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Relationship Between Vasa Vasorum and Blood Flow to Vein Bypass Endothelial Morphology

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• In order to define the respective roles of the vasa vasorum blood supply and intraluminal arterial blood flow in maintaining the endothelial integrity of in situ vein bypasses, we have carried out two separate but interrelated experiments in a canine model. In vivo studies on eight dogs demonstrated that even in the absence of intraluminal blood flow the vasa vasorum maintained endothelial integrity and also showed that the endothelium was very sensitive to the loss of the vasa vasorum blood supply. In a second group of experiments on 14 in situ vein bypasses we studied the effect of division of the vasa vasorum immediately after arterialization of the bypass. This experiment showed that arterialization of the vein maintained endothelial integrity despite division of the vasa vasorum.

(Arch Surg 1985;120:386-388)

The specific technique adopted for vein preparation prior to its use as an arterial bypass has significant implications with regard to its future performance.^{1,3} Preservation of the normal structure and function of endothelium is important in maintaining patency of vein bypasses especially in situations with limited runoff.^{4,5} In addition, subsequent occurrence of myointimal hyperplasia may be minimized or diminished by the avoidance of harvesting injuries.^{6,7} These factors have particular significance when small-diameter veins are used as a bypass conduit.⁵

We describe experimental work with a canine model on

the role of vasa vasorum in preserving venous endothelial integrity.

MATERIALS AND METHODS

Fifteen mongrel dogs weighing 20 to 30 kg were anesthetized with intravenous sodium thiopental, intubated, and maintained on a respirator (Harvard) with halothane anesthesia. In the first experiment, in eight dogs the external jugular veins were exposed on one side for a length of 10 cm. Half of this exposed length of vein was completely dissected free of all vasa vasorum and in the other half the vasa vasorum were left intact. Sharp dissection was utilized and care was taken to avoid direct venous trauma. Venous side branches were ligated away from the main vein so as not to disturb the vein wall. In order to completely exclude transit of venous blood through the lumen, both ends of the vein were clamped. The exposed outer surface of the vein was kept moist with 0.9% sodium chloride solution at room temperature. One hour after occlusion of the vein 2 mL of 25% fluorescein was injected into the ipsilateral forelimb vein. Immediately following fluorescein injection an ultraviolet light (Wood's lamp) was used to detect fluorescence of the exterior of the vein wall. The entire 10-cm segment of vein was then opened longitudinally and the interior examined for fluorescence. Small segments of vein were excised 1 cm from either side of the interface of the part of the vein with and without vasa vasorum. This area was termed the junctional area. Specimens were placed in fixative and examined histologically by scanning and transmission electron microscopy as well as by light microscopy using hematoxylin-eosin.

In the second experiment, 14 external jugular vein-to-carotid artery in situ bypasses were performed in seven dogs. The artery was initially mobilized and the anastomoses were constructed end-to-side with a continuous monofilament suture. The carotid artery between the anastomoses was ligated after bypass flow was instituted. No instrument was passed through the vein lumen and the veins were not irrigated or mechanically distended and no

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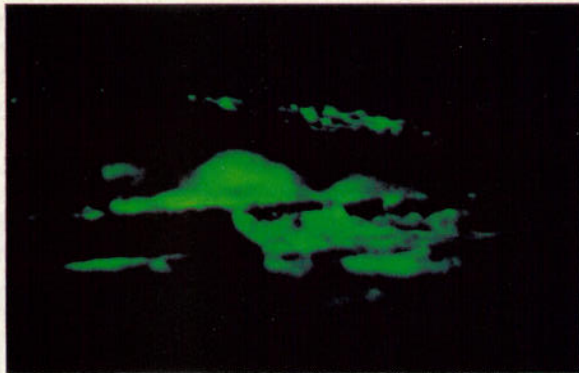


Fig 1.—Dark exposure showing on left, lack of fluorescein in mobilized vein segment. Fluorescein is also seen on portion of vein with intact vasa vasorum and in surrounding tissue.

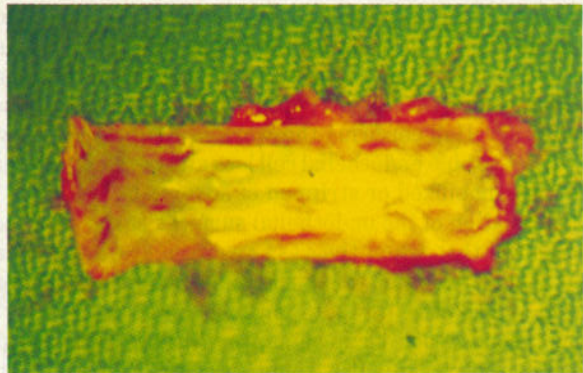


Fig 2.—This view of intimal surface of canine external jugular vein shows fluorescence on surface corresponding to portion of vein with intact vasa vasorum. Fluorescence extends for 5 mm into junctional zone.

heparin was used. On both sides of the neck, the bypasses were done in situ with the vasa vasorum initially left intact. After arterialization of the bypasses, the vasa vasorum along the complete length of one vein were interrupted by sharp dissection while in the contralateral vein the vasa vasorum were left intact. Continuous blood flow was reestablished in each vein within 25 minutes.

These vein bypasses were harvested at 2, 4, 6, 8, 10, 14, and 21 days after implantation and the middle sections of each vein were studied by scanning and transmission electron microscopy.

RESULTS

Following fluorescein injection in the first eight dogs, the entire external surface of the 5-cm nonmobilized (ie, intact vasa vasorum), in situ vein fluoresced (Fig 1). This fluorescence was seen within eight seconds of injection. On opening the veins the fluorescence was seen to extend on the endothelial surface only for 5 mm into the junctional area of the vein (Fig 2). The actual presence of fluorescein on the endothelial surface was confirmed by scraping off the endothelium with a fine steel wool pledget and examining the pledget for fluorescence. In the mobilized vein segments, (ie, those lacking vasa vasorum), the luminal surface was covered with fibrin and platelet aggregates overlying a largely denuded endothelial layer. Both light and transmission microscopy showed that considerable subendothelial swelling was present in the mobilized segments. By contrast, the endothelium of all nonmobilized in situ veins appeared intact without any subendothelial swelling.

In the second set of experiments, at each of the seven time intervals at which the veins were harvested, the endothelium was normal and equally well preserved in the arterial bypasses irrespective of whether the vasa vasorum were interrupted or not.

COMMENT

A major difference between a reversed saphenous vein bypass and the in situ saphenous vein bypass is that the vasa vasorum are left intact on the in situ vein bypass for the majority of its length. The superior results of the in situ bypass have been attributed both to the more ideal hemodynamic taper and also to improved endothelial preservation in these veins.^{5,8} Bush et al⁴ have provided biochemical

evidence of endothelial preservation by demonstrating a more favorable balance of prostacyclin and thromboxane A₂ in in situ-prepared human saphenous veins compared with reversed human saphenous veins. Preliminary work in our own laboratory has also confirmed this important finding. The present study focuses on the relationship of vasa vasorum blood flow in maintaining morphologic endothelial integrity in an in vivo setting.

Previous work on venous vasa vasorum done with India ink or micropaque injections or with benzidine or orthotolidene stains in vitro has shown that the vasa vasorum enters the media through the adventitia.^{9,12} However, there is controversy in the literature as to the depth of penetration of the vasa vasorum into the media.¹³ O'Neill¹³ using a benzidine dye technique to stain red blood cells in the lumen of the vasa vasorum did not show evidence of vasa vasorum in the media. However, he points out that this may be due to a lack of red blood cells in the deeper vasa vasorum or due to the lack of stain penetrating the deeper layers of the vein wall. Brook⁹ using a similar technique with orthotolidene instead of benzidine demonstrated vasa vasorum within 36 μm of the endothelial layer in a human long saphenous vein that was 208 μm thick. At this level the vasa vasorum were situated within the deepest layers of the tunica media but peripheral to the internal elastic membrane. Most authors agree that there is no communication of the vasa vasorum with the lumen of the vein.^{9,12,13}

Our initial series of experiments with canine jugular vein using intravenous fluorescein confirms that blood flow through the vasa vasorum is capable of supplying nutrients to the entire vein wall including the endothelium. Brook's work⁹ suggests that diffusion must be required for the latter portion of this process and that this occurs for only a short distance through the vein wall. However, in the first experiment, the presence of fluorescein in the endothelial cells was confirmed by scraping off only this layer and proving that it exhibited fluorescence. We must, therefore, conclude that fluorescein reached the endothelium via the vasa vasorum. The marked differences in endothelial morphology of the portions of the vein with and without vasa vasorum also suggest that endothelial integrity can be maintained solely by vasa vasorum blood flow for at least

one hour after interruption of the intraluminal blood flow. After one hour of interrupted intraluminal blood flow loss of the vasa vasorum blood supply causes such significant endothelial changes that fibrin platelet deposition takes place. This observation confirms the work of Krupski et al⁴ who found 25% to 50% endothelial cell loss after one hour in veins that were mobilized or stripped of adventitia.

Examination of the endothelial surfaces of the junctional zone shows that when the vasa vasorum are interrupted, fluorescence does not reach the endothelium except for a limited length of vein wall (5 mm).

The second series of experiments with arterialized in situ vein bypasses with and without vasa vasorum shows the importance of continuous arterial blood flow in the vein bypass. The similarity of the scanning electron micrographs at each time interval confirms that intraluminal arterialized blood can maintain endothelial morphology even in the absence of vasa vasorum. Thus, the endothelial surface of an arterialized vein bypass is probably nourished by both the luminal blood and through the vasa vasorum blood supply.

The results of these canine studies would appear to be particularly relevant when preparing the lower, smaller end of the saphenous vein for an in situ bypass. When using an in situ vein as a bypass conduit the focus should be, therefore,

on maintenance of the vasa vasorum blood supply to the vein and in establishing arterial flow in the bypass as quickly as possible before mobilization to avoid ischemic injury to the vein wall and hereby preserve the endothelial monolayer with its antithrombotic properties.⁴ Ideally, the vein should be arterialized and not be transected or detached from its bed until the recipient artery is readied for anastomosis. Side branches having arteriovenous fistulas should be left intact in order for continuous blood flow to be maintained through the vein lumen while the distal anastomosis is being completed. All of these measures will serve to limit ischemic injury to the distal part of the mobilized vein. This lower distal part of the vein is at the greatest risk for ischemic injury; hence when this segment of the vein is mobilized we attempt to dissect the vein en bloc with its surrounding fat and accompanying vasa vasorum. The use of loupe magnification is particularly helpful in identifying and avoiding transecting the vasa vasorum and only the distal few millimeters of the vein should be cleaned of surrounding tissue in order to perform the distal anastomoses accurately. The vasa vasorum blood supply should be carefully preserved over as much of the mobilized segment of an in situ vein as possible in order to minimize ischemic injury to the vein.

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