

Apoptosis in Human Embryonic and Diseased Skeletal Muscle

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Abstract

Muscle cell death by apoptosis is a physiological process which seems to play an important role in the regulation of muscle cell population during myogenesis. Our observations of human fetal muscle indicate that muscle apoptosis appears at different stages of development. Initially at the myotube stage, a number of unwanted cells are eliminated by apoptosis, reducing the final number of muscle fibres. In the later stage between 20-22 weeks gestation a single cells are removed from clusters which seemingly is responsible for final muscle fibre size and shape. Loss of muscle cells as a result of enhanced apoptosis has been observed also in pathological muscle tissue in postnatal life.

In neonates with acute, fatal form of Werdnig-Hoffmann disease partially immature muscle cells undergo deletion by apoptosis. The ultrastructural features of this phenomenon are essentially the same as those found in human fetal muscle between 20-22 weeks of gestation. Affected muscle cells die rapidly with the characteristics of programmed cell death.

Key words: muscle cell apoptosis, fetal muscle, Werdnig-Hoffmann disease.

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Muscle apoptosis is an active form of programmed cell death depending upon internal machinery of the muscle cell. Programmed muscle cell death is usually engaged in a precisely regulated way to achieve the final muscle fibre size and shape. The best example is a muscle apoptosis during embryonic development, where muscle tissue is re-shaped by natural cell death getting rid unwanted cells. Particularly in the mammalian muscle tissue a striking excess of cells is initially formed which are later eliminated. At the early stages of fetal muscle development up to 40% of the cells are eliminated by apoptosis [10, 11]. Characteristic morphological features of apoptosis were originally described by Kerr et al. 1972 [6]. These include nuclear chromatin and sarcoplasm condensation, cell fragmentation into a number of fragments which are phagocytosed by neighbouring cells. Loss of muscle cells as a result of enhanced apoptosis has never been observed in neonatal stage except of two cases with acute form of Werdnig-Hoffmann disease [3, 4].

In the present study special attention has been put on the ultrastructure of dying muscle cells in Werdnig-Hoffmann disease and comparison if they display the same morphological characteristic as apoptotic cells in embryonic stage.

Material and Methods

Normal fetal muscle - the material consisted quadriceps femoris muscle specimens from 20 human fetuses at 9, 10, 14, 18, 20 and 24 weeks of gestation. Details of how the fetuses were obtained and fixed have already been published [1, 2].

Werdnig-Hoffmann disease - quadriceps femoris muscle specimens from two neonates at 3 and 6 weeks of age with Werdnig-Hoffmann disease were used for this study [3, 4].

The specimens for electron microscopy were fixed in glutaraldehyde in 0.1 M phosphate buffer at pH 7.4 post-fixed in 1% osmium tetroxide in the same buffer, dehydrated and embedded in Spurr-resin. Semithin sections were stained with methylene blue for light microscopy. Thin sections were prepared from selected regions, stained with uranyl acetate and lead citrate and examined in a JEM 1200 X electron microscope.

Results

Our observations of human fetal muscle indicate that muscle apoptosis appeared at two different stage of development. Initially at the myotube stage (9 to 11 weeks of gestation) a number of unwanted cells were eliminated by apoptosis. A dying myotubes had a tendency to be arranged in a small groups, although they were also found scattered

among normal myotubes. The first sign of degeneration was observed in sarcoplasm rather than in the nucleus. There was an increase in density of myofibrils and their homogenization. The myofibrils were clumped in dense amorphous masses without any recognizable sarcomeric pattern (Fig. 1). The nuclei of myotubes which in normal cells were oval, large and euchromatic became irregular and heterochromatic. Dying cell fragments with dense sarcoplasm were observed in extracellular space but they were not found within sarcoplasm of neighbouring cells.

In the later stage between 20 and 22 weeks of gestation, apoptotic cells were found within clusters of immature muscle fibres (Fig. 2). Such cells retracted from its neighbours within a cluster and showed markedly denser appearance than unaffected cells. The nucleus showed considerable condensation with clumping of chromatin. At more advanced stage of apoptosis the nuclear chromatin was aggregated in dense masses (Fig. 2). In the final stage of degeneration, electron dense apoptotic bodies were seen within sarcoplasm of neighbouring cells (Fig. 3). Some bodies contained nuclear fragments, others harboured only electron dense sarcoplasmic elements (Fig. 3).

In both cases of Werdnig-Hoffmann disease ultrastructural findings were identical. Muscle cells undergoing apoptosis were scattered among immature muscle fibres. They were contracted, their sarcoplasm was very dark. The nuclear chromatin was densely aggregated into a large compact granular masses (Fig. 4). Sometimes the nuclei were broken up into several parts. Apart from the dark muscle cells, numerous muscle cell fragments were observed in extracellular space as well as in the cytoplasm of intact neighbouring muscle cells. Cellular fragments ap-

peared as round or oval membrane-bound bodies identifiable as apoptotic bodies (ABs). The number, size and composition of these ABs varied widely, some of them contained one or more nuclear fragments whereas others contained sarcoplasmic elements alone. Observation of a number of specimens at different magnifications showed that ABs could be divided into early and late bodies.

Early ABs can be presented as membrane-bound structures containing condensed sarcoplasm with recognizable organelles (Fig. 5) and sometimes nuclear fragments. Most of the ABs appeared to be broken down to a form in which the organelles were no longer visible (Fig. 6). At this stage they are known as late ABs or residual bodies and contained fibrillar or amorphous electron dense material. Remnants of degraded ABs were also observed within the cytoplasm of immature muscle fibres.

Discussion

Our results indicate that in human ontogenesis programmed muscle cell death appears in two successive waves of cell degeneration. The first, which occurs at the myotube stage probably determines a number of functional muscle fibres [10, 11]. Cell death in second wave which occurs later, at 20 weeks of gestation, probably regulates the muscle cell shape and size matching the proper number of fusing cells forming immature muscle fibres [2]. Morphology of apoptotic myotubes is quite different than the morphology of the majority of immature muscle fibres undergoing apoptosis. While sarcoplasm of dying myotubes showed increased electron density and condensation, little accumulation of nuclear chromatin was observed.

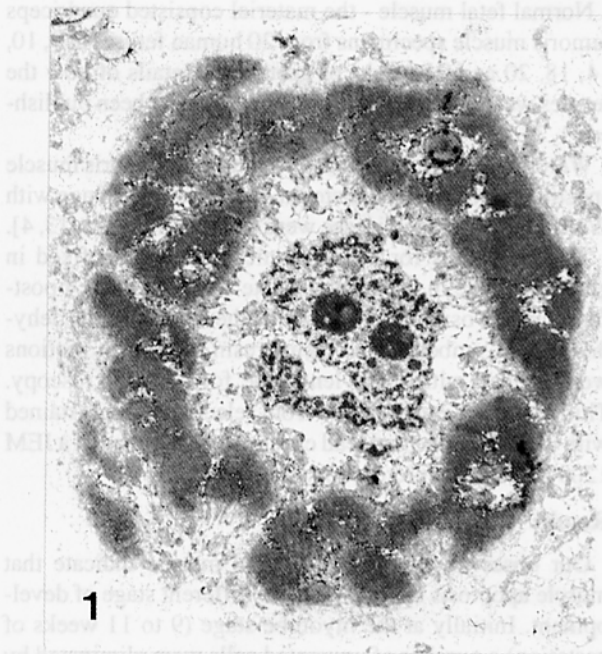


Figure 1. Ten weeks old human fetus. Dying primary myotube. x 5000.

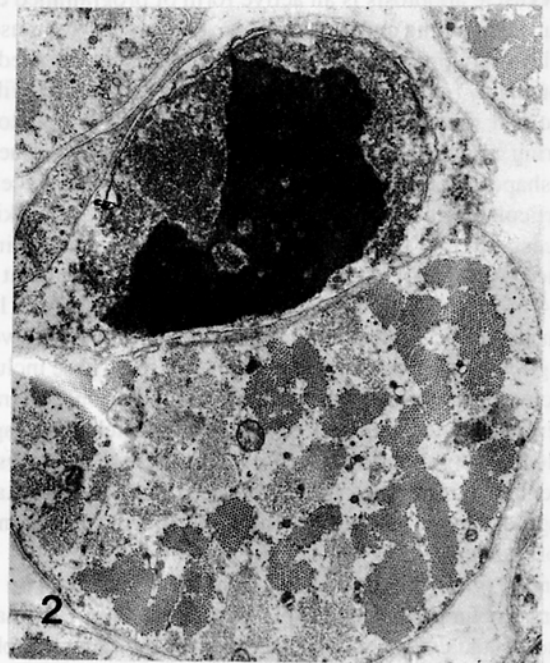


Figure 2. Twenty weeks old human fetus. Satellite myofibre undergoing apoptosis within cluster. Note its condensed nuclear chromatin. x 10000.

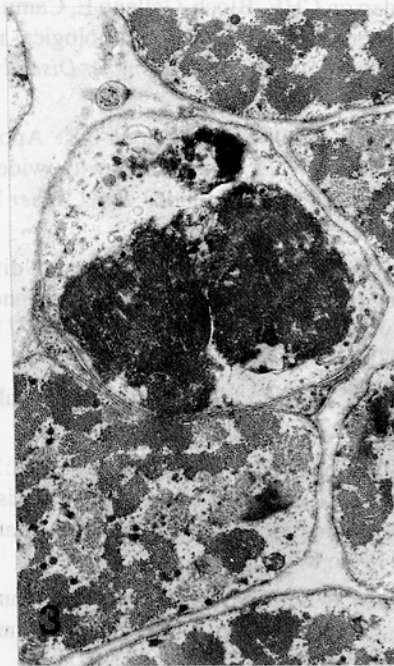


Figure 3. Twenty weeks old human fetus. The two apoptotic bodies within sarcoplasm of immature muscle fibre. Note their compact sarcoplasm in contrast to density of immature muscle fibre. x 7500

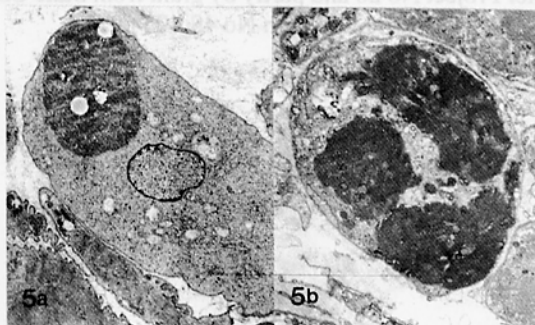


Figure 5. Neonate with fatal form of W-H disease. Early apoptotic bodies. a. x 3800 b. x 5000.

Degenerating cells in immature fibres show prominent condensation and aggregation of nuclear chromatin and slight increase in electron density of sarcoplasm. In contrary to immature muscle fibres, apoptotic bodies were never seen within sarcoplasm of myotubes. These two morphologically distinct types of programmed cell death seen in our material, have been also described in the bird's nervous system and were termed "cytoplasmic" and "nuclear" type [7].

It is difficult to explain these morphological differences however the reason for this discrepancy may depend on muscle immaturity and different cell composition. Myotubes in comparison with well differentiated immature muscle fibres are relatively very immature when cell death occurs [2].

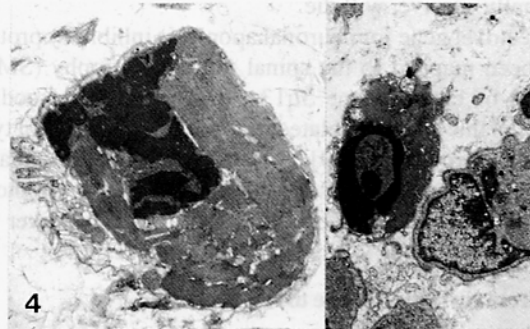


Figure 4. Neonate with fatal form of W-H disease. Muscle cells undergoing apoptosis. The sarcoplasm is condensed. Chromatin is aggregated in a compact granular mass. x 5000.

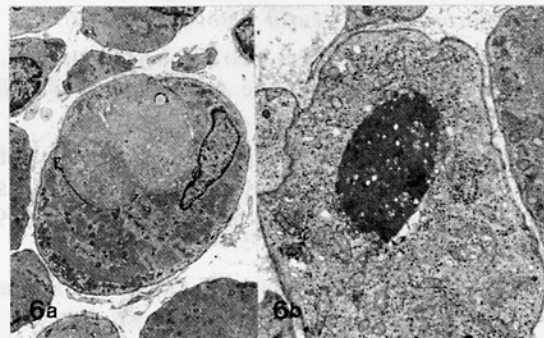


Figure 6. Neonate with fatal form of W-H disease. Late apoptotic bodies. a. x 3500 b. x 6000.

Muscle apoptosis, frequent in embryonic stage, to our knowledge was never observed in normal neonatal muscle. The appearance of muscle apoptosis in neonates with acute fatal form of Werdnig-Hoffmann disease is the most intriguing finding. In severely retarded muscle of affected children, numerous fibres undergo elimination by apoptosis. The morphological features of this phenomenon were essentially the same as those observed in second wave of embryonic apoptosis. These include nuclear condensation, muscle cell fragmentation into a small membrane-bound bodies subsequently digested by surviving normal muscle cells. The similarity between these two physiological and pathological types of muscle cell death could be explained by sudden deficit of neurotrophic factors. It is well known that muscle maturation depends on specific trophic factors [5] which promote survival and muscle cell differentiation. Muscle deprived of such signals die in typical programmed cell death.

There are also some evidence that apoptosis may be caused by activation of suicide program. Gene acting as a brake on the suicide program has been recently identified [8]. If its function is activated, the cell death does not occur.

If its function is inactivated by mutation, many cells that normally survive, will die.

The novel gene for neuronal apoptosis inhibitory protein has been mapped to the spinal muscular atrophy (SMA) region of chromosome 5q13 [9]. The two first coding exons of this gene are deleted in approximately 67% of type 1 SMA chromosome [9]. In such cases this prolonged process of massive muscle cell elimination by apoptosis observed in our cases can be morphological marker for fatal acute form of Werdnig-Hoffmann disease.

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References

- [1] Fidziańska A: Human ontogenesis. 1. Ultrastructural characteristics of developing human muscle. *J Neuropath Exp Neurol* 1980; 39: 475-486.
- [2] Fidziańska A, Goebel HH: Human ontogenesis. 3. Cell death in fetal muscle. *Acta Neuropath (Berlin)* 1991; 81: 572-579.
- [3] Fidziańska A, Goebel HH, Warlo I: Acute infantile spinal muscular atrophy. Muscle apoptosis as a proposed pathogenetic mechanism. *Brain* 1990; 113: 433-445.
- [4] Fidziańska A, Zelazowska E, Glinka Z: Fatal neonatal hypotonia and muscle apoptosis. In press.
- [5] Henderson CHE, Bloch-Gallego E, Camu W, et al: Motoneuron survival factors: biological roles and therapeutic potential. *Neuromusc Disord* 1993; 3: 455-458.
- [6] Kerr JFR, Wyllie AH, Currie AR: Apoptosis: a basic biological phenomenon with wide-ranging implication in tissue kinetics. *Br J Cancer* 1972; 26: 239-257.
- [7] Pilar G, Landmesser L: Ultrastructural differences during embryonic cell death in normal and peripherally deprived ciliary ganglia. *J Cell Biol* 1976; 68: 339-356.
- [8] Raff MC: Social controls of cell survival and cell death. *Nature* 1992; 356: 397-400.
- [9] Roy N, Mahadevan MS, McLean M, et al: The gene for neuronal apoptosis inhibitory protein is partially deleted in individuals with spinal muscular atrophy. *Cell* 1995; 80: 165-178.
- [10] Webb JN: The development of human skeletal muscle with particular reference to muscle cell death. *J Pathol* 1972; 106: 221-228.
- [11] Webb JN: Cell death in developing skeletal muscle: histochemistry and ultrastructure. *J Pathol* 1977; 123: 175-180.

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