin-embedded, and 4- μ m serial cross sections were cut for all staining procedures with the exception of Sirius red, for which 7- μ m sections were used. Tissue sections were attached to Super Frost Plus slides (Menzel Glaser GmbH, Braunschweig, Germany). Hematoxylin and eosin staining was used to examine vessel wall morphology, Alcian Blue/Verhoeff's van Gieson to identify areas of medionecrosis and elastic fibers, respectively,^{19,20} and Sirius red for assessment of collagen content, specifically type 1 and type 3.²¹

Immunohistochemical labeling was used to assess cell growth (PCNA; Dakocytomation, Glostrup, Denmark), ET-1/ET_A/ET_B expression (Sigma Aldrich, St Louis, Mo), and monocyte/macrophage presence (CD68, Dakocytomation). After tissue sections were rehydrated and washed in Tris saline buffer (TBS, 1X), citrate heat retrieval (0.1 mol/L citrate) was used to enhance epitope binding. Endothelin receptor and CD68 antigen retrieval was performed with 0.8% and 0.4% pepsin in 0.1N HCl, respectively.

Sections were then washed in 0.1% sodium azide and 2% H₂O₂, blocked with 4% powdered milk solution, and then incubated in 10% normal goat serum. Primary antibody was diluted in TBS (ET-1 1:250; ET_A and ET_B 1:100; PCNA 1:50; CD68 1:100) and incubated in a humidified chamber overnight at room temperature. Slides were then incubated for 1 hour inclusive at room temperature with an indirect peroxidase reaction with a biotinylated secondary antibody (Jackson Immunoresearch Laboratories Inc, West Grove, Pa). Peroxidase activity was visualized after the addition of 3,3'-diaminobenzidine (Zymed, San Francisco, Calif). Sections were lightly counterstained with Mayer's hematoxylin, dehydrated with ethanol and xylene, and mounted with DePx (BDH Laboratories, Poole, England).

Histological Assessment and Quantitative Image Analysis

The cellular remodeling variables (VSMC disorganization and intimal hyperplasia) were semiquantitated and graded on a scale

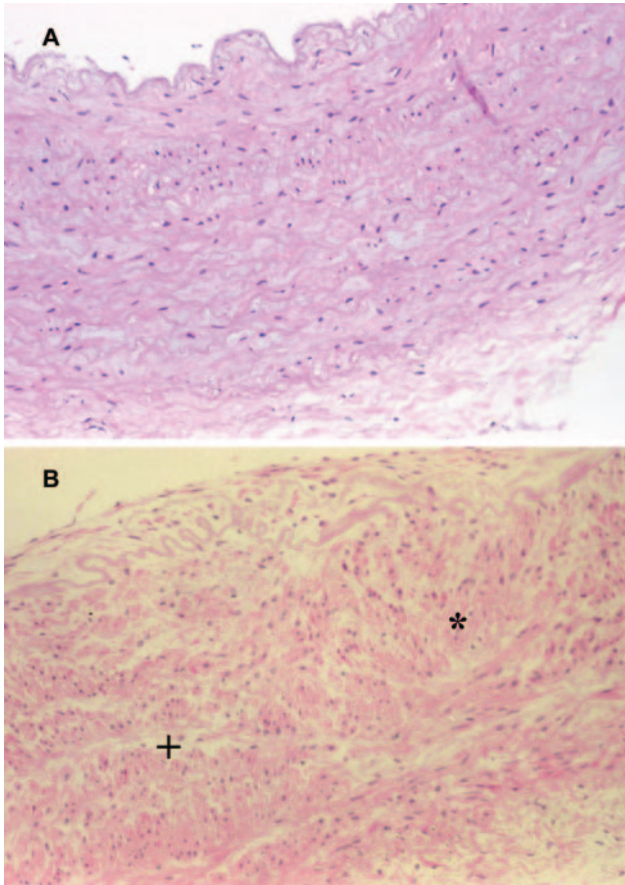


Figure 1. Control stained with hematoxylin and eosin ($\times 200$), demonstrating well-organized, circumferentially aligned VSMCs in the tunica media, interspersed by multiple layers of elastic lamellae (A). B, UIMA section with evidence of moderate medial VSMC disorganization (*) combined with extensive elastic lamellae disruption and fragmentation (+).

between 1 and 4 depending on pathological change. VSMC disorganization was scored as follows: 1=well-organized distribution and alignment of VSMCs in the tunica media (Figure 1A); 2=mild VSMC disorganization without evidence of elastic lamellae or internal elastic lamina disruption; 3=moderate VSMC growth and proliferation in the media in conjunction with increased loss and fragmentation of elastic fibers (Figure 1B); or 4=severe pathological remodeling of the tunica media and intima characterized by gross VSMC growth and disorganization including complete loss of elastic lamellae.

Intimal hyperplasia assessment was graded as follows: 1=no VSMC infiltration between the endothelial basement membrane and the internal elastic lamina; 2=some evidence of VSMC or monocyte infiltration in the tunica intima; 3=moderate synthetic VSMC content and inward distension of the intima; or 4=gross synthetic VSMC proliferation and monocyte/macrophage infiltration of the intima leading to eccentric remodeling. Inflammatory cell assessment was performed by counting the number of positively stained cells in 1 whole cross section of the tunica media.

Immunochemical and histochemical staining for ET-1, ET_A, ET_B, medionecrosis, and collagen presence was measured quantitatively with image-analysis software connected to a light microscope. In the case of Picrosirius-stained collagen, sections were imaged for total collagen staining with standard light microscopy procedures and additionally were imaged with polarized light microscopy to detect variations in type 1 and 3 content. A Nikon Eclipse E600 (Nikon, Tokyo, Japan) light microscope fitted with a Spot RT slider cooled CCD camera (Diagnostic Instruments Inc, Sterling Heights, Mich)

captured digital images, which were then analyzed with Image Pro Plus version 4.5.1.29 (Media Cybernetics, Silver Spring, Md). Twenty fields were captured per slide at $\times 200$ magnification. The border of the tunica media was traced and referred to as an area of interest and measured in micrometers squared (Area A). Positive areas of staining were then selected and independently calculated in micrometers squared (Area B). To determine the percentage of medial area of staining, the following calculation (Equation 1) was applied:

$$(1) \quad \text{Percentage area of staining} = \left\{ \frac{\text{area B}}{\text{area A}} \right\} \times 100\%$$

PCNA staining was determined as a percentage ratio of PCNA-positive medial VSMCs to total medial VSMCs per field (Equation 2), for 20 fields at $\times 200$ magnification, as follows:

$$(2) \quad \text{Percentage PCNA positive cell count} = \left\{ \frac{\text{PCNA positive}}{\text{total cell count}} \right\} \times 100\%$$

Image Analysis Reproducibility

Reproducibility of quantitative image analysis studies was assessed by measuring 15% of the total number of histology samples 3 times, that is, at 3 different time points. Measurements were repeated with the protocol described above for quantitative image analysis. The average coefficient of variation for intraobserver variability was determined to be 1.94%, where mean area percentage of staining for repeat measures was 47.33%, 46.88%, and 46.49%.

Statistical Analysis

Data were analyzed with SPSS version 11.5 (SPSS Inc, Chicago, Ill). Results are presented as mean \pm SD, and comparisons between control and CABG patient tissue sections were determined with a Student *t* test, corrected for variance. Statistical significance was assumed when $P < 0.05$. The correlation coefficient (*r* value) was calculated when we reviewed associations between 2 data sets.

The authors had full access to the data and take full responsibility for its integrity. All authors have read and agree to the manuscript as written.

Results

Four distinct elastic patterns have been documented in the IMA.¹ These include the elastic pattern having >8 lamellae (Figure 2A); elastomuscular pattern with from 5 to 7 lamellae (Figure 2B); muscular type 1 (Figure 2C) with 3 to 4 irregular lamellae, all inclusive of the internal and external elastic lamellae; and muscular type 2, which has diffuse and disorganized elastic fibers not including the internal and external elastic lamina (Figure 2D). Only 2 CABG patients had multiple specimens collected because of allowances in the surgical procedure at the time. The first had a segment of proximal right UIMA collected, and the other had a mid-distal section collected in addition to the distal left UIMA. No differences were noted in the elastic pattern between different regions in these cases. No correlations were found to exist between vascular remodeling variables and elastic pattern, with *r* values of 0.009 and -0.206 for intimal hyperplasia and VSMC disorganization, respectively (Table 1).

The percentage area of medionecrosis as defined by Alcian blue staining was quantitated for both CIMA and UIMA sections. There was a significant increase ($P = 0.0006$) in the area of medionecrosis in UIMA specimens (Figure 3A) compared with the control vessels (Table 2). Furthermore, no significant correlation was observed between age and percentage of area of medionecrosis in the control group

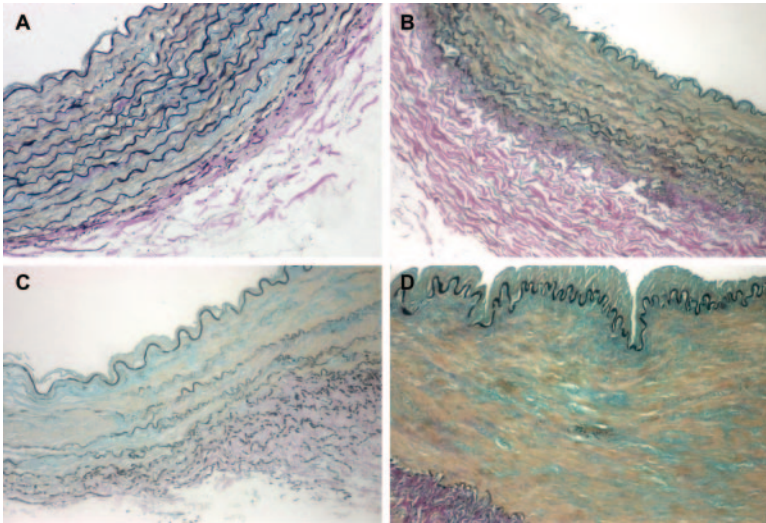


Figure 2. An elastic CIMA stained with Alcian blue/Verhoeff's van Gieson (A), elastomuscular CIMA (B), muscular type 1 CIMA section (C), and (D) muscular type 2 vessel from a CABG patient. All images $\times 200$.

($r=0.458$), which suggests that the higher average age of the CABG group was not contributory to this result.

Immunohistochemical investigation of chronic inflammation in the IMA wall with a specific immunolabel for monocytes and macrophages demonstrated a high variability in the number of macrophages infiltrating the UIMA tunica media, with a range from 0 to 203 in 1 whole-vessel cross section. Although not statistically significant, twice as many macrophages were present in the UIMA specimens as in controls (9.8 ± 29.5 versus 5.9 ± 5.3 , respectively). Monocytes/macrophages in the tunica media of these vessels were predominantly located at or near the medial/adventitial junction as opposed to the intimal/medial junction. Presence was intense in the vasa vasorum and the adventitia, with a very mild presence associated with the endothelium and subendothelial regions (Figure 3B).

In the control and UIMA specimens, the adventitia was dominated by thick, organized collagen fibers that were a mixture of type 1 and type 3 fibers. The tunica media consisted primarily of long, thin, well-organized type 3 fibers that were organized concentrically within the elastic lamellae (Figure 4A). Specimens collected from the CABG patients also had a tunica media composed predominantly of type 3 collagen; however, there was also gross localization of thick, disorganized type 1 fibers in some sections that was commonly associated with areas of intimal thickening (Figure 4B). Quantitative image analysis identified statistically significant increases in total, type 3, and type 1 collagen area in the tunica media of UIMA relative to CIMA (Table 2).

Abnormal and excessive VSMC growth is a feature of atherogenesis, so PCNA was applied to assess cell cycling in the tunica media (Figures 4C and 4D). The number of S-phase-

positive cells in the CABG patient group was >3 times greater than in the CIMA specimens ($P=0.0001$; Table 2).

ET-1 immunostaining localization in the CIMA and UIMA was noted in luminal endothelial cells and vasa vasorum (Figure 5A), VSMCs of the tunica media (Figure 5B) and thickened intima (Figure 5C), fibroblasts in the adventitia, and monocytes/macrophages in the vasa vasorum (Figure 5D). Quantitative image analysis of ET-1-stained area in the tunica media of the UIMA was significantly greater than control (Table 2). To account for any confounding acute effects of the papaverine, the vasodilating agent used on the IMA before grafting, a small subgroup of UIMAs were collected before papaverine treatment. Interestingly, there was a significant decrease in the percentage area of ET-1 labeling in the specimens collected after a 45-minute papaverine infusion with respect to those that were collected before perfusion ($42.90 \pm 13.12\%$ versus $52.07 \pm 7.27\%$, $P=0.005$).

ET_A receptor staining in both CIMA and UIMA sections was principally located in the tunica media. Additionally, positive staining was identified in adventitia (specifically, fibroblasts and macrophages in this region) and also in the vasa vasorum. Most noteworthy, though, was ET_A localization in the luminal endothelium (Figure 6A). When no nuclear counterstain was applied, the nucleus labeled strongly for the ET_A receptor (Figure 6B, arrow), both in the medial VSMCs and endothelial cells. Quantitative review of ET_A receptor area in the tunica media of CIMA and UIMA sections demonstrated a significantly higher percentage area of staining in the UIMA specimens (Table 2) than in control specimens. ET_B localization, as with the ET_A receptor, also produced some unexpected results in which staining appeared to be associated with the connective tissue (specifically, the

TABLE 1. Histology Review of the UIMA

Cellular Remodeling	Elastic (n=7)	Elastomuscular (n=24)	Muscular Type 1 (n=7)	Muscular Type 2 (n=17)	r
Intimal hyperplasia	2.4	1.9	1.8	2.3	0.009
VSMC disorganization	2.7	2.6	2.4	2.3	-0.206

No correlations were present when the pathological cellular remodeling variables were compared with artery structure type.

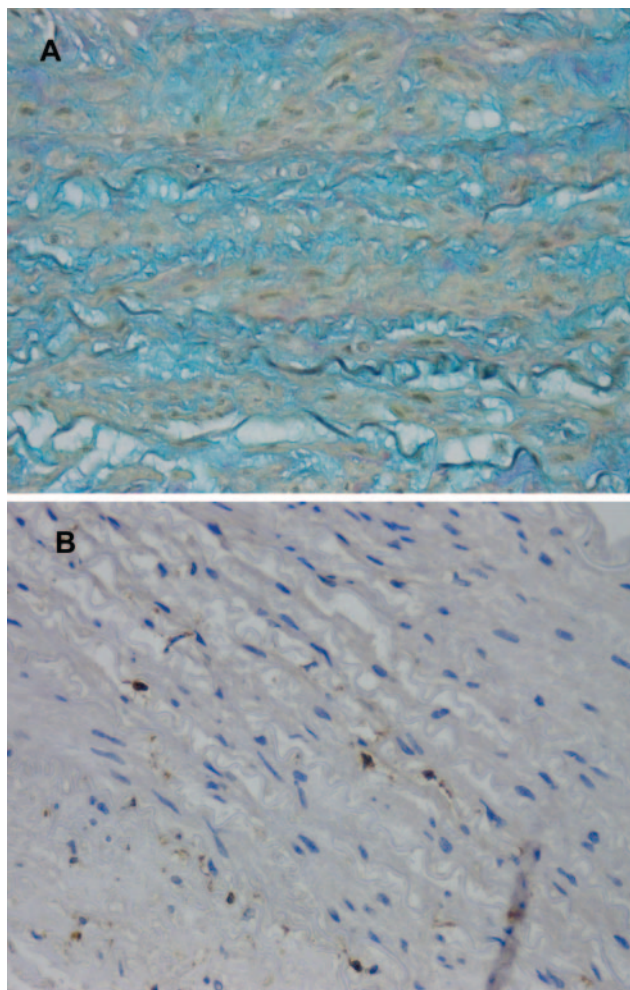


Figure 3. Medionecrosis in the UIMA wall (A) leading to fragmentation and loss of elastic lamellae and (B) macrophage infiltration in the tunica media via the adventitia. Images $\times 400$.

collagen fibers in the adventitia and in the media of both control and patient vessels). Regions of intimal hyperplasia and the luminal surface also stained strongly for ET_B (Figure 6C). Quantitative assessment of ET_B receptor presence was

TABLE 2. Quantitative Analysis of Vascular Remodeling Parameters and In Situ ET-1, ET_A, and ET_B Presence

Remodeling Variable	CIMA (Area or Cell %)	UIMA (Area or Cell %)	<i>P</i>
Medionecrosis	17.10 \pm 9.96	31.85 \pm 9.96	0.0006
Total collagen	36.12 \pm 10.48	65.65 \pm 15.34	0.0001
Type 1	5.22 \pm 3.33	15.18 \pm 8.50	0.0001
Type 3	20.14 \pm 6.61	32.39 \pm 11.31	0.0001
PCNA	11.06 \pm 8.18	37.29 \pm 12.88	0.0001
ET-1	8.56 \pm 8.42	46.27 \pm 18.46	0.0001
ET _A (n=24)	18.58 \pm 7.65	46.88 \pm 11.52	0.0001
ET _B (n=19)	34.73 \pm 5.20	42.98 \pm 7.08	0.0067

Pathological structural, extracellular, and growth markers were all significantly elevated in the CABG patient artery tissue compared with control specimens.

significantly greater in the UIMA specimens than in CIMA, although the nature of these receptors and how they may be involved in pathological remodeling are uncertain given their peculiar location (Table 2).

CABG patient risk factor demographics included hypertension, hyperlipidemia, diabetes mellitus, family history, and cardiovascular history (Table 3). From this group, 8% of patients were taking an ACE inhibitor at the time of surgery, 3% were taking a calcium channel blocker, 25% were taking a β -blocker, and 55% were taking various combinations of the above.

Discussion

Although the IMA is generally considered to be a small elastic artery, various histological patterns were observed in this study, with a tendency for most specimens to fall into the category referred to as elastomuscular. In the histological investigations of the UIMA, no difference in the elastic pattern was observed in the 2 cases in which multiple sections were collected from different anatomic positions, that is, the proximal right UIMA and distal left UIMA as well as the mid-distal and distal left UIMA.

No trend was observed between increasing evidence of intimal thickening or medial VSMC disorganization and muscular histological pattern. Additionally, the presence of medionecrosis was not age-dependent, because there was no correlation between age and Alcian blue staining in the controls ($r=0.458$). Correlations between age and medionecrosis were not investigated in the UIMA specimens because hypertension, a significant factor in this group, is also associated with medionecrosis and would therefore become a confounding factor in the analysis. Contrary to Schlattmann and Becker,²² who suggested that medionecrosis²³ in the vessel is not pathological and simply a phenomenon of aging, medionecrosis in the UIMA tunica media of patients undergoing cardiac revascularization surgery was almost twice that of the control specimens, 31.85 \pm 14.52% versus 17.10 \pm 9.96% ($P=0.0006$), respectively.

There was no significant monocyte or macrophage infiltration identified in the UIMA specimens (9.8 \pm 29.5) or in the control specimens (5.9 \pm 5.3). Interestingly, infiltration of monocytes/macrophages into the vessel wall did not appear to be via the endothelium, because their presence was far greater in the medial/adventitial junction and vasa vasorum.

Coupled with the medionecrosis, there were also significant elevations in total, type 3, and type 1 collagen distribution within the tunica media of UIMA compared with the CIMA ($P=0.0001$, all respective comparisons). Although both type 3 and type 1 collagens were elevated in CABG patients, it is the increased deposition of type 1 collagen that has been directly related to adverse pathological remodeling and is indicative of severe fibrosis in the artery wall.

Remodeling of the extracellular matrix and vessel structure is generally related to VSMC growth and proliferation,²⁴ because VSMCs are known to manipulate their microenvironment as a result of mechanical and chemical stimuli.⁸ It is not surprising given the significant fibrotic changes present on average in the UIMA specimens that there were equally apparent differences in the levels of VSMC growth in this

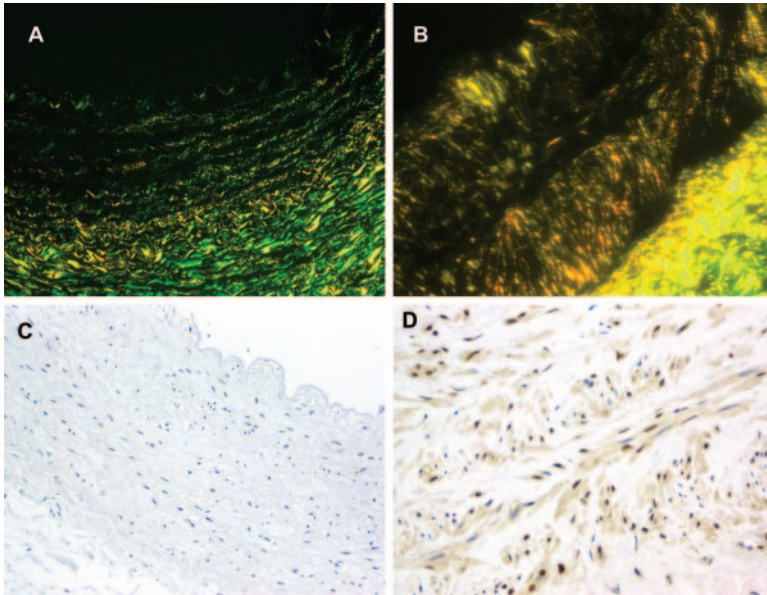


Figure 4. IMA collagen profiles in a CIMA (A, $\times 200$) and UIMA (B, $\times 200$) where long, thin, wavy fibers as opposed to thick, disorganized collagen structures are apparent, respectively. PCNA labeling indicates VSMC cycling in a CIMA section (C, $\times 200$) and UIMA (D, $\times 400$).

group with respect to CIMA. PCNA immunolabeling was more than 3 times greater in the UIMA VSMCs ($37.29 \pm 12.88\%$) than in controls ($11.06 \pm 8.18\%$).

In normal human arteries, ET-1 protein expression is localized to luminal endothelial cells²⁵; however, in active coronary and aortic atherosclerotic plaque, ET-1 immunoreactivity is associated with areas of extensive macrophage infiltration,²⁶ as well as endothelial cells and VSMCs,²⁷ respectively. In the present study, ET-1 presence was noted in endothelial cells, VSMCs, fibroblasts, and macrophages, as well as endothelial cells of the vasa vasorum and neutrophils. Staining intensity overall was strongest in the fibroblasts and inflammatory cell types, with consistently moderate to strong ET-1 presence in the IMA VSMC nuclei and, to a lesser extent, albeit more diffusely, in the cytoplasmic area of the VSMCs and endothelium. The percentage area of ET-1 was significantly higher in the UIMA specimens ($46.27 \pm 18.46\%$ versus $8.56 \pm 8.42\%$, $P=0.0001$). Additionally, patients whose UIMA was dilated with the vasorelaxant drug papav-

erine had a 10% decrease in endogenous ET-1 expression from the tunica media ($P=0.005$) compared with those vessels that were collected before papaverine infusion. Hence, papaverine stimulation may interfere with acute ET-1 expression by increasing endothelium-independent nitric oxide levels.

Plasma ET-1 has been reportedly increased in subarachnoid hemorrhage²⁸ and has been suggested to be a major cause of cerebral vasospasm afterward. Despite subarachnoid hemorrhage being the leading cause of death in the control group, it is unlikely that this would have influenced levels of in situ ET-1 in the CIMA owing to the rapid onset of this condition.

ET_A receptors are known to exist in both healthy and atherosclerotic vessels, and physiologically, they are important in maintaining vascular function. However, in pathological conditions such as atherosclerosis, elevations in ET_A subtype density have been implicated, with disease progression resulting from its contribution to VSMC mitogene-

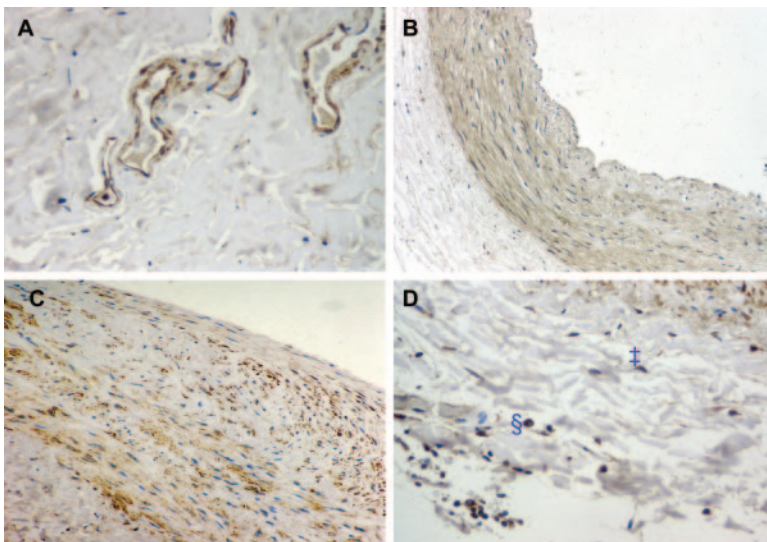


Figure 5. ET-1 expression was noted in the vasa vasorum (A, $\times 400$), luminal endothelium and tunica media VSMCs (B, $\times 200$); areas of intimal hyperplasia in a UIMA specimen (C, $\times 200$); fibroblasts (†) and macrophages (§) in the adventitia (D, $\times 400$).

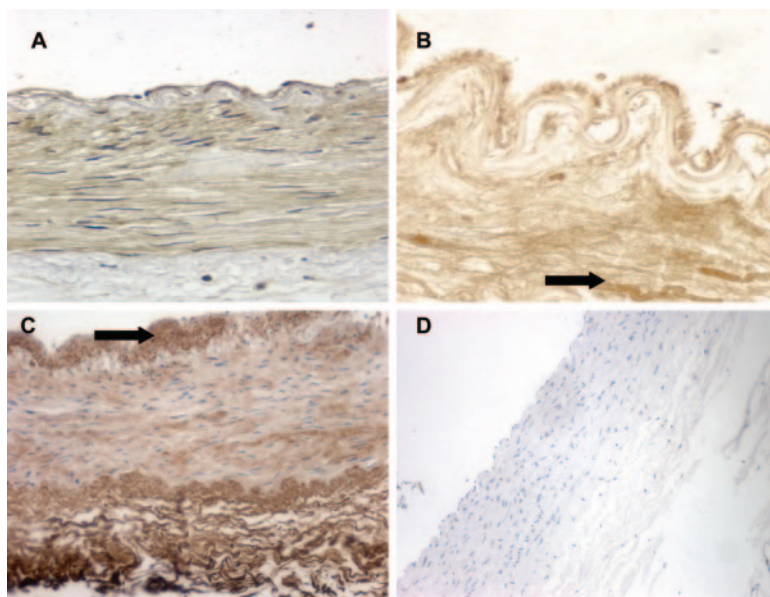


Figure 6. A, ET_A receptor expression in the UIMA tunica media and endothelium ($\times 200$); B, nuclear ET_A expression in UIMA VSMCs (arrow) and in the endothelium ($\times 1000$); C, UIMA ET_B expression demonstrating strong staining of connective tissue structures (arrow, $\times 200$); and D, rabbit IgG control ($\times 200$).

sis.^{29,30} In vivo models have indicated that ET_A receptor signaling is directly involved in mediating ischemia in advanced atherosclerosis and that ET_A receptor antagonism could prevent myocardial infarction.³¹ Importantly, Barton et al³² have demonstrated that long-term ET_A blockade reduces the development of atheroma independently of serum cholesterol and blood pressure changes using an apolipoprotein E-deficient murine model.

ET_A receptor area in the tunica media of the UIMA sections was highly elevated compared with the control ($46.88 \pm 11.52\%$ versus $18.58 \pm 7.65\%$; $P=0.0001$, respectively). ET_A localization was apparent in chronic inflammatory cells and fibroblasts. ET_A receptors were present in the mammary endothelial cells of the lumen and vasa vasorum, which has not been reported previously. In particular, ET_A receptors were observed on the nuclear surface of VSMCs in the IMA tunica media, which suggests the effect of ET-1 stimulation is both cellular and nuclear.

Staining for ET_B presence was highly unusual and produced some unexpected findings in that this receptor subtype

appeared to be colocalized to the connective tissue in the media and adventitia. Areas of intimal hyperplasia commonly included strong collagen type 1 staining and also had an increased presence of ET_B receptors. ET_B receptor labeling area was significantly elevated in the UIMA compared with the control tissue ($P=0.0067$). There are some authors who have suggested this receptor subtype is equal to or more important in neointimal formation than the ET_A receptor.¹⁷ ET_B area was not assessed in the tunica intima because the CIMA had no evidence of intimal thickening, whereas a reasonable number of UIMAs had some degree of intimal hyperplasia. Additionally, of the UIMA specimens that were assessed, strong ET_B staining was apparent in areas of mild to moderate intimal hyperplasia. Therefore, it is probably a marker that should be considered equally as important as the ET_A receptor in terms of its potential contribution to the development of atherogenesis.

Consideration should be given to the fact that the vast majority of the patient cohort was taking either an ACE inhibitor or a β -blocker, in addition to statin therapy. ACE inhibitors and statins have been documented to inhibit ET-1 expression in in vivo³³ and in vitro models,³⁴ respectively. These results are not in agreement with the present findings; however, in other research, we have demonstrated that plasma ET-1 is not different between a CABG patient group and a control group,³⁵ which potentially reflects the fact that these medications affect the level of circulating ET-1 but not the more chronic and stable source present in the tunica media.

Conclusions

This study is novel for multiple reasons, because it has combined an in-depth review of UIMA histology and quantitatively assessed pathological remodeling variables with ET-1, ET_A, and ET_B receptor expression in this unique vessel. Although the IMA was originally considered to a passive conduit, these histological findings concur with physiological studies that indicate this artery is strongly responsive to

TABLE 3. CABG Patient Risk Factor Status

Risk Factor/Comorbidity	% of Group Total (n=55)
Smoking, current	9
Smoking, reformed	58
Hypertension	85
Hyperlipidemia	75
Diabetes mellitus, type 1	5
Diabetes mellitus, type 2	31
Family history of CVD	71
CVD history	87

CVD indicates cardiovascular disease.

CVD history included angina, acute myocardial infarction, previous CABG or angioplasty, cerebrovascular accident, transient ischemic attack, or carotid atherosclerotic disease. Family history of CVD was defined as the occurrence of either a cerebrovascular accident or acute myocardial infarction event in a biological parent under the age of 65 years.

exogenous vasoactive peptides, particularly ET-1. High endogenous levels of ET-1 peptide expression and ET_{A/B} receptor presence, coupled with pathological cellular and extracellular remodeling in the vascular tissue of patients with severe ischemic heart disease, are related to deleterious processes that are essentially a prelude to atherosclerosis. Hence, this research supports previous findings that ET-1 is a strong preatherogenic marker of abnormal vascular function and that the elevated presence of both receptor types suggests that the clinical use of endothelin receptor antagonists in high-risk groups may prevent pathological tissue remodeling that results from elevated ET-1 expression in ischemic heart disease, pulmonary hypertension, and congestive heart failure.

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Disclosures

None.

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CLINICAL PERSPECTIVE

Endothelin-1 (ET-1), a vasoconstricting mitogen, is ubiquitously expressed by the vascular endothelium, where the majority of the secreted peptide is released abluminally, binding to the endothelin A receptor subtype (ET_A) in the tunica media of the artery and thus mediating vasoconstriction. This system starts to go amiss when vascular smooth muscle cells (VSMCs) in the wall of the vessel start to synthesize and express ET-1, which generally acts in an autocrine/paracrine manner. It is the upregulation of ET-1 and angiotensin II as a result of chronic mechanical and/or chemical trauma to the vessel wall that has been suggested to activate the deleterious mechanisms that lead to abnormal VSMC growth or dedifferentiation. This report reviews the histopathology of the internal mammary artery graft in CABG patients and a control group. It specifically investigates the associations between cellular and structural pathological remodeling parameters and ET-1. In particular, we have identified increased levels of VSMC cycling and collagen deposition in the ungrafted internal mammary artery from the CABG patients, in conjunction with significant elevations of ET-1 expression in the tunica media. These changes in VSMC growth behavior, coupled with remodeling of the extracellular matrix, are characteristic of hypertension and predispose to atherogenesis.