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Apoptosis, Paraptosis, Necrosis, and Cell Regeneration in Posttraumatic Cerebral Arteries

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Rezumat

Apoptoza, paraptoza, necroza și regenerarea celulară în arterele cerebrale posttraumatice

Acest studiu a fost realizat pentru a înțelege natura și semnificația funcțională a programelor activate de moarte celulară și a semnelor de reabilitare în timpul modificărilor vasculare survenite după injuria creierului. Noi am folosit microscopia optică și electronică de transmisie pentru a descrie modificări ale celulelor în endoteliul vascular și în tunica medie a unei artere corticale la 4 săptămâni de la traumatismul craniocerebral. În tunica medie a arterei posttraumatice vătămate au fost identificate ultrastructural fenotipurile apoptotic și paraptotic, cât și unele semne timpurii ale regenerarii celulelor musculare netede în nucleu și citoplasmă. Surprinzător, unele celule endoteliale au arătat o dezvoltare amplă a reticulului endoplasmic rugos, în timp ce alte celule endoteliale au arătat necroză tipică. În concluzie, în același perete vascular lezional posttraumatic au fost întâlnite două grupe de celule sinucigașe – apoptotice și paraptotice, dar și celule cu semne de regenerare subcelulară. Semnificația patofiziologică a coexistenței programelor duble de moarte celulară și a regenerării celulare pare să fie în legatură cu supraviețuirea celulelor după un anumit timp de la agresionarea arterială, când unele celule dispar iar altele încearcă prin adaptare să supraviețuiască, suferind injuria reversibilă.

Cuvinte cheie: artere cerebrale posttraumatice, apoptoza, paraptoza, necroza, regenerare celulara, ultrastructura

Abstract

This study is to understand the nature and functional significance of the activated cell death programs and rehabilitation signs during late vascular changes after brain injury. We used light and transmission electron microscopy to describe changes of cells within the vascular endothelium and tunica media of the cortical arteries four weeks after craniocerebral traumatism. Within tunica media of the posttraumatic damaged artery, apoptotic and paraptotic phenotypes were identified as well as some early ultrastructural signs of smooth muscle cells regeneration, these cell highlighting a remarkable degree of plasticity. Surprisingly, some endothelial cells showed an extensive rough endoplasmic reticulum development, whereas other endothelial cells showed typical necrosis. In conclusion, two groups of suicidal cells – apoptotic and paraptotic cells – were encountered in the same lesional vascular wall after neurotrauma, showing also signs of cell regeneration. The pathophysiologic significance of the coexisting double cell death programs and cell regeneration seems to be in relation with late cell survival, after arterial damage when some cells disappear and other cells try to survive undergoing reversible injury.

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Key words: posttraumatic cerebral arteries, apoptosis, paraptosis, necrosis, cell regeneration, ultrastructure

Introduction

Considerable work over the past three decades has determined a number of programmed cell death forms in health and disease, but the nomenclature pertaining to cell death is now confusing, with over 50 terms used for various forms/features of cell death (1,2,3,4,5,6,7,8,9,10).

The role of ultrastructure in differential cell death diagnosis is now increasing. We can compare ultrastructurally different cellular events in relation to cell differentiation and adaptation, or conversely, with some pathways of programmed death in various environmental conditions. The purpose of this investigation was to examine comparative morphofunctional changes of cell population within the well-integrated and dynamic tissue, i.e., the vascular wall, after arterial damage due to the craniocerebral traumatism to understand the pathophysiologic significance of changes into the endothelium versus tunica media, i.e., behaviour of endothelial cells versus smooth muscle cells in the same vascular segment after prolonged ischemia.

Material and Method

We obtained by surgery, in accordance with ethical guidelines, a vascular segment from a patient (male, aged 51) operated on for left internal carotid artery thrombosis, four weeks after a craniocerebral injury. Multiple blocks from a damaged cortical artery were processed for light and transmission electron microscopy (TEM). Samples observed under a light microscope were fixed with 2.5% buffered glutaraldehyde, post-fixed with 1% buffered osmium tetroxide, dehydrated and embedded in resin epoxy (Epon 812). Sections with a thickness of 0.5 to 1 μm were cut with an ultramicrotome, mounted on glass slides, stained with 1% toluidine blue, and examined under a light microscope. Samples prepared for TEM were also treated with glutaraldehyde and osmium tetroxide, dehydrated in graded alcohols, and then embedded in resin epoxy. Ultrathin sections, 70 nm thick, were cut with an ultramicrotome, then mounted on specimen grids covered with electron-transparent plastic films made from formvar and twice contrasted with uranyl acetate and lead citrate aqueous solutions. After drying, the specimens were examined under a JEM 1200 EX (JEOL) transmission electron microscope. The electron micrographs were then processed on a computer and converted into images using Digital Micrograph (Gatan) software.

Results

Examination of the posttraumatic cerebral artery after 4 weeks from the craniocerebral injury by light and transmission electron microscopy offered insight into the differential response of the cell populations in the vascular wall. By light microscopy, despite the multiple disarrangements that took place in the damaged vascular wall, the striking histological feature was a bizarre configuration of cortical artery in cross section, with distorted contour of the vessel, fibrous and amorphous aspect of the vascular wall, corrugated internal elastic lamina, numerous vacuolated cells and a dense thrombus, thrombosis being encountered at different sectioning levels (*Fig. 1A*). Ultrastructurally, large varieties of phenotypical changes were seen into the endothelium and tunica media with significant morphological differences from one compartment to another. These ultrastructural features may be divided into four main categories: apoptotic, paraptotic, necrotic, and regenerative cell changes. Typical apoptotic cells showing decreased cell bodies, condensed nuclei and densified cytoplasm were located in the thickness of the tunica media, but not in the endothelium (*Fig. 1B*). As a rule, the smooth muscle cells of apoptotic phenotype were still surrounded by a basal lamina (*Fig. 2*). However, in the extracellular spaces the collagenous matrix was degraded, with degenerated and fragmented collagen fibrils or replaced by electron vide spaces illustrating variable degrees of edema and a significant reduction of cell population. Basal lamina itself is degenerated around these modified cells which appear isolated. Also, within the tunica media, a significant number of smooth muscle cells with paraptotic phenotype was observed. These cells are mainly characterized by well-known cytoplasmic vacuolization, with numerous small vacuoles created in cytoplasm, dense chromatin in the nucleus, widened perinuclear space, and mitochondrial vesiculation (*Fig. 3*).

The paraptotic smooth muscle cells were isolated and oriented in different planes, displaying different microscopic features and separated by a variable collagenous extracellular matrix or, conversely, densely packed together, characterized by distinctive features of their physiologic condition. The main distinctive feature of these cells remains a progressive cytoplasmic vacuolization. Further, confluent vacuoles are often seen in these cells. If the two distinctive programmed cell death pathways were activated in the posttraumatic artery within the tunica media, on the contrary, in the endothelium, we did not observe typical apoptotic or paraptotic cells, most of them being necrotic in aspect. However, a few endothelial cells were characterized by decreased body, pyknotic nucleus, and little cytoplasm. Most of endothelial cells and even smooth muscle cells showed ultrastructurally a uniform granular aspect of the nucleus and cytoplasm. Some endothelial cells were neither apoptotic/paraptotic nor necrotic in aspect, but nonfunctional cells suggested a slow evolution to mortification. Other endothelial cells presented characteristically an extensive rough endoplasmic reticulum (RER) occupying almost the entire cytoplasm and having dilated cisterns filled with amorphous material recently deposited, while other endothelial cells exhibited the same extensive RER but with empty cisterns. In addition, some subendothelial cells showed graded dilation of the RER component (*Fig. 4*). This extensive development of the RER compartment may be induced by a dedifferentiation/transdifferentiation

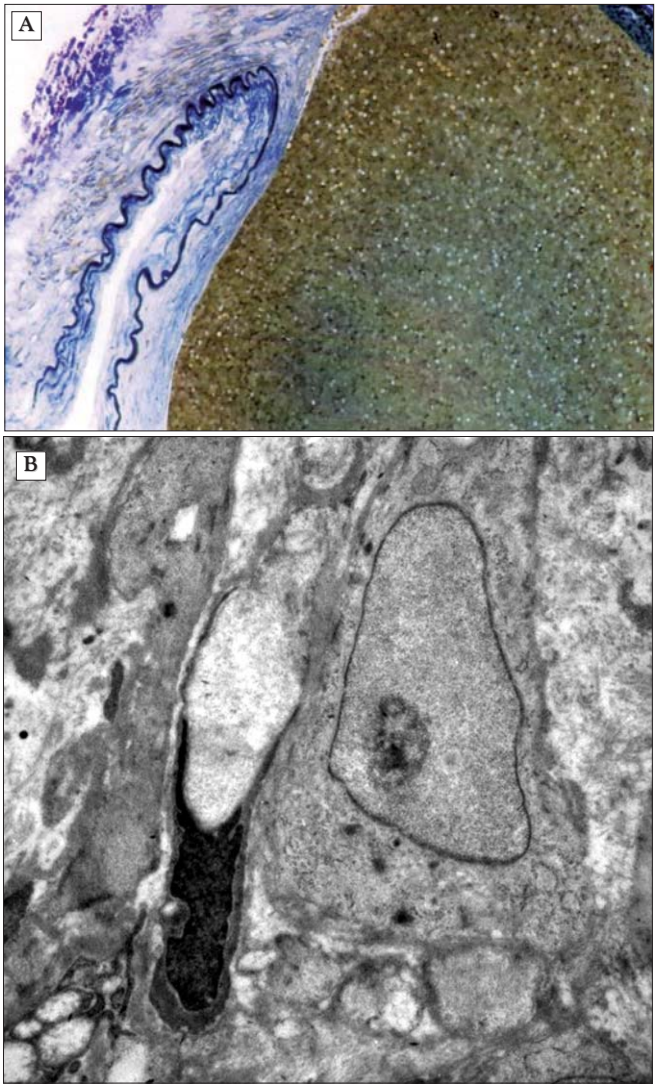


Figure 1. *(A) Cross section of a distorted cortical artery showing perivascular thrombus and fibrous arterial wall, four weeks after cranial traumatism. x200;* ***(B)*** *Typical apoptotic cell showing a small body, condensed nucleus and densified cytoplasm and a neighbouring cell of regenerative type showing euchromatinized nucleus, prominent nucleolus and cytoplasmic organelles, both cells located within the tunica media. x20,000*

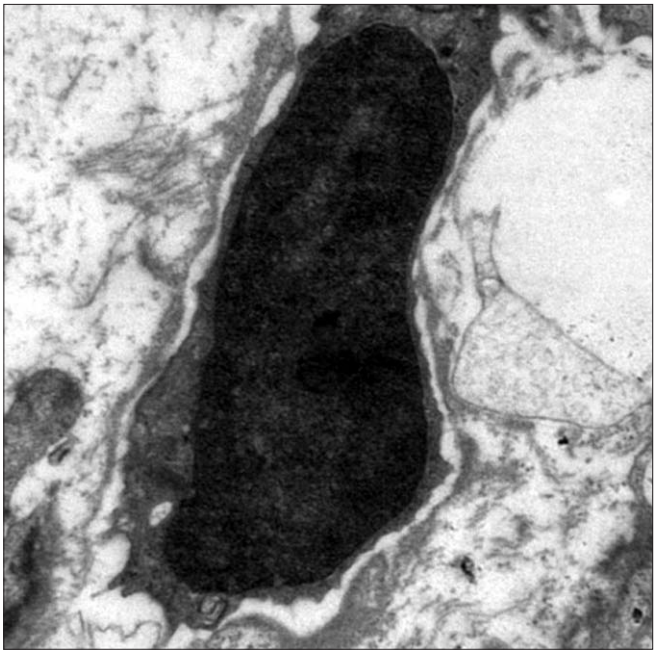


Figure 2. *Smooth muscle cell of apoptotic type containing a dense nucleus and cytoplasm, and surrounded by a basal lamina. x25,000*

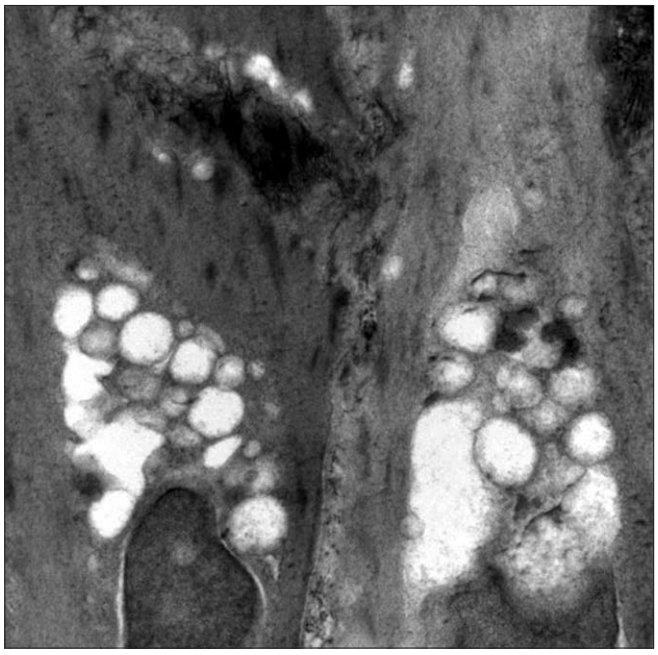


Figure 3. *Two typical paraptotic smooth muscle cells showing the same features: dense chromatin, widened perinuclear space, confluent vacuoles in cytoplasm and mitochondrial vesiculation. x15,000*

phenomenon in the endothelium. Conversely, both in the tunica media and the endothelium a small fraction of cells showed signs of regeneration with a large spectrum of modifications, from cells containing a large nucleus and a thin rim of cytoplasm to cells with an abundant cytoplasm and smaller nucleus (*Fig. 5*). In the tunica media, a synthetic smooth muscle cell phenotype was observed during the regeneration steps. Ultrastructurally, cells of genuine regenerative phenotype were characterized by the following features:

- functional nucleus with euchromatin and areas of genic activation;
- prominent nucleolus with fibrillar and granular compartments, migrating under the nuclear envelope;
- increased cytoplasmic compartment with little mitochondria, ribosomes, rough endoplasmic reticulum profiles, and a few cisterns filled with recently synthetized amorphous material;
- cytoskeletal components such as microtubules, intermediate filaments, microfilaments and attachment plaques.

Therefore, when the nucleus becomes active and the

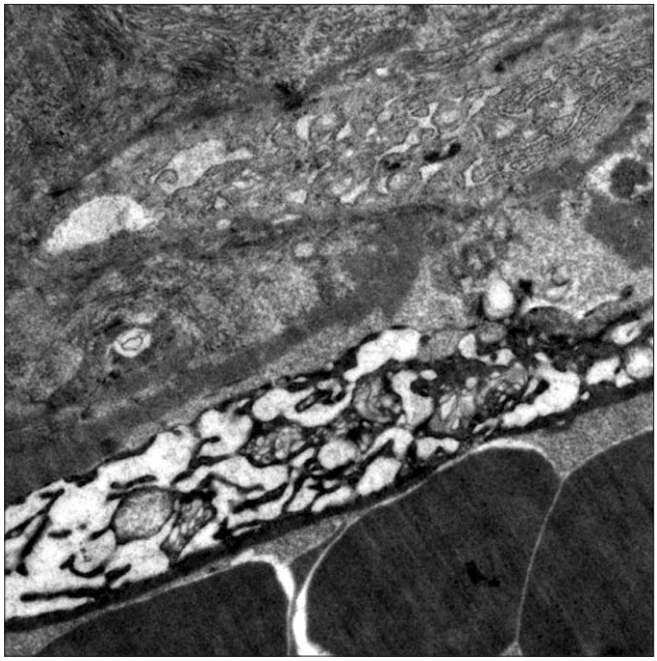


Figure 4. *Prenecrotic endothelial cell showing an extensive RER with dilated cisterns, and subendothelial cells showing various degrees of RER dilation, also suggesting necrosis. x10,000*

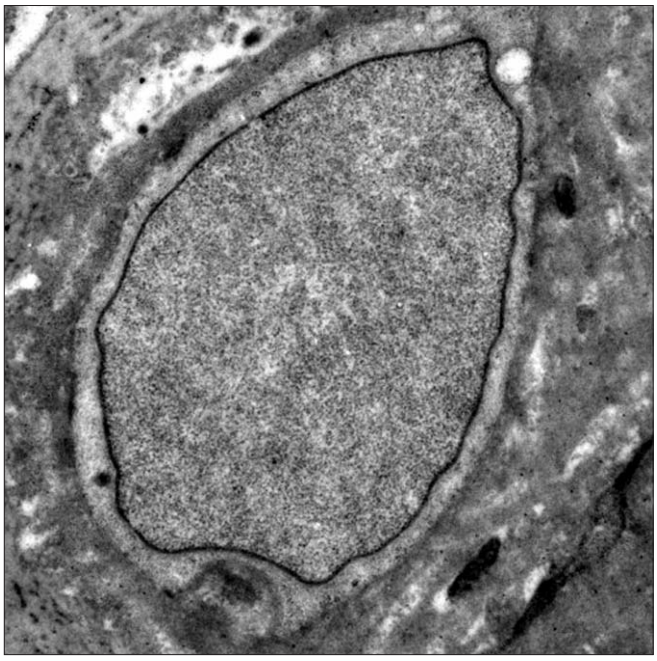


Figure 5. *Smooth muscle cell containing a large nucleus and little cytoplasm suggesting an inactive metabolic state. x20,000*

cytoplasm abundant at a cellular pole with increased number of organelles and cytoskeletal elements, the cells undergo a new ultrastructural pattern (*Fig. 6*). In the thickness of the vascular wall after cerebral traumatism one can see a complex ultrastructural pattern due to the degenerative and regenerative cell events.

Discussions

The vascular changes seen by microscopy in the first four weeks after the traumatism showed a simultaneous complexity of damage, degeneration, regeneration, and repair processes within the wall of thrombosed cortical arteries. The cells showed a different ability to respond to damage, and two forms of programmed death (apoptosis and paraptosis) were observed in addition to intramural necrosis. Paradoxically, in the same lesion, a few cells showed ultrastructural cellular signs of regeneration in certain cytoplasmic areas. In *Table 1* we listed the main cell characteristics revealed by TEM in the post-traumatic arterial wall. Additionally, cell injury (caused by diminished blood supply and inadequate nutrition) results from functional and biochemical abnormalities in one or several essential cellular components.

The most important targets of injurious stimuli are the following:

- mitochondrion, the sites of ATP generation;
- cell membranes, on which the ionic and osmotic homeostasis of the cell and its organelles relies on;
- protein synthesis;
- the cytoskeleton; and

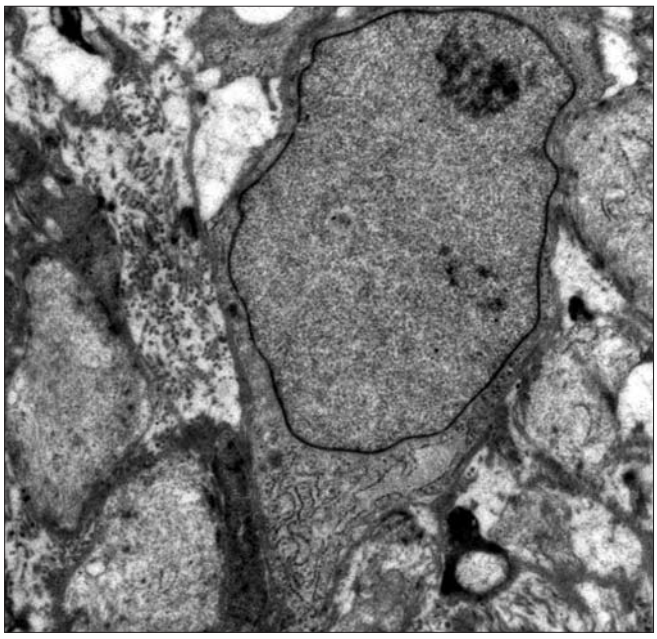


Figure 6. *Cell of regenerative type displaying an euchromatinized nucleus with areas of genic activation, a prominent nucleolus, and abundant cytoplasm with mitochondria, ribosomes, RER profiles, and attachment plaques. x12,000*

- the genetic apparatus of the cell.

DNA damage and accumulation of misfolded proteins lead to cell death mainly by apoptosis, whereas depletion of ATP, membrane, and cytoskeleton damage lead to necrosis.

Table 1. *Ultrastructural features of apoptosis, paraptosis, necrosis, and regeneration in the posttraumatic cerebral arteries*

Morphology	Apoptosis	Paraptosis	Necrosis	Cell regeneration
Cell body	Shrinking	Sometimes enlarged	Swelling	Increasing size, with developing RER and other organelles
Cell membrane	Intact, blebbing (zeiosis)	Intact	Broken	Intact
Cytoskeleton	Collapse before death	Collapse before death	Collapse after death	In formation
Lysosomes	Intact	Intact	Leaking	Small, a few
Endoplasmic reticulum	Dilated, loss of ribosomes from external membranes	Sometimes dilated	Dilated	In developing, with dilated cisterns
Mitochondria	Intact, with cytochrome c release and ATP synthesis	Late swollen, without ATP synthesis	Broken, without ATP synthesis	A few
Cytoplasm	Compact and condensed, with intact organelles	Vacuolised, with numerous vacuoles	Vacuolised, swollen and broken membranes	In developing, electronlucent
Nucleus	Shrinking and fragmented, disintegrating nucleolus	Without fragmentation	Enlarged and broken	Rounded, with developing nucleolus
Chromatin	Condensation and internucleosomal DNA fragmentation	With or without condensation	Clumped, randomized fragmentation	Euchromatin, with areas of genic activation
Apoptotic bodies and their removal	Common, heterophagy (rapid) by other cells	Absent	Absent	Absent

Mitochondrial damage may lead to reversible injury and death by necrosis and apoptosis. Cells have mechanisms that repair the damage to DNA, but if this damage is too severe to be corrected (e.g. after oxidative stress), the cell initiates its suicidal program and dies by apoptosis. A similar reaction is triggered by inappropriately folded proteins, which may be the result of external triggers such as free radicals.

Oxidative stress leading to calcium accumulation, mitochondrial dysfunction, and the production of reactive oxygen radicals is an important mechanism of cell death following both ischemic and traumatic insults (11). If ischemia persists and oxygen is not restored, irreversible injury and death are ensured. Irreversible injury is associated with severe swelling of mitochondria and extensive damage of the plasma membrane, and a massive influx of calcium into the cell may occur. Death is mainly induced by necrosis, but apoptosis and paraptosis also contribute to this. The apoptotic pathway is activated probably by release of proapoptotic molecules from leaky mitochondria, whereas the paraptotic pathway remains to be determined.

However, when cells and their organelles die, certain molecular aggregates remain functional. Apoptotic response to trauma in the brain is regionally distinct and may be involved in both acute and delayed patterns of cell death (12). The exact percentage of cells dying by apoptosis and paraptosis versus necrosis depends upon several factors including ischemic severity and duration. Genetic and gender factors have also been showed to be important mediators of pathomechanisms present in loss of cellular integrity and tissue destruction. Apoptosis versus necrosis was described in the vascular wall (13,14). It is known that adverse environmental conditions such as lack of oxygen or essential nutrients in case of ischemia after stroke or excessive mechanical strain such as trauma are potent necrosis initiators. Also, it is known that cellular swelling is the first manifestation of injured cells, but difficult to appreciate with the light microscope. On the other hand, it is crucial to understand both the molecular mechanism of regeneration and the intrinsic and extrinsic signals that induce and promote this process emphasized ultrastructurally in our case. Identification of genes that are differentially expressed during the early stages of vascular wall regeneration was not yet studied, and a de novo transcriptome assembly was not yet generated. The transcriptome (i.e., the set of genes that are actively expressed by the genome) is a dynamic determinant of the cellular phenotype and tissue function. Because vascular occlusions may lead to irreversible damage of the central nervous system, a new therapy is necessary in the field of regenerative medicine. Transcriptome profiling is a powerful tool for the characterization and functional analysis of endothelial progenitor cells in health and disease (15). At present it is known that the comparison of transcriptomes could help identify specific pathways that are involved in and/or regulating certain processes (16). In this respect, it is necessary to know better the molecular processes involved in both the vascular endothelium and the tunica media because TEM observations showed a different behaviour within the two tunicae (cell layers). Increased pinocytotic activity within the endothelial cells, endothelial microvilli projections, large vacuoles and craters are reported after brain trauma injury (17,18). An extensive development of the RER compartment was described only in endothelial cells, although in the tunica media a synthetic phenotype was evidenced (19, 20). Moreover, it is known that smooth muscle cells become non-contractile after a few minutes of ischemia (21).

The spatiotemporal events from the vascular wall after brain trauma merit much interest in future studies for the cell plasticity, functional adaptation mechanisms, atrophy/ death and regenerative potential in this new neuro-restorative era,

both in the endothelium and the tunica media.

A recent report (10) showed ultrastructurally how the plasmalemma of endothelial cells could be involved in an opposite phenomena – cell demise and survival. Understanding the multidimensional cascade of secondary brain injury and cell recovery abilities offer differentiated therapeutic options.

In future research, apoptosis and other cell death programs should be utilized as therapeutic tools (22), in connection with brain and vascular-specific tissual organization and function (23).

Conclusions

This study is the first demonstration of the coexistence of multiple programs of cell death with a simultaneously rehabilitation potential in the cerebral arterial wall after brain injury. Now it is known that adverse environmental conditions such as lack of oxygen or essential nutrients in case of excessive mechanical strain such as trauma are potent apoptosis/paraptosis and necrosis initiators. The percentage of different cell death forms in the thickness of vascular wall was evidently increased in tunica media versus tunica intima. Ultrastructural features of endothelial cells versus smooth muscle cells demonstrated significant characteristics of their modulated phenotypes in the damaged vessel in relation to different gene expression and spatiotemporal events. Our ultrastructural observations revealed the biological potential of the medial smooth muscle cells, those functional adaptation mechanisms that support not only survival, but certain rehabilitation subcellular signs, concomitantly with numerous dying cells.

In conclusion, further studies at molecular level are expected to be performed to better understand the differential behaviour of cells in the same tissue and micro-environmental conditions after neurotrauma.

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