Acceleration of Impairment of Endothelium-dependent Responses Under Poor Runoff Conditions in Canine Autogenous Vein Grafts

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Objectives: To assess the effects of changes in shear stress on endothelium-dependent responses.

Materials and methods: Autogenous vein grafts were implanted in poor or normal distal runoff limbs of 10 mongrel dogs. Six weeks after grafting the vein grafts were removed, cut into rings, and suspended in organ chambers for isometric tension recording.

Results: The average value of intimal thickening was 110.7 ± 45.2 μm in poor runoff limbs and 65.5 ± 27.9 μm in control limbs, respectively. There was a significant difference between the two groups. Acetylcholine caused comparable endothelium-independent contractions in both groups. In the control group, adenosine diphosphate, thrombin and A23187 caused endothelium-dependent relaxations. In the poor runoff group, the endothelium-dependent relaxations caused by adenosine diphosphate and thrombin were impaired, while A23187 caused comparable endothelium-dependent relaxations. Direct relaxations in response to sodium nitroprusside were comparable between the two groups.

Conclusions: This dysfunction of the endothelium under conditions of abnormal flow may accelerate intimal thickening of the vein graft and result in late graft failure.

Key Words: Endothelium-derived relaxing factor; Vein graft; Poor runoff; Adenosine diphosphate (ADP); Thrombin.

Introduction

Saphenous veins remain the most durable conduit for small vessel arterial bypass. Despite the excellent results achieved with saphenous veins for coronary and peripheral bypass, approximately 25% of these autogenous grafts occlude within five years.1,2 Late graft failure is usually attributed to graft thrombosis based on intimal hyperplasia or progressive athrosclerotic vascular disease.3,4 In particular, in cases with poor distal runoff vessels, late graft failure frequently occurs.6–9

In previous studies we found that the outcome of vascular repair of the lower extremities could be predicted by an electromagnetically determined flow waveform of the reconstructed artery8,10 and that this state can be defined according to the variation of the wall shear stress in a cardiac cycle. In addition, wall shear stress variation is an important haemodynamic factor linked to the intimal thickening of vein grafts.11,12

Endothelium modulates the responsiveness of the underlying vascular smooth muscles by releasing the endothelium-derived relaxing factor (EDRF),13,14 which has been identified as nitric oxide (NO).15 NO is not only a vasodilator but also a potent inhibitor of platelet aggregation.16–18 EDRF-NO is known to be modulated by alterations of the blood flow.19–21 A chronically elevated blood flow is known to result in increased endothelium-dependent relaxations. Recently, our data has demonstrated that endothelium-dependent relaxations caused by adenosine diphosphate were impaired under poor runoff conditions in canine femoral arteries.22

Some previous studies have reported the impairment of endothelium-dependent relaxations in the autogenous vein graft.23–27 However, the effect of poor distal runoff conditions, including low blood flow and low wall shear stress linked to late graft failure,6,11,12 on EDRF-NO in autogenous vein graft is still unknown.

The present studies were designed to examine whether endothelium-dependent responses were
altered in poor runoff conditions of canine autogenous vein grafts by using a poor runoff model.12

Materials and Methods

Animal model

Ten mongrel dogs of either sex, weighing 15–20 kg, were anaesthetised by infusing pentobarbital (30 mg/kg) intravenously. A canine poor runoff model was prepared according to Morinaga’s method.12 All tributary arteries distal to the saphenous artery in one posterior limb were ligated and severed, except for a superior branch of the posterior femoral artery. Thus, a condition of poor runoff was created.

Four weeks after the first surgical procedure, the collateral vessels seemed to be fully developed in the right lower limb (the poor runoff limb).12,28 The femoral artery and vein were exposed at the proximal site to the previous operation, and a 4 cm segment of femoral vein was interposed into the femoral artery in end-to-end fashion with 7-0 polypropylene monofilament sutures. Grafts implanted in the same way into the contralateral limb under conditions of normal blood flow served as the control group.

Assessment of haemodynamics

Before harvesting 6 weeks after operation, a flow probe connected to an electromagnetic flowmeter was applied on the femoral artery to obtain the mean blood flow rate, and flow waveforms were recorded. The recorded waveforms were traced with a digitiser (K-150, Kanto Denshi Co., Tokyo, Japan) connected to a personal computer (PC-9801RX, Nippon Electric Company, Tokyo, Japan) to calculate the wall shear stress and its variation. Regarding the intraluminal velocity profile, changes in the flow of the boundary layer adjacent to the vessel wall reflected the flow waveform patterns; stagnation in the boundary layer adjacent to the wall is observed under conditions of a gentle-sloping flow waveform, while a remarkable fluctuation is observed under a flow waveform with a steep acceleration and deceleration.29 In regard to this change, we recreated the blood flow waveforms by calculating the integral of time differential of shear stress during a cardiac cycle (shear stress variation, ζ-variation), using a computational simulation method.29,30

In vitro experiments

The isolated vein grafts from the poor runoff portion and from the control portion were cleaned of peri-vascular tissue while taking care not to touch the luminal surface. Rings 4–5 mm in length were then cut. In some rings the endothelium was removed (rings without endothelium) by rubbing the luminal surface gently with a cotton swab.26 Each ring was suspended for measurement of isometric force (U gauge, Shinko Co., Tokyo) and was suspended individually in a 20 ml volume organ chamber filled with oxygenated Krebs–Henseleit solution at 37°C.31 The composition of the solution was as follows (mM): Na+ 137.4, K+ 5.9, Ca2+ 2.6, Mg2+ 1.2, HCO3− 15.5, H2PO4− 1.2, Cl− 134.0 and glucose 11.5. The solution was aerated with 95% O2-5% CO2 and the pH was adjusted to 7.3–7.4.12,31

The rings were progressively stretched and contracted with a submaximal contraction of norepinephrine (3 × 10−7 M) at each level of stretching until the active tension was maximal (optimal length).31,32 They were then allowed to equilibrate for 45 min before the experiments.

Measurement of intimal thickening

Semi-thin sections were also stained with haematoxylin-eosin or the elastic van Gieson’s method. Intimal thickening was measured by using an ocular cytometer placed on the ocular lens of a light microscope. The average of intimal thickening of eight randomly selected points of each sample was taken, then the average of the three segments each graft was assessed as the degree of its intimal thickening.31

Protocol

Relaxations were examined in rings contracted with norepinephrine (3 × 10−7 M), which caused about 60% of 3 × 10−5 M norepinephrine-induced maximal contractions.31 The order of the drugs tested was as follows: (1) ACh (10−9–3 × 10−5 M), (2) ADP (10−8–3 × 10−5 M), (3) thrombin (0.1, 0.3 and 1 U/ml), (4) A23187 (10−5–10−6 M), (5) sodium nitroprusside (10−2–10−5 M). A pair of rings either with and without an endothelium were incubated with indomethacin (10−5 M) for 40 min to prevent the synthesis of endogenous prostanoids.31 Endothelium-derived relaxing factor is not a product of cyclooxygenase, and its release is not affected by
indomethacin. Therefore, in the present study, the endothelium-dependent responses were examined in the presence of indomethacin to focus on the endothelium-derived relaxing factor.

**Drugs**

The following drugs were used: acetylcholine (ACh), thrombin, adenosine diphosphate (ADP), norepinephrine, indomethacin (Sigma Chemical Company, St. Louis, U.S.A.), the calcium ionophore A23187 (Calbiochem, La Jolla, CA, U.S.A.) and nitroprusside (Wako, Co., Osaka). Unless otherwise specified, the drugs were prepared daily in distilled water, kept on ice and added to the organ chambers. The calcium ionophore A23187 was dissolved in dimethyl sulfoxide and diluted with distilled water. Indomethacin was dissolved in an equal molar concentration of Na₂CO₃ (10⁻⁵ M). The drug concentrations are reported as the final molar concentration (M) in the bath solution.

**Calculations and statistical analysis**

The results are expressed as mean ± SEM. Unless otherwise specified, n means the number of animals from which the rings were taken. In the rings contracted with norepinephrine (3 × 10⁻⁷ M), responses are expressed as the percent changes from the contracted levels. For relaxations, the effective concentration of vasodilators causing 50% inhibition (ED₅₀) or 30% inhibition (ED₃₀) of the contractions to norepinephrine (3 × 10⁻⁷ M) was calculated from each concentration-response curve, and the means of these values were presented as the negative logarithm of the molar concentration. For contractions evoked by norepinephrine, the effective concentration was that producing 50% (EC₅₀) of the contractions to the maximum contractions evoked by 3 × 10⁻⁵ M norepinephrine. A statistical evaluation of the data was performed by Student's t-test for paired observations. Values were considered to be statistically significant when p was less than 0.05.

**Results**

**Haemodynamic data and histology**

Flow rate and ζ-variation in the poor runoff group were significantly lower than in the normal flow group (Table 1). Namely, an abnormal flow condition in the poor runoff limbs were characterised by low blood flow and low wall shear stress variation. Typical actual blood flow waveforms are shown in Fig. 1.

The average value of intimal thickening was 110.7 ± 45.2 μm in poor runoff limbs and 65.5 ± 27.9 μm in the control limbs, respectively. There was a significant difference between the two groups.

**Characteristics of smooth muscle (Table 2)**

There was no statistically significant difference in the optimal tension or contractions evoked by norepinephrine between the control and the poor

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**Table 1. Haemodynamic data.**

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 6)</th>
<th>Poor runoff (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean flow rate (ml/min)</td>
<td>67.4 ± 22.2</td>
<td>23.4 ± 10.5*</td>
</tr>
<tr>
<td>Shear stress variation (dyne/cm²)</td>
<td>97.9 ± 9.5</td>
<td>44.1 ± 14.0*</td>
</tr>
</tbody>
</table>

* Denotes a significant difference (p<0.05) between the two groups.

**Table 2. Smooth muscle characteristics.**

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 6)</th>
<th>Poor runoff (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optimal tension (g)</td>
<td>1.1 ± 0.1</td>
<td>1.1 ± 0.2</td>
</tr>
<tr>
<td>EC₅₀ to NE (−log M)</td>
<td>6.7 ± 0.2</td>
<td>6.8 ± 0.1</td>
</tr>
<tr>
<td>Relaxation to SNP ED₃₀ (−log M)</td>
<td>6.4 ± 0.3</td>
<td>6.7 ± 0.5</td>
</tr>
<tr>
<td>Maximal relaxation (%)</td>
<td>114 ± 8.4</td>
<td>110 ± 10.2</td>
</tr>
</tbody>
</table>

NE = norepinephrine; SNP = sodium nitroprusside; EC₅₀ = effective concentration producing 50% of the contraction to maximum contractions evoked by 3 × 10⁻⁵ M; ED₃₀ = effective concentration producing 30% inhibition of the contractions caused by norepinephrine (3 × 10⁻⁵ M).
runoff group. Sodium nitroprusside (10^-9 to 10^-5 M) caused comparable concentration-dependent relaxations in the rings without an endothelium between the two groups.

Endothelium-dependent relaxations (Table 3)

Control group: In the rings precontracted with norepinephrine (3 x 10^-7 M), ACh caused endothelium-independent contractions (Fig. 2). ADP, thrombin and A23187 caused endothelium-dependent relaxations in the control group (Figs 3-5).

Poor runoff model: ACh caused endothelium-independent contractions (Fig. 2). There was no significant difference between the control and poor runoff group. A23187 caused endothelium-dependent relaxations which did not differ significantly from those obtained in the vein graft from the control group, whereas the endothelium-dependent relaxations caused by ADP and thrombin were significantly reduced in the poor runoff model (Figs 3-5).

Table 3. Endothelium-dependent relaxations.

<table>
<thead>
<tr>
<th></th>
<th>Control (n=6)</th>
<th>Poor runoff (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relaxation to ADP maximal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>relaxation (%)</td>
<td>51.9 ± 3.9</td>
<td>43.6 ± 3.7</td>
</tr>
<tr>
<td>$E_{D50}$ (-log M)</td>
<td>5.8 ± 0.3</td>
<td>5.2 ± 0.2</td>
</tr>
<tr>
<td>Relaxations to A23187</td>
<td></td>
<td></td>
</tr>
<tr>
<td>maximal relaxation (%)</td>
<td>81.6 ± 4.9</td>
<td>78.3 ± 0.3</td>
</tr>
<tr>
<td>$E_{D50}$ (-log M)</td>
<td>7.3 ± 0.4</td>
<td>7.2 ± 0.3</td>
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</tbody>
</table>

ADP = adenosine diphosphate; $E_{D50}$ = effective concentration producing 50% inhibition of the contractions caused by norepinephrine (3 x 10^-7 M); $E_{D30}$ = effective concentration producing 30% inhibition of the contractions caused by norepinephrine (3 x 10^-7 M).

Fig. 2. Cumulative concentration-response curves to ACh in canine vein grafts without endothelium taken from control and poor runoff models during a contraction evoked by norepinephrine (3 x 10^-7 M) in the presence of indomethacin (10^-5 M). The squares show the control and the circles show the poor runoff group.

Discusssion

Haemodynamic factors such as a low flow velocity and low shear stress result in progression of late graft failure because of intimal thickening. In our previous studies we classified the electromagnetically measured blood flow waveform at reconstructive surgery into five types. We reported a close relationship between the ultimate results of the arterial reconstruction and intraoperative blood flow waveforms. In grafts with a type 0 or I flow wave pattern (normal flow group, characterised by steep acceleration and deceleration) had good long-term patency. In grafts with a type II, III or IV flow waveform pattern (abnormal flow group, characterised by a gentle sloping), graft failure was more frequent than in the normal flow group. In our present experiment, we used a poor runoff model in the canine femoral artery, which is similar to a human patient with peripheral vascular disease, because under this poor runoff condition, the intimal thickening of the autogenous vein graft was significantly thicker than that in the control group.

Some authors have demonstrated previously that, in the vein graft, endothelium-dependent relaxations were impaired under normal flow condition. A major finding of our present study is that the canine femoral vein graft in the poor runoff condition, which is linked closely to clinical late graft failure, results in the impairment of endothelium-dependent relaxations caused by ADP and thrombin in addition to the impairment of endothelium-dependent relaxations caused by ACh.
EDRF and Poor Runoff

Control

Thrombin 1 U/ml

With endothelium

Norepinephrine 3 x 10^-7 M

Thrombin 1 U/ml

Without endothelium

Norepinephrine 3 x 10^-7 M

Poor-runoff

Thrombin 1 U/ml

With endothelium

Norepinephrine 3 x 10^-7 M

Thrombin 1 U/ml

Without endothelium

Norepinephrine 3 x 10^-7 M

1 g

3 min

Fig. 4. Actual recording of endothelium-dependent relaxations to thrombin (1/ml). Thrombin-induced relaxations were significantly reduced in the poor runoff model compared to the control group. Wo = wash out.

Fig. 5. Endothelium-dependent responses to thrombin in canine vein grafts from control and poor runoff model during a contraction evoked by norepinephrine (3 x 10^-7 M) in the presence of indomethacin (10^-5 M). * Denotes a significant difference (p<0.05) between the two groups. Closed square (■) = poor runoff model; closed circle (○) = control.

relaxations in vein grafts under the abnormal flow condition. First, the responsiveness of vascular smooth muscle cells to the EDRF may be changed. This possibility is unlikely, because there was no difference in the endothelium-dependent relaxations caused by A23187 between the two groups. In addition, in rings without an endothelium, the direct relaxations caused by sodium nitroprusside and contractions caused by norepinephrine and ACh were not altered. Second, the diffusion of the EDRF from the endothelium to the underlying smooth muscle may be impaired. This possibility appears likely because the intimal thickening was more prominent in the poor runoff group than in the normal flow group. The intimal thickening could act as a diffusion barrier against the EDRF, which has a very short life. The role of intimal thickening in reducing the diffusion of the EDRF is, however, controversial.33 Finally, the production or release of the EDRF caused by ADP and thrombin may be depressed under an abnormal flow. Alternatively, the impairment of responses to ADP and thrombin may be due to the impairment of receptors, receptor-coupling mechanisms or the impairment of nitric oxide production. This has to be considered because endothelium-dependent relaxations can be evoked by the calcium ionophore, A23187, a substance that releases an EDRF by a process not associated with receptor activation.34,35 It is most likely that a combination of these two mechanisms contribute to the decreased endothelium-dependent responses under the poor runoff condition.

Endothelium-derived relaxing factor (EDRF) has been identified as nitric oxide (NO). NO is not only a vasodilator but also a potent inhibitor of platelet aggregation. NO mediates its physiological effect on smooth muscle cells by the elevation of intracellular c-GMP and may function by a c-GMP-mediated mechanism as a modulator of vascular smooth muscle cell mitogenesis and proliferation.37 In the canine femoral vein, endothelium-dependent relaxations were caused by NO, because Funahashi et al. demonstrated that endothelium-dependent relaxations were significantly inhibited by N^6-L-arginine methyl ester (L-NAME).38 Onohara et al. also reported the dysfunction of the endothelium under an abnormal flow using the same poor runoff model. The production of prostacyclin in the canine vein graft was lower in abnormal flow than that in normal flow conditions. Interactions between aggregating platelets and intimal thickening may be important, since antiplatelet drugs reduce intimal hyperplasia.39,40 The dysfunction of endothelium in terms of the decreased production of EDRF-NO as well as decreased prostacyclin production under

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abnormal flow conditions may increase platelet aggregation and eventually facilitate the development of peripheral arterial occlusive disease and thus the occurrence of late graft failure.

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References


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