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Pathophysiology of Cerebral Ischemia

Nilton B. A. Junior, Ricardo J. Del Carlo, Lukiya S. C. Favarato, Evandro S. Favarato, Vanessa G. Pereira, Aline R. Murta, Daise N. Q. da Cunha*

Veterinary Department, Universidade Federal de Viçosa, Av. PH Rolfs, zip code: 36570-000, Viçosa, MG, Brazil

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Corresponding Author:
Daise N. Q. Cunha
daisenunes@gmail.com

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ABSTRACT

Cerebrovascular accident (CVA) is the sudden interruption or decrease of blood supply (oxygen and glucose) to the brain resulting in cerebral infarction, permanent neurological damage, severe functional limitations and death. Stroke is the second most common cause of death worldwide and the leading cause in Brazil. The risk factors for CVA include systemic arterial hypertension and other vascular diseases, diabetes mellitus, sedentarism, dyslipidemia, and smoking. These risk factors are at high prevalence, globaly, increasing the prospects for new incidents of the disease. Currently, the treatment options for CVA are limited, partially because many promising medicines presented intolerable side effects or limited therapeutic effects in the clinical trials. In the acute and subacute phases of the CVA the therapeutic goals are to protect the neurons at risk, increase the endogenous capacity of the central nervous system (CNS) to regenerate itself, and diminish functional sequelae. The knowledge regarding the role of the molecular mechanisms underlying CVA is the key for new therapeutic discoveries aiming at neuroprotection, neuroregeneration and neurogenesis.

Key words: Cerebral Ischemia, Primary Brain Lesion, Secondary Brain Lesion.

INTRODUCTION

Currently, the ischemic cerebrovascular accident (ICVA) is the leading cause of death in Brazil (Camargo *et al*., 2005; Minelli *et al.*, 2007), the second most common cause of death globally, and the major cause of adult disability in the United States (Feigin, 2005). Among the risk factors are systemic arterial hypertension and other cardiovascular diseases, diabetes mellitus, sedentarism, dyslipidemia, and smoking, increasing the prospects for new incidents of the disease (Campos-Souza *et al*., 2007). The encephalic lesion extends to adjacent areas where the reperfusion mechanisms are trying to repair the damage, hence diminishing subsequent sequelae (Dinargl *et al*., 1999).

Recently, many treatment strategies are being proposed, and despite the improvement of patients suffering from acute neurological damage, there are no specific treatments for CVA capable of preventing the progress of the cerebral disorder, ultimately resulting in permanent neurological damage, severe functional limitations, coma and death (Hankey, 1999; Muntner *et al.*, 2002). Therefore, the knowledge and understanding of the physiopathological mechanisms in cerebral ischemia are essential to create new strategies for tissue repair and neuroprotection. The goal of this review is to present the cellular events triggered in the CNS after cerebral ischemia.

Pathogenesis of cerebral ischemia

OICVA is caused by the interruption of the blood supply, in a particular arterial branch, by thromboembolic or hemodynamics mechanisms, causing metabolic imbalance (high demand versus low supply of oxygen and glucose) in the brain ultimately determining cell death (Feigin, 2005).

At the cellular level, the reduction of cerebral blood flow (CBF) and subsequent oxygen depletion trigger biochemical events resulting in phosphorylation and anaerobic metabolism. The anaerobic glycolysis is insufficient and determines the depletion of phosphate reservoir, including ATP accumulating lactic acid (Wyatt *et. al*., 1989), calcium (Ca+), and water. Further, the cell membrane depolarize and excitatory neurotransmitters are released, particularly glutamate in the axonal endings. Meanwhile, in the cytoplasm, will occur accumulation of free fatty acids, arising from the disintegration of the phospholipidic membrane undergone oxygen peroxidation, free radicals which in turn promotes the formation of more free radicals inside the mitochondria, with the aid of prostaglandins, xanthine and uric acid, and finally in some neurons Ca+ will induce nitric oxide (NO) production (Shalak and Perlman, 2004).

The effects from disruption of cellular energy balance, Ca+ accumulation, lipid peroxidation, acidosis, glutamate release, intense production of free radicals, and NO neurotoxicity culminate in cell death (Figure 1).

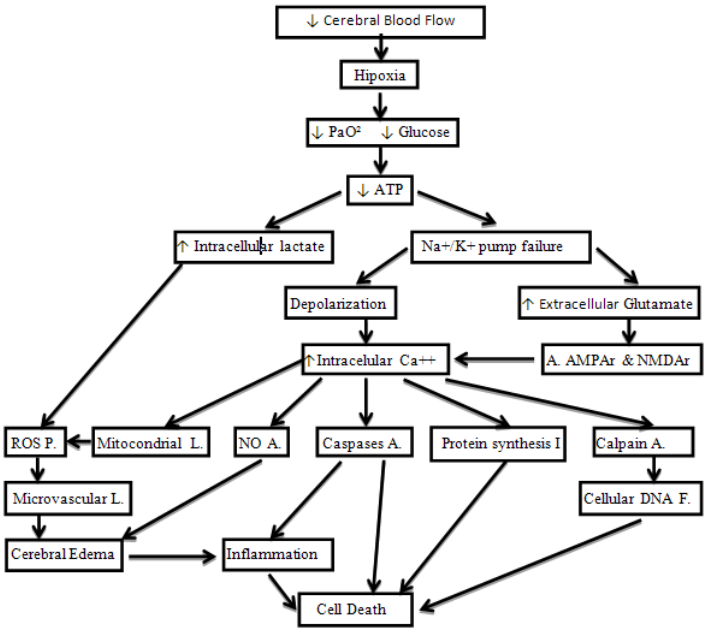

Figura 1. Physiopathological evolution of cerebral ischemia. (P) Production. (L) Lesion. (A) Activation. (I) Interruption. (F) Fragmentation. (ROS) Reactive oxygen species. (NO) Nitric oxide. (DNA) Deoxyribonucleic acid (Modified from Shalak and Perlman, 2004).

Secondary cerebral lesion

Neuropathological analysis reveals that brain ischemia leads to two distinct areas of ischemia: the core zone which is an area of severe ischemia, and the penumbra zone, the term used to describe ischemic, but still viable cerebral tissue. In the central zone, severe reductions of blood supply to the brain causes metabolic collapse, reduction of cell energy and ionic homeostasis, subsequent loss of cellular integrity resulting in cell death in a few minutes. Meanwhile, in the penumbra zone there will be hypoperfusion, i.e., low blood flow compensated by collateral arteries anastomosing with branches of the occluded vascular tree resulting in neurophysiological functional losses, but the cellular metabolism and structure will remain preserved (Sharp *et al*., 2000 and Hossmann, 2009). An ischemic event is dynamic (Figure 2).

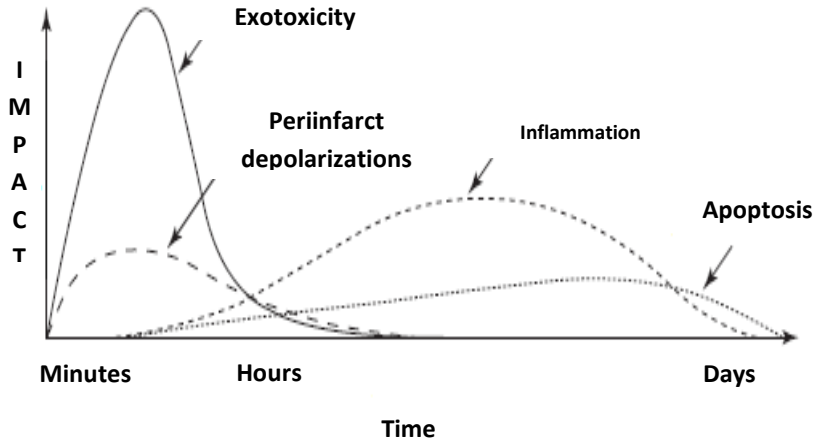

Figure 2. Cascade of harmful events in focal cerebral ischemia. Minutes after deficits in focal perfusion, exotoxic mechanisms lead to lethal damage to neurons and glial cells. In addition, exotoxicity triggers a series of events that contribute to enhancing tissue injury. Such events include periinfarct depolarization and mechanisms that promote inflammation and apoptosis. The x-axis reflects the evolution of the cascade over time, while the Y axis shows the impact of each event in the cascade of neuronal death.

While blood supply is low, but sufficient to attend the demands of the ionic channels electrical activity the cerebral tissue remains alive. Paradoxally, reperfusion initiates an inflammatory cascade with free radicals, released from dead cells; worsening the ischemic injury.

It is estimated that initially, the area of penumbra corresponds to 50% of the tissue that later will progress into infarct (Dinargl *et al*., 1999). The understanding of these mechanisms in the penumbra zone is essential to preserve function and promote the survival of nervous cells after reperfusion, and could potentially be an area of new discoveries of more effective treatments.

Glutamate release

Astrocytes regulate neuronal excitability and synaptic activity by releasing gliotransmitters such as glutamate, which is the most important excitatory neurotransmitter of the CNS. The neurons are only exposed to small amounts of these neurotransmitters because of efficient mechanisms of removal and absorption responsible to free the neurons from its toxic effects (Kostandy, 2012). During cerebral ischemia, blood flow is severely diminished along with adenosine triphosphate (ATP) resulting in energy failure and collapse of the ATP-dependent

cell metabolism, such as ionic channels (Kimelberg and Mongin, 1998). Depolarization of the cell membrane, stimulated by Ca^{++} entry, release the glutamate retained in the intracellular vesicles, which is then eliminated by exocytose. The extracellular glutaminase released by the injured neurons increases the hydrolysis of glutamine producing extracellular glutamate (Mena *et al*., 2000).

The disruption of the electrochemical gradient of the astrocytes causes the glutamate transporters to operate in the reverse direction leading to excess extracellular glutamate. The accumulation of extracellular glutamate stimulates the neuronal receptors *N*-Methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAr) and kainite. These receptors are linked to ionic channels in the membrane and determine the influx of Na^{+} and Ca^{++} together with water resulting in cytotoxic edema (Furukawa, 1997).

Activation of the NMDAr is sufficient to destroy the majority of the neurons, being preponderant in the mediation of at least some of the glutamate neurotoxicity, because allows Ca^{++} influx and its entry determines mitochondrial dysfunction, caspase-3 activation through the action of calpain, and production of NO and ROS leading to neuronal death (Dobrek and Thor, 2011).

Intracellular Calcium accumulation

The intracelular Ca^{++}, in low concentrations, participate in many cytoplasmic reactions. During an ischemic injury Ca^{++} will influx the cells of nervous tissues through NMDA receptors stimuli, a Ca^{++} agonist regulated by glutamate, and the Ca^{++} efflux is paralyzed. Calcium is also released by the mitochondria and sarcoplasmic reticulum. These alterations, resulting in the increase of intracellular Ca^{++} interfere with many enzymatic reactions including the activation of lipases, proteases, endonucleases and phospholipases, and in the formation of ROS derived from xanthine and prostaglandins synthesis. The accumulation of cytoplasmic Ca^{++}, after ischemia, determines irreversible cerebral injury (Grow and Barks, 2002).

Increase of free radicals

The nervous tissue is too vulnerable to the action of free radicals because holds some peculiarities such as being rich in lipids and unsaturated fatty acids which may react with ROS to form peroxyl radicals that determine the lipid oxidation of the membrane of neurons (Porter, 1984). Additionally, the brain has low to moderate catalase, and glutathione activity which eliminate the hydrogen peroxidase (H^2O^2) reducing oxidation (Cooper and Kristal, 1997), and has elevated metabolic activity using up to 20% of the consumed O_2 by the body, although the brain constitutes only 2% of the total body mass. The combination of these factors makes the CNS totally vulnerable to oxidative damage (Dringen, 2000).

Reactive Oxygen Species

Reactive oxygen species (ROS) are molecules of short life span originated as part of the normal cell metabolism and defense system. At low levels they play a role in the regulation of cell growth, differentiation, proliferation and apoptosis through actions in different receptors, genes, ionic channels, enzymes, proteins and nuclear transcription factors (Poli *et al*., 2004; Liu *et al*., 2005). In cerebral ischemia the production of ROS is increased, but in reperfusion its production is accelerated because of cytotoxic events stemming from lipid peroxidation, protein oxidation and fragmentation of deoxyribonucleic acid (DNA) (Crack and Taylor, 2005). The ROS are produced through the reduction of molecular oxygen (O_2) to form

superoxide (O_2^-) mediated by NADPH oxidase, xanthine oxidase or mitochondrial electron transport chain (Droge, 2002). There is a balance between the production of ROS and antioxidant defense system which is essential for normal metabolism. In this balance, the cells produce superoxide dismutases (SODs) that convert the superperoxide to hydrogen peroxide (H_2O_2) and oxygen, and the catalase and glutathione peroxidase convert H_2O_2 to water. In the presence of transition metals, may produce the radical hydroxyl (OH^-). The accumulation of ROS, due to excessive production or depletion of antioxidant enzymes, generates a synergic cascade activating signals that could lead to cell injury. This damage results from DNA alteration, lipid peroxidation, protein oxidation and rupture of structural functions and cellular integrity, such as ability of molecular transportation, energy production, and ionic balance (Olmez and Ozyurt, 2012).

Nitric Oxide

The free radical, nitric oxide, is synthesised from l-arginine by the enzyme NO synthase (NOS) to l-citrulline. The NOS is heavily activated during ischemia, as well as during reperfusion, and its production lasts long periods of time in the neurons, glial and endothelial cells. The nitric oxide has neuroprotective and neurotoxic properties. The activation of NOS during the ischemia of neurons causes the death of neurons by combining with superoxide yielding peroxynitrite, a potent radical that activates lipid peroxidation and increases glutamate release. The activation of NOS in the endothelial cells is neuroprotective because NO production acts relaxing the adjacent smooth muscle cells leading to vasodilation and increase of blood flow in the affected cerebral region. Conversely, the NO production, in the astrocytes, occurs during ischemia and has uncertain effects, but it is believed that in excess can be both neurotoxic and neuroprotective (Bolaños and Almeida, 1999).

During reperfusion, the O_2 concentration may exceed the mitochondrial ability to reduce O_2 to H_2O, and therefore the production of superoxide anion (O_2^-) can be increased. The superoxide reacts with the ON and form peroxynitrite ($ONOO^-$), which is an irreversible inhibitor of mitochondrial function in addition to being a pro-oxidant that damages lipids, proteins and DNA, determining neuronal cell death (Radi *et al*., 1991).

Elevation of free iron

Iron (Fe) is essential to most living things because of its unique property of reverse cyclic oxidation and reduction. Easily donate and receive electrons, alternating between Fe_3^+ and Fe_2^+ ions and therefore is considered to be a source of free radicals, particularly the hydroxyl radical (OH^-). Iron is also an enzymatic co-factor and as such is a key participant in the mediated oxygen toxicity, being involved in the generation O_2 e H_2O_2, through the acceleration of the non-enzymatic oxidation of many molecules, including adrenaline and glutathione (Gutteridge and Halliwell, 2000).

The majority of the iron present in the body is found in the hemoglobin, nevertheless inside the cells, the ferritin is the main reservoir, in the form of iron oxide, and is utilized to synthetize cofactors in the respiration and DNA synthesis. The release of iron from the ferritin requires reduction from ferric to ferrous form in the serum by an chelating agent. The role of ferritin is controversial since it acts both as a neuroprotector, since in ischemia it is capable of chelating the iron released from proteins, but also ferritin donate free Fe which increases the oxidative stress contributing to a larger neurological deterioration. The ability to damage cerebral tissue is linked to increased systemic reservoir of iron, because when elevated the prognosis is worse in the initial phases of cerebral ischemia, increasing the oxidative stress and leading to necrosis of parts of the ischemic penumbra (Carbonell e Rama, 2007).

Inflammatory mediators

In ischemia, after the collapse of the blood-brain barrier (BBB) the membrane permeability changes and intensifies the leucocyte extravasation, and the production of ROS from damaged cells, triggers oxidative stress and inflammatory cascade. Endogenous molecules, known as damage-associated molecular patterns (DAMPs) are released from the injured tissue and activate the immune system infiltrated cells. In the ischemic brain, Heat shock proteins, β-amyloid (Aβ), hyaluronan, heparin sulfate, dNa or RNa immune complexes, oxidized low-density lipoproteins, and several other molecules, have been considered as possible DAMPs. Among them, high mobility group box 1 (hmGB1) is a well characterized damp in ischemic brain injury that increases Blood-Brain- Barrier (BBB) permeability or promote its breakdown. HMGB1, is localized in cell nuclei in the normal brain, translocates to the cytoplasm increasing the vascular permeability and promoting the disaggregation of BBB during ischemia (Zhang *et al*., 2011).

The DAMPs stimulate Toll-like receptors (TLRs), TLR2 and TRL4, involved in the innate immune-mediated response to non-infectious injury, including ischemic brain injury (Shichita *et al*., 2012). Several inflammatory cytokines and mediators are produced. Among them, IL-β signal or activates other molecules such as capase-1, a protein known to participate in the inflammasome complex and is present in the neurons, glial cells, microglia, and macrophages, which can be activated with hypoxia, ATP reduction, or through endogenous molecules produced by injured cells (Shichita *et al*., 2012). IL-1β is considered to be a neurotoxic mediator that promotes neuronal cell death and increases the chemokine in the microglia and astrocytes, and its inhibition is associated with reduction of ischemic lesion (Allan *et al*., 2005). The TNFα is also involved in ischemia; it is expressed in the cerebral tissue during hypoxemia within 1 hour after reperfusion and causes neuronal cell death through neurotoxic effects, but also, may be considered to a neuroprotector mediator because it participates in the mechanisms of suppression of inflammatory signs (Hallenbeck, 2002). The IL-6 is important in many types of inflammation, but in cerebral ischemia acts as a neuroprotective cytokine contributing to angiogenesis, favoring the survival of nervous cells (Jung *et al.*, 2011).

Chemokines are also important potentiating of inflammation post ischemia. The monocyte chemotactic protein-1 and IL-8 act in the leucocytes infiltration and increases the area of ischemic injury (Shichita, *et al.*, 2012). The intercellular adhesion molecule 1 (ICAM-1), produced by endothelial cells, is essential for chemotaxia and for leucocyte infiltration, it is elevated in cerebral ischemia favoring the augmentation of the inflammatory processes. The matrix metalloproteinases (MMPs) are important mediators of the inflammation post ischemia and intensifies the permeability of the BBB. The MMP-9 has neurotoxic action (Shichita, 2012).

The T-cells produced cytokines act in the inflammatory regulation during ischemic lesions. The IFN-γ is neurotoxic and acts in the neurons inducing their death. The release of IL-4 e IL-5 induce the production of neurotrophic factors by the astrocytes, which has a neuroprotetive signaling role through inhibition on the expression of cytokines induced by ONS (Butovsky et al, 2005). The IL-10 is an immune suppressor and exert a neuroprotective effect by suppressing the neurotoxic actions of TNF-α e IFN-γ (Liesz *et al*., 2009).

Cell Death

The neuronal cell death induced by ischemic injury has been, traditionally, characterized as necrosis. The high indices of extracellular glutamate stimulate the influx of Na^{+} e Ca^{++} through the NMDAr, AMPAr and kainite receptors and caring water with it to the inside of the cell resulting in cytotoxic edema (Furukawa, 1997). In necrosis there is edema, rupture of cytoplasmic organelles, loss of the membrane integrity, and lysis of the neuronal cells activating the inflammatory process (Shalak and Perlman, 2004). However, in models of

cerebral ischemic injury, there are morphological and biochemical evidences that the cell death occurs through apoptosis. Apoptotic neurons are more easily detected in the penumbra from the beginning of the ischemic lesion and during the reperfusion period and probably those nerve cells that maintains a minimum level of metabolic activity (Yuan and Yankner, 2000). The apoptosis, programmed cell death, is characterized by capases activation through extrinsic or mitochondrial via. The extrinsic via is induced by the activation of the death receptor on the surface of the cell FAS (FasR), also known also known as apoptosis antigen 1 (APO-1 or APT). The oligomerization of death receptors recruit adaptive molecules involved in caspase-8 activation. The intrinsic or mitochondrial activation, in ischemia, is initiated by high levels of glutamate, intracellular calcium, reactive oxygen species (ROS), and DNA damage. When the mitochondria receives proper apoptotic signaling or suffers an irreversible damage, pro-apoptotic molecules such as cytochrome C are released to the cytoplasm. Accompanied by the ATP, the cytochrome C forms the complex apoptotic protease activating factor 1 (Apaf-1), also known as apoptosome. This cleaves the procaspase-9, which in turn releases caspase-9. The caspases 8 and 9 activate, among others, the caspase-3 which cleaves the amyloid precursor protein (APP), resulting in increased production of the amyloid ß-peptide, which in turn increases caspase-3 activation, initiating the cell death by apoptosis (Carbonell and Rama, 2007).

CONCLUSION

At the cellular level, the reduction CBF and subsequent oxygen depletion trigger biochemical events responsible for the primary and secondary lesions that will overtake the CNS and perpetrate morphofunctional losses. An ischemic event is dynamic and the knowledge of the physiopathological mechanisms underlying the cerebral ischemia is essential to discover new pathways to re-establish the microenvironment and conditions needed for damaged brain neurons to inhibit intrinsic undesirable programmed processes of cell death, and thus offer more definite neuroprotection.

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