Institutional report - Experimental

Use of arterial patch to improve re-endothelialization in a sheep model of open carotid endarterectomy. An incentive to use internal thoracic artery as an on-lay patch following coronary endarterectomy?

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Abstract

To better understand the effect of the internal thoracic artery on endothelial growth after open coronary endarterectomy, we designed an experimental test of the hypothesis that closing an endarterectomized artery by an arterial patch improves re-endothelialization. The two carotid arteries were endarterectomized in nine sheep and were randomly chosen for closure by native arterial femoral (ART) patch or polytetrafluoroethylene (PTFE) patch. Three animals were randomly chosen for sacrifice at 8, 15 and 21 days each. The endarterectomized segments were studied macroscopically and microscopically. The endarterectomized area covered with adhesive thrombus was more extensive in the PTFE than in the ART group (P=0.0117). In the ART group, the regenerated endothelium was normal and sprouted from the edges of both the endarterectomy and the arterial patch towards the central endarterectomized area. In the PTFE group, it sprouted from the edges of the endarterectomy and never reached the central endarterectomized area, where abnormal endothelium was observed. The endarterectomized area covered with normal endothelium was more extensive in the ART than in the PTFE group at 8 days, at 15 days, and 21 days (P<0.001). Arterial patch closure of open-endarterectomized artery improved regenerated endothelium quality.

Keywords: Endarterectomy; Animal model; Autograft; Endothelium

1. Introduction

Coronary endarterectomy enables diffusely diseased arteries to be grafted. Concentric and uniform myofibro-intimal proliferation is systematically observed at the endarterectomy site five years post-surgery [1]. Previous studies reported poor late patency for endarterectomy grafts and reduced five-year actuarial survival in coronary endarterectomy patients [2].

We have previously described [3] a coronary artery reconstruction technique using the internal thoracic artery (ITA). When open endarterectomy is associated to coronary artery reconstruction, the long coronary arteriotomy is closed with an ITA onlay patch. The running suture fixes each edge of the ITA patch inside the coronary artery lumen; 75% of the endarterectomized coronary wall is thus excluded from the lumen of the reconstructed coronary artery. Our previous study [3] found neither coronary restenosis nor endarterectomized coronary artery obliteration at two years' follow-up. Increased five-year actuarial survival was reported in patients operated on with the same technique [4]. These improved results could perhaps be attributed to the use of the ITA instead of saphenous vein to close the long arteriotomy involved in open coronary endarterectomy. The relation between re-endothelialization and intimal hyperplasia has often been examined. Degree of re-endothelialization correlates inversely with neointima size [5]. To better understand the effect of the ITA patch, we designed an experimental test of the hypothesis that closing an endarterectomized artery by an arterial patch improves re-endothelialization.

2. Material and methods

2.1. Operative procedure

All animals received humane care in accordance with the European Convention on Animal Care. Nine Texel sheep, weighing between 30 and 40 kg, were used. Each animal was anesthetized with thiopental and inhaled halothane. The two carotid arteries and the right superficial femoral artery were exposed. After intravenously heparin injection
(100 IU/kg), the first carotid artery was opened along 5 cm and a 4-cm carotid endarterectomy was performed. The carotid endarterectomy was simulated by removal of the intima and the 3/3 inner layers of the media. The arteriotomy was closed either with an expanded polytetrafluoroethylene (PTFE) patch of 0.39 mm thickness or with an arterial (ART) patch. 4 cm of the right femoral artery was harvested and preserved in 0.9% saline at 18 °C during the few minutes needed to endarterectomize the carotid. Both PTFE and ART patches had the same size (5 × 0.5 cm), and they were sutured to the carotid artery with a 6.0 polypropylene running suture. The type of patch and the side of the open-endarterectomized carotid artery were randomized. After surgery, subcutaneous heparin (10,000 IU) was injected twice daily for the first week. Three animals were randomly selected for sacrifice at 8, 15 and 21 days after surgery. Then, the carotid arteries were surgically exposed and harvested for study.

2.2. Macroscopic assessment

Immediately after removal, carotid artery specimens were opened longitudinally following the medial longitudinal line of the patch. The endothelium surface was washed with a low flow of saline so as to remove fresh blood and conserve adhesive thrombi. Samples were then preserved for light microscopic study. The carotid arterial wall area covered with adhesive thrombus was measured from photographs of the specimen, and a grid paper was used to calculate it as a percentage of total endarterectomized area.

2.3. Light microscopic

For each carotid specimen, five arterial wall strips were cut, as shown in Fig. 1. The area covered with regenerated endothelium on each section was measured micrometrically and expressed as a ratio to the total area of each hematoxylin–eosin stained arterial section. Each transversal section was divided in two parts according to left or right side. Neointimal thickening was assessed as the ratio of intima area to media area measured in the hematoxylin–eosin stained longitudinal arterial sections in the samples harvested at 21 days post-surgery.

2.4. Electron microscopy

This study compared morphological aspect of the regenerated endothelium and the histological characteristics of their cells at 21 days post-surgery.

2.5. Statistics

The proportions of endarterectomized area covered with mural thrombosis, with regenerated endothelium, and the ratio of intima area to media area were expressed as median value. For each animal, each measurement on the endarterectomized carotid artery closed with the ART patch was paired to the correspondent measurement on the endarterectomized carotid artery closed with the PTFE patch and compared with the Wilcoxon test. A linear regression model was used to test the association between the percentage of endarterectomized area covered with mural thrombosis and with regenerated endothelium in the ART and PTFE groups; the differential association according to patch group was assessed by entering the percentage of endarterectomized area covered with each type of regenerated endothelium by patch type as an interaction term in the model.

3. Results

3.1. Macroscopic study

Animal no. 2 sacrificed at 15 days after surgery had total thrombosis of both carotid arteries and was excluded. The endarterectomized area covered with adhesive thrombus (Table 1) in the PTFE group was more extensive (P = 0.01) than in the ART group (Fig. 2).

<table>
<thead>
<tr>
<th>Date of sacrifice (days postoperatively)</th>
<th>Animal</th>
<th>Arterial patch group</th>
<th>PTFE patch group</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>no. 1</td>
<td>90%</td>
<td>95%</td>
</tr>
<tr>
<td>8</td>
<td>no. 4</td>
<td>80%</td>
<td>100%</td>
</tr>
<tr>
<td>8</td>
<td>no. 6</td>
<td>70%</td>
<td>100%</td>
</tr>
<tr>
<td>15</td>
<td>no. 2</td>
<td>Occlusion</td>
<td>Occlusion</td>
</tr>
<tr>
<td>15</td>
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<td>60%</td>
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</tr>
<tr>
<td>21</td>
<td>no. 9</td>
<td>5%</td>
<td>30%</td>
</tr>
<tr>
<td>Median value</td>
<td>5%</td>
<td>65%</td>
<td></td>
</tr>
</tbody>
</table>

*P = 0.01.
3.2. Morphological aspect of the regenerated endothelium

There were two types of regenerated endothelium covering the endarterectomized areas. E1 endothelium was regular and continuous. It grew from the edge of the arterial patch and from the edge of the endarterectomy into the center of the endarterectomized area. It appeared as normal endothelium found in the non-endarterectomized area. E2 endothelium did not grow from the edge of the endarterectomy; it formed small islands of endothelium area. E2 endothelium never reached the central part of the endarterectomized area. It appeared irregular and continuous. It grew from the edge of the endarterectomy into the center of the endarterectomized area. E1 endothelium did not grow from the edge of the endarterectomy area. E2 endothelium appeared rougher and showed a cobblestone-like appearance. There were several gaps between the regenerated endothelial cells, filled with platelets or inflammatory cells.

3.3. Measurement of the regenerated endothelium

Table 2 presents the ratios of regenerated endothelium area to the area of the longitudinal hematoxylin–eosin stained arterial sections. The regenerated endothelium was type E1 only, either in the ART group or in the PTFE group at each sacrifice with similar median value.

Table 3 presents the ratios of regenerated endothelium area to the area of the transversal hematoxylin–eosin stained arterial sections. In the ART group, the regenerated endothelium was type E1 only. In the PTFE group, the E1 endothelium never reached the central part of the endarterectomized area which was covered by E2 endothelium. The median value of the E1 endothelium-covered endarterectomized area was 25% in the ART group vs. 0% in the PTFE group ($P<0.0001$) at 8 days, 89% vs. 0%, respectively ($P=0.0005$) at 15 days, 100% vs. 25%, respectively, at 21 days post-surgery ($P<0.0001$).

The percentage of endarterectomized area covered with mural thrombosis correlated strongly (Fig. 3) with the percentage of endarterectomized area covered with regenerated endothelium in both the ART group ($R=-0.855$, $P<0.0001$) and the PTFE group ($R=-0.724$, $P<0.0001$). A significant difference in the association between the percentage of endarterectomized area covered with mural thrombosis and with regenerated endothelium was found according to patch type ($P=0.006$).

3.4. Electron microscopy

The scanning electron microscopy study showed differences between E1 and E2 endothelium (Fig. 4). In E1 endothelium, the regenerated cells were regular, oblong, attached to each other and oriented in the direction of the blood flow. In E2 endothelium, the regenerated cells were bloated, with a cuboid to polygonal morphology, and lacked the typical flow-wise alignment. The E2 endothelium appeared rougher and showed a cobblestone-like appearance. There were several gaps between the regenerated cells, filled with platelets or inflammatory cells.

Although E1 and E2 endothelium appeared markedly different, the transmission electron microscopy study disclosed Weibel-Palade bodies in the cytoplasm of both types, identified the cells as endothelium.
Section of carotid artery according to the Fig. 1 defined by firstly the date of the sacrifice, secondly the identification number of the animal, thirdly the site of the section, fourthly the width of the endarterectomized area on the section; E1 = normal endothelium; E1/s, the regenerated endothelium E1 area-to-section area ratio expressed in %; L–R, ratio on the left and on the right side of the transversal section; E2, abnormal endothelium; E2/s, the regenerated endothelium E2 area-to-section area ratio expressed in %; *P < 0.001; \( \text{y} \)P > 0.05.

### 4. Discussion

This study found that patching with autologous femoral artery accelerated the regression of adhesive thrombus and enhanced re-endothelialization in the endarterectomized area; the regenerated endothelium showed normal anatomic characteristics and its growth in the endarterectomized area was faster, compared to synthetic patching.

Loss of endothelial integrity is followed almost immediately by platelet adhesion, aggregation and activation on the exposed subendothelial tissue. Platelet-derived growth factor and other platelet-derived products modulating the vascular response to injury, are necessary to smooth muscle cell migration into the intima [6, 7]. A clear link exists between acute thrombus formation and release of growth factors from the activated platelets. These compounds elicit a cascade of events that culminates in the activation, phenotypic transformation, migration and proliferation of vascular smooth muscle cells. The most potent means of establishing vascular homeostasis after injury is by restoring the injured endothelium. Rapid re-endothelialization of arterial injury sites decreases subsequent platelet deposition [8] and smooth muscle cell proliferation [5]. The regression of the adhesive thrombus was faster in the ART group than in the PTFE group. Faster adhesive thrombus regression should decrease the interaction between platelets and vascular smooth muscle cells.

Some functions of the endothelium are critical for the prevention of stenosis by neointimal thickening. So, it is important to check that the neoendothelium has the characteristics able to limit neointimal thickening. This study investigated only the antithrombotic function of the regen-

### Fig. 3. Relationship between the endarterectomized areas covered by adhesive thrombus and by regenerated endothelium. Y = % of endarterectomized area covered by adhesive thrombus; X = % of endarterectomized area covered by regenerated endothelium. Arterial patch group, \( Y = 0.737–0.91X, R = -0.855, P < 0.0001 \). PTFE patch group, \( Y = 0.419–0.463X, R = -0.724, P < 0.0001 \).

### Fig. 4. Scanning electron microscopy under 4000× magnification. ART, the regenerated E1 endothelium in the arterial patch group. PTFE, the regenerated E2 endothelium in the PTFE patch group.
erated endothelium. The percentage of endarterectomized area covered by mural thrombosis showed a weaker correlation with the percentage of endarterectomized area covered by regenerated endothelium in the PTFE than in the ART group, whereas the percentage of endarterectomized area covered with regenerated endothelium was not different. The explanation for this would be an impaired antithrombotic function in E2 endothelium, found only in the PTFE group.

Endothelial regrowth appears to downregulate intimal smooth muscle cell proliferation; however, if the arterial lesion area is >3 cm, endothelial cells migrating and sprouting from the edges of the arterial injury never reach the central part of the treated arterial segment [9, 10] where smooth muscle cells at the luminal surface continue to proliferate [9]. In fact, recruitment of circulating endothelial progenitor cells plays an important role in vascular repair re-endothelialization [11] and could explain the aspect of the re-endothelialization observed in the PTFE group: E1 endothelium sprouting from the edge of the endarterectomy and E2 endothelium in the center of the endarterectomized area. The E2 endothelium appeared rougher, with a cobblestone-like aspect, resembling the regenerated endothelium derived from circulating endothelial progenitor cells [11]. Unfortunately, the regenerated endothelium derived from circulating endothelial progenitor cells seems to be functionally impaired with regard to vasomotor reactivity of the traumatized vessel [11]. It loses the ability to synthesize endothelial-derived relaxing factor [12] which can stimulate the proliferation of endothelial cells and suppress neointimal hyperplasia [13]. The long-term result could be continued intimal ingrowth. Our study failed to find any difference in neointima size between the two groups but, in the PTFE group unlike the ART group, the re-endothelialization of the endarterectomized area was incomplete. Smooth muscle cells at the luminal surface will continue to proliferate [9].

The present study had several limitations.

Sheep are less commonly used than rats or pigs for evaluation re-endothelialization after arterial injury. We don’t use anti-platelet therapy postoperatively as it has been recommended because the action of anti-platelet treatment is unknown in the sheep.

Our experimental model is not a coronary endarterectomy model. The carotid artery was chosen because open endarterectomy is easier and cheaper to perform there than on the coronary artery. Carotid arteries contain more elastic fibers and proportionately less smooth muscle cells than coronary arteries and, coronary and carotid arteries are subject to significantly different hemorheological conditions [14]; this may explain the moderate intimal hyperplasia observed.

We chose the PTFE patch as a control, although endarterectomized coronary arteries are classically closed by a saphenous vein patch. In fact, it has been shown that vein patching does not influence re-endothelialization or intimal hyperplasia [15] following carotid endarterectomy.

In this study, open carotid endarterectomy was performed in normolipemic young animals with normal arteries, and this may also be considered to have been a limitation.

5. Conclusion

Our findings would be an explanation of the improved results of the open endarterectomy associated to coronary artery reconstruction with an ITA and, at least, an incentive to use the ITA as an onlay patch in arteriotomy closure following open coronary artery endarterectomy [3, 4].

References