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ORIGINAL ARTICLE

Intimal regeneration after coronary endarterectomy and onlay grafting in coronary artery bypass grafting

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Abstract

Objectives Coronary onlay grafting, with or without endarterectomy, has been widely used for the treatment of diffuse lesions. Recent studies have demonstrated excellent long-term patency and favorable remodeling of onlay anastomosis; however, the underlying mechanisms remain unknown. Here, we describe the mechanism of intimal regeneration based on postmortem pathological evaluation of a patient who had undergone onlay grafting with coronary endarterectomy.

Methods The onlay anastomosis was analyzed using a combination of immunohistological stainings, namely, H&E, vimentin, α-SMA, factor VIII, and Ki-67, to identify the source and mechanism of intimal regeneration after onlay grafting with endarterectomy.

Results Our results suggest that the regenerated endothelium derives from the smooth muscle cells of the endarterectomized media of the coronary artery and that it circumferentially covers the internal lumen of the arterial graft.

Conclusions Intimal regeneration, derived from the smooth muscle cells of the endarterectomized coronary artery that proliferate toward the graft lumen, may be a key mechanism that underlies the observed favorable remodeling after onlay grafting during coronary endarterectomy.

Keywords Coronary artery bypass grafting · Onlay grafting · Endarterectomy · Intimal regeneration · Coronary remodeling

Introduction

Cardiovascular risk factors induce endothelial dysfunction through various complex mechanisms [1], and this applies even during coronary revascularization, as the nature of the vascular endothelium is a primary factor that affects long-term graft patency. Multiple studies have clearly demonstrated the long-term patency of coronary anastomosis with the internal thoracic artery (ITA), which yields physiological and metabolic effects that benefit not only the graft itself but also the recipient coronary system [2, 3]. However, in patients with diffuse coronary artery lesions, some ingenuity is necessary to achieve complete revascularization, such as onlay grafting with or without coronary endarterectomy (CE) [4]. Off-pump onlay grafting using the left-ITA (LITA) has shown excellent graft patency in patients with diffuse coronary artery disease [5], and favorable remodeling of the onlay anastomosis has been reported at 1 year after the surgery [6].

We have also previously demonstrated the safety of open onlay grafting, with or without CE, on a beating heart, in patients with diffuse lesions in all areas of the coronary artery, including in the secondary branches, with no 30-day mortality and excellent early patency (98.1%) [7]. We have also confirmed morphological remodeling of the onlay anastomosis using optical coherence tomography (OCT), which was used to directly observe re-endothelialization of the internal lumen (Fig. 1). However, the mechanism underlying such intimal regeneration after onlay with CE has not yet been characterized in detail. Therefore, here, we have examined the mechanism of intimal regeneration by performing

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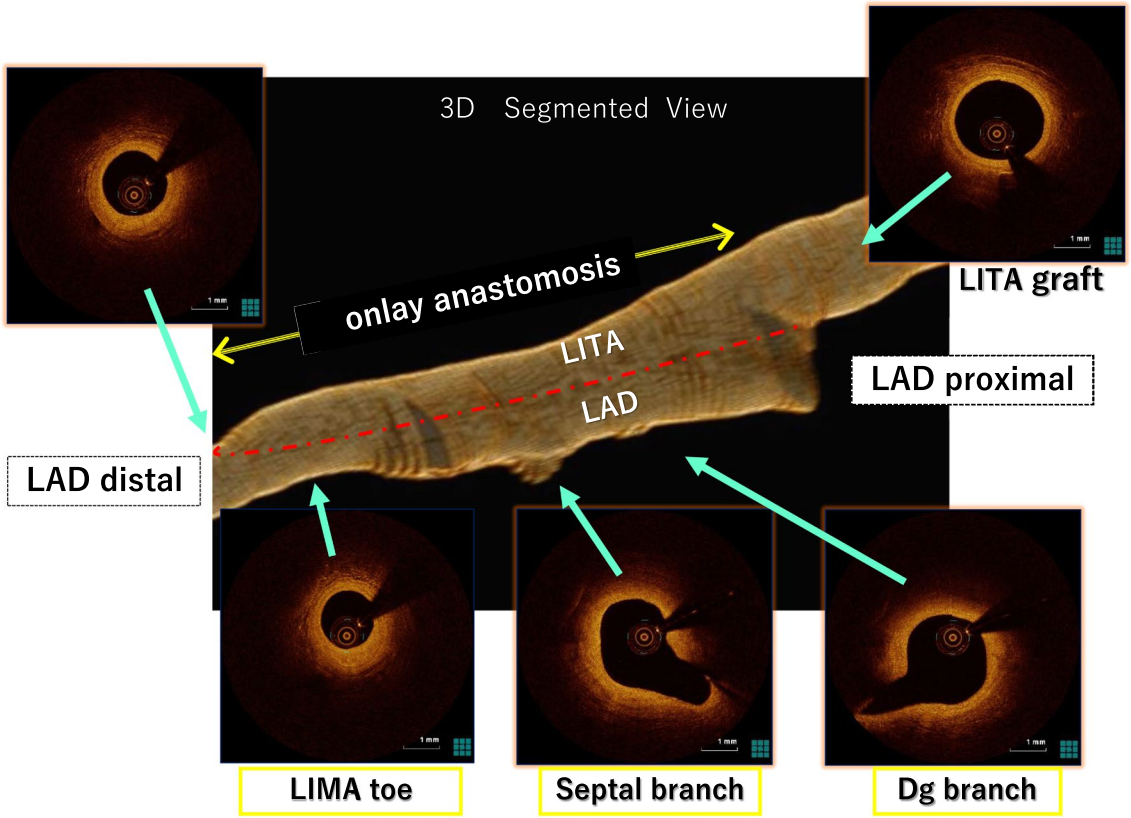

Fig. 1 3D and vertical section of optical coherence tomography (OCT) of the LITA–LAD onlay anastomosis (2 cm) without endarterectomy; post-op, 4 months (different case). The anastomosis exhibited three layers of the arterial structure with a high luminance intima on each vertical section. The anastomotic lumen is smooth, and its diameter is comparable to that of the native LAD. Septal and diagonal branches are preserved. Red dotted line, anastomotic line in a 2 cm length onlay anastomosis without endarterectomy. *LITA* left internal thoracic artery, *LAD* left anterior descending artery

a postmortem pathological assessment of tissues obtained from a patient who had undergone onlay grafting with CE.

Subject and methods

A 71-year-old man was suffering from effort angina due to severe two-vessel disease with 4th in-stent restenosis in the left anterior descending (LAD) artery at segments 6–8. He had multiple risk factors, such as diabetes, hyperlipidemia, hypertension, previous myocardial infarction, arteriosclerosis obliterans, and severely calcified atheroma in the arch and the descending aorta. He was also a current smoker and was undergoing hemodialysis for chronic renal failure. Surgical risk scores were calculated to be 7.1% mortality and 25.5% morbidity based on the Japan risk score [8], 4.82% based on the Euro score; and 7.26% mortality, and 28.2% morbidity based on the Online Risk Calculator of The Society of Thoracic Surgeons, USA.

The patient underwent on-pump beating coronary artery bypass grafting (CABG); specifically, open onlay grafting of the LITA–LAD at segment 8 for a distance of 4.0 cm after stent removal and CE, and a saphenous vein graft (SVG) anastomosis to the atrioventricular branch (AV). The onlay method used has been described in detail elsewhere [7]. The postoperative course was complicated by delayed occurrence of intestinal ischemia at day 35, followed by multiple organ failure. The patient died on postoperative day 42.

We evaluated the anastomotic lesion using immunostaining methods, including hematoxylin and eosin, and staining for α-SMA, factor VIII, and Ki-67. We verified the mechanism of neointimal regeneration using morphological and immunohistological analysis, such as α-SMA is expressed in the smooth muscle and in the vascular smooth muscle; factor VIII is a marker of the intimal endothelium in the circumferential vascular endothelium; and Ki-67 is a cell proliferation marker that is expressed both in the nucleolus of proliferating cells and in the chromosome during proliferation.

Results

Histopathological examination

Figure 2 shows macro- and microscopic images of the onlay anastomosis of the LITA to the LAD segment with CE. The onlay area of CE-section was found to be completely covered with neointima (Fig. 2c, d). This neointima also circumferentially covered the intima of the anastomosed LITA (Fig. 3). This observation implies that the regenerated endothelium was derived from the endarterectomized base of the LAD segment that had extended to and covered the intima of the anastomosed LITA. Characteristics such as continuity of the endothelial cells, constitution of the fibroblasts, and proliferative ability of the endothelial cells were assessed using immunohistological methods (Figs. 3, 4). Figure 3a shows continuous neointimal proliferation over the entire surface of the internal lumen of the onlay anastomosis. This neointima had α-SMA-positive cells (Fig. 3b), indicating the presence of smooth muscle cells within the neointima. Factor VIII staining revealed that the endothelial cells sequentially lined the innermost layer of the neointima and fully covered the original intima of the LITA (Fig. 3c, d). The endothelial cells of the LITA were positive for Factor VIII as they were present within the neointima. Additionally, the anastomotic lumen had a monolayer of neointimal tissue that was positive for Factor VIII (Fig. 3c). These morphological and immunohistological results suggest that the newly formed tissue is a continuous neointima from the LAD. Moreover, as a CE is always performed within the media, a thin layer of the medial tissue remains after the CE, particularly as a remnant base of the endarterectomized coronary artery and stumps of the media. Therefore, these results imply that the neointima, including the endothelial cells, was indeed derived from the medial layer of the endarterectomized coronary artery, i.e., the native LAD artery.

Staining for Ki-67, a cell proliferation marker, was less active in the endothelial cells of the anastomosed ITA but was higher on the LAD side, suggesting that the intima of the endarterectomized LAD segment had undergone greater proliferation compared to the ITA (Fig. 4).

In contrast, Fig. 5 shows end-to-side anastomosis between the SVG and the AV without angioplasty. The anastomosis showed good patency with no evidence of stenosis or thrombosis. Although intimal hyperplasia of the SVG was observed, it had originated from the SVG and

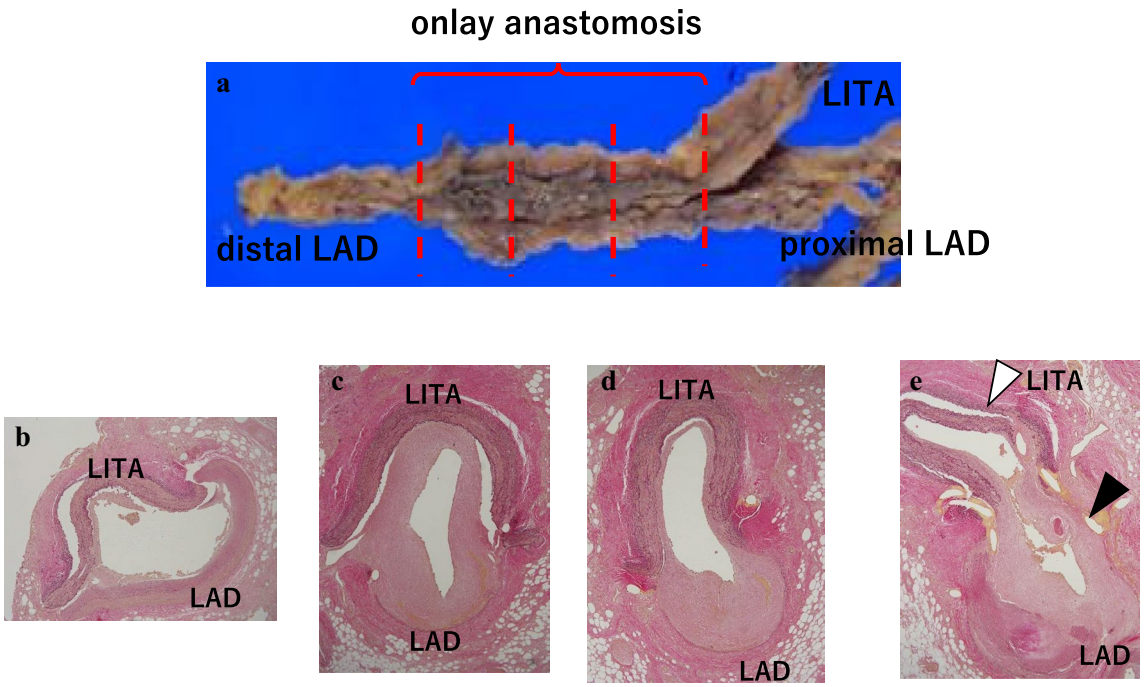

Fig. 2 Macro- and microscopic photographs of the onlay anastomosis of the LITA with the LAD segment with coronary endarterectomy. **a** Macroscopic view of the onlay anastomosis. The LITA was anastomosed to the LAD for a length of 2 cm without stenosis or thrombosis. **b–e** (H&E stain, ×10 magnification); Microscopic photographs of each section. The onlay area is entirely covered by the neointima, which was derived from the base of the endarterectomized LAD, it extends and covers the intima of the anastomosed LITA. **b** Distal end of the onlay anastomosis where the healthy native intima is preserved in the LAD; **c, d** center portions of the onlay anastomosis with endarterectomy; **e** proximal edge of the anastomosis between the LAD (white arrow) and LITA (black arrow). *LITA* left internal thoracic artery, *LAD* left anterior descending artery

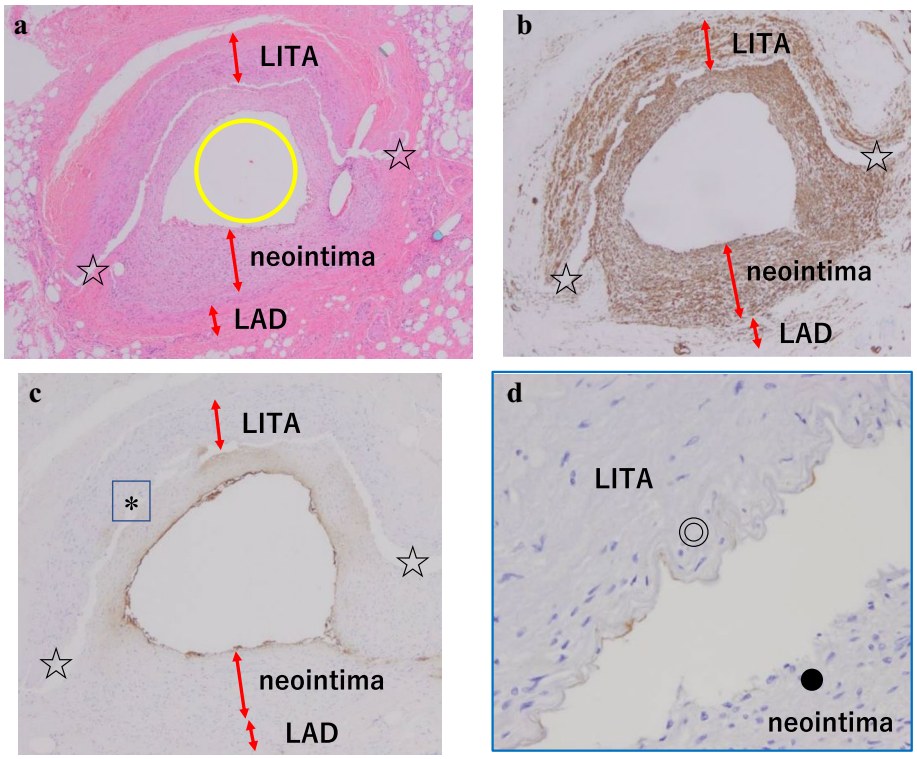

Fig. 3 The LITA–LAD onlay anastomosis with endarterectomy. **a** (H&E stain, ×20 magnification) The yellow circle shows circumferential neointimal formation from the LAD to LITA. **b** α-SMA stain (×20 magnification) showing a continuous layer of smooth muscle cells within the neointima. **c** Factor VIII stain (×20 magnification) showing a sequential lining of endothelial cells on the entire surface of the internal lumen of the onlay anastomosis. **d** Junction of the LITA and neointima (* **c**; ×200 magnification). The LITA intima (◎) is covered with the neointima (●). ☆ anastomotic site of the LITA–LAD; LITA, left internal thoracic artery; LAD, left anterior descending artery

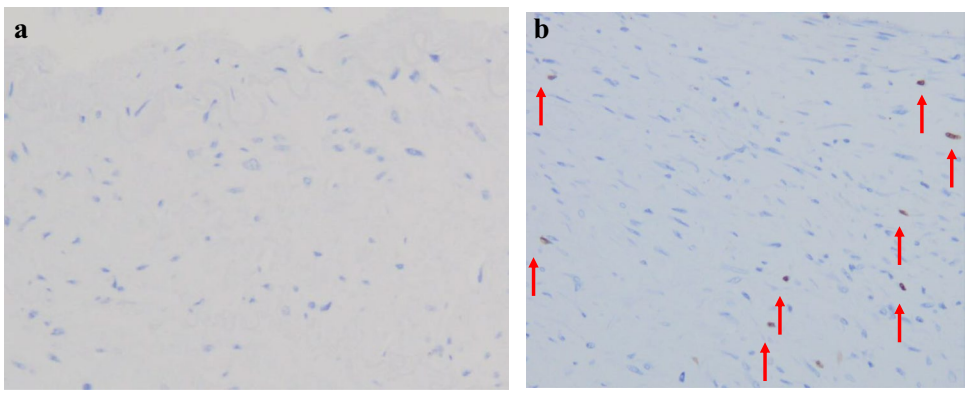

Fig. 4 Ki-67 expression in the neointima of the onlay anastomosis (×100 magnification). **a** Intima of the LITA with lower numbers of Ki-67-positive cells, indicating low cell proliferation. **b** Neointima with many Ki-67-positive cells, indicating high proliferation activity (red arrows). *LITA* left internal thoracic artery, *LAD* left anterior descending artery

circumferentially covered the native coronary artery lumen, which was confirmed by histological staining for α-SMA (Fig. 5).

Discussion

Basic evidence and surgical concept of onlay anastomosis with CE

Onlay grafting with or without endarterectomy has been previously adopted as part of CABG procedures. However, owing to technical difficulties and a lower patency rate, its clinical usage was limited in conventional CABG procedures [9], but recent reports have demonstrated improved outcomes [10]. We have also been safely and successfully performing this procedure on a beating heart for diffuse coronary artery lesions since 2001 [7].

Shimokawa et al. [6] have described favorable postoperative remodeling of the onlay anastomosis with the LITA, with the onlay anastomosis being entirely enlarged by the graft and its lumen seen to be rough in the early period after surgery. Importantly, by 1 year after surgery, the anastomosis had acquired a diameter identical to that of the coronary artery in the disease-free regions and the lumen had become smooth. OCT assessment showed re-endothelialization of the endarterectomized lumen, which may be compatible with vascular remodeling seen in the angiographic study

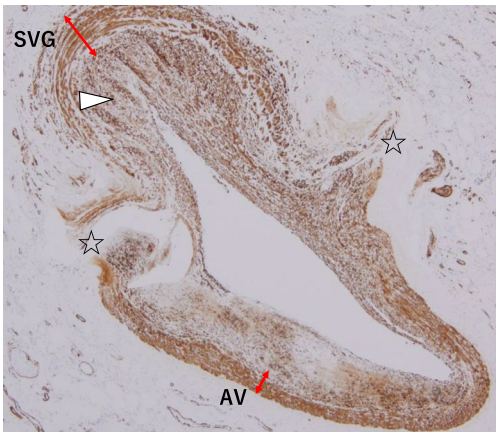

Fig. 5 Neointima formation at the ordinary end-to-side anastomosis site between the SVG and the AV. Axial section of the anastomosis (α-SMA stain, ×20 magnification). White arrow shows neointimal hyperplasia in the internal lumen of the SVG as it extends and covers the internal lumen of the AV. ☆ anastomotic site of the SVG–AV, *SVG* saphenous vein graft, *AV* atrio-ventricular branch

[10]; however, its histological components remain unidentified. If intimal regeneration had indeed caused the observed favorable remodeling, the origin of the endothelium and the mechanism of intimal proliferation need to be identified. Evidence from animal models has shown that vascular endothelial cells are spewed, branched, fused, invaded, and retracted during vascularization and angiogenesis. In a mouse model of interposing recipient carotid arteries with donor carotid arteries (end-to-end anastomosis), a neointima was formed with more than 90% of the cells being derived from the native common carotid artery [11].

Pathological findings and morphological implications of onlay anastomosis with CE

The mechanism of intimal regeneration in a porcine coronary endothelial injury model has been examined using α-SMA and desmin stainings at 35 days after coronary artery injury [12]. As the media and the neointima were positive for α-SMA and the neointima was negative for desmin, a cell extension model from the adventitial side was inferred. However, here, a different regeneration mechanism needs to be considered based on staining patterns obtained. Liang et al. [11] have reported that de-differentiation of smooth muscle cells from the arterial graft is necessary for neointimal formation. However, we show that de-differentiation of the smooth muscle cells from the endarterectomized base of the native LAD segment caused neointimal formation as the regenerated endothelium that circumferentially covered the internal lumen of the LITA was derived from the endarterectomized native LAD segment, accompanied by stronger intimal proliferation on the LAD side (Fig. 4).

Although the intravascular lumen after CE is initially irregular and dilated, favorable vascular remodeling would proceed thereafter to adapt to the flow in the distal coronary artery. A chronic increase in flow would result in the enlargement of the arterial lumen, while flow reduction would induce intimal thickening and a reduction in vessel lumen. This phenomenon has been demonstrated in the canine ITA after initiating flow reduction by ligating the side branch [13]. Normal vessel wall stress, a combination of blood pressure within the vessel and shear stress due to blood flow, acts on the blood vessel wall and induces deformation of endothelial and smooth muscle cells. Greater shear stress due to increased blood flow induces the release of nitric oxide (NO) from endothelial cells, affects the smooth muscle of the media, and restores blood vessel wall stress; in contrast, excessive shear stress induces a decrease in the blood vessel diameter to lower this shear stress. These changes are considered adaptive responses that help maintain shear stress on the vessel wall within certain limits.

The range of onlay anastomosis with CE depending on procedure

Structural analysis of onlay anastomosis with CE from the long-axis view

We ensure that a few intimal fixation stitches are added to the healthy native intima at the cut-edge of CE, and therefore, the proximal and distal edges of the onlay anastomosis with CE includes healthy native intima. In the case of onlay anastomosis with CE, the length of anastomosis should be longer than that of CE so that intracoronary blood flow remains less turbulent with lower shear stress, which results in favorable long-term patency.

Structural analysis of onlay anastomosis with CE from the short-axis view

Minato et al. [7] have reported that the anastomotic line should be made as straight as possible with consistent height along the entire anastomosis. This is because a wavy anastomosis, such as with deeper anastomotic lines but absent side branches or with shallow lines and side branches present, will result in an irregularly shaped anastomosis, increasing the risk of early thrombotic occlusion. For instance, in cases involving LAD, we construct anastomosis that are typically on a high, straight line on the left side to preserve the ostia of the diagonal branches while they are on a deep, straight line on the right side to protect the septal branches. Onlay anastomosis is beneficial because it facilitates proper reperfusion in each branch.

Structural analysis of onlay anastomosis without CE

An onlay anastomosis without CE requires long patch grafting with the usual procedure, but the general concept of the surgical procedure itself is potentially associated with inflammation because the autoregulatory and self-recovery process of intimal regeneration could be less active after onlay anastomosis without CE compared to that observed after a procedure with CE. In our case, healthy coronary intimal tissue was preserved in the distal anastomosis site of LAD and showed no neointimal formation, which is in contrast to the dome-like covering of the LITA. This may be because the autopsy tissue was obtained from an individual with a significant degree of systemic inflammtion. Thus, it is possible that the standard mechanism of tissue repair may differ from the mechanism outlined here. Additionally, it is assumed that this result does not apply to all anastomotic phenomenon after CE.

Molecular and pathophysiological mechanisms underlying favorable remodeling

Endothelial NO is the principal mediator of shear stress-induced vasoregulation [14]. NO activates soluble guanylate cyclase which elevates intracellular cyclic guanosine monophosphate in the endothelium, the smooth muscle cells, and the platelets, and results in inhibition of their shape change and aggregation. These pathophysiological mechanisms seem to be inevitable during vascular remodeling processes, such as during proliferation of medial smooth muscle cells, dysfunction of intimal endothelial cells, activation of adventitial fibroblasts, macrophage-mediated inflammation, and participation of extracellular matrix proteins [15]. This phenomenon may provide clues to the underlying physiology and biology of favorable remodeling during flow alterations in different types of anastomoses. We believe that these mechanisms are key factors that regulate proper intimal proliferation depending on flow alterations and that they are involved in the favorable remodeling of the onlay anastomosis with CE.

For remodeling after anastomosis without CE, we found no reports of autopsied cases, other than what is described here. We assumed that there is no starting point for the regeneration of the intima from the LAD lumen if onlay patch without CE was performed. However, in our case, the anastomotic part on the toe side resembles the condition of the onlay patch without CE procedure, and we show that the normal intima was preserved and covered with the LITA dome, suggesting no significant neo-endothelial regeneration. One possible initiation point is the cut-edge of the LITA, where normal intima-media is preserved in the anastomotic lumen. Thus, intimal regeneration may have arisen from the LITA and covered the sclerosed LAD lumen. Nevertheless, the actual mechanism of favorable remodeling in cases of onlay anastomosis without CE remain unclear, and further experimental investigation is essential.

Interestingly, in our study, the SVG itself showed neointimal hyperplasia, which extended circumferentially and covered the internal lumen of the native coronary artery. Basic research has shown that when the common carotid artery and the inferior vena cava are anastomosed in an end-to-end fashion, the carotid artery functions as the host-side artery and contributes to 60% of endothelial regeneration while it is 40% from the vein graft side [16].

In the case described here, inflammatory conditions such as postoperative stress and septicemia may have altered neointimal formation compared to that observed under ordinary surgical conditions. Intimal regeneration at the site of onlay anastomosis in our case may be an example of an overreaction due to concurrent active inflammation during septicemia.

Ait-Oufella et al. have reported that physiological function and microcirculatory failure during severe sepsis would dynamically affect the endothelium [17]. Although the inflammatory system cannot exclude the involvement of surgical stress and infections, this phenomenon warrants further investigation with respect to delay in the functional recovery of regenerated tissues.

Our results suggest the presence of significant differences in intimal regeneration patterns between onlay anastomosis with CE and ordinary SV anastomosis. We have used biomarker staining to confirm that intimal regeneration contributed to favorable remodeling of the onlay anastomosis with CE. However, further investigations are necessary to ascertain how favorable remodeling replaces intimal hyperplasia after onlay anastomosis.

Conclusion

Intimal regeneration appears to originate from smooth muscle cells of the endarterectomized media of the native coronary artery and proliferate toward the graft lumen. This phenomenon may underlie favorable remodeling after onlay grafting with CE.

Our study is limited by its retrospective design and inclusion of only one autopsy case. Further investigation is required to understand the mechanism of various patterns of intimal regeneration, for instance, after onlay without CE or onlay using other grafts, such as RA, gastroepiploic artery, or SVG.

Compliance with ethical standards

Conflict of interest None of the authors have any conflict of interest to declare.

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