Apoptotic Death of Dystrophic Muscle Fibers After Exercise: a New Hypothesis on the Early Events of Muscle Damage

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Abstract

Apoptosis is a specific form of programmed cell death that plays an important role in development, growth regulation and diseases. Skeletal muscles in adult animals are fully differentiated syncytial cells and apoptosis, which is known to be present in tissues that modulate their cellular homeostasis under the influence of growth and/or hormonal factors, should be, in theory, absent. On the other hand, programmed cell death has been recently described in early stages of myocardial infarct and cerebral ischemia. In any case, muscle regeneration after injury starts from satellite cells, which proliferate and fuse to substitute lost myofibers. Apoptosis could have a role at least in regulating myoblast proliferation during muscle regeneration, in particular in the progression of dystrophinopathies. The secondary pathogenetic process by which a lack of dystrophin/dystrophin associated glycoproteins leads to muscle degeneration in dystrophinopathies is unknown. Muscle damage is induced by exercise, but foci of myofiber death appear few days later. Recently we were able to show that after all-night spontaneous running in a wheel-cage *mdx* mice present increased signs of DNA muscle damage that can be interpreted as the initiation of the apoptotic process (Carraro U, Rizzi C, Rossini K, Podhorska-Okolov M, Arpesella G, Mikus P. 1994. In: Skeletal Muscle Assist (Salmons S, Ed) CA Heart, University of Twente, Enschede, The Netherlands pp 43-46; Podhorska-Okolov M, Sandri M, Bruson A, Carraro U, Massimino ML, Arslan P, Monti D, Cossarizza A and Franceschi C. 1995. Basic Appl. Myol. 5: 87-90). Evidence of apoptosis in dystrophic myoblasts and myofibers have been collected independently in other two muscle research laboratories (Tidball JG, Albrecht DE, Lokensgard BE, Spencer MJ. 1995. J. Cell Sci. 1995; 108: 2197-2204; Smith J, Fowkes G and Schofield PN. 1995. Cell Death Diff. in press). Furthermore, we have shown that ubiquitin, a protein whose appearance is also related to apoptosis, increases in both normal and dystrophic muscle after exercise (Sandri M, Carraro U, Podhorska M, Rizzi C, Arslan P, Monti M and Franceschi C. 1995. FEBS Lett. 373: 291-295). Taken together these findings could shed a light on the molecular pathogenesis of muscle fiber death in dystrophin deficiency. Moreover, our data suggest that exercise-induced muscle damage may be mediated by apoptosis. The occurrence of similar phenomena in patients affected by dystrophinopaties is a major open issue. If apoptosis has a pivotal role in the pathogenetic events leading to dystrophy in human muscle, these studies could be relevant not only for understanding the basic biological mechanisms of muscle damage in dystrophinopathies, but could also have a major impact on disease management.

Key words: apoptosis, dystrophinopathies, DNA fragmentation, TdT nick end labeling, Duchenne muscular dystrophy, exercise-induced muscle damage, hypothesis, *mdx* mouse, pathogenesis, programmed cell death, ubiquitin.

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The secondary pathogenetic process by which a lack of dystrophin/dystrophin associated glycoproteins (DAG) leads to muscle degeneration in dystrophinopathies is an open issue.

Since the Duchenne muscular dystrophy (DMD) gene was identified using a positional cloning strategy, a wealth of information has been accumulated about the complexity of the DMD gene and its protein product dystrophin [1]. It

is becoming increasingly clear that one of the prime consequences of a lack of dystrophin in muscle is a loss of the associated glycoprotein complex. The detailed function of this glycoprotein complex is currently unknown, but it appears that a loss of part of the glycoprotein complex alone is sufficient to cause dystrophic degeneration of muscle even in the presence of normal dystrophin content [11]. A number of possible mechanisms have received attention. These are: i) a lack of dystrophin/DAG leads to a change in plasma membrane permeability; ii) a lack of dystrophin/DAG leads to decreased mechanical stability of the muscle plasma membrane and of the sarcomer; iii) a lack of dystrophin/DAG leads to a specific defect in muscle calcium homeostasis. The data for and against these potential secondary pathogenetic mechanisms has recently been reviewed by Jackson [9]. Data concerning the possible role of the dystrophin/DAG proteins in stabilising the plasma membrane is currently contradictory. Supports for the theory initially were observations that the muscles of *mdx* mice were particularly susceptible to lengthening contractions and hypo-osmotic shock. Though contradictory data have been also provided, there is the general expectation that exercise-induced damage have a role in the myodystrophic process and that modifications of the manner of usage of muscles may have some role to play in the reduction of muscle degeneration in patients with Duchenne or Becker muscular dystrophy [20]. The variable time course of expression of muscle degeneration in dystrophic muscle appears to indicate that neither a change in muscle permeability nor a change in muscle calcium homeostasis are inevitable consequences of a lack of dystrophin/DAG. However it is clear that once initiated, muscle degenerative mechanisms are (at least in part) mediated by external calcium and associated with an increased permeability of the plasma membrane to external markers and calcium [4]. In summary, the secondary pathogenetic process by which a lack of dystrophin/DAG leads to muscle degeneration is unknown.

Apoptosis is a specific form of cell death that plays an important role in development, growth regulation and diseases [19]. Skeletal muscles in adult animals are fully differentiated syncytial cells and apoptosis, which is known to be present in tissues that modulate their cellular homeostasis under the influence of growth and/or hormonal factors, should be, in theory, absent. On the other hand, programmed cell death has been described in early stages of myocardial infarct [8, 21], and cerebral ischemia/reperfusion [10]. In any case, muscle regeneration after injury, if takes place, starts from satellite cells, which proliferate and fuse to substitute lost myofibers: apoptosis could have a role at least in regulating myoblast proliferation during muscle regeneration.

In a research proposal which was granted by Telethon-Italy in 1991 Claudio Franceschi, a leading immunologist of ageing at The University of Modena, proposed that apoptosis could have a role in the pathogenesis of dystrophinopaties [5, 6]. In the subsequent years this hypothesis was tested in collaboration with us, though several problems were encountered related to the peculiarities of skeletal muscle tissue, such as difficulties in obtaining cell or nuclei suspensions and relatively low number of apoptotic nuclei present in tissue sections of adult muscle. Only recently, enough sensitive techniques became available to observe and quantify apoptotic cells in muscle tissue sections [7]. Using this approach we were able to show that i) a small but reproducible number of apoptotic nuclei is present in *mdx* muscle; ii) this number increases dramatically in muscle after exercise; iii) this last phenomenon is more marked in muscle of dystrophic mice [3, 12]. In particular, we have shown that dystrophin-deficient mouse muscle shows a dramatic increase of apoptotic myonuclei after a night of spontaneous wheel-running. The techniques used to assess apoptotic cell death were the terminal deoxy nucleotidyl transferase (TdT) nick end-labelling [7], and DNA fragmentation assessed by gel electrophoresis in agarose and polyacrylamide. Furthermore, ubiquitin, a protein whose appearance is also related to apoptosis, increases in both normal and dystrophic muscle after exercise [13-15]. Similar observations have been done contemporarly and independently in other two well known muscle research laboratories. Tidball and collaborators at the UCLA Jerry Lewis Neuromuscular Research Center recently showed that in dystrophin-deficient mice muscle apoptosis precedes necrosis, and that apoptotic events continue into the stage of dystrophic pathology that is currently viewed as necrosis. Indeed they showed that condensed mitochondria, fragmented sarcoplasmic reticulum and nuclei with chromatin condensations resembling apoptosis appear in fibers that otherwise possess normal morphology. The results are substantiated by two molecular assays for apoptosis: i) TdT mediated end-labelling of DNA in nuclei in tissue section; and ii) assays for DNA ladders, analyzing prenecrotic, peak necrotic and regenerated hindlimb muscles of normal and *mdx* mice [17, 18]. Smith, Fowkes and Schofield, at the Department of Anatomy of The University of Cambridge, are showing that programmed cell death and apoptotic morphology is increased in dystrophic *mdx* muscle and in cultured muscle cells. They also show that the growth factor IGF-II, which is thought to play a role in mammalian myogenesis, reduces apoptosis in mammalian skeletal muscle myoblasts both *in vivo* and *in vitro* [16].

Altogether these findings could shed a light on the molecular pathogenesis of muscle fiber death in dystrophin deficiency. Moreover, our data suggest that exercise-induced muscle damage may be mediated by apoptosis. It is well known that exercise in an unaccustomed muscle provokes mild injury, soreness and lactic acid accumulation. In adult muscle the events observed after an eccentric muscle exercise reflect the typical sequence of acute inflammation which follows the unknown primary damaging event, usually related to the mechanical overloading of the intra-inter fiber components induced by the contractile machinery. The events are divided in time lapses of 24 h

and comprise accumulation of neutrophils in the first 24 h, release of lysosomal enzymes till 48 h, release of inflammation mediators and signs of repair at 72h [2]. Our observations that spontaneous running increases the number of apoptotic myonuclei in differentiated muscle fibers of adult mice [3, 12, 15] shed a light on the pathogenesis of the post-exercise muscle injury, since DNA double strand breaks, detectable after exercise, precede any sign of necrosis, indicating that DNA damage is present while inflammation is in progress. We consider this result in mice as the starting point for further investigation aimed to clarify the mechanism(s) whereby apoptosis occurs especially after exercise, and its role in the progression of the dystrophinopathies. To quantitate the minimum exercise and the consequent muscle fatigue which induces apoptosis of normal and *mdx* mouse muscle tread-mill tests could be used. Oxidative stress-induced myopathies ought to be analyzed to test the post-ischemia/reperfusion hypothesis, as suggested by studies in myocardiocytes [8, 21].

The occurrence of similar phenomena in human muscle from patients affected by dystrophinopathies is a major open question. If apoptosis has a pivotal role in the pathogenetic events leading to dystrophy in human muscle, these studies could be relevant not only for understanding the basic biological mechanisms of muscle damage in dystrophinopathies, but could also have a major impact on disease management.

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