Non-penetrating Vascular Clips Anastomosis Inhibited Intimal Thickening Under Poor Runoff Conditions in Canine Autogenous Vein Grafts

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Objective: Late graft failure is still a significant problem, particularly in cases with poor runoff vessels. The main cause of late graft failure is intimal thickening of the anastomotic region. Vascular closure system (VCS) clips may provide ideal anastomosis, since they do not penetrate the wall. Therefore, we examined whether the VCS clips affect intimal thickening under poor runoff conditions in the canine autogenous vein grafts.

Methods: A canine poor runoff model was prepared at both femoral veins. Four weeks after the first surgical procedure, two groups were established according to the two different methods of anastomosis employed. The right femoral vein graft was performed using polypropylene sutures, conventional surgical anastomosis (control group), while the left femoral vein graft was performed using VCS clips anastomosis (VCS group). Four weeks after grafting, the vein grafts were removed and the intimal thickening of proximal, distal anastomosis and midportion of the vein grafts were examined histologically.

Results: In the control group, flow rate and variation were 26 ± 8 ml/min and 51 ± 10 dynes/cm², respectively. In the VCS group, the flow rate and variation were 23 ± 11 ml/min and 44 ± 14 dynes/cm², respectively. There were no significant differences between the two groups. The average value of intimal thickening of both the anastomotic region and the midportion of the vein graft in the VCS group was significantly inhibited compared to that of the control group. The number of positive cells of masson trichrome stain in the VCS group was significantly less than that of the control group.

Conclusions: These experiments indicate that VCS clips significantly inhibit intimal thickening under poor runoff conditions in canine autogenous vein grafts to a greater extent compared to suture-constructed anastomosis. One mechanism that may account for the decreased intimal thickening is the inhibition of the expression of transforming growth factor-β (TGF-β), because the number of positive cells of masson trichrome stain in the VCS group was significantly less than that of the control group.

Key Words: Vascular clip; Vein graft; Intimal thickening; Poor runoff.

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 30% of these autogenous grafts occlude within 5 years.1,2 Late graft failure is usually attributed to graft thrombosis based on intimal hyperplasia or progressive atherosclerotic vascular disease.1,2 In particular, in cases with poor distal runoff vessels, late graft failure frequently occurs.3–7

A paramount concern of the cardiovascular surgeon is the integrity of a small-diameter, hand-sutured arterial anastomosis. This concern is justified based on the technical problems associated with the sutureting of small arteries: potential intimal injury, adventitial stripping, anastomatic bleeding and a persistent intimal luminal foreign body. All these factors contribute to both early and late anastomotic failure, particularly in the form of intimal hyperplasia.4,12

A non-suture vascular anastomotic technique consisting of the application of accurate-legged, non-penetrating titanium clips in an interrupted fashion to everted tissue edges at high compressive forces has been developed.13 The system enables rapid and precise microvascular reconstructions.13 Clips have proved to be at least equivalent, both biologically and technically, to needle-and-suture technique.14 However, little information is available about the effect of VCS clips on intimal thickening of autogenous vein grafts.

Therefore, the present studies were designed to examine whether the VCS clips affect the intimal thickening under poor-runoff conditions in canine autogenous vein grafts by using a poor-runoff model.15
Materials and Methods

Animal model

Eight 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: that is, all tributary arteries distal to the saphenous artery in the unilateral posterior limb were ligated and severed, except for a superior branch of the posterior femoral artery. Thus, a condition of poor runoff was completed at both femoral arteries.

Four weeks after the first surgical procedure, the collateral vessels seemed to be fully developed in both lower limbs (the poor runoff limbs). The femoral artery and vein were exposed at the proximal site of the surgical procedures described above, and a 4-cm segment of femoral vein was interposed into the femoral artery. Two groups were established according to the method of anastomosis employed. The right femoral vein graft was in end-to-end fashion with 7-0 polypropylene monofilament interrupted sutures (control group) and 12–16 sutures (median; 14) per anastomosis were applied. Then, the left femoral vein graft was anastomosed with VCS clips (VCS group). Four untied stay sutures were used prior to clipping and these were removed after the anastomosis was completed. Twelve to 16 clips (median; 14) per anastomosis were applied. The distance between the sutures are about 1 mm in length. The VCS clips were titanium clips (U.S. Surgical, Norwalk, Conn) donated by Auto Suture Japan Company.

All procedures were performed according to sterile techniques with a general anaesthetic, 30 mg/kg sodium pentobarbital, given intravenously plus maintenance dosages as required. During surgery, cephalosporin (100 mg/kg) was given intravenously.

Assessment of haemodynamics

In previous studies we found a close correlation between the outcome of the reconstructed arteries and intraoperative blood flow waveforms. In terms of the intraluminal velocity profile, we analysed flow waveforms and found that normal flow waveform patterns were characterised by a remarkable fluctuation of flow in the boundary layer adjacent to the vessel wall, whereas in abnormal flow waveforms stagnation of flow was seen in the boundary layer. Changes in flow in the boundary layer adjacent to the vessel wall were greater in the normal flow group, as compared with findings in the abnormal flow group. Thus, we prepared flow waveforms by using an integral of time differential of wall shear stress in a cardiac cycle (shear stress variation, τ-variation).

Before harvesting the vein grafts at 4 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 using a digitiser (K-150, Kanto Denshi Co., Tokyo, Japan) connected to a personal computer (PC-9801RX, Nippon Electric Company, Tokyo, Japan), which displayed a computational simulated intraluminal velocity file and calculated the wall shear stress adjacent to the vessel wall and the integral of time differential of the wall shear stress in a cardiac cycle (shear stress variation, τ-variation). The computational method was based on the assumption that: (1) blood is a Newtonian fluid; and (2) vessels to be studied are rigid, straight, long tubes. A detailed description of the method has been published elsewhere.

Measurement of intimal thickening

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

Masson trichrome stain

All histopathological sections of each animal were examined using a 3CCD colour video camera (FUJIX Digital Camera HC-2500 3CCD; Fujifilm, Japan) mounted on a standard microscope (BX50; Olympus, Japan). Drawings of the limits of the vessels were made on the screen of a multiscreen colour computer display (Diamondtron RD17GZ; Mitsubishi, Japan) and then digitised with a two-dimensional analysis system (Mac SCOPE, Mitani Corporation, Japan) connected to a Macintosh computer system (Power Macintosh G3; Apple Computer Inc, California, USA). Histopathological examination of the vein grafts was performed.
Table 1. Haemodynamic data.

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<th>Control (n=8)</th>
<th>VCS (n=8)</th>
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<tr>
<td>Mean flow rate (ml/min)</td>
<td>26 (18–35)</td>
<td>23 (13–37)</td>
</tr>
<tr>
<td>Shear stress variation (dyne/cm²)</td>
<td>51 (40–61)</td>
<td>44 (30–65)</td>
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Data are expressed as median (range). VCS: Vascular closure system clips.

To assess the fibrosis of the vein grafts, trans-sectional images of the area of the total intimal lumen and the area of the total medial lumen were studied. The inner border of the lumen and the outer border of the tunica media were traced on each arterial image with Massons trichrome staining at ×50 magnification, and the areas encircled by the tracings were calculated. During the quantification, non-round vessels resulting from oblique transection were discarded; only round vessels were studied. The area of fibrosis in the intima was calculated, and the intimal fibrosis was determined as the ratio of the area of fibrosis to the total area of the intima. The intimal fibrosis measurements were performed using a computer package.

Calculations and statistical analysis

The results are expressed as mean±SD or median (range) as appropriate. Unless otherwise specified, n means the number of rings taken from different dogs. A statistical evaluation of the data was made by the Kruskal–Wallis test. If the value was statistically significant, Scheffes test for multiple comparisons was used to identify differences among the groups. Values were considered to be statistically significant when p was less than 0.05.

Results

All vein grafts (n=16) were patent.

The time for performing each of the two anastomosis of vein grafts in the VCS group (4±1 min) was significantly shorter than that of control group (13±4 min).

Haemodynamic data

Flow rate and τ-variation in both groups were similar (Table 1).

Histology

(1) Intimal thickening

The development of intimal thickening of the autogenous vein graft is shown in Table 2. The average value of intimal thickening in the proximal, distal anastomotic region and the midportion of the vein grafts in control group was significantly thicker than those of VCS group.

(2) Histological examination of the distal anastomotic region of the vein grafts

Figure 1 shows the histopathological findings for the longitudinal tissue section from the canine femoral vein graft in the control group (conventional surgical procedure). A remarkable anastomotic hump was observed (Fig. 1a). The anastomotic hump was composed of fibromuscular intimal hyperplasia (I), which was observed by a high-powered view of the squared area of (a) (Fig. 1b).

Massive hyalinised necrotic tissue is apparent, which is surrounded by vascular rich inflamed granulation tissue, which was observed by a high-powered view of the squared area of (a) (Fig. 1c).

Figure 2 shows histopathological findings for the longitudinal tissue section from the canine femoral vein graft in the VCS group. As seen in Figure 2(a), in contrast to the control group there is no apparent anastomotic hump. The amount of hyalinised necrotizing tissue is minimal, and fibrous vascular rich tissue, suggesting scar of granulation tissue, is associated. Fibromuscular hyperplasia is recognised, similar to Figure 1(b), but there was no apparent humping formation when observed by high-powered view of squared area of a (Fig. 2b).

(3) Histological examination of Masson trichrome stain

The positive area of masson trichrome stain in the intimal thickening is expressed as a percentage of the total area. The control group and VCS group were 99±5 % and 75±5 %, respectively. There was significant difference between the two groups.
Table 2. Intimal thickening (μm).

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<tr>
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<th>Control (n=6)</th>
<th>VCS (n=6)</th>
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<tr>
<td>Anastomotic region of the vein grafts (proximal)</td>
<td>62 (48–72)</td>
<td>28 (20–40)*</td>
</tr>
<tr>
<td>Anastomotic region of the vein grafts (distal)</td>
<td>64 (54–76)</td>
<td>22 (12–36)*</td>
</tr>
<tr>
<td>Midportion of the vein grafts</td>
<td>47 (35–55)</td>
<td>14 (8–26)*</td>
</tr>
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Data are expressed as median (range).

VCS: Vascular closure system clips.

The asterisk denotes a significant difference (p<0.05) between the two groups.

**Discussion**

Grafting with an autologous vein as a vascular substitute in the treatment of peripheral arterial occlusions is the surgical procedure that yields the best long-term results. However, graft failure is still a significant problem and the main cause of graft failure is intimal thickening. Intimal thickening is not a cause of early failure of human autogenous vein bypasses. It is a cause of mid-term failure at between 1 and 2 years. Its aetiology is not totally understood, but appears to be related not only to the characteristics of the arterial anastomosis in its wall but also to turbulence, platelet damage and release of various fibroblast stimulating substances. In addition, late graft failure is a significant problem, particularly in cases with poor runoff vessels. Haemodynamic factors such as a low flow velocity and low shear stress result in progression of late graft failure due to intimal thickening. In our previous studies we classified the electromagnetically measured...
Vascular Clips and Intimal Thickening

Fig. 2. Histological examination of the distal anastomotic region in the canine femoral vein graft by VCS clips: (a) Histopathological findings of the longitudinal tissue section of clipped canine femoral vein graft. Note marked absence of an anastomotic hump. The amount of hyalinised necrotising tissue is minimal (H: arrow), and fibrous vascular rich tissue, suggesting scar of granulation tissue, is associated (G). (b) High-powered view of squared area of (a). Fibromuscular hyperplasia is recognised (I), similar to Fig. 1(b), but no apparent humping formation. I = intima, M = media.

blood flow waveform at reconstructive surgery into five types. We reported a close relationship between the ultimate results of the arterial reconstruction and intra-operative blood flow waveforms. Grafts with a type 0 or I flow pattern (normal flow group, characterised by steep acceleration and deceleration) had a 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 canine femoral vein grafts. The canine femoral vein in dogs weighing 15–20 kg is not a particularly small vessel. This is an anastomosis of 2–3 mm in diameter, end to end, which would be expected to have good patency. Then 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.

There is an increasing demand for an easier, quicker, less damaging, but reliable procedure to create a vascular anastomosis. This demand is not new, but is revitalised by the movement of vascular procedures in various specialities, including cardiovascular surgery, toward minimally invasive procedures.

The key principle in surgical application of the clips in the ability to evert tissue and special everting forceps facilitate this manoeuvre. The non-penetrating accurate-legged clip confers technical advantages to the surgeon engaged in vascular surgery. The clip provides the capability of improved dual reconstructions, rapid and reliable bloodtight vascular anastomoses, and the capability of controlling haemorrhage from arterial and venous sources.

The striking evidence of the present study is that VCS clip anastomosis significantly inhibited the in-operative blood flow waveform. Grafts with a type 0 or I flow pattern (normal flow group, characterised by steep acceleration and deceleration) had a 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 canine femoral vein grafts. The canine femoral vein in dogs weighing 15–20 kg is not a particularly small vessel. This is an anastomosis of 2–3 mm in diameter, end to end, which would be expected to have good patency. Then 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.

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as collagen, fibronectin and proteoglycan and inhibits ECM degradation.\textsuperscript{25–27} Increased expression of TGF-\(\beta\) has been shown in human and experimental cardiac hypertrophy/ fibrosis.\textsuperscript{28–30} In addition, the increased impression of TGF-\(\beta\) in cultured cardiac fibrosis, vascular smooth muscle cells and renal mesangial cells was observed.\textsuperscript{31–33} Thus, the net effect of TGF-\(\beta\) leads to fibrosis, which results in vascular smooth muscle cell proliferation. Therefore, the present experiment suggested that VCS clips suppress the impression TGF-\(\beta\), which results in the inhibition of intimal thickening of the vein grafts. However, previous reports have demonstrated the comparative intimal thickening between the VCS clip and conventional suture in the pig model\textsuperscript{34} and dog arteriovenous fistula.\textsuperscript{35} In addition, a recent report demonstrated that vascular clips have no significant effect on the cellular proliferation, intimal changes or peak systolic velocity at anastomoses in rabbit juglar vein grafts.\textsuperscript{36} The precise mechanism of the different results were unknown. Further experiments were required to elucidate the mechanism of the suppression of the intimal thickening of vein grafts under poor runoff conditions.

We concluded that VCS clips anastomosis significantly inhibited the intimal thickening under the poor-runoff conditions in the autogenous vein grafts compared to those of conventional sutured anastomosis. VCS clip anastomosis could be beneficial in preventing intimal hyperplasia, which results in good patency rate after arterial reconstruction.

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