Degeneration of Astrocytic Processes and Their Mitochondria in Cerebral Cortical Regions Peripheral to the Cortical Infarction

Heterogeneity of Their Disintegration Is Closely Associated With Disseminated Selective Neuronal Necrosis and Maturation of Injury

Umeo Ito, MD, PhD, FAHA; Yoji Hakamata, DVM, PhD; Emiko Kawakami, BS; Kiyomitsu Oyanagi, MD, PhD

Background and Purpose—Astrocytes support neuronal functions by regulating extracellular ion-homeostasis and neurotransmitters, and by providing energy substrates such as lactate to the neurons via their astrocytic processes (APs). Whether injured APs are associated with neuronal survival/death is still an unanswered question. We investigated APs in the neuropil, especially those around astrocytes and normal-appearing, degenerating, and dead neurons in cerebral cortical regions peripheral to the cortical infarction (RPI).

Methods—Stroke-positive gerbils were euthanized at various times after the ischemic insult. Ultrathin sections were obtained from the RPI sectioned coronally at the infundibular level. We counted the number of normal-appearing, degenerated, and dead neurons and astrocytes in paraffin sections, the number of cut-ends and mitochondria in APs in the neuropil on electron-microscopic photographs, and determined the percent-volume of APs by Weibel point-counting method. We compared the number of cut-ends and mitochondria and percent-volume of APs around astrocytes at 5 hours and 48 hours, and around normal-appearing, degenerated, and dead neurons at 12 hours.

Results—Although the number of astrocytes did not change (average of 12.3±0.20%) during 0 to 48 hours, that of the dead neurons increased from 9.71±1.34 to 44.39±1.40% during 5 to 48 hours postischemia. The number of normal-appearing APs and mitochondria in APs decreased respectively from 13.49±0.65 to 1.61±0.14/28.20 μm² and from 1.86±0.18 to 0.61±0.07/28.20 μm² in the neuropil during 0 to 48 hours. The number of normal-appearing APs around astrocytes decreased from 12.3±0.19 to 1.7±0.05/38.33 μm² with an increase in percent-volume of degenerated APs from 1.17±0.04 to 11.45±0.23%, from 5 to 48 hours postischemia. The number of normal-appearing APs decreased from 4.36±0.52 to 1.56±0.17/38.33 μm² with an increase in percent-volume of degenerated APs, from 2.41±0.52 to 12.55±1.0%, from around the normal-appearing to dead neurons, at 12 hours.

Conclusions—In the RPI, heterogeneous degeneration of APs was closely associated with disseminated selective neuronal necrosis and the maturation phenomenon seen in ischemic neuronal injury. (Stroke. 2009;40:2173-2181.)

Key Words: astrocytic process • mitochondria • ischemic penumbra • disseminated selective neuronal necrosis • maturation phenomenon • delayed neuronal death

The study of cell death after ischemia has focused primarily on the neurons. Astrocytes are known to support neuronal functions by regulating extracellular ion-homeostasis and neurotransmitters, and by providing energy substrates such as lactate to the neurons via their astrocytic processes (APs). As astrocytes are also damaged by ischemia, dysfunction or loss of these cells and their APs is thought to be closely associated with postischemic neuronal survival/death. However, little is known about the neuronal dysfunction induced by morphological changes in the APs in the neuropil and surroundings or in those directly in contact with the neurons.

Electron-microscopically, APs pervade the neuropil and are recognized by their irregular contours. Some of them form perivascular end-feet that make a complete layer of various widths that is imposed between the nerve fibers and the endothelial cells. In the APs, glial fibrils occur in bundles, and the mitochondria are oriented parallel to the long axis of the APs. Glycogen granules are observed, more in end-feet and APs near the neuronal perikaryon than in other areas. In

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From the Department of Neuropathology (U.I., E.H., K.O.), Tokyo Metropolitan Institute for Neuroscience, Tokyo; and the Department of Basic Science (Y.H.), School of Veterinary Nursing and Technology, Faculty of Veterinary Science, Nippon Veterinary and Life Science University, Japan. Correspondence to Umeo Ito, MD, PhD, FAHA, Department of Neuropathology, Tokyo Metropolitan Institute for Neuroscience, 2-6 Musashidai, Fuchu-shi, Tokyo 183-8526, Japan. E-mail umeo-ito@nn.iij4u.or.jp
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the smaller processes, the only structures found are ribosomes, glycogen granules, and bundle of fibrils.

The degeneration of APs has been shown in vitro to be attributable to energy failure and acidosis.5,6 There are many observations made in vitro5,7 and ultrastructural ones made in vivo8–10 of astrocytic swelling, particularly of the cytoplasm, end-feet, and APs around the neurons. However, the changes in the APs in the neuropil are still obscure in the RPI.

By giving a threshold ischemic insult to induce cerebral-cortical focal infarction, we devised a model in which a slowly developing large RPI appeared, where only DSNN occurred, around a focal infarction in the unilateral cerebral cortex of Mongolian gerbils.9,11–13 In this model, by dividing the ischemic insult into 2 parts, the mortality rate of the animals attributable to epileptic seizure was decreased drastically; and the infarction size became uniform so that focal cerebral cortical infarction evolved only in the coronal face sectioned at the chiasmatic level (Face A), and only DSNN matured in the coronal face sectioned at the infundibular level (Face B; see Supplemental Figure I, available online at http://stroke.ahajournals.org).

In the present study, using Face B of this model we investigated the ultrastructural temporal profile of APs in the neuropil in correlation with that of neuronal degeneration and death, aiming to elucidate the time-course of morphological changes, including the changes in astrocytes and their processes and end-feet associated with neurons in different stages of the cell death process in the RPI.

Materials and Methods

The animals used here were handled in accordance with the “Guidelines for the Experiment of Tokyo Metropolitan Institute for Neuroscience,” and adequate measures were taken to minimize pain and discomfort to the animals.

Animals were numbered and grouped by using a table of random numbers. Also using this table, an outsider numbered each specimen for LMS and EM pictures in each of the settings. Measurement was performed, in each of the settings, starting with the smaller number.

Under anesthesia with 2% halothane, 70% nitrous oxide, and 30% oxygen, a midline cervical incision was made in adult male Mongolian gerbils (60 to 80 g); and the left carotid artery was then exposed and occluded twice with a Heifetz aneurismal clip for 10 minutes each time, with a 5-hour interval between the 2 occlusions. Anesthesia was discontinued immediately after each cervical surgery, and the behavior of the animals was observed in the wakefulness state for 10 minutes during the carotid occlusion. Ischemia-positive animals were selected based on having a stroke-index score of over 13 points.14 These animals were euthanized at 0 (sham-operated animals), 5, 12, 24, and 48 hours after the second ischemic insult, by intracardiac perfusion with diluted fixative (1% paraformaldehyde, 1.25% glutaraldehyde in 0.1 mol/L cacodylate buffer) for 5 minutes, followed by perfusion with concentrated fixative (4% paraformaldehyde, 5% glutaraldehyde in 0.1 mol/L cacodylate buffer) for 20 minutes for electron microscopy (EM, 3 animals for each time group) or with 10% phosphate-buffered formaldehyde fixative for 30 minutes for light microscopy (LMS, 5 animals for each time group).

We used 3 animals for each time point in the EM study; and from each of them, 2 adjacent ultrathin sections, including the 2nd to 4th cortical layers along a 2-mm path of the cortex, were obtained from a point one-third of the distance from the rhinal (RF) to interhemispheric fissures (IF) of the left cerebral cortex on Face B (Supplemental Figure I). The sections were double stained with uranyl acetate and lead solution, and observed with an electron microscope (H9000, Hitachi). Paraffin-sections were separately stained with hematoxylin-eosin (HE) or periodic acid Schiff (PAS) for LMS. We performed the following morphometries:

Percentage of Normal-Appearing, Degenerating, and Dead Neurons, and of Astrocytes

Using an eye-piece micrometer (U-OCMSQ10/10) under 200-power magnification, we calculated the percentage of the normal-appearing, degenerated, and dead neurons in the 2nd to 4th cortical layers, by laterally moving the specimen along a 2-mm path of the cortex at a point one-third of the distance from the rhinal to interhemispheric fissures on HE-stained Face B of each of 5 animals (Supplemental Table I A, available online at http://stroke.ahajournals.org). The average number of all neurons...
counted was 10,098.4±437.5 per time point. The following criteria were used to classify the neurons: for normal-appearing neurons, a clear cytoplasm containing a large round nucleus with homogenous chromatin and a centrally located large nucleolus; for degenerating neurons, an eosinophilic-dark cytoplasm surrounded by a clear halo (swollen APs) along with nuclear chromatin condensation or aggregation; and for dead neurons, an asterisk-shaped condensed eosinophilic cytoplasm surrounded by a clear halo and a nucleus showing karyorrhexis or pyknosis.

In the same way, under 400-power amplification, we determined the percentage of astrocytes in the 2nd to 4th cortical layers, by laterally and vertically moving the specimen along a 1-mm path of the cortex at a point one-third of the distance from the rhinal to the interhemispheric fissures on PAS-stained Face B of each of 5 animals. The average number of all cells counted except for endothelial cells was 6612±193.9 per time point. The following criteria were used to identify astrocytes: those cells with a round or elliptical or occasionally polygonal nucleus surrounded by a conspicuous nuclear membrane and having clear homogenous chromatin with an eccentrically located single small nucleolus, and an irregular foamy cytoplasm filled with PAS-positive glycogen particles. Endothelial cells were defined as those cells surrounding the vascular lumen and having an elliptical basophilic nucleus.

**Area of and Number of Mitochondria in the Astrocytic Cytoplasm and End-Feet**

Using a computer-assisted digitizer (Measure 5, System Supply), we measured the area (μm²) of astrocytic cytoplasm in each of 3 animals at 0, 5, 12, 24, and 48 hours postischemia, and expressed them as the average obtained from 22.4±1.8 evenly distributed EM photographs of astrocytes at 8010 to 10,680 times magnification per time point (Supplemental Table II). In the same way, using the same animals we measured the area (μm²) of the end-feet and number of mitochondria in them, expressing them as the average obtained from 20.4±0.7 evenly distributed EM pictures of small vessels and end-feet at the same magnification per time point.

**Percent-Volume and Numbers of Cut-Ends and Mitochondria in APs in the Neuronal**

Placing a 1.0×1.0 cm quadratic lattice on 40.2±2.1 evenly distributed EM photographs (magnified 18,690 times) of neurons (Supplemental Figure II) for 3 animals in each time group, we determined the percent-volume of the APs in the neuropil by using the point-counting method12,15 (Supplemental Table II). By this method we counted the number of intersecting points of the lattice touched by the cut-ends of the APs among an average of 17,163.2±60 times magnification per time point. The following criteria were used to classify the neurons: for normal-appearing neurons, a clear cytoplasm containing a large round nucleus with homogenous chromatin and a centrally located large nucleolus; for degenerating neurons, an eosinophilic-dark cytoplasm surrounded by a clear halo (swollen APs) along with nuclear chromatin condensation or aggregation; and for dead neurons, an asterisk-shaped condensed eosinophilic cytoplasm surrounded by a clear halo and a nucleus showing karyorrhexis or pyknosis.

**Area of and Number of Mitochondria in the Astrocytic Cytoplasm and End-Feet**

Using a computer-assisted digitizer (Measure 5, System Supply), we measured the area (μm²) of and number of mitochondria in the astrocytic cytoplasm in each of 3 animals at 0, 5, 12, 24, and 48 hours postischemia, and expressed them as the average obtained from 22.4±1.8 evenly distributed EM photographs of astrocytes at 8010 to 10,680 times magnification per time point (Supplemental Table II). In the same way, using the same animals we measured the area (μm²) of the end-feet and number of mitochondria in them, expressing them as the average obtained from 20.4±0.7 evenly distributed EM pictures of small vessels and end-feet at the same magnification per time point.
We also measured the numbers of mitochondria and cut-ends of APs in an average of 15 912.8 ± 966.2 evenly distributed areas of 1.0 cm² in the neuropil of the EM pictures for 3 animals in each time group, and converted the counted number in each EM picture to the number in a 100 cm² area of the each EM picture (28.2 µm², by real size).

**Ultrastructural Criteria**

**Normal-Appearing APs**

Those containing normal astrocytic micro-organelles such as endoplasmic reticulum, rough-surfaced endoplasmic reticulum, ribosomes, glial fibrils, mitochondria, and glycogen granules, but negative for components of neurites such as microtubules, neurofilaments, synapses, synaptic vesicles, and spine or thorn with spine apparatus; they showed various degrees of swelling (cytotoxic edema).

**Degenerated APs**

Those with sparse glycogen granules in an empty cytoplasm with loss of the micro-organelles described in "1," occasionally containing degenerated mitochondria or simple membranous structures, and also negative for the neurite components described above; they also showed various degrees of swelling.

**Degenerated Mitochondria**

Swollen mitochondria with disoriented, disintegrating, and sometimes peripherally located cristae with decreased density and cavitation of the matrix.

**Percent-Volume and Number of Cut-Ends and Mitochondria in the APs Around the Normal-Appearing, Degenerated, and Dead Neurons at 12 Hours Postischemia**

Placing a 0.5 cm × 0.5 cm quadratic lattice on 20.5 ± 1.9 evenly distributed EM photographs (8010× magnification) of the neuropil around astrocytes (Supplemental Figure II) for 3 animals in each neuronal group, we determined the percent-volume of the APs in the neuropil by using the point-counting method²,¹⁵ (Supplemental Table III). By this method we counted the number of intersecting points of the lattice touched by the cut-ends of the APs among an average of 27 520.5 ± 1102 evenly distributed points in the neuropil around astrocytes of the EM pictures for 3 animals in each time group, and converted the counted number in each EM picture to the number among 100 intersecting points of the lattice of each EM picture (percent volume). (According to the equation for the relative error for different volumetric proportions, all values had less than 5% error.)

We also measured the number of cut-ends of APs in 24 916 ± 1028.2 evenly distributed areas of 0.25 cm² in the neuropil surrounding astrocytes of EM pictures for 3 animals in each time group, and converted the counted number in each EM picture to the number among a 25 cm² area of each EM picture (38.33 µm², by real size).

**Percent-Volume and Number of Cut-Ends of APs Around Astrocytes at 5 hours and 48 hours**

Placing a 0.5 cm × 0.5 cm quadratic lattice on 20.5 ± 1.9 evenly distributed EM photographs (8010× magnification) of the neuropil around astrocytes (Supplemental Figure II) for 3 animals in each time group, we determined the percent-volume of the APs in the neuropil by using the point-counting method²,¹⁵ (Supplemental Table IV). The number of intersecting points of the lattice touched by the cut-ends of the APs was counted among an average of 27 042.7 ± 2523.2 evenly distributed points in the neuropil around various neurons of the EM pictures for 3 animals in each neuronal group, and we converted the number counted in each EM picture to the number among 100 intersecting points of the lattice for each EM picture (percent volume); (according to the equation for the relative error for different volumetric proportions, all values had less than 5% error).

We also measured the number of mitochondria and cut-ends of APs in 24 671.7 ± 2311.6 evenly distributed areas of 0.25 cm² in
thereafter, degenerating and dead neurons increased in number among the normal-appearing neurons during 0 to 12 hours postischemia, and then decreased toward 48 hours; whereas that of the degenerated neurons increased during 5 to 48 hours (Figure 2A).

On the other hand, the average percentage of astrocytes among all cells except for endothelial cells (Figure 1D) was 12.3±0.20 during 0 to 48 hours postischemia; and there were no statistical differences in this percentage at the various time points (Figure 2A).

In EM pictures, the astrocytic cytoplasm (Figure 3A) and end-feet (Figure 3B) were swollen and showed an increased number of glycogen granules at 5 hours postischemia. Mitochondrial number and size were also increased by elongation, branching, and budding of them (Figure 3A, inset). The spaces between cristae were dilated with an electron-dense matrix. During 12 to 24 hours postischemia, swelling of the astrocytic cytoplasm and the number of mitochondria in it did not change, whereas the end-feet gradually thinned out and showed a decreased number of mitochondria during that time period.

At 48 hours postischemia, the APs around astrocytes degenerated and were swollen, and the number of mitochondria, glycogen granules, and glial fibrils increased in the enlarged swollen cytoplasm (Figure 3C). Some of the astrocytes became globular and lost their APs, but were connected to the vascular wall by their foot-process (Figure 3E). Some astrocytic cytoplasm made direct contact with the vascular wall (Figure 3F). At 48 hours postischemia, the end-feet were thinned-out, with a diminished number of mitochondria and surrounded by degenerated APs. (Figure 3D). In the present study, neither ultrastructural evidence of extravasation of plasma fluid into the extracellular space nor disruption and detachment of the end-feet from the vasculature were confirmed. On morphometry, the area of and mitochondrial number in the astrocytic cytoplasm increased significantly during 0 to 5 hours and remained unchanged until 24 hours postischemia; and then both values increased at 48 hours (Figure 2B and 2C). The area of and mitochondrial number in end-feet also increased significantly during 0 to 5 hours, and then decreased gradually up to 48 hours postischemia (Figure 2B and 2C).

Firstly, we compared APs in the neuropil of the RPI at 0, 5, 12, 24, and 48 hours. Compared with those at 0 hours, APs in the neuropil of the RPI at 5 hours were swollen and showed an increased number of glycogen granules (Figure 4A and 4B). The number of normal-appearing mitochondria was not changed, with occasional evidence of their proliferation (Figure 4B, inset). Swollen degenerated APs with sparse glycogen granules increased in number during 12 to 48 hours, whereas the number of normal-appearing APs decreased, along with increased swelling of neurites (Figure 4C and 4D). The number of normal-appearing mitochondria in the APs decreased, whereas that of degenerated ones increased during 12 to 48 hours (Figure 4C and 4D). On morphometry, the percent-volume of the normal-appearing and total APs in the neuropil drastically increased during 0 to 12 hours postischemia, and then decreased toward 48 hours; whereas that of the degenerated APs increased during 12 to 48 hours postischemia (Figure 2D). The number of cut-ends of the normal-appearing and total APs decreased during 0 to 48 hours, whereas that of the degenerated APs increased during 5 to 12 hours postischemia and then decreased by 48 hours (Figure 2E). The number of the normal-appearing mitochondria in APs...
decreased during 0 to 48 hours, whereas that of the degenerated mitochondria increased during 5 to 12 hours postischemia and then remained at the same level toward 48 hours (Figure 2F).

Secondly, we compared APs around the astrocytes at 5 hours and 48 hours postischemia. At 5 hours postischemia, the normal-appearing APs (traced in blue) of almost the same size were disseminated homogenously around the astrocytes. The degenerated APs (traced in red), some of which were swollen, were scanty (Figure 5A). On the contrary, at 48 hours, variously-sized normal-appearing and swollen degenerated APs containing degenerated mitochondria were observed surrounding the astrocytes (Figure 5B). On morphometry, the percent-volume of normal-appearing APs around the astrocytes decreased from 5 to 48 hours, whereas that of degenerated and total APs increased (Figure 6A). The number of cut-ends of normal-appearing and total APs around the astrocytes decreased from 5 to 48 hours postischemia, whereas that of the degenerated APs increased (Figure 6B).

Finally, we compared APs around the normal-appearing, degenerated, and dead neurons in the neuropil at 12 hours postischemia. Normal-appearing APs of almost the same size (traced in blue), and scanty degenerated APs (traced in red), some of which were swollen, were attached to and scattered around the normal-appearing neurons (Figure 5C). Around the degenerated neurons, there were normal-appearing and degenerated APs, some of which showed marked swelling, which was especially prominent in APs attached to the neurons. The swollen normal-appearing APs contained mitochondria and were rich in glycogen granules (Figure 5D). Dead neurons showed necrotic hallmarks such as mitochondrial flocculent densities and disruption of the nuclear or cellular membrane and were surrounded mainly by swollen degenerated APs containing degenerated mitochondria and scanty glycogen granules, especially prominent in APs attached to the neurons (Figure 5E and 5F). On morphometry, the percent-volume of the normal-appearing APs around neurons decreased around degenerated to dead neurons, whereas that of the degenerated and total APs increased around the normal-appearing, degenerated, and dead neurons (Figure 6C). The number of cut-ends of the normal-appearing APs around neurons decreased around these same 3 kinds of neurons; whereas that of the degenerated APs increased around the normal-appearing, degenerated, and dead neurons (Figure 6D). The number of the normal-appearing mitochondria decreased in the APs around the degenerated to dead neurons, whereas that of the degenerated mitochondria increased around the normal-appearing, degenerated, and dead neurons (Figure 6E).

Discussion

Previously, we found that postischemic neuronal injuries matured hours or days after the ischemic insult and that the speed and grade of the injury depended on the intensity of ischemia and vulnerability of the neurons; and so we designated this phenomenon as the “maturation phenomenon of ischemic injuries.” Seven years later, the same findings confined to the hippocampus in the bilateral carotid occlusion model using the same animals was designated as “delayed
neuronal death.”17 During the maturation period, cells may resume various functions if adequate reperfusion follows the ischemia.2 It has been thought that toxicity caused by excitatory amino acids leads to selective neuronal necrosis and that acidosis leads to pan-necrosis, damaging both astrocytes and neurons. However, the mechanisms governing the induction of neuronal death/survival is still obscure.1

In the present study, the maturation of neuronal injury and DSNN progressed during 0 to 48 hours postischemia in the RPI. Whereas the number of astrocytes in the RPI did not change, as evaluated by LMS, and the astrocytic cytoplasm and mitochondria were not damaged, as judged from EM observation. Also the end-feet were swollen and showed an increased number of mitochondria and glycogen granules at 5 hours postischemia. During 12 to 48 hours postischemia, they gradually decreased in thickness. It has been thought that end-feet are bound together to form a contiguous coat that is imposed between the nerve fibers and the endothelial cells, contributing to the glia-limitans of the blood-brain barrier. As breakdown of the blood-brain-barrier occurs only in the infarction, not in RPI, extravasation of the plasma protein-bound Evans blue and plasma fluid in the extracellular space was not found in the previous6 or present study. Detachment and rupture of the end-feet from blood vessels were not detected, either.

However, it is obvious that astrocytes are also damaged by ischemia and that their dysfunction can lead to neuronal death or dysfunction.2–4,18 We could not find any literature on ultrastructural studies on APs in relation to the dying neurons in the RPI. Therefore, we focused on the ultrastructural changes in the APs in the neuropil and correlated them with the maturation of neuronal injury and DSNN.

Chronological and comparative ultrastructural observations of APs suggested that the degenerated APs first appeared around the degenerated/dead neurons, spread in the neuropil by their shrinkage, and surrounded astrocytes at 48 hours postischemia (Figure 7). The number of the astrocytic mitochondria increased at 48 hours, partly because of proliferation in loco and partly to those coming from the degenerated and shrinking APs and enlarging the astrocytic cytoplasm. Some of the astrocytes had become globular, losing their APs and extending 1 foot-process to or attaching their processes to the dying neurons. Some of the astrocytes had become globular, losing their APs and extending 1 foot-process to or attaching their processes to the dying neurons. As breakdown of the blood-brain-barrier occurs only in the infarction, not in RPI, extravasation of the plasma protein-bound Evans blue and plasma fluid in the extracellular space was not found in the previous6 or present study. Detachment and rupture of the end-feet from blood vessels were not detected, either.

Figure 6. A, Percent-volume of APs around the astrocytes, comparison at 5 and 48 hours postischemia: “+”*P<0.05 vs 5 hours. B, Number of cut-ends of APs around the astrocytes, comparison at 5 hours and 48 hours postischemia: “+”*P<0.05 vs 5 hours. C, Percent-volume of APs, comparison among the APs around the normal-appearing, degenerated, and dead neurons, at 12 hours postischemia: “P<0.05 vs normal-appearing, degenerated; “+P<0.05 vs normal-appearing, dead; “#P<0.05 vs degenerated, dead. D, Number of cut-ends of APs, comparison among the APs around the normal-appearing, degenerated, and dead neurons, at 12 hours postischemia: “P<0.05 vs normal-appearing, dead; “+P<0.05 vs normal-appearing, dead; “#P<0.05 vs degenerated, dead. E, Mitochondrial number of APs, comparison among the APs around the normal-appearing, degenerated, and dead neurons, at 12 hours postischemia: “P<0.05 vs degenerated; “+P<0.05 vs normal-appearing, dead. “#P<0.05 vs degenerated, dead.
After temporary ischemia, all neurons in the RPI are still normal-appearing but in a necro-biotic state induced by neuronal hyper-excitation. The heterogeneous degeneration of the APs around the neurons of various degrees of viability induces further neuronal injuries and death by the secondary decrease in the energy supply to the necro-biotic neurons and by derangement of the glutamate-glutamine cycle and ion-homeostasis.1–3,18 This heterogeneous degeneration of APs was tightly associated with DSNN and the maturation phenomenon of neuronal injury (Figure 7). The APs attached to the degenerated neurons showed remarkable swelling,8,9 but normal-appearing mitochondria and glycogen granules were still preserved in these APs. No necrotic hallmarks were observed in the slightly darkened degenerated neurons with slight nuclear chromatin condensations. These neurons may have later recovered during the maturation period.2,16

These degenerated APs indicated their dysfunction and ultrastructural changes induced by (1) impaired metabolic trafficking of the fuel between APs and neurons,1–3 (2) a metabolic shift from aerobic to anaerobic with an increase in lactate production and a pathological decrease in pH,1–3,5,7,19 (3) a deranged glutamate-glutamine cycle,3,18 (4) potassium ions produced by neuronal hyper-excitation,1 and (5) a disrupted Donnan equilibrium attributable to injury to the energy-consuming ion-pump at the injured cell membrane of APs.1,20

In the present study, the degeneration and decrease in the number of APs coincided well with those of mitochondria in APs. Oxidative stress and accumulation of excessive Ca2+ after an ischemic insult induces opening of mitochondrial permeability transition (MPT) pore in the mitochondrial inner membrane, which induces the release of cytochrome-c with edematous intermembranous matrix through the ruptured outer membrane and inhibits oxidative respiration. This induces a metabolic shift from aerobic to anaerobic and excessive lacto-acidosis in APs and also inhibits metabolic trafficking between astrocyte and neurons. Cyclosporin A (Cs A) can protect mitochondria in astrocytes, but not those in neurons, from a Ca2+ insult and inhibit the opening of the MPT pore.1,2,5,7 Acetyl-L-carnitine (ALCAR) improves post-ischemic neurological outcome by reducing lactate levels and...
oxidative stress, acting on formation acetyl-CoA in the mitochondria of APs.1,2,5,7

The development of a new therapy to ameliorate the deranged interaction between neurons and surrounding/attached APs and in turn promote the maturation toward neuronal recovery so as to decrease the progression of DSNN and minimize neurological impairment in wide cerebral cortical regions peripheral to the cortical infarction after ischemia, especially in the case of carotid occlusion, is mandatory.

Conclusion

With a constant astrocytic density from 5 to 48 hours postischemia, the number of dead neurons increased in concert with a decrease in the numbers of normal-appearing APs and their mitochondria in the neuropil. Also, the numbers of normal-appearing APs and their mitochondria decreased, and the number of swollen degenerated ones increased, around the degenerated and dead neurons. Heterogeneous degeneration of APs of the normal-appearing astrocytes in the RPI is tightly associated with DSNN and the maturation phenomenon.

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Disclosures

None.

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Umeo Ito, Yoji Hakamata, Emiko Kawakami and Kiyomitsu Oyanagi

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