

Vascular Delay, Angiogenesis and Cardiomyoplasty

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Abstract

Vascular delay is a surgical technique that renders a skin flap sub-lethally ischemic and produces significant alterations in the characteristics of the tissue perfusion. The enhanced perfusion is associated with decreased distal flap necrosis and increased viability. Applying this technique to skeletal muscle flaps produces similar vascular changes and results in enhanced perfusion and contractile function. The changes in vascular architecture and total blood flow for both skin and muscle flaps represent alterations in basic arteriogenic and angiogenic activity. The recruitment of previously collapsed collateral vessels and growth and development of new vessels occurs over a period of days. The optimal period of vascular delay for rat latissimus dorsi muscle (LDM) is 3-14 days and 14 days has been used for human LDM. The mechanism(s) underlying the changes in angiogenic activity are likely to involve vascular growth factors such as basic fibroblast growth factor (b-FGF) and vascular endothelial growth factor (VEGF). The use of such adjuncts have significant potential benefits to the use of the LDM in cardiomyoplasty.

Key words: perfusion, vascularization, vascular architecture, muscle contraction, surgical delay.

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Vascular delay confers an increased survival to skin flaps after tissue transfer and has become an important surgical technique in reconstructive surgery. The surgical technique of vascular delay also significantly improves perfusion and function of the latissimus dorsi muscle when this muscle is employed as a contractile assist in cardiomyoplasty. This review will address the literature background to support this statement and emphasize the role of our experiences in solidifying this conclusion. This review will be focused and limited to the role of vascular delay, the resulting angiogenic activity, and the future of cardiomyoplasty.

Vascular Delay

The technique of vascular delay has been practiced for several hundred years. Gaspare Tagliacozzi (1545-1599) was renowned in Europe for his surgical abilities in reconstructive rhinoplasty and thus presaged a hint of modern plastic surgery techniques [19]. Tagliacozzi used a skin flap from the forearm to reconstruct the nose. This skin flap was "staged" so that the procedure allowed a fourteen day delay period. Only through this staging or delay procedure could one obtain properly

viable skin tissue for coverage of the new nose. The staging of vascular delay involves a surgical reduction of the vascular supply to tissue, usually by undermining the flap and dividing the perforating vessels [5]. This produces a tissue flap that is sub-lethally ischemic. After a designated period of time, the flap is transferred to its new site as a free or pedicled flap (i.e., a neurovascular pedicle remains intact) in a second operation. Through mechanisms to be discussed and still being elucidated, the tissue reacts to the ischemia in a manner that improves perfusion and reduces the risk of necrosis after transfer.

Reconstructive surgeons have used the term "delay" for over 300 years to describe a surgical procedure in which a pedicled flap is elevated in two or more stages, separated by a period of delay of 1 to 3 weeks. This technique, which we term "vascular delay", produces a period of ischemia in the tissue and stimulates reorientation or growth of the tissue vasculature. The technique of vascular delay should not be confused with the common practice of surgical delay. It is often necessary that a surgical procedure incorporate a period of wound

healing prior to manipulation of the surgical site [11]. For example, in cardiomyoplasty (CMP) the latissimus dorsi muscle (LDM) is lifted as an uni-pedicled muscle flap and wrapped around the myocardium but the LDM is not stimulated to contract (i.e., begin a training regimen) for a period of two weeks. This “delay” following surgery has been confused in the literature and by journal reviewers/editors with the surgical technique of vascular delay.

The optimal period for vascular delay has been the question of extensive study, particularly for skin flaps. Tagliacozzi used a period of about two weeks and this was accepted as part of the staged procedure. In more contemporary times, Morris and Taylor [20] studied rabbit skin flaps and determined that the increased perfusion of the flaps was due to widening of choke vessels between adjacent vascular territories. They determined that the maximal effect of this perfusion change occurred between 48 and 72 hours after the surgical procedure. This same group has also shown that similar results are obtained with a three week surgical or vascular delay of several different skeletal muscles, including the LDM [7].

We have investigated the optimal period of vascular delay using the latissimus dorsi muscles (LDM) of Sprague-Dawley rats [24]. We studied vascular delay periods of 0, 3, 7, 10, and 14 days and determined the percent necrosis that developed. In anesthetized animals, muscles were subjected to a vascular delay on one side and the contralateral LDM was sham operated. The vascular delay consisted of separating the muscle from the external oblique muscles and freeing the LDM from all intercostal-perforating vessels. In the second stage, both muscles (i.e., vascular delayed and control) were elevated as pedicled LDM flaps, surrounded in silicone sheeting, repositioned, and sutured into its original position. The skin was then closed and the animal allowed to recover. Four to five days after elevation, the experiment was terminated and the muscles inspected and quantitatively analyzed for percent necrosis. As Figure 1 clearly shows, any period of vascular delay is superior to acute flap elevation and a delay period as brief as 3 days is adequate to provide some protection to the muscle.

Angiogenesis

Vascular delay of the latissimus dorsi muscle is associated with improved tissue perfusion. Vascularization of a tissue occurs by vasculogenesis, arteriogenesis, and angiogenesis [6, 13]. Vasculogenesis is the process of vascular development that occurs exclusively during embryonic development and therefore does not apply to vascular delay. Arteriogenesis is the proliferation of pre-existing arteriolar connections or collateral vessels. Under normal conditions these interconnecting arterial vessels are patent but not providing perfusion to tissues.

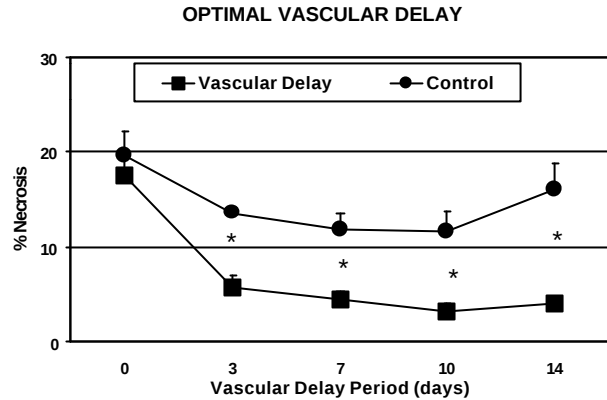


Figure 1. Optimal period of vascular delay as defined by minimal necrosis of distal LDM flap. Quantifying the area of the rat LDM and the area of necrosis determined the percent necrosis and the data is presented as mean±sem. Asterisks indicate significant differences in percent necrosis between control (non-delayed) flaps and vascular delayed LDM flaps. The n for each group is 8-10 animals and each animal served as its own control.

They can be recruited however to bypass a site of occlusion. The vessels associated with arteriogenesis are composed of endothelial lining, internal lamina, and a few layers of smooth muscle cells. Arteriogenesis is a process of active remodeling of the vessels and not a simple passive dilatation of closed conduits [6]. In vascular delay, ligation or interruption of perforating vessels alters the pattern of blood flow such that flow is forced along the longitudinal axis of the muscle flap. Existing collateral vessels expand, enabling the tissue to accommodate an enhanced longitudinal blood flow [20].

Angiogenesis is a fundamental physiological process through which new capillary blood vessels sprout from pre-existing vessels. The term was introduced in 1935 to describe placental blood vessel growth and adopted by Folkman to describe the new blood vessel development associated with tumor growth [12]. Endothelial migration and proliferation, extracellular matrix breakdown, endothelial differentiation and vascular wall remodeling characterize angiogenesis [6]. The mechanism responsible for the vascular changes which occur during vascular delay have not been elucidated, but clearly arteriogenesis and angiogenesis are likely to be participants.

Capillary blood vessels consist of two cell types, endothelial and pericytes, both containing all the genetic information necessary to form the capillary network. Angiogenesis is regulated by the continuous opposing actions of activator and inhibitor molecules capable of interacting to maintain the microvasculature [12]. The genetic and molecular mechanisms that control this development and turnover of the microvasculature have begun to be revealed through research initially focused upon tumor angiogenesis. In particular, two proteins

were identified that were mitogenic to vascular endothelial cells: basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF) [13]. The role of these two proteins in vascular development is the subject of intense investigation. Basic FGF, the first endothelial growth factor to be isolated and sequenced, stimulates the growth of endothelial cells, smooth muscle cells, fibroblasts and some epithelial cells while VEGF is primarily a mitogen for vascular endothelial cells [12]. VEGF appears essential for vasculogenesis to occur embryonically. Disruption of the genes for VEGF receptors results in interference with vasculogenesis and death, while a deficiency of VEGF is associated with delayed differentiation of endothelial cells and impairment of vasculogenesis and angiogenesis [13]. Several studies have shown the VEGF is transiently upregulated in tissues engaged in active angiogenesis while VEGF receptors are expressed on target endothelial cells in nearby blood vessels. Hypoxia has been shown to be a powerful stimulus to the upregulation and release of bFGF [3, 21] and increased VEGF through stabilization of VEGF mRNA [22,23]. The end result is angiogenesis resulting from a hypoxic stimulus.

Vascular Delay and CMP

In the surgical procedure of CMP, it is necessary to lift the entire LDM as a single neurovascular-pedicled flap. As a consequence, the distal LDM becomes ischemic because the thoracodorsal arterial supply does not normally perfuse the distal two-thirds of the LDM. The LDM is then wrapped around the heart and stimulated to contract in a training protocol, which leads to fiber type conversion, and ultimately produces skeletal muscle mechanical assistance to the myocardium. The distal LDM is severely stressed by this protocol which first induces ischemia and then exacerbates the stress by causing the ischemic muscle to perform work.

Most anatomical and surgical reference texts depict the LDM as receiving the majority of its vascular supply from the thoracodorsal neurovascular pedicle and only a small portion from the peripheral perforating branches of the intercostal vessels [e.g., 5, 17, 18]. In fresh human cadavers, Tobin et al. [25] demonstrated that two-thirds of the LDM is supplied by the intercostal vessels. The well documented ischemia and necrosis that occurs as a result of the acute un-staged lifting of the muscle flap is likely to compromise the ability of the LDM to assist myocardial contractility [15]. This would also account for the variability in results seen with CMP patients and the lack of significant hemodynamic improvement characteristic of CMP [14]. To address these concerns our research group has studied the use of vascular delay as an approach to improving muscle viability and contractile function. The mongrel dog model was utilized in these studies because the architecture of

the LDM and its vasculature is similar to the human [25].

Carroll et al. [8] compared the perfusion and contractile function of the LDM after a ten day vascular delay period and after a sham delay operation on the contralateral side. Vascular delay significantly improved LDM perfusion (as determined by laser-Doppler perfusion imager), especially in the distal segment of the muscle previously shown to be at risk for ischemia and necrosis. The contractile properties, specifically the peak force generation and fatigue resistance, were also significantly improved in the vascular delayed muscle. A second experimental protocol was developed to more closely mimic the temporal events occurring in CMP. Thus, animals were treated as previously described with vascular delay of the LDM on one side and a sham delay on the other. Following a ten day vascular delay period, the muscles were lifted as unipedicled free-flaps, wrapped around silicone stents and covered with silicone sheets to prevent re-vascularization. The silicone stents were bilaterally fixed via stainless-steel wires to ribs at their normal resting length and the skin was closed in layers. Following a two-week "healing" period (as is the practice in clinical CMP), the muscles and stents were freed from their rib attachments and studied for resting perfusion and contractile function. In four of the nine animals studied the distal LDM on the non-delayed side had atrophied and necrosed to the point that neither perfusion nor contraction could be measured. The prevention of re-vascularization by the silicone stent and sheeting resulted in significant necrosis of the distal non-delayed LDM. Prevention of re-vascularization of the vascular delayed muscle did not diminish perfusion of the distal LDM (See Figure 4 of reference 9). Since the non-delayed muscles were so compromised by the acute flap elevation and prevention of re-vascularization, the contractile properties of the non-delayed muscles were also significantly inferior to the vascular delayed LDM's [9].

Basic FGF has been isolated from the chronically stimulated latissimus dorsi muscles of goats which were used for cardiac assist in cardiomyoplasty [4]. The authors noted an increased collateral vessel formation from the LDM to the myocardium, implicating bFGF or other angiogenic growth factors in the improvement of perfusion. Since exogenous administration of bFGF had been observed to enhance the release of endogenous bFGF in hypoxic tissue in an autocrine-like fashion [20], we hypothesized that the administration of bFGF immediately following a vascular delay procedure would further enhance perfusion and therefore contractile function. Using the animal previously described, both LDM's were subjected to a bipedicle vascular delay procedure followed immediately, on one side, by injection of 100 µg of human recombinant bFGF into

the thoracodorsal artery and by injection of vehicle on the other side. Ten days later, both LDM's were elevated as pedicled flaps and prepared for perfusion and contractile function studies. Muscle biopsy samples were taken at the termination of the experiment for determination of bFGF expression. Administration of exogenous bFGF increased expression of native bFGF and significantly increased perfusion by 20% and fatigue resistance by 300% over the control vascular delay muscle [10]. The results of this study clearly indicate that administration of growth factors may serve as an important adjuvant to surgical procedures like cardiomyoplasty that require the use of well perfused contractile muscle.

Conclusion

The end result of these many investigations is the conclusion that vascular delay of the latissimus dorsi muscle significantly improves the perfusion of the muscle and the ability of the muscle to generate force and contract with diminished fatigue. The results and techniques developed in acute studies have been applied to chronic dynamic CMP studies in dogs [1, 2]. Use of vascular delay in chronic CMP studies produced significant increases in numerous hemodynamic parameters compared to CMP without the preconditioning effect of vascular delay (see Santamore et al., this monograph). These changes are likely due to angiogenic growth factors as well as arteriogenic vascular changes. Based upon these acute and chronic CMP studies, the first human CMP utilizing vascular delay to condition the LDM was performed in St. Antonio Hospital, Nieuwegein, The Netherlands in August 1997 [24]. The vascular delay procedure and the CMP were successful. Biopsies obtained at the time of the CMP indicated that the contractile apparatus and muscle cell structure of the distal LDM had been preserved. In addition, endothelial and capillary changes consistent with angiogenesis were observed in the distal LDM. The patient had an uneventful recovery and remains active and productive. Follow-up studies have not been made available to the authors, but we can report that the patient has returned to the daily bicycling activities of a Nederlander.

The future of cardiomyoplasty clearly is tied with the improved surgical approach afforded by preconditioning the LDM with vascular delay, improved stimulation paradigms and the exciting prospects of gene therapy [16] as a mechanism to transfer angiogenic properties to both the myocardium and the assisting latissimus dorsi muscle.

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