Caspase 3 Expression Correlates With Skeletal Muscle Apoptosis in Duchenne and Facioscapulo Human Muscular Dystrophy. A Potential Target for Pharmacological Treatment?

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Abstract. Apoptosis was detected in different muscular diseases, including severe dystrophin deficiency, but apoptotic mechanisms are not completely described in adult skeletal muscle. Studying patients affected by Duchenne muscular dystrophy (DMD) and by facio-scapulo-humeral dystrophy (FSHD) we showed an increase of apoptotic myonuclei, bax, and bcl-2-positive myofibers. Positive correlation was detected between apoptotic nuclei and bax expression (p < 0.01). Expression of caspases was analyzed by RNase protection. Caspase transcript was not detected in normal skeletal muscles. DMD muscles expressed caspase 8, 3, 5, 2, 7 and Granzyme B mRNAs. Low levels of caspase 6, 3, and Granzyme B transcripts were detected in FSHD patients. Tissue levels of caspase 3 protein significantly correlated with apoptotic myonuclei (p < 0.05) and with bax expression (p < 0.01). In all DMD cases the activity of caspase 3 was increased, while the FSHD samples were heterogeneous. These data indicate that human skeletal muscle fibers, during the dystrophic process, modulate the expression of caspases and that caspase 3 is involved in myofiber cell death, opening new perspective in the pharmacological treatments of muscular dystrophies, such as the use of caspase inhibitors.

Key Words: Apoptosis; Caspase; Dystrophy; Skeletal muscle.

INTRODUCTION

In the past 5 years, several studies showed that apoptosis occurs in skeletal muscle tissue both at myoblast and myofiber level. Apoptosis was observed in different animal models of muscle diseases (1) and its involvement in the development of human myopathies has been also intensively investigated. The first studies on apoptosis in muscle derived from the description of the developmentally regulated death of larval muscles (2, 3). We know that mammalian skeletal muscle undergoes apoptosis during development (1). In fact, in vitro experiments demonstrate that skeletal muscle myoblasts undergo apoptosis as a consequence of protein synthesis inhibition (4), oxidative stress (5), metabolic perturbation (6), use of DNA damaging agents, such as UV (7) or cis-platinum (8), and, in general, by all conditions that alter the proliferation/differentiation processes (1). Differentiated myotubes, in vitro, also undergo apoptosis when perturbation of G0 state occurs (9) or when dystrophin (8), α-dystroglycan (10), γ-sarcoglycan (11), merosin, and β1 integrins (12) are absent or altered. Several studies recently showed significant apoptotic DNA fragmentation in skeletal muscles from animal models for muscular dystrophies, such as those involving dystrophin (13, 14), merosin (15), γ-sarcoglycan (11), and α 5 integrin deficiency (16). From these studies, it appears that apoptosis always preceded necrosis, although necrosis appears to be the main cause of cell death at later stages. Increasing evidence suggests that apoptosis occurs in part of the multinucleated muscle cell as a segmental process. This is particularly important during muscle atrophy in which myonuclei and cytoplasm loss are parallel events (17, 18). Recently, apoptosis has been detected in some human muscle diseases such as Duchenne dystrophy (DMD) (19), α-sarcoglycan- and merosin-deficient muscular dystrophy (20), spinal muscle atrophy (21, 22), and limb-girdle muscular dystrophy type 2A (23). However little is known about the molecular mechanisms that regulate skeletal muscle cell death.

Apoptosis is a specific form of cell death. Quantification of apoptosis has been underestimated for decades, since the apoptotic cells shrink and are rapidly phagocytized by neighboring cells (3). They do this by activating genetically controlled cell suicide machinery that leads to apoptosis. A number of mechanistic pathways to apoptosis have been defined. A common end-point for these pathways is the activation of a series of cysteine proteases collectively known as caspases. Caspases are activated by sequential processing by family members. Thus caspase 3, one of the farthest downstream in this cascade, is activated by processing of procaspase 3 (p32) into its active form (p20/p17 and p12) by upstream caspases during apoptosis (i.e. caspase 9 and 8). Procaspase...
8 and 9 are activated earlier by the death-inducing signaling complex (DISC) and Apaf-1/cytochrome c complex, respectively. It has been proposed that the caspase family be divided into at least 2 groups: the “effectors”, caspases that are directly responsible for proteolytic cleavage that lead to cell disassembly (i.e. caspase 3, 6, 7); and the “initiators”, caspases that are involved in upstream regulatory events (i.e. caspase 2, 8, 9, 10) (24, 25). Activation of this caspase cascade results in proteolytic cleavage of a large number of cellular proteins with diverse biological functions.

At present there is no convincing data about caspase recruitment and the caspase cascade pathway in differentiated myocytes. We recently demonstrated using staurosporine (a protein kinase inhibitor known to induce apoptosis in a variety of cell types including skeletal muscle [26]) that multinucleated skeletal muscle undergoes apoptosis via the caspase 9-caspase 3 pathway in vitro (Sandri et al, unpublished observation). In the present paper, we show for the first time that different caspases are expressed in DMD and (facio-scapulo-humeral dystrophy) FSHD, but not in normal skeletal muscle. Moreover, the correlation between apoptosis and caspase 3 expression, and the finding that caspase 3 activity is only in dystrophin-deficient muscle fibers, suggests that apoptosis occurs through a caspase 3 pathway in human skeletal muscle. These findings open new pharmacological approaches to retard progression of muscular dystrophies.

MATERIALS AND METHODS

Human Biopsies

We studied muscle biopsy specimens from 15 patients with clinical features of a progressive muscular dystrophy. Five children (age: 7.4 ± 1.5 years) were affected by DMD. Ten patients (37.0 ± 11.3 [19–69] years) were affected by FSHD, demonstrated by presence of EcoRI fragments of less than 35 kb. Three patients (36.5 ± 17.2 [24–44] years) without clinical and histological evidence of a neuromuscular disorder served as normal controls. Diagnosis was based on the clinical and genomic DNA analyses on lymphocytes for dystrophin gene deletion or for the presence of EcoRI fragments shorter than 35 kb belonging to a region of chromosome 4 long-arm containing a 3.3 kb KpnI tandem repeat for FSHD. Histochemistry and immunohistochemistry were performed on muscle biopsy specimens frozen in a 3.3 kb KpnI tandem repeat for FSHD. Histochemistry and immunohistochemistry were performed on muscle biopsy specimens to confirm the diagnosis. Muscle biopsies were frozen in liquid nitrogen immediately after excision and stored at −80°C. Serial transverse 8-μm-thick sections were cut on a cryostat microtome, dried at room temperature, and stored at −20°C until staining.

Myogenin Construct, Probes, and In Situ Hybridization

The plasmid containing cDNA for myogenin was constructed as follows: The Pfu polymerase (Stratagene, La Jolla, CA) was used to amplify a region of the human myogenin mRNA (EMBL accession number X17651) from human skeletal muscle cDNA using the primer sequences 5’-GCA GGC TCA AGA AGG TGA AT-3’ and 5’-ATG GAT GAG GAA GGG GAT AG-3’. The PCR product was blunt-end cloned into the Smal site of the vector pBlueScript II SK(+). The plasmid was linearized with PstI before reverse transcription with T3 RNA polymerase and “S-UTP. In situ hybridization with the 32P-labeled cRNAs for myogenin, MHC1 and MHC emb was performed as previously described (27).

Immunohistochemical Analysis for Myofibers

Cryosections were fixed in 2% paraformaldehyde for 10 min, washed in PBS, quenched in 50 mM NH4Cl for 10 min, washed in PBS, and permeabilized with 0.1% Triton X-100 for 10 min. Slides were incubated overnight at 4°C with primary antibodies using the following dilutions: rabbit anti-bax, rabbit anti-bcl-2 and rabbit anti-caspase 3 polyclonal antibodies (Santa Cruz Biotechnology, Santa Cruz, CA) 1:50; mouse anti-laminin, 1:100 in 1% rat serum. After washing twice with PBS (5 min each), sections treated with anti-bax, bcl-2 and caspase 3 were incubated with biotin-conjugated goat anti-rabbit Ig (1:200 in 1% rat serum) for 1 h at 37°C, rinsed in PBS, and incubated with streptavidin cyanine3-conjugate (1:250 in 1% rat serum) for 1 h at 37°C. The slides processed for anti-laminin, were incubated with Cy3-conjugated anti-mouse Ig (1:100 in 1% rat serum) for 1 h at 37°C. Negative controls were performed by omitting the primary antibody. After washing 3 times in PBS and staining nuclei with Hoechst 33258, slides were mounted in Elvanol and observed under an epifluorescence microscope (Carl Zeiss, Oberkochen, Germany). Bax, bcl-2 and caspase3-positive fibers were quantified and expressed as percentage of the number of fibers. Three sections at different levels from the same block were analyzed. At least 500 fibers were counted in each section. All data are expressed as mean ± SD.

Double Labeling TUNEL/Immunohistochemistry

Some sections were double-labeled for the presence of apoptotic nuclei and laminin as previously described (19). Briefly, the slides were processed for laminin as above described; after incubation with secondary antibody, the slides were rinsed in PBS and then labeled for fragmented DNA using terminal deoxynucleotidyl transferase with fluorescein-conjugated nucleotides as described by the manufacturer (In Situ Cell Death Detection Kit-POD distributed by Roche). Sections were viewed under epifluorescence microscope. Fibers containing TUNEL-positive nuclei were counted and expressed as a percentage of the total number of myofibers per sections. Three sections at different levels from the same block were analyzed. At least 500 fibers were counted in each section. All data are expressed as mean ± SD.

RNA Extraction and RNase Protection Assay

Total RNA was prepared from 20 cryosections by Ultraspec™ RNA isolation system (Biotex, Houston, TX), p7hApo1 Multi Probe Template set (Pharmingen, San Diego, CA) human plasmid was used to synthesize caspase 8, granzyme B,

caspase 3, caspase 6, caspase 5, caspase 2, caspase 7, caspase 1, caspase 9, L32, and GAPDH biotin labeled cRNA probes; the probes were then treated with DNaseI (2U) at 37°C for 30 min. Ten μg of total RNA was hybridized at 56°C for 12 h to biotin labeled cRNAs using RPA III™ Ribonuclease Protection Assay Kit (Ambion, Austin, TX). The probes and the protected fragments were analyzed on a denaturing urea 5% polyacrylamide gel and electroblotted onto nylon membranes. Detection of biotin labeled probes was performed by BrightStar™ BioDetect™ Nonisotopic detection kit (Ambion). The relative content of protected transcripts was determined by densitometry using a Bio-Rad Gel Doc 2000 and the Bio-Rad Multi-ANALYST version 1.1 software.

Caspase 3 Activity Assay

Twenty cryosections of biopsy samples were lysed and caspase 3 activity was measured by CaspACE Assay System Colorimetric (Promega, Madison, WI). Lysates were incubated with a specific substrate, Ac-DEVD-pNA at 37°C for 4 h, and caspase activity was measured spectrophotometrically at 405 nm. As a control for caspase 3 specificity, lysates were preincubated with 10 μM Z-DEVDfmk, caspase 3 inhibitor, at 37°C for 30 min.

Clinical Grading of FSHD

Clinical grading of FSHD was done according to the criteria of a FSHD consortium: Degree I: slight weakness of the shoulder girdle (able to raise arms more than 45°) or of the lower limb muscles (gait with proximal or distal deficit). Degree II: slight weakness of the shoulder girdle and of the lower limb muscles (± weakness of the facial muscles). Degree III: mild weakness of the shoulder girdle (able to raise arms between 45° and 90°) or of the lower limbs muscles (ambulation is possible but patient unable to get up from floor) (± weakness of the facial muscles). Degree IV: mild weakness of the shoulder girdle and of the lower limb muscles (± weakness of the facial muscles). Degree V: severe weakness of the shoulder girdle (impossible to raise arms more than 45°) (± mild weakness of the lower limbs, ± weakness of the facial muscles). Degree VI: severe weakness of the lower limb muscles (ambulation only with support) (± severe weakness of the shoulder girdle, ± weakness of the muscles). Degree VII: severe weakness of the lower and severe weakness of the shoulder girdle (± weakness of the facial muscles).

Statistical Analysis

Mean value ± Standard Deviation was used. Student's t-test and linear regression were used when appropriate. A value of p < 0.05 was considered statistically significant.

RESULTS

Activation of Satellite Cells Mainly Occurs in DMD

Turnover of myonuclei was monitored by in situ hybridization technique using a probe specific for myogenin. Duchenne patients showed a massive activation of satellite cells (Fig. 1A, B, G), while only 1 of the 4 FSHD cases showed myogenin-positive cells. Control muscle did not show any sign of myoblast replication (Fig. 1G). In the FSHD samples, which did not show myogenin-positive cells, MHCemb transcripts revealed by in situ hybridization were also absent (data not shown). Interestingly, some areas of DMD samples revealed that most of the interstitial cells were myogenic (ranging from 20% to 75% of interstitial cells) (Fig. 1C, D). Activation of satellite cells also occurs in areas where either myofiber damage or inflammatory cells were absent (Fig. 1E, F).

Level of Apoptosis in Human Skeletal Muscles

To determine the level of apoptosis in dystrophic muscles before analyzing caspase expression, we studied apoptotic DNA fragmentation by using the TUNEL method. Double labeling TUNEL/laminin showed that a small amount of nuclei positive for DNA fragmentation was detected in a subsarcolemmal position both in DMD and FSHD samples (Fig. 2). Quantitative measurement of apoptotic nuclei revealed that the process was present not only in interstitial cells but also in few myofibers in DMD patients compared to control samples (Fig. 2; Table 1). On the other hand, FSHD samples showed a very low level of apoptosis in interstitial cells (Table 2). The apoptotic process is significantly elevated in the DMD patients (0.31% ± 0.14% vs 0.02% ± 0.02%, p < 0.01). Although some FSHD samples had many apoptotic fibers, 3 of the 10 patients did not show any apoptotic nuclei (0.26% ± 0.24% vs 0.02% ± 0.02%, p = NS).

We used immunocytochemical techniques to analyze muscle content of bcl-2 and bax. The analyses were performed on FSHD samples, which showed levels of apoptosis higher than 0.15%. Normal muscle was characterized by a negative pattern for both bcl-2 and bax (Fig. 2; Table 3). In DMD and FSHD, bax expression significantly increased (p < 0.01 vs control and p < 0.05 vs control, respectively), while bcl-2 was upregulated mainly in DMD patients (p < 0.01 vs control).

Correlation Between Apoptosis and bax/bcl-2 Levels

The trend of bax levels seems to correlate with apoptotic myonuclei, especially in DMD muscles (i.e. muscles with high apoptotic myonuclei show high level of bax). When we correlate the number of apoptotic fibers and bax-positive fibers, a positive correlation was found (r² = 0.44, p < 0.01) (Fig. 3). We did not find a relation between apoptotic fibers and bcl-2 (p = NS). Because apoptosis is regulated by the balance of pro- and anti-apoptotic products, we calculated bax/bcl-2 ratio and looked for a correlation with apoptotic nuclei. Skeletal muscles did not show a significant trend for bax/bcl-2 ratio (r² = 0.4, p = 0.1).
Fig. 1. Myogenin expression detected by in situ hybridization in human skeletal muscles. A-B: Dark-field of 2 DMD samples at low magnification (Magnification ×100). C-D: Area in which most of the interstitial cells were myogenic (C: dark-field, D: bright-field; magnification ×200). E-F: Example of Myogenin-positive myoblast in an area of normal appearance (E: dark-field, F: bright-field; magnification ×400). G: Quantification of myogenin-positive myoblast in the dystrophic and normal human skeletal muscles (data are expressed as mean ± SD, n = 3).
Correlation between Apoptosis and Activation of Satellite Cells

Because of the significant increase of apoptosis and the presence of myogenin-positive myoblasts in the DMD group we investigated the presence of a correlation between myofiber cell death and myoblast activation. Despite the small number of patients analyzed to rule out a statistically significant conclusion (n = 4), a negative trend was detected between apoptotic myonuclei and myogenin-positive myoblasts ($r^2 = 0.90, p < 0.05$) and between apoptotic interstitial cells and activated myoblasts ($r^2 = 0.99, p < 0.01$) (Fig. 3).

Correlation between Apoptosis and Clinical Grading in FSHD

Because FSHD patients showed heterogeneity in the apoptotic process, we looked for a correlation with clinical grading to understand if the difference could be ascribed to a different degree of severity of muscle pathology. A positive correlation was not found between clinical grading and apoptotic myofiber ($r^2 = 0.33, p = 0.08$).

Fig. 2. Examples of Laminin/TUNEL, bax, bcl2 and caspase 3 staining in normal (C) and dystrophic muscles (DMD: Duchenne dystrophy, FSHD: facio-scapulo-humeral dystrophy). Total nuclei are counterstained by Hoechst 33258 (blue). Bax, bcl2, and caspase-3 immunoreaction are revealed by Cy3-conjugated secondary antibody (red) (Magnification ×200). Laminin is detected in red while TUNEL-positive nuclei are revealed in green. (Magnification ×400).
Caspases Are Upregulated Differently in Dystrophic Muscles than in DMD and FSHD

We extracted RNA from the samples that showed DNA fragmentation in at least 0.3% of fibers. After hybridization with probes for 9 different caspases we detected that the effector caspases 3/7 and the regulatory caspases 8/5/2/granzyme B were expressed in DMD patients. The effector caspases 3/6 and regulatory granzyme B were upregulated in FSHD (Fig. 4). Among all the caspases analyzed, only caspase 3 is commonly expressed and the highest level is present in the DMD group (Table 4). Since caspase 3 is a potent “effector” caspase, we analyzed the protein level and the enzymatic activity in muscles.

Caspase 3 Localization and Correlation with Apoptotic Myonuclei

We analyzed the pattern of expression of caspase 3 by immunohistochemistry. The polyclonal antibody that we used recognized both the inactive form (procaspase) and the active heterodimer (Caspase p20/17 and p12). In accordance with the mRNA analysis we detected a higher level of caspase 3 protein in DMD (Fig. 2; Table 3) (14.6 ± 9, p < 0.05 vs control) than in FSHD muscles (3.6 ± 3.3, p = 0.1 vs control). Moreover, we found a positive correlation between myofibers positive for caspase 3 and myofibers with apoptotic nuclei (r² = 0.33, p < 0.05) (Fig. 3). Since the expression of caspases was different between the 2 dystrophies, the correlation caspase3-apoptotic fiber greatly improved when we analyzed each group (r² = 0.93, p < 0.001 in DMD) (r² = 0.88, p < 0.001 in FSHD) (Fig. 3). Caspase 3 also correlates with bax expression (r² = 0.56, p < 0.01) (Fig. 3).

Caspase 3 Is Enzymatically Active

The presence of the active form of caspase3 was assessed by using a specific substrate (Ac-DEVD-pNA) by a colorimetric assay. Muscle lysates of the patients with

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the highest level of caspase 3 were tested. A significant increase in caspase 3 activity was detected in DMD lysates but not in lysates of control muscles. The activity of caspase 3 was completely inhibited by DEVD-fmk, the specific inhibitor of the protease (Fig. 5).

DISCUSSION

This study confirms that apoptotic cell death occurs in dystrophic muscle and provides the first description of caspase 3 activation in human skeletal muscle tissue. The first experiments that suggested caspase could play a role in muscle cell death were performed in vitro. Skeletal muscle explants were treated with staurosporine and this resulted in apoptotic DNA fragmentation, which was inhibited by a pancaspase inhibitor (zVAD-fmk) (28, 29). However, these data were inconclusive since the investigators did not distinguish myonuclei from interstitial cells (i.e. fibroblast, endothelial, phagocytic, and satellite cells). In vitro experiments strongly support a role of caspase 3 in myofiber cell death. A recent study on staurosporine-treated C2C12 myotubes using western blotting also reported a cleavage of pro-caspase 3 within 4 h of staurosporine treatment (26). The authors studied the time course of staurosporine-induced cell death and they detected externalization of phosphatidylserine residues at 2 h, positive TUNEL labeling at 4 h, DNA ladder at 8 h, and apoptotic ultrastructural appearance of chromatin condensation at 24 h after staurosporine treatment. The time course results of myotube cell death suggest that caspase 3 play a role in the early events of myotube death. We recently confirmed that caspase 9/3 activation is an early event during myotube cell death. Furthermore, we found that DEVD-fmk (caspase-3 inhibitor) and LEHD-fmk (caspase 9 inhibitor) increase myotube survival and inhibit DNA fragmentation in vitro experiments (unpublished observations).

The absence of caspase 3 activity in normal muscle reflects the expected myofiber resistance to undergo apoptosis. Our results on normal muscles are in agreement with recent data that show a lack of a direct Apaf-1/Cytc/dATP-mediated activation of caspase 3 in homogenates of human skeletal biopsies from healthy subjects (30). The authors concluded that human skeletal muscle lack the ability to commit apoptosis via the caspase-9 pathway. However, our results show that myofiber could modulate the expression of caspases depending from its healthy status. In this way it becomes relevant to compare our in vivo results to those seen on pathologic muscles where apoptotic DNA fragmentation was detected. The first important results that suggested a caspase 9/3 pathway during skeletal muscle cell death were obtained by studying immunolocalization of cytochrome c in human dystrophies. In limb-girdle muscular dystrophy type 2A, apoptosis was detected in 0.4% of myonuclei and a diffuse sarcoplasmic cytochrome c fluorescence was detected in TUNEL-positive fibers (23). Since cytochrome c is known to leak out of the mitochondrial space during pro-apoptotic events and to activate caspase 9/3 cascade by binding apaf-1/dATP, the possibility that a mitochondrial apoptotic pathway is present in muscle cell death is strongly suggested. This hypothesis is strengthened by
animal models of muscle degeneration. Burn injury induces muscle weakness that occurs as a result of the loss of muscle mass. Apoptotic myofibers were detected 3–7 days after damage and at sites distant from burn injury; conditions that suggest a systemic effect of pro-apoptotic factors. In this model caspase-3 and -9 were activated (31). Similar results were obtained on dystrophic mice, like laminin α2-deficient mice (dy/dy). Most of the myofibers co-stained for active caspases 3/9 and apoptotic DNA fragmentation detected by TUNEL (15, 32). Further evidence for a caspase-dependent pathway arises from a case report of a novel congenital myopathy that showed apoptotic myonuclei and activation of caspase-3 and-9 (33).

Our results showed that human myofibers activated caspase-3 mRNA transcription and protein synthesis during disease. The difference between apoptotic fibers and the percentage of caspase-3-positive fibers was expected since the antibody we used recognizes the active and inactive pro-form of caspase 3. Fibers expressing caspase-3 do not necessarily undergo DNA fragmentation since they first need to activate caspase-3. Not all caspase-3 fibers undergo cell death because caspase 3 may be blocked by protective factors such as IAP or bcl-2 family (25). However, correlation between caspase 3 and DNA fragmentation suggests that caspase 3 is not repressed during muscle cell death. This was strengthened by evidence of caspase-3 activity in caspase-3-positive muscles.

**Fig. 3.** Graphics of linear regression statistical analyses.
Fig. 4. RNase protection for the detection of caspase mRNAs in control and dystrophic muscles. Note the shift of caspase probes after RNase treatment. Caspases are absent in control muscles and expressed at different levels in DMD and FSHD samples.

The majority of the bax, bcl-2 and caspase-3-positive myofibers were normal in morphology in both DMD and FSHD. In DMD the majority of muscle fibers were positive for both bax and caspase-3. Correlation between caspase-3 and bax expression suggests a role of mitochondria in this cell death pathway. In fact bax is known to induce apoptosis through the release of cytochrome c from mitochondria and activation of the caspase 9/3 pathway (24, 25).

Caspases are crucial mediators of apoptosis in many mammalian cells and previous studies have demonstrated that peptide-based caspase inhibitors, such as bocDfmk, are capable of abrogating apoptosis induced by different agents in a variety of systems including brain and heart (24, 25). Pretreatment with caspase inhibitors protects cardiac muscle and neural cells from ischemia-induced cell death (24).

Several authors showed that apoptosis occurs as a segmental process in the multinucleated skeletal muscle fiber (17, 19, 21). The activation of an apoptotic process in a particular region of myofiber could be necessary to repair muscle cell without loosing the viability of the entire fiber and to prevent dangerous inflammatory lesions, which could be harmful for neighboring myofibers. In this way only a small number of satellite cells could be activated to restore myonuclear loss. This is in agreement with the physiological role of apoptosis, that is to eliminate damaged cell without disturbing neighboring cells in order to maintain tissue function. Our preliminary results suggest this hypothesis. Further studies are needed to establish if the low activation of satellite cells in tissues with the highest amount of DNA fragmentation suggest that apoptosis acts to preserve myofibers.

To our knowledge this is the first report that demonstrates the activation of a caspase-dependent cell death in differentiated human skeletal muscle cells. Since apoptotic death is observed in several myopathies, identification of drugs that can effectively inhibit muscle apoptosis could lead to development of therapeutic strategies in the treatment of these disorders. On the other hand, before caspase inhibitors are used in the management of muscular dystrophies, it remains to be established by in

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TABLE 4
Densitometry of RNase Protection Assay
Fig. 5. Caspase 3 activity in normal and dystrophic muscles. Note the presence of activity in dystrophic muscles and the inhibition of caspase 3 obtained by DEVDfmk pretreatment (caspase 3 inhibitor) (data are expressed as mean ± SD, n = 3).

vivo experiments whether apoptosis plays a noxious or protective role.

REFERENCES

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