Role of endothelial cell denudation and smooth muscle cell dedifferentiation in neointimal formation of human vein grafts after coronary artery bypass grafting: therapeutic implications
Sasaki, Y.; Suehiro, S.; Becker, A.E.; Ueda, M.

Published in:
Heart

DOI:
10.1136/heart.83.1.69

Citation for published version (APA):
Role of endothelial cell denudation and smooth muscle cell dedifferentiation in neointimal formation of human vein grafts after coronary artery bypass grafting: therapeutic implications

Y Sasaki, S Suehiro, A E Becker, H Kinoshita and M Ueda

Heart 2000;83:69-75
doi:10.1136/heart.83.1.69

Updated information and services can be found at:
http://heart.bmjjournals.com/cgi/content/full/83/1/69

These include:

References
This article cites 25 articles, 11 of which can be accessed free at:
http://heart.bmjjournals.com/cgi/content/full/83/1/69#BIBL

6 online articles that cite this article can be accessed at:
http://heart.bmjjournals.com/cgi/content/full/83/1/69#otherarticles

Rapid responses
You can respond to this article at:
http://heart.bmjjournals.com/cgi/eletter-submit/83/1/69

Email alerting service
Receive free email alerts when new articles cite this article - sign up in the box at the top right corner of the article

Topic collections
Articles on similar topics can be found in the following collections

Histopathology (994 articles)
Other Cardiovascular Medicine (2040 articles)

Notes

To order reprints of this article go to:
http://www.bmjjournals.com/cgi/reprintform

To subscribe to Heart go to:
http://www.bmjjournals.com/subscriptions/
Role of endothelial cell denudation and smooth muscle cell dedifferentiation in neointimal formation of human vein grafts after coronary artery bypass grafting: therapeutic implications

Y Sasaki, S Suehiro, A E Becker, H Kinoshita, M Ueda

Abstract

Objective—To provide better insights into the genesis of neointimal thickening in human vein grafts early after surgery.

Design—Retrospective study.

Setting—Tertiary referral centre.

Subjects—18 distal anastomotic sites of patent grafts, obtained at necropsy from eight patients who died over differing periods (ranging from two days to nine months) after the procedure.

Main outcome measures—Immunohistochemical evaluation of smooth muscle cell phenotype modulation in relation to proliferative activity.

Results—The earliest changes are characterised by loss of surface lining endothelial cells and insudation of blood corpuscular elements admixed with fibrin-platelet thrombus. At sites of injury vimentin positive and actin negative spindle shaped cells appear in the intima, while the related pre-existent media shows focal absence of actin positive smooth muscle cells. Proliferative activity colocalises at these sites. With time distinct neointimal thickening occurs, associated with disappearance of proliferative activity and a phenotypic shift of the smooth muscle cells.

Conclusions—The observation that luminal endothelial cell denudation, with insudation of the intima with blood elements, occurs in the very early stages suggests that these phenomena are responsible for the observed dedifferentiation of pre-existent smooth muscle cells, known to be a prerequisite for cell proliferation and the evolution of intimal thickening. It is likely, therefore, that platelet released growth factors play a pivotal role, which thus may provide a target for preventive pharmacological intervention.

(Heart 2000;83:69–75)

Keywords: smooth muscle cell proliferation; vein graft stenosis; platelet derived growth factor; platelet receptor inhibitors

Late (> 1 year) obstruction of human vein grafts following coronary artery bypass grafting (CABG) has been extensively documented in various studies. The earliest change, whereas Kockx and colleagues documented insudation of blood constituents into the vein intima as early as the first week after surgery. Furthermore, the observation that luminal endothelial cell denudation, with insudation of the intima with blood elements, occurs in the very early stages suggests that these phenomena are responsible for the observed dedifferentiation of pre-existent smooth muscle cells, known to be a prerequisite for cell proliferation and the evolution of intimal thickening. It is likely, therefore, that platelet released growth factors play a pivotal role, which thus may provide a target for preventive pharmacological intervention.

Methods

PATIENTS

Hearts were obtained at necropsy from eight patients who had died within one year of surgery, veins contained smooth muscle cells (SMCs) in the media with reduced expression of α smooth muscle actin, suggesting a synthetic phenotype. These are intriguing observations which need further evaluation, since they may provide a better insight into the genesis of neointimal thickening and, hence, may open gateways to novel therapeutic interventions. This was the main purpose of our study.

Table 1 Relevant clinical data

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Reason for CABG</th>
<th>Anastomotic sites examined*</th>
<th>Interval CABG/death</th>
<th>Cause of death</th>
<th>Risk factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>74</td>
<td>Female</td>
<td>Unstable AP</td>
<td>RCA (3) OM (12)</td>
<td>2 days</td>
<td>Abdominal aortic aneurysm rupture</td>
<td>Hypercholesterolaemia</td>
</tr>
<tr>
<td>2</td>
<td>66</td>
<td>Male</td>
<td>AMI</td>
<td>LAD (8) RCA (3) OM (12)</td>
<td>3 days</td>
<td>Perioperative myocardial infarction</td>
<td>Diabetes mellitus, Hypertension</td>
</tr>
<tr>
<td>3</td>
<td>82</td>
<td>Male</td>
<td>Unstable AP</td>
<td>LAD (8) OM (12)</td>
<td>6 days</td>
<td>Renal failure</td>
<td>Diabetes mellitus, hypertension; smoking</td>
</tr>
<tr>
<td>4</td>
<td>64</td>
<td>Male</td>
<td>Unstable AP</td>
<td>RCA (3) DX (9)</td>
<td>7 days</td>
<td>Mediastinitis</td>
<td>Diabetes mellitus, hypertension; smoking</td>
</tr>
<tr>
<td>5</td>
<td>55</td>
<td>Male</td>
<td>OM + CHF</td>
<td>LAD (7)</td>
<td>9 days</td>
<td>Perioperative myocardial infarction</td>
<td>Diabetes mellitus, hypertension; smoking</td>
</tr>
<tr>
<td>6</td>
<td>61</td>
<td>Male</td>
<td>AMI</td>
<td>LAD (7) OM (12)</td>
<td>33 days</td>
<td>Cardiac failure</td>
<td>Diabetes mellitus; hypercholesterolaemia; hyperuricaemia; smoking</td>
</tr>
<tr>
<td>7</td>
<td>77</td>
<td>Female</td>
<td>Unstable AP</td>
<td>LAD (7) RCA (3) DX (9)</td>
<td>4 months</td>
<td>Cerebral infarction</td>
<td>Hypertension; smoking</td>
</tr>
<tr>
<td>8</td>
<td>82</td>
<td>Female</td>
<td>Unstable AP</td>
<td>RCA (3) PL (14)</td>
<td>9 months</td>
<td>Cardiac failure</td>
<td>Hypertension</td>
</tr>
</tbody>
</table>

*Segments of the coronary arteries are shown in parentheses (according to American Heart Association committee report).
following CABG surgery. The interval between operation and death ranged from two days to nine months. From these eight patients a total of 18 distal anastomotic sites of the patent grafts were used for pathological analyses and immunocytochemical investigations. The relevant clinical data are summarised in table 1.

All hearts were fixed in methanol-Carnoy’s fixative (60% methanol, 30% chloroform, and 10% glacial acetic acid).

Five non-implanted saphenous vein segments, harvested at surgery and fixed in methanol-Carnoy’s fixative, were used as controls. The vein grafts, together with the coronary arteries which contained the sites of anastomoses, were removed from the epicardial surface. The anastomotic sites were cut serially at 1 mm intervals, perpendicular to the long axis, and four slices from each anastomotic site were obtained. A total of 72 slices from the 18 anastomotic sites were examined. All slices, from the anastomotic sites and from normal saphenous vein segments, were processed for pathological and immunocytochemical investigations.

### Table 2: Monoclonal antibodies used in the study

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Specificity and reactivity</th>
<th>Reference</th>
<th>Source</th>
<th>Working dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>HHF-35</td>
<td>Muscle actin</td>
<td>Gown et al</td>
<td>Dako</td>
<td>1:50</td>
</tr>
<tr>
<td>CGA-7</td>
<td>Smooth muscle cell actin</td>
<td>Gown et al</td>
<td>Enzo</td>
<td>1:20</td>
</tr>
<tr>
<td>Anti-vimentin</td>
<td>Smooth muscle cells, fibroblasts, macrophages, endothelial cells</td>
<td></td>
<td>Dako</td>
<td>1:100</td>
</tr>
<tr>
<td>HAM-56</td>
<td>Macrophages, some endothelial cells</td>
<td>Gown et al</td>
<td>Dako</td>
<td>1:70</td>
</tr>
<tr>
<td>PGM-1</td>
<td>Macrophages</td>
<td>Endothelial cells</td>
<td>Dako</td>
<td>1:100</td>
</tr>
<tr>
<td>Anti-vWF</td>
<td>Endothelial cells</td>
<td>Dako</td>
<td>1:50</td>
<td></td>
</tr>
<tr>
<td>UCHL-1</td>
<td>T lymphocytes</td>
<td>Dako</td>
<td>1:150</td>
<td></td>
</tr>
<tr>
<td>L-26</td>
<td>B lymphocytes</td>
<td>Dako</td>
<td>1:250</td>
<td></td>
</tr>
<tr>
<td>Anti-PCNA</td>
<td>Proliferating cells</td>
<td>Dako</td>
<td>1:50</td>
<td></td>
</tr>
</tbody>
</table>

Antibodies were obtained from: Dako, Dako Laboratory, Glostrup Denmark; Enzo, Enzo Biochemicals, New York, USA.

Figure 1  Micrographs of an anastomotic site, taken two days after grafting (patient 1). Panels A–G are serial sections. (A) Elastic tissue stain. The site of anastomosis, indicated by arrowheads, with the right coronary artery. The luminal surface of the vein graft shows a cellular response. Details of the cellular response, indicated by the arrow, are shown in panels B–G. (B) Haematoxylin and eosin stain. At the luminal surface endothelial cells have been denuded. The earliest cellular response of the grafts is demonstrated by an accumulation of polymorphonuclear leucocytes and mononuclear round cells, amid a fibrin-platelet thrombus, partially covered by spindle shaped cells (arrows). (C) Vimentin stain. Spindle shaped cells and round cells at the response site are positive. (D) HHF-35. The spindle shaped cells and round cells are negative. (E) HAM-56. Some round cells stain with this macrophage marker. None of the spindle shaped cells at the luminal site stain positive. (F) UCHL-1. Some small round cells stain with this T lymphocyte marker. (G) PCNA. Positive cells are seen at the site of cellular response and in the adjacent pre-existent media. Original magnification: (A) × 18; (B) × 580; (C–G) × 360.
From each slice 20 sections were cut serially at a thickness of 5 µm. Every first and second section was stained with haematoxylin and eosin and with Weigert's elastic van Gieson's stain, respectively; the other sections were used for immunocytochemical staining.

**IMMUNOCYTOCHEMISTRY**

The monoclonal antibodies used were directed against α isotypes of muscle specific actin common to all muscle cells (HHF-35), and the γ actin isotypes specific only for SMCs (CGA-7), vimentin, endothelial cells (vWF), macrophages (HAM-56 and PGM-1), T lymphocytes, B lymphocytes, and proliferating cell nuclear antigen (PCNA). PCNA is expressed in the late G1 (presynthetic), S (DNA synthetic), and G2 (premitotic) phase of the cell cycle. Anti-PCNA thus serves as a marker for proliferating cells. The source, specification, and working dilution of the antibodies used are shown in table 2.

For the evaluation of the phenotypic characteristic of SMCs in relation to PCNA positivity, we performed double immunostaining for HHF-35 and PCNA in patients 1 and 2, according to the procedure previously reported. Alkaline phosphatase was visualised with fast blue BB (blue, HHF-35) and the peroxidase with 3-amino-9-ethylcarbazole development (red, PCNA).

**MORPHOMETRY**

Morphometric analysis was performed using two sections through a midportion of the anastomotic area from each site. The thickness of the neointimal tissue was measured using computer aided planimetry. The number of macrophages within the surface area of the neointimal thickening was quantified, again using computer aided planimetry, and expressed as the absolute number of macrophages per 0.01 mm² surface area.

**STATISTICAL ANALYSIS**

Statistical comparisons of the thickness of the neointimal tissue were performed by multiple comparisons test, using Scheffe’s method. Student’s t test was used for the comparisons of the number of macrophages.
Results

IMMUNOCYTOCHEMISTRY

In normal saphenous veins used as controls, almost all SMCs within the media were positive for both HHF-35 and CGA-7. In the normal saphenous vein the intima was hardly discernible, but when distinctly present contained HHF-35 and CGA-7 positive SMCs. Some macrophages were scattered in the media. T lymphocytes were not found in these specimens. There were no PCNA positive cells in either media or intima.

Although all saphenous vein grafts harvested were patent, all 72 slices obtained from anastomotic sites showed neointimal tissue, albeit to varying degrees.

The earliest cellular response was encountered at five anastomotic sites (20 slices) in patients 1 and 2, each with an interval of < 3 days. At these sites the luminal surface of the vein showed an accumulation of polymorphonuclear leucocytes and mononuclear round cells with occasional spindle shaped cells, amid a fibrin-platelet thrombus. Immunocytochemically, the mononuclear round cells were identified as macrophages and T lymphocytes. The spindle shaped cells, which had a basophilic cytoplasm and a pale euchromatic nucleus with a large nucleolus, were positive for vimentin, but none of these cells stained for the actin markers HHF-35 and CGA-7. Double immunostaining for PCNA and HHF-35 revealed that the PCNA positive cells in these areas were actin negative (fig 2). Moreover, these PCNA positive areas in the media did not co-localise with the sites where macrophages were present.

The five anastomotic sites ranging from six to nine days after CABG (patients 3, 4, and 5) showed more prominent neointimal tissue at the luminal surface of the venous side. At this stage, the neointimal tissue was composed of macrophages and spindle shaped cells, intermingling with a fibrin-platelet thrombus. There were no polymorphonuclear leucocytes or T lymphocytes. The number of spindle shaped cells appeared to be increased compared to the number at two days after grafting. These cells were positive for vimentin, but negative for both actin markers HHF-35 and CGA-7. PCNA positivity in the actin negative spindle shaped cells was more evident (fig 3), while PCNA positive macrophages were observed only occasionally. The anti-vWF antibody was negative at the luminal side.

The two anastomotic sites at 33 days (patient 6) showed a distinct layer of neointimal tissue. Most neointimal cells stained positive with HHF-35; macrophages and T lymphocytes were found scarcely within the neointima. SMCs in the deeper layers of the neointima also stained positive for CGA-7, whereas those close to the luminal surface were negative for CGA-7 (fig 4). Anti-vWF revealed no positivity at the luminal surface of the neointima. PCNA positivity in the neointima was observed only in
a few spindle shaped cells among those closest to the lumen.

At four months after CABG (patient 7), the four anastomotic sites presented a more prominent neointima, but otherwise the findings were similar to those at 33 days. Approximately two thirds of the SMCs at the deeper side of the neointima stained positive suggesting that most cells have the phenotype of fully differentiated SMCs (compare to panel B). (D) Anti-vWF. Regenerated endothelial cells line the luminal surface. Original magnification: (A) × 30; (B–D) × 90.

Table 3  Results of immunophenotypic expression of neointimal smooth muscle cells

<table>
<thead>
<tr>
<th>Patient</th>
<th>Interval CABG/death</th>
<th>Immunophenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2 days</td>
<td>−−+++</td>
</tr>
<tr>
<td>2</td>
<td>3 days</td>
<td>−−+++</td>
</tr>
<tr>
<td>3</td>
<td>6 days</td>
<td>−−+++</td>
</tr>
<tr>
<td>4</td>
<td>7 days</td>
<td>−−+++</td>
</tr>
<tr>
<td>5</td>
<td>9 days</td>
<td>−−+++</td>
</tr>
<tr>
<td>6</td>
<td>33 days</td>
<td>++++ ++ ++++</td>
</tr>
<tr>
<td>7</td>
<td>4 months</td>
<td>++++ ++ ++++</td>
</tr>
<tr>
<td>8</td>
<td>9 months</td>
<td>++++ ++ ++++</td>
</tr>
</tbody>
</table>

+++, almost all cells positive; ++, about two thirds of cells positive; +, approximately half of all cells positive; −, negative.

Figure 5  Micrographs of an anastomotic site, nine months after grafting (patient 8). Panels A–D represent serial sections. (A) Elastic tissue stain. Note distinct neointimal tissue at the anastomotic site (arrowhead). The neointima at the site of the asterisk is shown at higher magnification in panels B–D. (B) HHF-35. Spindle shaped cells in the neointima are positive. (C) CGA-7. Most spindle shaped cells are positive suggesting that most cells have the phenotype of fully differentiated SMCs (compare to panel B). (D) Anti-vWF. Regenerated endothelial cells line the luminal surface. Original magnification: (A) × 30; (B–D) × 90.

Figure 6  Thickness of the neointima.

MORPHOMETRY
The morphometric findings regarding neointimal thickness related to the time interval between CABG and death are shown in fig 6. There was no significant difference (p = 0.998) between the thickness of the neointima at 2–3 days and at 6–9 days. However, there were significant differences (p < 0.0001) in thickness of the neointima between 6–9 days and 33 days, between 33 days and four months, and between four months and nine months.
Morphometric analysis of the number of macrophages within the neointima showed a significantly (p < 0.0001) higher number in cases with an interval of < 3 days than in those 6–9 days after grafting (fig 7).

**Discussion**

This investigation reveals that human vein grafts very soon after CABG show distinct changes. These changes are characterised by loss of surface lining endothelial cells, insudation of blood corpuscular elements such as polymorphonuclear leucocytes and monocytes, admixed with a fibrin-platelet thrombus, the appearance of vimentin positive, actin negative spindle shaped cells, and absence of actin positive SMCs in the pre-existent media. Moreover, with an increase in the time interval between CABG and death a decrease in PCNA activity was noted. These findings support previous experimental studies of neo-intimal cellular kinetics and phenotypic changes of SMCs, suggesting that the decline in cell proliferative activity is part of the evolution of the neointima and relates to the state of neo-intimal SMC differentiation. However, in humans the role of cell proliferation in the setting of neo-intimal formation remains controversial.

Our study provides novel observations with respect to the proliferative activity related to injury. We observed PCNA positivity of both inflammatory cells within the reactive tissues at the site of intimal injury, as well as in the de-differentiated areas within the pre-existent media. Moreover, with an increase in the time interval between CABG and death a decrease in PCNA activity was noted. These findings support previous experimental studies of neo-intimal cellular kinetics and phenotypic changes of SMCs, suggesting that the decline in cell proliferative activity is part of the evolution of the neointima and relates to the state of neo-intimal SMC differentiation. However, in humans the role of cell proliferation in the setting of neo-intimal formation remains controversial.

Our study provides novel observations with respect to the proliferative activity related to injury. We observed PCNA positivity of both inflammatory cells within the reactive tissues at the site of intimal injury, as well as in the de-differentiated areas within the pre-existent media. Moreover, with an increase in the time interval between CABG and death a decrease in PCNA activity was noted. These findings support previous experimental studies of neo-intimal cellular kinetics and phenotypic changes of SMCs, suggesting that the decline in cell proliferative activity is part of the evolution of the neointima and relates to the state of neo-intimal SMC differentiation. However, in humans the role of cell proliferation in the setting of neo-intimal formation remains controversial.

Our study provides novel observations with respect to the proliferative activity related to injury. We observed PCNA positivity of both inflammatory cells within the reactive tissues at the site of intimal injury, as well as in the de-differentiated areas within the pre-existent media. Moreover, with an increase in the time interval between CABG and death a decrease in PCNA activity was noted. These findings support previous experimental studies of neo-intimal cellular kinetics and phenotypic changes of SMCs, suggesting that the decline in cell proliferative activity is part of the evolution of the neointima and relates to the state of neo-intimal SMC differentiation. However, in humans the role of cell proliferation in the setting of neo-intimal formation remains controversial.

Our study provides novel observations with respect to the proliferative activity related to injury. We observed PCNA positivity of both inflammatory cells within the reactive tissues at the site of intimal injury, as well as in the de-differentiated areas within the pre-existent media. Moreover, with an increase in the time interval between CABG and death a decrease in PCNA activity was noted. These findings support previous experimental studies of neo-intimal cellular kinetics and phenotypic changes of SMCs, suggesting that the decline in cell proliferative activity is part of the evolution of the neointima and relates to the state of neo-intimal SMC differentiation. However, in humans the role of cell proliferation in the setting of neo-intimal formation remains controversial.

Our study provides novel observations with respect to the proliferative activity related to injury. We observed PCNA positivity of both inflammatory cells within the reactive tissues at the site of intimal injury, as well as in the de-differentiated areas within the pre-existent media. Moreover, with an increase in the time interval between CABG and death a decrease in PCNA activity was noted. These findings support previous experimental studies of neo-intimal cellular kinetics and phenotypic changes of SMCs, suggesting that the decline in cell proliferative activity is part of the evolution of the neointima and relates to the state of neo-intimal SMC differentiation. However, in humans the role of cell proliferation in the setting of neo-intimal formation remains controversial.
Genesis of human vein graft neointimal thickening

The observation that luminal endothelial cell denudation, with insudation of the intima with blood corpuscular elements and adherence of a fibrin-platelet thrombus at the site of injury, are the most prominent changes in the early stages of human vein grafts after CABG, suggests that these phenomena trigger the repair process; eventually this may lead to neointimal formation which has been shown to be the most common underlying pathology of obstructed human vein grafts. This then could provide a target for pharmacological intervention at the stage of the CABG procedure itself, for instance by suppressing the irreversible attachment of platelets to the sites of injury using inhibitors of receptor glycoprotein IIb/IIIa. A suppressed or mitigated SMC response may also have a beneficial effect on the eventual development of atherosclerosis in these vein grafts, since SMCs also play an important role under these circumstances. At present we are not aware of any studies documenting the effects of preventive measures as alluded to above in patients undergoing CABG.

STUDY LIMITATIONS

Obviously, the present study does not allow for a time related sequence of events in human vein grafts after CABG. The number of observations is too small to allow further statistical analysis. Nevertheless, the observations are intriguing, not least since they relate very well to observations made at other sites, such as intracoronary angioplasty, where atherectomy tissue. However, for that purpose one has to handle material that comes available in a different manner. For this a strict protocol should be employed and basically fresh material should be sampled. This is extremely difficult, if not almost impossible, in the setting of patients undergoing a CABG procedure.