Duchenne muscular dystrophy (DMD) is a progressive muscle-wasting disorder of skeletal and cardiac muscle. Mutations in the dystrophin gene produce DMD, but much is still unknown about the pathophysiology of DMD. Dystrophic muscle is defined by replacement of normal myofibers by connective tissue and adipocytes. Normal skeletal muscle is highly regenerative and, in the face of continued degeneration, muscle regeneration in DMD is robust, although insufficient to match the pace of degeneration. The dystrophin protein is a subsarcolemmal, spectrin repeat–containing protein of skeletal muscle myofibers and cardiomycocytes. Dystrophin associates tightly with a constellation of transmembrane proteins forming the dystrophin-glycoprotein complex (DGC). Extracellularly, the DGC binds to laminin 2, and intracellularly, the DGC binds to cytoplasmic γ-actin, a myocyte-specific form of filamin and neuronal nitric oxide synthase (nNOS). Positioned at the plasma membrane, the DGC is a mechanosignaling complex that connects the extracellular matrix with the cytoskeleton, and dystrophin is central to this role. When dystrophin is absent, as is the case in DMD, the entire DGC is destabilized. The dissolution of the DGC produces plasma membrane instability associated with increased intracellular calcium and myofiber degeneration.

The ongoing and widespread muscle degeneration in DMD produces a number of systemic responses. In the present issue of Circulation, Straino and colleagues now show that arteriogenesis is enhanced in mdx mice, a mouse model of DMD. Under ischemic conditions, an increase in hindlimb perfusion was seen in mdx as compared with control mice. As an explanation of increased perfusion, muscle from mdx mice was found to have longer arterioles compared with control muscle. Interestingly, capillary density was not increased, and the observed difference in blood flow was attributed solely to arteriole length differences consistent with enhanced arteriogenesis. Given the prominent regeneration that is a feature of mdx muscle, it was hypothesized that there may be systemic enhancement of regeneration. Supporting this, the authors showed accelerated wound healing in the skin of mdx mice compared with normal control skin. Because full-length dystrophin is not expressed in skin, enhancement of wound healing must arise from a systemic, non–cell-autonomous mechanism or systemically mediated stimulation of regeneration. Consistent with this hypothesis, serum from mdx mice produced increased vascular growth in vitro. This growth was, in part, regulated by stromal-derived factor-1, as antibodies to its receptor, CXCR4, partially suppressed in vitro vessel growth. Finally, it was shown that there was an increase in the number of c-kit+ cells in the muscle of mdx mice after hindlimb ischemia and that c-kit+ cells were found preferentially associated with vessels, in some cases expressing markers consistent with vascular smooth muscle cell differentiation. Together, these data support the hypothesis that widespread muscle degeneration, as occurs in dystrophin-deficient muscle, provides a stimulus for arteriogenesis.

Given the complexity of signals necessary for arteriogenesis, it is likely that additional factors beyond stromal-derived factor-1 contribute to the enhanced arteriogenesis seen in mdx muscle. Likely candidate growth factors include basic fibroblast growth factor and vascular endothelial growth factor. Interestingly, Straino et al found that these factors were not increased in the serum of mdx mice. Basic fibroblast growth factor has been shown to be increased in serum from DMD patients and therefore may stimulate arterial growth. Nitric oxide (NO) also supports arteriogenesis. In experimentally produced arteriovenous malformations, NO inhibition demonstrated that NO was required for normal vascular hyper trophy. Exercise is known to increase collateral blood flow, and this increase arises from arteriogenesis that also requires NO. More recent studies have suggested that endothelial NO may be a critical regulator of arteriogenesis.

nNOS is mislocalized from the plasma membrane in the skeletal muscle of DMD patients and mdx mice, and the loss of nNOS from the plasma membrane has clear consequences for regulating blood flow into dystrophin-deficient muscle. Normal skeletal muscle requires NO for attenuation of α-adrenergic–mediated vasoconstriction during contraction. In contrast to normal muscle, blood vessels in contracting mdx remain constricted in response to α-adrenergic stimulation, and this response was also seen in DMD patients. Mice lacking nNOS also fail to modulate vasoconstriction during contraction, proving that it is the loss of nNOS from muscle sarcolemma, and not its translocation to the cytoplasm, that mediates this effect. These data have been further supported by studies on muscle lacking α-syntrophin. Through its PDZ domain, α-syntrophin scaffolds nNOS at the plasma membrane, and mice lacking α-syntrophin have mislocalized nNOS. Displacement of nNOS is not sufficient to produce muscular dystrophy, but mislocalization of nNOS may lead to comparative ischemia during contraction, and
this partial ischemic state may exacerbate muscular dystrophy and provide a stimulus for new vessel growth.

Despite good evidence that NO is decreased at the sarcolemma of mdx skeletal muscle, pockets of higher-density NO may be present, leading to unexpected consequences such as increased arteriogenesis. Regionally upregulated NO is a feature of γ-sarcoglycan– and δ-sarcoglycan–null hearts. The sarcoglycans are components of the DGC, and sarcoglycan and dystrophin gene mutations genetically complement in mice and share phenotypic overlap in humans. Although total levels of NO were not different among sarcoglycan-null and control hearts, focally increased endothelial NOS and NO were found distributed throughout sarcoglycan and mdx hearts. In sarcoglycan-null hearts, focally upregulated NO has functional consequences because inhibition of NO rescued sarcoglycan-null animals from an otherwise lethal dose of carbachol. Focal NO excess also affects cardiac microvessels because inhibition of NO paradoxically decreases vascular spasm. The DGC may differ between skeletal and cardiac muscle with regard to NO and NOS response, and disruption of dystrophin may have effects beyond disruption of the sarcoglycan complex. Supporting this, nNOS does not appear to be as reduced from sarcoglycan-mutant muscle as it is from dystrophin-deficient muscle. γ-Sarcoglycan–null muscle has 68% of normal nNOS levels, and δ-sarcoglycan–null muscle displays 42% of normal nNOS levels, whereas mdx muscle has 18% of nNOS levels (A.H. and E.M.M., unpublished results, 2002). Therefore, it remains to be seen to what degree enhanced arterial growth is a product of nNOS mislocalization, a consequence of ischemia, or purely a systemic response to the stimulus provided by widespread muscle degeneration.

Muscle-specific overexpression of nNOS produces an improved outcome in mdx mice. One mechanism by which enhanced nNOS expression may lead to reduced muscle degeneration is through a direct effect on α-adrenergically mediated vasoconstriction during contraction, although this has not been directly examined. Relief from contraction-induced ischemia may alleviate the dystrophic phenotype but also may reduce the stimulus for new vessel growth. Alternative mechanisms are likely to mediate the improved phenotype associated with nNOS overexpression in mdx mice. Muscle-specific expression of NO alters the ability of immune cells to mediate cellular destruction in dystrophin-deficient muscle. Indeed, the role of inflammation within degenerating skeletal muscle is likely a key contributor to the dystrophic process. On histopathology, focal degeneration in DGC-mutant muscle is accompanied by a mononuclear cellular as well as a gene transcription profile consistent with an inflammatory picture. mdx Mice treated with prednisolone display a reduction in inflammatory infiltrate and cytokines and, importantly, reduced sarcolemmal damage. The use of steroids in the treatment of a DMD patient is routine in many parts of the world, but the use of steroids remains controversial, largely because of the side effect profile associated with prolonged steroid exposure.

Whether the enhanced arterial growth now described in mdx mice is a feature of human DMD is not yet known. The time course of muscle degeneration in the mdx mouse differs from DMD patients. In the mdx mouse, there is a peak of muscle necrosis from 2 to 10 weeks of age. It has been argued that the mdx mouse has unique features of regeneration and that the apparent reduction in necrosis that characterizes older mdx mice reflects an enhanced regenerative potential compared with humans. Straino et al found that the number of hematopoietic progenitor cells in peripheral blood was increased in mdx mice. Serum from mdx mice supported in vitro vessel growth, confirming that enhanced arteriogenesis is, in part, mediated by a muscle-extrinsic process. Enhanced arterial growth may not be related to the loss of dystrophin per se but may instead be a systemic response to widespread muscle degeneration and cytokine-mediated bone marrow activation. Finally, it is unclear whether enhanced arteriogenesis in mdx mice is pathological or protective for the process of muscle degeneration and muscle regeneration. It is reasonable to assume that accelerated muscle regeneration may result from enhanced vascular growth. On the other hand, enhanced arteriogenesis may accelerate cellular infiltration and cytokine release, ultimately stimulating myofiber destruction. The timing, species specificity, and molecular details of enhanced arterial growth in degenerative muscle disease require further exploration to determine their significance in DMD pathogenesis and treatment.

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References


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