Abstract. Intracerebral hemorrhage (ICH) represents the most lethal form of stroke and has deleterious consequences. Primary injuries are generated by the blood collection itself and consist in local tissue destruction. They are prolonged and amplified by a combination of secondary injuries involving the toxic effects of blood, oxidative stress, recruitment of activated microglia/macrophages and neutrophils and excitotoxicity. Brain tissue is particularly vulnerable oxidative attack due to its rich content in polyunsaturated fatty acids which favors lipid peroxidation. Different from other tissues, brain presents only reduced amounts of antioxidant enzymes – catalase (CAT), glutathione peroxidase (GPX) and superoxide dismutase (SOD) and their activity decreases with age. It also contains elevated amounts of non-heme iron. The study of neuroprotection in ICH use as targets pathological processes like oxidative stress, inflammation, cerebral edema. In this context, the role for oxidative stress in and potential antioxidant therapies were analysed.

Keywords: oxidative stress, intracerebral hemorrhage, antioxidant

Physiopathological background

Brain has a particular predisposition to oxidative aggression, due to an increased oxygen consumption; even though it represents 2% from body weight, brain benefits of an important blood supply- 20% from cardiac output. It uses 20% from total body oxygen consumption, receiving 3.5 ml oxygen/100 g. of cerebral tissue/min. It is also characterized by a rich content in polyunsaturated fatty acids which favors lipid peroxidation. Brain contains elevated amounts of non-heme iron. Specific brain territories (globus pallidus, substantia nigra, circumventricular organs) contain large amounts of iron which may amplify lipid peroxidation. In the event iron becomes liberated and encounters H2O2, it generates, via the Fenton reaction, the highly reactive and destructive _OH. In addition to iron, certain areas of the brain are rich in ascorbic acid (vitamin C). In the presence of free iron, vitamin C becomes a potent pro-oxidant [1]. In normal brain, the mitochondrial respiratory chain represents the major intracellular source of reactive oxygen species (ROS). During mitochondrial metabolism, 4% to 5% from consumed oxygen is converted into reactive oxygen species, mainly superoxide anion (O2-). Superoxide dismutase reduces O2- to hydrogen peroxide H2O2 which can be further converted into hydroxyl radicals HO· via the Fenton reaction. Hydroxyl radicals and superoxide anions can further react with other molecules in biological systems, leading to the formation of other free radicals and changes in intracellular signaling [2].

Intracerebral hemorrhage

Intracranial hemorrhage comprises bleeding within the cranial vault and includes ICH, subdural hematoma, epidural bleeds, and subarachnoid hemorrhage (SAH). From these, ICH is a stroke subtype accounting for about 10% of all strokes [3]. Causes of ICH are represented in most cases (70 – 80%) hypertension. Bleeding is associated...
with intraparenchymal small vessel degenerative processes such as hypertensive small vessel microaneurysms or amyloid angiopathy (primary ICH). Other causes (secondary ICH) are represented by structural lesion like tumours, arteriovenous malformations, intracranial aneurysm bleeding or amyloid angiopathy (primary ICH).

Intracerebral hemorrhage (ICH) is a bleeding that occurs directly into the brain parenchyma which occurs twice as common as SAH and is equally as deadly. The hematoma locations in descending order are: ganglionic, lobar, cerebellar, and brainstem[7,8,9].

ICH evolves in two phases: the first one is believed to cause damages due to the local mass effect of an expanding haematoma. Even though animal models have failed to reveal such a local pressure, it was showed that blood mass induce a mechanical disruption of the neurons and glia, accompanied by mechanical deformation[10].

Dependent on the severity of mitochondrial dysfunction, the results of injury range from temporary metabolic suppression (hibernation phase) to cellular swelling and necrosis. Because ICH triggers a multitude of deleterious cascades in both white and gray matter, neuroprotection has a complex meaning. Blood extravasation into the brain parenchyma compress brain tissue, increases intracranial pressure and produce ischemia and destruction. Ischemia activates inflammatory and oxidative pathways leading to tissue necrosis, apoptosis, breakdown of the blood–brain barrier, edema and by this closing a positive feedback loop with further increases in intracranial pressure [11].

Studies regarding cerebral blood flow described a risk area of decreased perfusion around the cerebral hemorrhage. This territory is also characterized by an ondulatory evolution as long as it represents a subject of an intense metabolic flux due to ischemia, inflammatory cascade and oxidative stress activation. Transient ischemia elevates cerebral levels of both excitatory neurotransmitters including glutamate and hydroxyl radical formation. Hemoglobin release from lysing erythrocytes may contribute to oxidative injury to tissue surrounding a hematoma. Hemoglobin toxicity is mediated its heme which oxidizes to hemin and acts on surrounding cells. Astrocytes respond to hemoglobin exposure by rapidly inducing ferritin. Instead, cortical neurons express very little ferritin after hemoglobin treatment [12].

The study of Perez de la Ossa et al identified the high-serum ferritin levels as predictors of poor outcome in patients with ICH. In their study, high ferritin levels were also related to poor outcome in patients with acute ischemic stroke and with a higher risk of hemorrhagic transformation in patients treated with reperfusion therapies.

The hemoglobin release represents a notable event and its removal begins immediately, due to plasma haptoglobin and local phagocytes. More, a haptoglobin overexpression was identified in preclinical studies in cerebral tissue surrounding haematoma. The toxicity of blood is continued by free iron release due to extravascular hemolysis. Iron released may augment oxidative stress, glutamate release, and inflammatory response. In experimental models of ICH it was shown that iron chelators may reduce brain edema and improve neurological function [13]. This role is also sustained by another article which found that iron chelator deferoxamine, reduced lipid peroxidation, improved post-ischemic vasoreactivity, cerebral perfusion and facilitate ATP recovery [14]. A phase I study assessing the feasibility, safety, and maximum tolerated dose of deferoxamine in ICH patients was successfully completed.

However, another clinical trial using a previously, preclinical successful free radicals neutralizer - NXY-059, showed inefficacy in ICH patients [15].

The selective cyclo-oxygenase-2 inhibitor celecoxib demonstrated efficacy in reducing edema and inflammation and improving functional recovery in a rat ICH model [16].

The evaluation of antioxidant enzymes showed that Mn-SOD and CuZn-SOD brain contents are decreased, protein carbonyl levels increased and apoptotic marker appeared in brain tissue [17, 18].

In order to stimulate endogenous antioxidant capacity of the brain, nuclear factor erythroid 2-related factor 2 Nrf2 – a transcription factor which regulates the expression of many antioxidant enzymes (SOD, CAT, GTS, GPX and others) was successfully tested in preclinical studies. Nrf2 act as an effective regulator of oxidative stress and blood detoxification components [19]. Nrf2 also mediates GSH biosynthesis and release from astrocytes and through this it protects neurons from oxidative stress. Experimental, Nrf2 overexpression specifically in astrocytes leads to neuroprotection in in vivo models [20].

The ability of SOD to buffer superoxide excess may be increased by SOD overexpression and potentially by treatment with SOD mimetic compounds. Several major classes of SOD mimetics have been reported: Mn(II) cyclic polyamines, Mn(III) salen derivatives, Mn(III) porphyrins and stable cyclic nitroxides. All neutralize superoxide in catalytic pattern [21].

Melatonin (N-acetyl-5-methoxytryptamine) released by the pineal gland, was shown to exert neuroprotection in models of brain trauma cerebral ischemia and excitotoxicity. In these models, oxidative stress is a common element and the ne-
uroprotective effects of melatonin as antioxidant was claimed [22]. Melatonin neuroprotection is mediated via potentiation of other brain antioxidants (ascorbic acid) thus altering the redox state of the cell and consequently attenuating NF-κB activation.

More, melatonin is an endogenous molecule and this enhance its safety. Surgical removal of the pineal gland, has been shown to aggravate the amount of oxidatively damaged molecules which implies that physiological concentrations of melatonin, are effective in scavenging toxic reactants [23]. Peroxisome proliferator-activated receptor (PPARg) is a member of the nuclear receptor super-family of ligand-activated transcription factors and is involved in the lipid and carbohydrate metabolism regulation. During intracerebral hemorrhage, the activation of nuclear factor kappa-B (NF-κB) takes place which leads to oxidative stress and secondary brain damage. Transcription factor peroxisome proliferator activated receptor-gamma (PPARg) via inhibition of NF-κB and induction of antioxidative defense components reduces inflammation and oxidative stress and protect ICH-affected brain. In addition, PPARg stimulates phagocytosis mediated hematoma cleanup, thus facilitating removal of hematoma, the source of toxicity and inflammation. The peroxisome proliferator-activated receptor–agonist pioglitazone is thought to act in ICH through interruption of matrix metalloproteinase activation, microglial activation and resulting inflammation [24].

Rosiglitazone, a synthetic, high-affinity PPARg ligand clinically used for management of type 2 diabetes mellitus, reduced oxidative stress and lowered blood pressure in patients and animal models with both diabetes mellitus/metabolic and nondiabetes/metabolic syndrome. It promotes a central antihypertensive effect by decreasing sympathetic vasomotor activity via PPARg–dependent transcriptional upregulation of the mitochondrial antioxidant UCP2 [25].

In an animal model, it was demonstrated that the presence of apoE4 is associated with enhanced neuroinflammatory responses and cerebral edema after ICH. Instead, the protective effect of the apoE3 isoform can be simulated by the administration of an apoE-mimetic peptide. This therapeutic approach improves outcomes in model animals, suggesting that it may be a rational strategy for targeted pharmacogenomic therapy humans after ICH [26,27].

The success of antioxidant strategies depends largely upon timely administration and the rate of penetration of the antioxidant into the site of tissue injury [28,29]. Best medical management of the ICH keeps in the first line the stabilization of airway, breathing function, and circulation. These are followed by specific measures to decrease secondary neurological damage and to prevent both medical and neurological complications. The control of coagulopathy when present is of the essence. Blood pressure management can be key and continues as an area of debate and ongoing research. Surgical evacuation of ICH is of unproven benefit though a subset of well-selected patients may have improved outcomes.

To date neuroprotectants need to be addressed in controlled randomized studies. Administration of antioxidants affords significant neuroprotection especially in ICH preclinical models and may hold promises for future clinical applications.

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