Ischemic Cerebral Process: Risk Factors, Angiogenesis and Neuroprotection

Processo Cerebral Isquêmico: Fatores de Riscos, Angiogênese e Neuroproteção

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ABSTRACT

Stroke is considered the main cause of incapacities in adults, and the third cause of death around the world; besides, many factors has been associated with this elevated prevalence. It results from an acute occlusion in a cerebral blood vessel, triggering hypoxia and ischemia in nerve tissue, which causes different kinds of lesions and responses when triggers a complex sequence of events that results in cell death. Otherwise, brain develops mechanisms that trigger neuronal resistence, inhibits cell death, and also promoves recuperation on injured tissue mediated by growth factors such as vascular endothelial growth factor (VEGF) and erythropoietin. Then, these interventions on nervous tissue are responsable by angiogenesis and neuroprotection, minimizing damage on brain.

Keywords: ischemia cerebral, neuroprotection, angiogenesis.

RESUMO

O acidente vascular encefálico (AVC) é considerado a principal causa de incapacidades em adultos e a terceira maior causa de mortes em todo o mundo, outrora, diversos fatores têm sido atualmente associados à elevada incidência desta patologia. O AVC isquêmico resulta de uma oclusão aguda em vasos sanguíneos cerebrais, desencadeando hipóxia e isquemia no tecido nervoso e levando conseqüentemente a diferentes tipos de lesões e respostas ao iniciar uma seqüência complexa de eventos que resulta em morte celular. Em contrapartida, o cérebro desenvolve mecanismos que desencadeiam resistência neuronal, inibem a morte celular e ainda promovem a recuperação do tecido lesado. Esses processos são mediados por vários fatores, tais como o fator de crescimento vascular endotelial (VEGF) e a eritropoietina, os quais por diversos mecanismos promovem a angiogênese e a neuroproteção, minimizando sobre maneira os danos ao tecido cerebral.

Unitermos: isquemia cerebral, angiogênese e neuroproteção.

1. ISCHIMIC CEREBRAL PROCESS (ISCHEMIC STROKE)

1.1 Definition, epidemiology and classification

The World Health Organization (WHO) defines stroke as a clinical syndrome typified by rapidly developing signs of focal or global disturbance of cerebral functions, lasting more than 24 hours or leading to death, without another apparent causes than of vascular origin. It is the third most common cause of death in developed countries, exceeded only by coronary heart disease and cancer, with 5 million people dying every year[1]. Projections to the year 2020 indicate that the number of people suffering from cerebrovascular disease each year will increase substantially, and that the majority of these will be in developing countries[2].

More often disabling than fatal, stroke is the leading cause of severe neurologic disability and results in enormous costs measured in both health-care dollars and lost productivity. Annually, 15 million people worldwide suffer a stroke. Of these, 5 million die and another 5 million are left permanently disabled. Also, stroke is the second leading cause of death above the age of 60 years, and the fifth leading cause in people aged 15 to 59 years old, but it is uncommon in people under 40 years and when it does occur, the main cause is high blood pressure [1].

In addition to duration, stroke can be classified as ischemic or hemorrhagic based on the type of pathologic injury. Ischemic strokes can be further classified as thrombotic or embolic in origin. Overall, ischemic stroke is 3 to 4 times as frequent as hemorrhagic stroke, accounting for 70% to 88% of all strokes[3]. Embolic strokes account for about 20% of ischemic strokes[4], but its frequency depend on the sample from which cases are drawn, the geographic region of the study, and the design of investigator-driven diagnostic algorithms.

1.2 Risk Factors

Some factors have been associated with increase incidence of ischemic stroke (Table 1), but age is the strongest risk factor[5]. Less than 15 per cent of stroke patients are aged less than 45, and at least two-thirds are over 60[6], moreover, the risk of stroke doubles in each successive decade after 55 of age[7,8].

Stroke incidence rates are generally higher in men than women[7], however, stroke-related case-fatality rates are higher in women than men[9] with the female sex accounting for about 61.5% of stroke fatalities in the United States in 2002 [3]. Circumstances such as oral contraceptive use and pregnancy uniquely contribute to the risk of stroke in women[10,11,12], but the fact that women have a lower incidence of transient ischaemic accident and of stroke after transient ischaemic attack compared with men may contribute to a lower incidence of stroke in female sex[13].

Otherwise, both maternal and paternal history of stroke may be associates with increase stroke risk[14,15]. This increased risk could be mediated through a variety of mechanisms, including genetic heritability, familial sharing of cultural/environmental and lifestyle factors, and the interaction between them[16,17]. One study using offspring of the original Framingham Study cohort members showed that both paternal and maternal histories of stroke or transient ischaemic attack were associated with an increased relative risk of 2.4 and 1.4, respectively[18].

Smoking is also a strong risk factor for ischemic stroke. Pathophysiological effects of smoking are multifactorial, affecting both the systemic vasculature and blood vessel distensibility and compliance by leading to increase arterial wall stiffness[19]. Also, smoking increase hematocrit, platelet aggregation and fibrinogen levels and decrease high-density lipoprotein level[20]. The relative risk of stroke among formers smokers (compared with nonsmokers) was 1,34 and 1,26 according to Nurses' Health Study[21] and Physicians Health Study, respectively[22]; besides, a prospective estimate from Framingham Heart Study showed an increase of 1.8-fold in stroke risk associated with smoking[23]. Otherwise, ex-cigarette smokers have a sustained excess risk of stroke for some years[24, 25]. Data from Framingham Study displayed stroke risk to be at the level of nonsmokers at 5 years from cessation[26]

Hypertension is the major treatable risk factor for both cerebral infarctation and intracerebral hemorrhage[27]. Most prospective studies observed increased incidence of stroke with increased blood pressure, both systolic and diastolic, without threshold[28]. The relashionship is direct, continuous and apparently independent[29]. In the Framingham Study, the relative risk of stroke in hypertensive patients was 3.1 in men, 2.9 in women, and 1.5 in patients with borderline hypertension[30]. In healthy populations, in both sexes and allowing for the association with age, increasing blood pressure is strongly associated with all the main pathological types of stroke[31, 32]. A meta analysis of 18 long-term randomized trials that both beta-blocker therapy (relative risk 0.71; 95% CI 0.59 to 0.86) ant treatment with high-dose diuretics (relative risk 0.49; 95% CI 0.39 to 0.62) were effective in preventing stroke[33]. In addition to this, the randomized Syst-Eur Trial of 4695 patients with isolated systolic hypertension aimed to lowering the systolic blood presure 20 mmHg, reached 42% of stroke reduction in the actively treated group compared to the group treated with placebo[34].

Sickle Cell Disease (SCD) is a genetic disorder autosomal dominant with clinical manifestations variable that usually manifests early in life as a severe hemolytic anemia. Stroke prevention is most important for patients with homozygous SS disease because prevalence of stroke by age 20 in these patients is at least 11%[35] and a substantial number of patients also have "silent" strokes on brain[36]. The highest stroke rates occur in early childhood with risk about 1% per year, but patients with transcranial Doppler evidence of high cerebral blood flow velocity rates have stroke rates in excess of 10% per year[37].

Insulin-dependent diabetics have both an increased susceptibility to atherosclerosis and an increased prevalence of atherogenic risk factors, notably hypertension, obesity and abnormal blood lipids. Glucose intolerance raises the risk of thromboembolic but not haemorrhagic stroke. In USA, among Hawaiian Japanese men in the Honolulu Heart program relative risks of thromboembolic stroke were 2.5 for patients with diabetes and 1.4 for subjects with high plasma glucose levels without a known history of diabetes, after adjustment for other risk factors[38]. In the Framinghan Heart Study the impact of glucose intolerance in brain infactation was greater in women than men, reaching significance as an independent contributer only in older women, but overall persons with glucose intolerance had double the risk of brain infarctation compared with nondiabetics[39].

About ischemic stroke, it remains yet equivocal whether hypercholesterolaemia is a risk factor. No clear influence of total cholesterol on atherothrombotic brain infarction was observed in the 36-year follow-up data in the Framingham Study[30], but the Honolulu Heart Program demonstrated a relative risk of 1.6 of elevated serum cholesterol for thromboembolic stroke in Japanese Hawaiian men aged 60 to 74[40].

The most frequent potential cardiac source of embolism to the brain is atrial fibrillation (AF), by virtue of clot forming in the left atrium and its appendage[41]. Embolism of cardiac origin accounts for about 20% of ischemic strokes[42]. More often the embolis origin in the left atrium due to an atrial fibrillation[43], but occasionally they can arise from left ventricle as a consequence of an acute miocadic infarct[44, 45, 46] or from deep vein thrombosis in the legs[47]. In the very elderly AF is the single most important single cause of stroke[48] and the most important risk factor for first-ever stroke in older people[49] due to an increase incidence of AF with age: 5% in people older than 65 years and 9% in those aged 80 or more[50]. AF is present in about 20% of all ischaemic stroke patients who, anyway, may have some other, possibly more likely, cause of stroke, such as carotid stenosis or intracranial small vessel disease[51,52, 53].

Although modest consumption of alcohol might even be protective for ischemic stroke[54, 55] it is difficult to disentangle any causal pathway from alcohol consumption to stroke, because alcohol almost certainly raises blood pressure[56, 57], affects blood lipids[58], and can cause atrial fibrillation and cardiomyopathy.

Epidemiologic evidence, animal studies, angiographic and ultrasound studies in humans, and a limited number of clinical trials suggest that comsumption of a diet rich in food with vitamin E, vitamin C, beta-carotene and flavonoids have all been proposed as protective vascular factors since they are antioxidants and protect the arterial intima from oxidative damage to DNA and lipoproteins. However, these hypotheses require testing before widespread use of supplementary vitamins can be generally recommended[59]. Besides, incresed Na intake is associated with hypertension, and reduction in salt consumption may significantly lower blood pressure and may reduce stroke mortality. Moderately elevated homocysteine levels may be associated with stroke and are associated with deficiency of dietary intake of vitamins B6 and B12 and folate. There is also evidence that a low serum albumin may be causally linked to stroke risk and outcome and that a significant number of stroke patients are undernourished on admission and their nutritional status deteriorates further whilst in hospital (60).

Finally, other factors can contribute with increase incidence of ischemic stroke as race/ ethnicity[60, 61], carotid stenosis[62], obesity[63], physical inactivity[64], hyperhomocysteinemia[65], drug abuse[66], hormone replacement therapy[67] and inflammatory processes[68].

1.3 Pathophysiology of stroke

An acute obstruction in medium cerebral artery (MCA) can produce an immediate reduction of the cerebral flow on a correspondent irrigation area (focal ischemia). Following typical embolic vascular occlusion in humans, occurs a spontaneous thrombolysis and a spontaneous recanalization, but at variable times following initial occlusion. Angiographic controlled studies in humans have shown that spontaneous recanalization can occur around 17% of the time within the first 6 to 8 hours of stroke and that approximately half of the vessels will reopen in 3 to 4 days[69].

Consequences of a cerebral ischemic are dependent of the seriousness and duration of blood flow reduction. Brain injury and neuronal death necessitate at least 1 to 2 minutes of focal vascular occlusion, so a less serious, but prolonged ischemia can produce damages as a short and serious ischemia[70]. In animal models, the pattern of the ischemic process is performed by a greatly reduced blood flow in the central region of infarct (“core”) and in a graded fashion centrifugally from the core (“penumbra”). In the area of core, cerebral blood flow decreases to less than 15% of baseline, which leads to reductions in adenosine triphosphate (ATP) levels to 25% of baseline. And in penumbral regions, cerebral blood flow decreases to between 15% and 40% of baseline, with ATP levels decreasing to between 50% and 70% of control within minutes of vessel occlusion[71].

Within minutes of vascular occlusion, brain tissue is deprived of glucose and oxygen, and the acidic by products of metabolism accumulate. This loss of substrate and decrease in pH level leads to cessation of the electron transport chain activity within mitochondria, which results in a rapid decline in ATP concentration. Loss of ATP leads to failure of the Na^+,K^+-ATPase, which results in a marked intracellular increase in intracellular Na^+ concentration. Persistent depolarization allows Ca^{2+} entry, and the resulting influx of Ca^{2+} damages the mitochondria, which further exacerbates energy failure[72,73,74]. Increased Ca^{2+} also induces nitric oxide synthase activity and expression, which favors the formation of peroxynitrate, a highly reactive free radical species[71].

Cells die by means of two major methods: necrosis and apoptosis. Necrotic cell death is an energy-passive process independent of protein synthesis that is characterized by loss of cellular architecture and ultimately culminates in cytoskeletal breakdown, with edema formation within 12 to 24 hours of ischemia.[71, 75]. The morphologic features of apoptotic cell death are quite different, with DNA laddering and regular clumping of chromatin (apoptotic bodies). This is followed by a stereotypical loss of cellular architecture (which takes several days) that involves the activity of caspases (family of cysteine proteases) and other enzyme systems[71,76].

Experimental focal cerebral ischemia has demonstrated gene induction and proapoptotic factors, as tumoral necrosis factor (Fas and Apo-2L)[77], TR3 receptor[78], [kappa]B nuclear-factor[79] and apoptosis-linked gene 2[80]; besides, there are antiapoptotic factors detected, like tumoral growth factor $[beta]_1$ (TGF-$[beta]_1$)[81], Bcl-omega protein[82], transforming growtn factor-[alpha] (TGF-[alpha])[83] and erythropoietin[84].

During cerebral ischemia, microglial cells, a group of cells with a mesodermal origen and derived from monocytes which migrated while embryologic precocious phase, has a faster activation than astrocytes and participate in inflammation process and system nervous repairing in adult. These cells has a function of a fagocyte, and liberate elastase, free radicals and proinflammatory or antiinflammatory interleukines (IL-1, IL-3, IL-5 and IL-6), neuronal growth factor, transformation and growth factor and tumoral necrosis factor[75,76].

Finally, ischemia also damages the brain's capillaries and endothelium and incites an inflammatory response whereby leucocytes infiltrate regions of infarct. The contribution of white blood cells to the process of secondary damage is controversial but white blood cells (chiefly neutrophils) appear within the infarct within 24 hours, at the appropriate time to cause damage[85].

Neutrophils are neurotoxic in several ways, including generation of free radicals from nicotinamide adenine dinucleotide phosphate oxidase, nitric oxide production from inducible nitric oxidesynthase within neutrophils, and formation of arachidonic acid leading to more free radical formation. It does not appear that intravascular sludging by white blood cells exacerbates ischemia[86]. Although, cerebral tissue can protect itself from repeated ischemic insults. A rat brain exposed to transient MCA occlusion will be protected against ischemic cell death within the conditioned zone after several days and lasting up to 7 days[87].

When arterial obstruction is removed, is unleashed an increase in the blood flow in an ischemic territory, also called as post-ischemic hyperemia. It is caused by vasoactive metabolites, reduction on sanguineous viscosity and neurogenic vasodilator[88]. This post-ischemic hyperemia is followed by a long period of post-ischemic hypoperfusion[89]. However, if the blood flow is not established on some areas of tissue, the post-ischemic reperfusion is incomplete; generally, it is a consequence of some factors, like sanguineous viscosity increment, intravascular coagulation, microvascular edema caused by podocyte, endothelial edema and venous obstruction[88].

2 ANGIOGENESIS

Angiogenesis is a physiopathologic process where new vessels arise from pre-existing ones within different phases: sprouting and maturation. Angiogenesis is also defined as a vascular neoformation usually of capillary origin. The development of new blood vessels is essential to embryonic growth and throughout life for physiological repair processes such as wound healing, post-ischaemic tissue restoration, The formation of the vascular system is fashioned by three processes. During embryogenesis, there is differentiation of embryonic mesenchymal cells (the endothelial precursor cells or angioblasts) into endothelial cells resulting in de novo development of blood vessels (vasculogenesis)[90]. Secondly, angiogenesis refers to the formation of new blood vessels by sprouting from pre-existing small vessels in adult and embryonic tissue (sprouting angiogenesis) or by intravascular subdivision (intussusception). The existing vasculature can be transformed into a mature network by processes of pruning and remodelling. Thirdly, arteriogenesis is defined as rapid proliferation of pre-existing collateral vessels. Angiogenesis also seems to be an organ-specific process reliant on the stage of microvascular network[91].

2.1 Post-embryonic angiogenesis

In post-embryonic development the main form of vasculature expansion is angiogenesis, also referred to as neovascularization. Post-embryonic angiogenesis follows the pattern of embryonic angiogenesis, and as tissue grows expansion of the vasculature is essential. This process includes growth and disappearance of capillaries and formation of arterioles and venules[91].

Angiogenesis also involves the differentiation and organization of endothelial cells into capillary tubes and the interplay between growth factors and cytokines. Cell adhesion molecules generally mediate innumerable cell–cell and cell–matrix interactions. These, in conjunction with the recruitment of supporting pre-endothelial cells that encase the endothelial tubes, provide maintenance and modulatory functions to the vessel. Supporting cells usually include pericytes in small capillaries and smooth muscle cells in larger vessels[92, 93].

2.2 Ischemic cerebral and angiogenesis

Under hypoxic conditions, both infiltrating macrophages and host cells produce angiogenic factors and cytokines that directly or indirectly control new capillary growth[94]. Early events involve activation of early response genes, c-fos and c-jun, which further regulate gliosis and angiogenesis in infarcted tissue. Fos expression colocalizes with the expression of basic fibroblast growth factor, being highest at the infarct periphery[95]. The areas in which neurons tend to survive longer are the same as those demonstrated to be highly angiogenic[96,97,98]. Thus, angiogenic factors might be neuroprotective and crucial determinants of neuronal survival after stroke.

Many studies show that after ischemia, due to hypoxia in injured tissue, upregulated promoter factors, such as VEGF, bFGF, that supports angiogenesis and development of a collateral circulation in penumbra area. Exists a modulation of promoters molecules; many of them are upregulated in acute ischemia and are responsible by a microvascular increase and a instability on blood–brain barrier (BBB) during angiogenesis. This microvascular system on BBB allows a correct hemostasis in cerebral parenchyma, prevents passage of undesired molecules proceeding from blood and provides neuroprotection.

Angiogenic growth factors. The existence of angiogenic factors was first observed with the isolation of a tumour factor that generated mitogenic activities in endothelial cells and later found to be a member of the FGF family. Angiogenetic growth factors are produced by a variety of different cells, and their functions include close involvement in developmental as well as tumour angiogenesis[99].

The first angiogenic growth factor to be discovered, Fibroblast growth factor (FGF) currently comprises at least 20 molecules with extensive mitogenic potentials representing some of the most potent angiogenic peptides. They are produced by vascular endothelial and smooth muscle cells, hence their almost omnipresent distribution. They stimulate fibroblast as well as endothelial cell growth and are therefore of vital importance in the process of angiogenesis, and also play a significant part in at least three of the four phases of wound healing: inflammation, repair and regeneration[100]. Further important functions of FGFs include tumour development and Progression.

Another factor is angiopoietin, a further family of growth factors involved in the early processes of angiogenesis and vasculogenesis are the angiopoietins. One isotype, angiopoietin 1 (Ang1) is present in tissues adjacent to blood vessels suggesting a paracrine mode of action, whilst another, angiopoietin 2 (Ang2) is only found at sites of tissue remodeling. In vitro neither Ang1 nor Ang2 have mitogenic effects mediated via Tie-2.1 However, Ang1 facilitates endothelial cell sprouting and vascular network maturation. Ang2 antagonises Ang1 by blocking Ang1-induced phosphorylization of Tie-2. On the other hand Ang2, in combination with VEGF, promotes neovascularization[101].

3.0 NEUROPROTECTION

Neuroprotection is considered an intervention (not essencialy pharmalogical) aiming to limit the volume of an infarct, and the death of vulnerable cells surrounding an infarct, mainly by involving inhibition of a cascade of pathological molecular events occurring under ischemia and leading to calcium influx, activation of free radical reactions and cell death[102].

It has been well documented that abrupt deprivation of oxygen and glucose to neuronal tissues deduces a series of pathological cascades, leading to spread of neuronal death. Of the numerous pathways identified, excessive release of excitatory amino acids, especially glutamate, accumulation of intracellular calcium cations by activation of voltage-operated calcium channels, abnormal recruitment of inflammatory cells, excessive production of free radicals, and initiation of pathological apoptosis are believed to play critical roles in ischemic damage, especially in the penumbral zone. Consequently, it is obvious to suggest that if one is able to interrupt the propagation of these cascades, at least part of the brain tissue can be protected[103].

Depending on interfered molecular event, neuroprotection strategies have different moments. Primary neuroprotection occurs when a medicine develops a neuron resistence against ischemic, hypoxic, excitotoxic or metabolic injury. Calcium channel blockers, sodium channel blockers, antioxidants, NO-sintase inhibitors, glutamate site antagonists and platelet activating factor (PAF) antagonists can reduce cerebral damage whether rapidly initiated[104].

Besides, secondary neuroprotection refers to a pharmacological intervention in pathogenic process that occurs after tissue lesion is installed, which are lately responsible by neuronal necrosis and apoptosis. In this group, there are substances that can reduce a late necrosis, such as inhibitor enzymes of inflammation inductors and cytokine blocker substances, and others that can reduce apoptosis (enzyme inhibitors of apoptosis). Even now tertiary neuroprotection, there are substances able to improve capacity of recovery a injury tissue and reduce diaschisis; for instance, medicines which increase disponibility of biogen amine. Trophic factors such as fibroblast growth factor, endothelial growth factor and erythropoietin improve neovascularization and have an immediate trophic effect on neurons through genes that facilitate neuronal repair and survival[104].

Activation of glutamate receptors leads to a further increase in intracellular calcium, activation of intracellular enzymes, and consequently neuronal death. Successive exploration of the complex pathophysiology of cerebral ischemia has resulted in the development of a great number of candidates for neuroprotective intervention. Some neuroprotective agents show a benefit in post hoc subgroup analyses, e.g. citicoline (phosphatidylcholine precursor), and some studies are still ongoing, e.g. magnesium (NMDA channel blocker), benzodiazepines (GABA agonists), YM-872 (AMPA receptor antagonist), NXY-059 (free radical scavenger), and repinotan (serotonin agonist)[102].

3.1 Vascular protection after stroke

Vascular protection can be defined as an augmentation of endothelial function to prevent vascular smooth muscle cell proliferation, thrombosis, inflammation, and endothelial apoptosis; mechanisms serving as targets for vascular protection should be evaluated in the context of ischemic stroke pathophysiology. These mechanisms can be separated into acute (hours), subacute (hours to days), and chronic (days to months) events[105].

Acute. This phase is characterized by hemodynamic and metabolic changes, resulting in dysregulation of cerebrovascular tonus and disruption of the blood–brain barrier (BBB)[106]. There are several factors that play important roles in regulation of vascular tone and structure, such as oxygen radicals as well as vasoactive factors including NO, endothelin-1 (ET-1), VEGF, and angiopoietin I[86,106]. The basal cerebrovascular tonus favors partial vasoconstriction, and because of it, plays an important role in regulation of local blood flow in response to changes in perfusion pressure as well as to alterations in metabolic and endothelial factors by adjusting vessel caliber[107, 108], contributing to the functionality and integrity of cerebral arteries that are critical to minimize brain injury during reperfusion.

Subacute. A number of proinflammatory genes, including interleukin-1[beta] (IL-1[beta]), TNF-[alpha], and transcription factors such as hypoxiainducible factor 1, nuclear factor [kappa]B, and interferon regulatory factor-1, are activated in response to the hypoxia, superoxide radical formation, and intracellular Ca^{+2} influx that occur during the acute phase[109,110]. These proinflammatory products influence expression of adhesion proteins and enzymes that degrade the components of extracellular matrix that are critical for integrity of vascular endothelium[111,112]. As an example, cytokines such as TNF-[alpha] and interleukin-1[beta] are closely linked[113,114] with activity and the expression of matrix metalloproteinases (MMPs), proteolytic enzymes that degrade basal lamina and permits leukocyte migration into the brain and leads to vasogenic edema. So, MMPs is probably correlated with the opening of the BBB[115,116] and with the extent of neuronal injury[117], and its important sources in the brain include microvascular endothelial cells, neutrophils, monocytes/microglia and natural killer cells[118,119].

Inflammation could contribute to vascular injury by several mechanisms. Besides microvascular obstruction caused by increase leukocyte adhesion[120], neutrophils can produce a number of potentially harmful substances, including toxic oxygen metabolites, destructive enzymes, and proinflammatory cytokines with neurotoxic properties. Nitric oxide is an example of a toxic oxygen metabolite that damages neurons produced by neutrophils through inducible nitric oxide synthase[121,122] although inhibition of poly (ADP-ribose) synthase, a nuclear protein that may mediate the toxicity of nitric oxide, decreases infarct volume, reduces neutrophil recruitment, and attenuates the generation of toxic oxygen metabolites[123]. Also, neutrophil depletion, as well as inhibition of the destructive enzymes that they secrete, such as elastase, decreases infarct size and cerebral edema and reduce endothelial cell death in the chronic phase[105,124].

At least, gene activation involves not only expression of proteins that cause vascular injury, but also induction of proteins that typically have a protective function. Studies on regulation of VEGF, angiopoietin, and bFGF systems demonstrated that these proteins and respective receptors are also activated within 2 to 4 hours of ischemia[125].

Chronic. This phase of ischemic stroke involves induction of genes that participate in the regulation of apoptosis as well as stimulation of angiogenic factors in endothelial cells. Programmed cell death is triggered by activation of cell surface receptors via several factors, including TNF-[alpha], superoxide, and IL-1[beta], all of which are stimulated in the acute phase of ischemic stroke. In response to these stimuli, a cascade of proteolytic enzymes known as caspases and other proteins such as B-cell lymphoma-leukemia 2 (Bcl2)-associated X protein (Bax) and transformation related protein 53 (Trp53) as well as antiapoptotic proteins including Bcl2 and inhibitor of apoptosis protein (Iap), are activated[126, 127]. Therefore, inhibition of apoptotic gene expression and stimulation of antiapoptotic proteins may offer a vascular protection strategy. In addition to its angiogenic and antiproliferative effects as described above, VEGF also stimulates endothelial cell survival[128].

3.2 Neuroprotectants in cerebral ischemia

In stroke, VEGF is a key mediator of angiogenesis, is considered a potent vascular permeability factor and also is an inducer of neuroprotection and promotes neurogenesis. Furthermore, it has been suggested that erythropoietin acts indirectly on endothelial cells via activation of the VEGF/VEGF receptor system and promotes brain vessels growth; it is also produced under conditions of local hypoxia and has a neuroprotective function. This cytokine is a potencial neurotherapeutic agent that opens a novel way for clinical investigations in protection to the developing brain[129].

3.2.1 Vascular endothelial growth factor (VEGF)

VEGF is upregulated after hypoxic injury to the brain that can occur during cerebral ischemia or high-altitude edema, and has been implicated in the blood-brain barrier breakdown associated with these conditions. VEGF is known to be a multifunctional peptide capable of inducing receptor-mediated endothelial cell proliferation and angiogenesis both in vivo and in vitro. In addition to its crucial role in embryonic vascular development, VEGF has been implicated in the process of neovascularization in adult pathophysiology[130].

VEGF has direct mechanisms that influence neurons by stimulating axonal outgrowth and improving survival[131,132]. Under critical conditions for neuronal cells it becomes a mediator of multiple molecular reactions leading to the inhibition of programmed cell death and the stimulation of neurogenesis[133,134]. VEGF is now known to be a multifunctional peptide capable of inducing receptor-mediated endothelial cell proliferation and angiogenesis both in vivo and in vitro. In addition to its crucial role in embryonic vascular development, VEGF has been implicated in the process of neovascularization in adult pathophysiology[130].

Several factors including oxygen radicals as well as vasoactive factors such as NO, endothelin-1 (ET-1), vascular endothelial growth factor (VEGF), and angiopoietin I play important roles in regulation of vascular tone and structure in this acute phase of stroke[135].

VEGF promotes endothelial integrity by stimulating NO production. However, VEGF increases BBB permeability in the acute phase after schemic stroke. Therefore, VEGF administration in this phase may worsen BBB leakage. Although VEGF-induced

NO production may be considered a means of improving endothelial integrity after ischemia, as discussed above, NO reacts strongly with superoxide to generate peroxynitrite that causes tissue damage. A recent study demonstrated that NO generated by neuronal NO synthase (NOS) during ischemia may be detrimental in addition to endothelial NO produced at reperfusion, causing damage via peroxynitrite formation[136].

VEGF also stimulates endothelial cell survival. It has been demonstrated that VEGF induces the antiapoptotic pathway through phosphatidylinositol 3-kinase, resulting in inhibition of endothelial apoptosis. These findings suggest that VEGF may offer additional protection in the chronic phase of ischemic stroke[137,138].

3.2.2 Erythropoietin

Erythropoietin receptor is specifically modulated during ischemia; in this context, this cytokine presents a indirect protection of brain tissue expressed by promoting angiogenesis and a direct protective effect on neuronal cells during stroke[139]. During cerebral ischemia, the cellular expression pattern of erythropoietin and its receptor is specifically modulated as a function of the duration of ischemia in neurons, endothelial cells, and glial cells[140]. Studies with primary cultures shown that astrocytes and neurons has an erythropoietin production[140,141]; also, astrocytes can be considered the main producers in the brain[142]. In normal adult human brain, weak erythropoietin/ erythropoietin receptor immunoreactivity is mainly neuronal[143].

Erythropoietin binding sites were found in neurons, brain endothelial cells and glial cells[140,141], in various areas of the brain, including cortex, hippocampus, and midbrain. It suggests that erythropoietin may have a whole range of paracrine and/ or autocrine actions in the central nervous system[143] Besides, erythropoietin and its receptor expression change significantly during brain development, with high expression of them, demonstrating that this hormone is a general morphogen and inducer of neurogenesis during early development[142].

The biologic effects of erythropoietin in the CNS involve activation of its specific receptor and corresponding signal transduction pathways, and consequently presents different mechanisms of neuroprotection. It includes generation of neuronal antiapoptotic factors and antiapoptotic mechanisms[144], prevention of oxidative damage[145] with direct antioxidant effects via activation of antioxidant enzymes and inhibition of lipid peroxidation, decrease of NO-mediated injury by inhibition of NO production[146], stimulation of angiogenesis[147], modulation of neurogenesis[148], reduction of glutamate toxicity[149], and reduction of inflammation with antiinflammatory effects[150].

Erythropoietin produced on the brain can protect neurons by direct and indirect mechanisms. Regarding direct pathway, can be taken many hypothesis: (1) erythropoietin can represses apoptosis in neurons by maintaining expression of Bcl-2 and Bcl-xL, in analogy with the case of erythroid precursor cells, or by other mechanism, as inactivation of caspases[151]; (2) by upregulating enzymes, which scavenge oxygen radicals as well as by downregulating enzymes that consume large amounts of ATP.[85]; and (3) eventually inducing transient calcium influxes which mediates erythropoietin-induced blockage of glutamate-induced neurotoxicity[152]. It has been shown that erythropoietin protects primary cultured hippocampal and cortical neurons against glutamate toxicity[140,152,153]. Erythropoietin also ameliorates hypoxia and glucose deprivation-induced neuronal damage and reduces the toxic effects of a glutamate agonist on cultured neurons[154].

In the case of indirect neuronal protection, angiogenesis might be stimulated in the brain by hypoxia/ischemia-induced erythropoietin. Endothelial cells carry the erythropoietin receptor, what can be explained by the fact that these cells and hematopoietic cells become from the mesenchymal precursor hemangioblast. As it does in erythroid precursors and neurons during ischemia, erythropoietin might also be a survival factor for endothelial cells preventing apoptosis[83].

4.0 CONCLUSIONS

The mechanism of vascular changes after ischemic stroke involves induction of genes that participate in the regulation of apoptosis as well as stimulation of angiogenic factors in endothelial cells. Therefore, inhibition of apoptotic gene expression and stimulation of antiapoptotic proteins may offer a vascular protection strategy.

A large number of studies provided evidence that VEGF induces re-endothelialization of blood vessels independent of its angiogenic effects, Although these past studies provided evidence that VEGF could play an important role in vascular protection, pleiotropic and potentially protective effects of VEGF on the vasculature in the ischemic stroke setting should be evaluated in further detail and may offer an important protective strategy in the subacute and chronic phase of ischemic stroke.

Protection of the cerebral vasculature after ischemic stroke is a strategy that appears to be a logical approach to prevent edema and hemorrhage and enhance recovery. The optimal end points of clinical trials of vascular protection may include development of cerebral edema or hemorrhagic transformation along with overall neurologic function. Acute vascular protection needs to be explored in humans as a strategy to improve neurologic outcomes after an acute ischemic stroke.

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