Chapter 16

Free Radicals and Neuronal Recovery from an Ischaemic Penumbra: A Review

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Additional information is available at the end of the chapter

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Abstract

Stroke remains the second leading cause of death worldwide. The major problem is that the therapeutic window is short and no accepted treatment is completely efficient. Even though there is evidence of free radical participation in the pathophysiology of stroke, no beneficial effects of antioxidants have been demonstrated in clinical assays. Moreover, some reports paradoxically indicate that antioxidants could be harmful and that oxidative stress preconditioning could reduce the long-term effects of stroke. There are two major areas within the ischaemic zone: (1) the core, where neuronal necrosis develops in minutes, and (2) the penumbra surrounding the core, where some neurons could eventually be recovered over an extended time. The present review specifically focuses on the role of free radicals in the life or death of brain cells (mainly neurons) within the ischaemic penumbra. It also analyses the effects of oxidative stress on blood-brain barrier disruption. In conclusion, we postulate a cascade of events that follow cerebral ischaemia and what type of therapeutic approach could eventually change the effect of free radicals on neuronal recovery from an ischaemic penumbra.

Keywords: free radicals, ischaemic penumbra, oxidative stress, antioxidants, stroke

1. Introduction

According to the Global Burden of Disease Study (GBD 2010), stroke was the leading cause of mortality and the third leading cause of disability-adjusted life-years (DALYs) worldwide [1]. The highest incidence rates of ischaemic stroke are in Eastern Europe, Central Asia, East Asia and North Africa/Middle East [2]. Using the GBD 2013 methods it was determined that from

1990 to 2013 there was a significant increase of prevalent cases, total deaths and DALYs due to haemorrhagic and ischaemic stroke in younger adults (20–64 years), mainly in developing countries [3].

Intravenous thrombolysis remains the standard therapy for ischaemic stroke, but the window for therapy is narrow (3–4.5 hours after a stroke) and not completely effective, primarily in patients with large vessel thromboses. Some clinical trials suggest that intra-arterial thrombolysis plus a stent application could be the best therapy in large artery thrombosis. However, such treatment requires a thorough examination of the patient (24 hour computed tomography angiogram service), trained radiologists and quick location of the clot, as well as a rapid triaging of the patients to the stroke intervention centre [4]. The main limitations of thrombolysis are [5] (1) the long delay from the onset of stroke to treatment, (2) the irreversible cell damage caused by ischaemia over time, (3) that ischaemia can result in haemorrhaging caused by thrombolytics and (4) collateral effects of thrombolytics. Time and an understanding of the pathophysiology of neuronal and brain cell death in stroke are critical.

The role of oxidative stress in stroke has been intensively explored. References in the PubMed database alone ("oxidative stress" AND stroke, abstract/title) reveal a total of 1,852 papers (from two in 1991 to 234 in 2015). Even though several studies show beneficial effects of antioxidant supplements for stroke, no meta-analyses (clinical studies) thus far have strongly validated the protective effects of any post-stroke antioxidant supplement. Moreover, there is no meta-analysis supporting the beneficial effects of antioxidant preventive supplementation [6–8]. Indeed, antioxidant supplements actually could be dangerous [9]. A meta-analysis including 4,875 subjects with a follow-up period of 7–15 years demonstrated that the daily intake of more than 300 mg of vitamin C was associated with an increased risk of cardiovascular diseases, coronary artery disease and stroke in diabetic patients [7]. However, there are controversies regarding the effects of dietary antioxidants on stroke. A meta-analysis including 116,117 participants and 1,989 cases demonstrated that circulating lycopene, not simply dietary lycopene, was associated with a significantly decreased stroke risk [10], whereas high flavonol intake, compared to low intake, was associated with a higher stroke risk [11].

Exercise can increase the endogenous antioxidant defence for changing stroke risk. Hooker et al. published an interesting prospective study performed in a total of 46,406 men and 15,282 women who had no cardiovascular disease at the beginning of the study and who carried out a treadmill test to evaluate their cardiorespiratory fitness. The subjects were followed from 1970 to 2001. There was an inverse relationship between cardiorespiratory fitness and the incidence of fatal or non-fatal stroke [12]. An interesting study performed with 31,696 Swedish women who were followed for 10.5 years concluded that a low-risk lifestyle was related to a low stroke risk. A low-risk lifestyle was defined as a healthy diet (to 50% of Recommended Food Score), moderate alcohol consumption (5–15 g/day), not smoking, physical activity (walking/bicycling ≥ 40 min/day and exercising ≥ 1 hour/week) and a body mass index < 25 kg/m^2 [13].

Stroke has been significantly associated with environmental pollution in different countries. Short-term exposure to air pollution has been related to stroke in Denmark [14] and in Sweden [15]. Recent studies in Israel and Germany concluded that long-term exposure to fine-particle

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dust was associated with a higher risk of stroke [16, 17]. It is relevant that the study in Israel found a relationship of pollution to stroke in young adults [17]. A large epidemiologic study performed with 114,537 women (Nurses' Health Study) in the USA found that the association between long-term pollution exposure and stroke was stronger in diabetic women [18]. Experimentally (though not fully demonstrated in people naturally exposed to pollution), the effects of pollution do seem to be related to oxidative stress. Oxidative stress could simply be defined as the condition where the balance between antioxidants and free radicals tilts in favour of free radicals [9].

2. The ischaemic penumbra

Depending on the location and diameter of the obstructed vessel, the interruption of blood flow and the consequent hypoxia and lack of nutrients produces an ischaemic core where Adenosine triphosphate (ATP) levels reach around 15% of the basal values within minutes. This results in neuronal death from which is impossible to recover [19]. The area that surrounds the ischaemic core is termed the penumbra (from the Latin *paene*, almost; *umbra*, shadow). ATP levels in the penumbra are around 50–70% of the basal values [19], indicating that neuronal death is delayed, and this is where therapeutic strategies should be directed to ensure that neurons survive and recover. Mechanisms leading to neuronal death in the penumbra seem to be triggered by hypoxia and the fall of glucose. Actually the ischaemic penumbra is highly dynamic with a rapid evolution that leads to cell death.

Dirnagl et al. [20] published an interesting paper in 1999 based on experimental and clinical (autopsy histopathological) studies of patients who died from stroke. The paper showed a time-course of damaging events in focal ischaemia. The author updated that time-course in 2012 [21]. Everything starts with blood interruption, and within minutes there is a high wave of excitotoxicity and peri-infarct depolarisation with an accumulation of glutamate, calcium and reactive oxygen species (ROS); in the following hours the mediators that were liberated and induced in the first stages trigger inflammation through the activation of cyclooxygenase-2, caspases, metalloproteinases (MMPs) and the liberation of inflammatory interleukins. After several days apoptosis takes place and the penumbra area becomes part of the death core, although repairing processes are also activated; and there are scar formation, neurogenesis and vasculogenesis [20, 21].

It has been experimentally demonstrated that there are two flow thresholds during stroke evolution, the first being a functional threshold that leads to reversible functional changes and the second producing irreversible membrane failure and morphological damage. The penumbra is between these thresholds [22].

Returning to the time-course concept, when the decrease of blood flow reaches about 20% of preocclusion values, there are a tremendous ionic disequilibrium, osmotic swelling and, finally, within minutes, terminal depolarisation at the core occurs. The blood flow around the core reaches 20 to 50% of preocclusion values within 6 hours, and by that time irreversible damage expands around the core. It is calculated that infarct volume increases more than 20% (from

core to penumbra) within the first 3 hours after occlusion [22]. Blood flow-dependent damage explains the short therapeutic window and the lack of thrombolytic therapeutic efficacy. It has been demonstrated experimentally that the biggest effect of thrombolytics occurs within 90 min because acute arterial occlusion and the reduction of blood flow can be tolerated only 1–2 hours before irreversible damage occurs [23]. Optimal treatment time is supposed to be around 3 hours, and between three and 4.5 hours, they only provide small effects [4, 23].

3. Role of oxidative stress in death and survival of brain cells in an ischaemic penumbra

The penumbra area includes neurons and other brain cells and structures such as astrocytes, endothelial cells, pericytes, smooth muscle cells, microglia, basement membrane, as well as extracellular matrix, all of which become activated by the lack of blood flow and thereafter interact to produce free radicals, inflammation and a cascade of events leading to cell death but also regenerative processes that could eventually limit the core progression [5]. It is important to recognise that the cascade of events triggered by stroke goes beyond the core and penumbra and includes the contralateral hemisphere and the whole organism. Cells of the neurovascular unit (see above), cells that migrate to the insult area (neutrophils, monocytes, T cells, B cells) and stem cells from the brain and blood participate in the damage and recovery associated with cerebral ischaemia [21].

Free radicals are produced soon after ischaemia and contribute to both the expansion of neuronal death and neuronal recovery from the ischaemic penumbra. The primary and rapidly produced free radical is probably superoxide, generated by mitochondrial dysfunction induced by hypoxia. Some time thereafter superoxide is produced from other sources at the penumbra, such as the nicotinamide adenine dinucleotide phosphate oxidase (NADPHox) complex, xanthine oxidase, lipoxygenase, cyclooxygenase (COX), cytochrome p450 and, probably (though not yet demonstrated), uncoupled endothelial nitric oxide synthase (eNOS) [24, 25]. Superoxide dismutase (SOD) catalyses the dismutation of superoxide into hydrogen peroxide that is then decomposed into water and oxygen by catalase or glutathione peroxidase (GPx) [9]. Hydrogen peroxide could eventually produce hydroxyl radical (more toxic than superoxide) if it reacts with iron (Fenton reaction). It is well known that hydrogen peroxide stimulates nuclear factor kappa B (NF-kB), which in turn induces the transcription of SOD genes [26]. Even though the redox stimulation of NF-kB has been long known, it is not clear if the stimulation is provided by hydrogen peroxide and/or superoxide itself.

The stimulation of SOD synthesis by ROS does not guarantee antioxidant protection. The continuous production of superoxide in cerebral ischaemia (first stimulated by hypoxia and later by other sources) could eventually turn SOD toxic. If superoxide production exceeds catalase activity and/or glutathione sources are exhausted, SOD could lead to the accumulation of the hydroxyl radical. This is precisely one of the handicaps of experimental models of cerebral ischaemia. The latter are usually produced in normal animals that have been subjected to occlusion of cerebral arteries. This is far from the clinical setting, where cerebral ischaemia

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usually develops in patients with comorbidities or environmental conditions that constitute a risk for stroke (e.g., diabetes, hypertension, obesity, atherosclerosis, smoke, air pollution). In such conditions there is already an elevated basal oxidative stress with probably poor endogenous antioxidant defence. Moreover, strategies to protect from cerebral ischaemia are far from realistic (e.g., antioxidant supplements are often given before stroke occurs). There are other circumstances that could interfere in the attempted extrapolation of experimental results to a clinical therapeutic design. Usually the experiments on cerebral ischaemia involve a combination of ischaemia/reperfusion, with the rationale being the aggravation of oxidative stress during reperfusion [21]. It would be exceptional to believe that an occluded vessel becomes permeable within a short time, i.e., reperfusion to an ischaemic territory is usually given by neoformation of vessels (angiogenesis). Another drawback of experimental assays is that measures to prevent bias are not common: e.g., randomisation of the animals, appropriate inclusion/exclusion criteria, a low number of cases and thus a low positive predictive value [21]. Even with those disadvantages mentioned, there is experimental evidence which helps to understand the events that lead penumbra cells to either die or survive.

Responses to superoxide production in the penumbra are fast. It has been shown that copper zinc superoxide dismutase (CuZnSOD), the cytosolic form, is increased in the penumbra 1 hour after middle cerebral artery occlusion (MCAO)/reperfusion (MCAOR), whereas manganese SOD (MnSOD, mitochondrial) increases in the penumbra and core 3 hours after MCAOR [27]. Nitric oxide (NO) is another free radical liberated in the ischaemic penumbra. The source of the earlier production of NO in the penumbra seems to be the neuronal nitric oxide synthase (nNOS). This enzyme has been shown to increase in the penumbra 1 hour after MCAO in the rat [28]. Later on, when the inflammatory reaction is present, the inducible NOS (iNOS) is responsible for the high concentrations of NO in the ischaemic penumbra [29]. The coincidence of large quantities of superoxide and NO in the proximity of the core (where the pH is low) facilitates the interaction of those free radicals to form peroxynitrite. Indeed nitrotyrosine (a marker of peroxynitrite production) has been detected in the penumbra 4 hours after MCAOR in the rat [29]. Tyrosine nitration by peroxynitrite can actually inactivate SOD [30].

Besides SOD, other antioxidant defence mechanisms are activated after cerebral ischaemia although they take a longer time. Nuclear factor (erythroid-derived 2)-like 2 (Nrf2), which is an important brain defence against oxidative stress, is upregulated in neurons [31, 32], microglia [31] and astrocytes [31, 32] of the ischaemic penumbra (not in the core), 24 hours after MCAOR in the rats and mice. Activated Nrf2 induces what is termed "phase II defence enzymes and antioxidant stress proteins" through its interaction with the antioxidant response element in the promoter region of target genes [33]. As a result Nfr2 activation during cerebral ischaemia provides protection through the modulation of microglia dynamics, the protection of astrocytes and neurons and regulation of the expression of antioxidant enzymes and anti-inflammatory mediators [33].

Free radicals produced during cerebral ischaemia contribute to a breakdown of the blood-brain barrier (BBB), an event that has been related to cerebral oedema, and has an important role in the pathophysiology of cerebral ischaemia by allowing the arrival of migrating cells that contribute not only to inflammation but also to eventual angiogenesis, neurogenesis and

partial regeneration. The BBB is a complex structure integrated by particular endothelial cells: (a) that do not have fenestrations, (b) that have limited pinocytic activity and (c) that have a high content of mitochondria; tight-junction proteins such as occludin, claudin 5 and zonula occludens 1; and pericytes, astrocytes, extracellular matrix and transporter systems, such as the receptor for advanced glycation end products (RAGEs) [34–37]. Protein leakage (evidence of BBB breakdown) has been demonstrated as early as 30 min after MCAOR [25, 38]. In some studies (but not all), such leakage is prevented by an antioxidant treatment [25]. An important source of free radicals during cerebral ischaemia is the vascular endothelium [25, 39]. Apparently the main contributor to superoxide production in the vessels of the penumbra is NADPHox [39]. Superoxide and other ROS activate MMPs, mainly MMP9 and MMP2, that degrade extracellular matrix and basal lamina around vessels, contributing to BBB disruption during ischaemia [38, 40]. It has been demonstrated that MMP9 is upregulated in the penumbra from 24 to 72 hours after ischaemia [41].

Delayed production of free radicals contributes to blood vessel disintegration in an ischaemic penumbra. The subacute phase of cerebral ischaemia (from 24 to 72 hours after blood flow interruption) is characterised (as mentioned above) by an explosion of free radicals produced by the inflammatory reaction induced during the events that occur in the acute phase. It has been estimated that this second wave of free radical generation coincides with a second opening of the BBB [42]. The main source of inflammatory mediators and free radicals in this phase is the microglia (resident macrophages of the brain and spinal cord). Microglia accumulate in the penumbra several hours after cerebral ischaemia [43], but apparently its active role in ischaemia begins earlier. Jolivel et al. published an interesting paper in 2015 [41] showing data on the time-course of microglia activation in the penumbra during ischaemia: (1) soon after reperfusion microglia become activated in the penumbra; (2) an association of activated microglia with endothelial cells of the penumbra begins as soon as 4 hours after reperfusion; (3) at 24 hours after reperfusion CX3CL1 (a chemokine involved in migration of microglia) significantly co-localises with microglia in the penumbra; (4) at the same time the microglia are associated with claudin 5 in the penumbra, which indirectly indicates the disruption of the BBB; (5) MMP9 is activated 3 hours after reperfusion and it peaks at 24 hours (another indirect evidence of BBB disruption) in the penumbra; and (6) at 24 hours after ischaemia neutrophils invade the penumbra (direct evidence of BBB disruption). Microglia activity seems to last only a few days, from 72 hours after ischaemia the microglia decline and the monocytes migrate to the penumbra; apparently those monocytes also have an inflammatory phenotype and serve to clean up the area [44].

Astrocytes play also an important role in the second wave of free radical production; it seems that they are activated by free radicals to protect and rescue neurons at the ischaemic penumbra. Astrocytes are abundant in the brain and have an important role in potassium homeostasis, synapse formation and BBB regulation [45]. Activated astrocytes give protection in ischaemia through glutathione regulation [33, 45]: (1) liberating glutathione, which has a free radical scavenger function; (2) facilitating the enzyme γ glutamyltranspeptidase (present on the surface of astrocytes) to hydrolyse extracellular glutathione, producing glycine and cysteine, that are then captured by neurons to synthesise their own glutathione; and (3) liberating

glutamine that is also captured by neurons to synthesise glutathione. Astrocytes also provide protection by removing excess glutamate, producing neurotrophic factors and transforming dehydroascorbate to ascorbate, which is liberated to neurons and works as an antioxidant [33, 46]. **Figure 1** shows an illustration of a hypothetical thrombosis of the middle cerebral artery (superior division) in humans with high-risk factors. **Figure 1** represents a cascade based on the data described above.

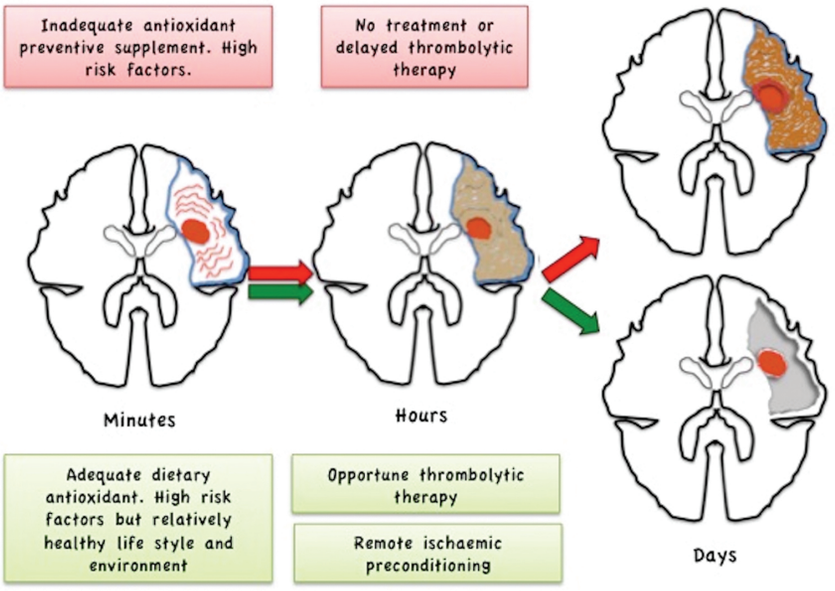

Figure 1. An illustration of a hypothetical thrombosis of the middle cerebral artery (superior division) in humans with high-risk factors. If the patient had received inadequate treatment with antioxidant supplements before cerebral ischaemia and/or the thrombolytic treatment is delayed, the ischaemic penumbra will be part of the core. If the patient has a relatively healthy lifestyle (see Ref. [13]) in a beneficial environment and receives opportune thrombolytic therapy and possibly remote ischaemic preconditioning, the consequences of the cerebral ischaemia will be smaller as compared with the conditions mentioned previously.

4. Preconditioning and survival in the ischaemic penumbra

Preconditioning can be defined as a biphasic response to a noxious stimulus, with a low dose (often accumulated low doses) providing protection when a higher dose is applied [47]. Preconditioning beneficial effects are usually explained by adaptive mechanisms such as changes in homeostasis thresholds or an increase of the endogenous defence. Oxidative preconditioning explains the protective effects of exercise [48]. Preconditioning has been used to increase neuronal survival at the ischaemic penumbra [49].

Several varieties of preconditioning have been used in cerebral ischaemia, such as hyperbaric oxygen (HBO), normobaric hyperoxia (NBH), hypoxia, ischaemic preconditioning and limb remote ischaemic preconditioning (RIsP). In the rat model of MCAOR HBO has been demonstrated to reduce oxidative stress as well as apoptosis in the penumbra, and it increases survival

time [50, 51]. Several mechanisms have been related to the beneficial effects of HBO preconditioning. HBO (2.5 ATA, 100% oxygen, 1 hour, every 12 hours, four times before MCAOR) increases SOD and catalase (not GPx) in the penumbra [50]. Longer HBO preconditioning (5 days before MCAOR) increases SirT1 (a class III histone deacetylase whose activity promotes lifespan in lower organisms) in the penumbra [52]. Interestingly, the protective effects of physical activity on cardiovascular ageing have been precisely associated with the increase of SOD and SirT1 [48].

NBH (100% oxygen, 1.0 ATA) has been also used for preconditioning to ameliorate cerebral ischaemia. NBH applied 30 min after ischaemia (MCAOR in a rat model, with 90 min of ischaemia) significantly reduced oxidative stress and apoptosis in the penumbra (with the greatest effect after 8 hours of NBH) [53]. It is important to mention that there are indications of potential harmful effects of HBO and NBH on cerebral ischaemia. Rink et al. [54] published an interesting paper in 2010 showing that HBO and NBH applied just during ischaemia in rats and mice submitted to MCAOR (90 min of ischaemia and 90 min of reperfusion) significantly decreased the infarct volume and also decreased oxidative stress, but increased pO_2 in the penumbra. However, when those therapies were administered during reperfusion the effect was the opposite. The findings are important because in the clinical setting it is difficult to establish the time-course of cerebral ischaemia and that administering HBO or NBH could be dangerous.

Hypoxic preconditioning has been used to promote angiogenesis in the ischaemic penumbra. In an interesting study, Li et al. [55] submitted mice to 30 min of hypoxia before cerebral ischaemia. Twenty-four and 72 hours later they demonstrated protection (a reduction of infarct volume and the neurophysiological index) and a significant increase of vascular endothelial growth factor (VEGF) as well as platelet endothelial cell adhesion molecule 1 (CD31, an index of angiogenesis) in both neurons and the vascular cells of the ischaemic penumbra.

Brain ischaemia preconditioning (BIP) has been extensively studied. Classically the model consists of submitting animals to a short period of transient MCAO, and then 1 day later, the animals are subjected to permanent MCAO or MCAOR [56]. Different mechanisms have been associated with BIP such as adaptation to glutamate activation of the N-methyl-D-aspartic acid (NMDA) receptor and the signal transduction pathways stimulated by this receptor including phosphorylation of cyclic AMP-responsive element binding protein (CREB) in the penumbra, persistent activation of protein kinase B (Akt) and decreased apoptosis in the penumbra [49]. Recently, Liu et al. [57] showed that rats submitted to BIP (one and 4 days before MCAOR) showed smaller infarct volumes and increased brain immunoreactivity to VEGF 7 days after MCAOR, which indicates similarities to the hypoxia preconditioning mechanisms.

Preconditioning, as described above, has been used in a number of experimental studies. In the last few years, a therapeutic strategy based on preconditioning principia has been applied not only in experimental but in clinical studies as well. RIsP simply consists of producing transient ischaemia in the upper or lower members, by inflation or deflation of a blood pressure cuff [58]. RIsP has been used in humans in a variety of applications such as in sports [59], for subarachnoid haemorrhages [60], and in cardiovascular surgery [61, 62]. There are experimental validations of the utility of RIsP in recovering the penumbra following cerebral

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ischaemia. Rats were subjected to MCAOR (90 min of ischaemia), immediately after ischaemia RIsP was applied in three cycles of occlusion and release, lasting 10 min each. After 48 hours it was demonstrated that the treatment (1) diminished the infarct volume, (2) improved the neurologic deficit and (3) decreased BBB breakdown (demonstrated by preventing an occludin protein decrease, Evans blue extravasation and MMP9 activation) [40]. Different mechanisms have been suggested for the protective effects of RIsP. Interestingly, in a clinical study of acute mountain sickness prevention by RIsP [59], it was clearly demonstrated (by electron paramagnetic resonance) that protection was not associated with the reduction of systemic oxidative stress. It seems that RIsP effects are associated with the liberation of the hypoxia-inducible factor α [58] and stimulation of eNOS [63]. **Figure 2** shows the modified **Figure 1**, adding molecular mechanisms and RIsP.

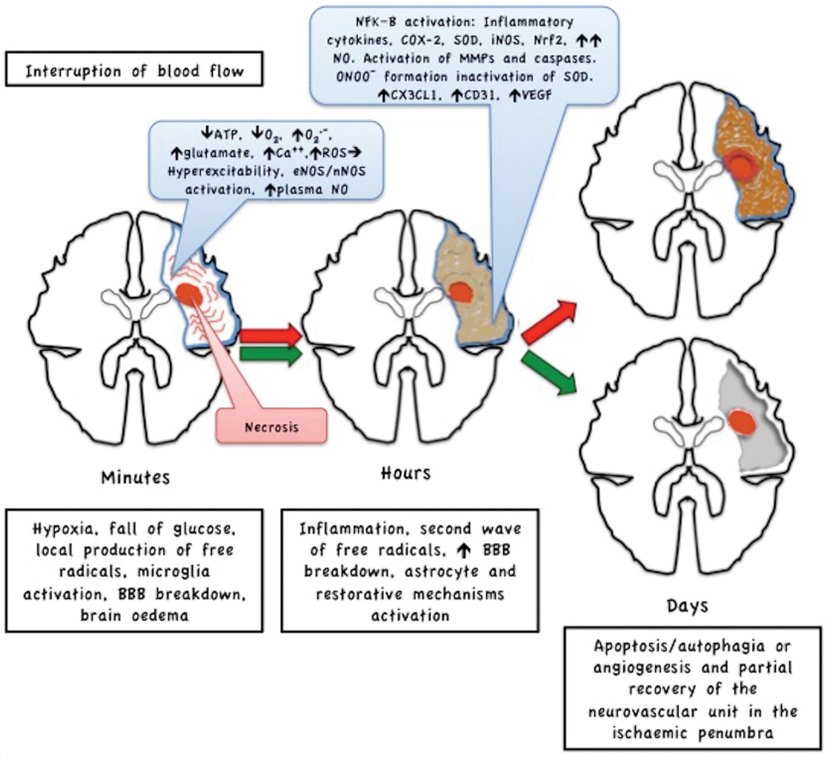

Figure 2. A hypothetical cascade of events leading to cell death or survival in the ischaemic penumbra. The cascade begins with an interruption of the blood supply that leads to hypoxia and the fall of glucose and consequently ATP. Within minutes there is necrosis in the core, and the subsequent ischaemic penumbra is characterised by an increase of superoxide ($O_2^{\bullet -}$), glutamate, calcium (Ca^{2+}) and other ROS, leading to hyperexcitability. eNOS and nNOS are activated (possibly not exclusively in the ischaemic penumbra) and nitric oxide (NO) levels increase in the plasma. Those phenomena produce a blood-brain barrier (BBB) breakdown and consequently brain oedema. From 1 to 7 days after blood interruption, there are a tremendous inflammatory reaction and a second wave of free radical production, activation of the NF-kB stimulating the synthesis of cyclooxygenase 2 (COX-2), superoxide dismutase (SOD), inducible NOS (iNOS) and the (erythroid-derived 2)-like 2 factor (Nrf2). NO increases additionally and interacts with $O_2^{\bullet -}$, producing peroxynitrite ($ONOO^-$), which in turn inactivates SOD through tyrosine nitration. Caspases and metalloproteinases (MMPs) are activated. The CX3CL1 and the endothelial cell adhesion molecule (CD31) increase. The restorative mechanisms begin with astrocyte activation. Part of the restorative mechanism is the induction of the vascular endothelial growth factor (VEGF) and CD31. If therapy is not effective or appropriate, the cells in the ischaemic penumbra will die from apoptosis or autophagy, which extends the core area. Otherwise the ischaemic penumbra is partially recovered.

The organism not only adapts to noxious stimuli but also to protective treatments, which is precisely one of the pitfalls of "preventive" antioxidant supplements. In an ongoing study (not yet published), our group found that daily doses (10 mg/kg per day for 7 days) of (−)-epicatechin ((−)-Epi) (one of the flavonols contained in chocolate) significantly increased the infarct volume 18 hours after permanent MCAO was produced in rats (**Figure 3**). It is important to note that cerebral ischaemia was produced in healthy (10-week-old) male Wistar rats, which could explain the results. We think that the treatment decreased the endogenous antioxidants and, when the ischaemia was produced, there was insufficient defence. We also think that the results would have been different if the treatment had been administered to animals that previously had high oxidative stress.

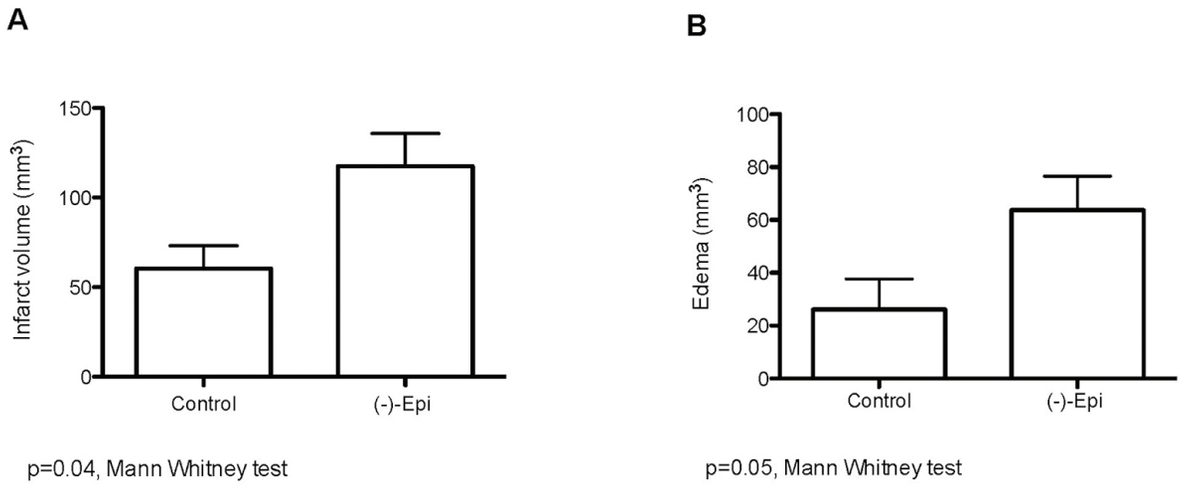

Figure 3. The significant increment of infarct volume (A) in rats treated with (−)-epicatechin ((−)-Epi) that were submitted to permanent occlusion of the middle cerebral artery (MCAO). And even though there was no significant difference in cerebral oedema (B), there was a tendency for the group treated with (−)-Epi to be higher.

5. Conclusions

Cerebral ischaemia remains a challenge in medical therapeutics because of the narrow window of time during which treatment can be applied, as well as its low efficacy. Brain cells in the ischaemic core cannot be recovered; they die within minutes from necrosis, whereas the death of those in the ischaemic penumbra is briefly delayed; and the mechanism of death is apoptosis and autophagy. Therapeutic strategies should be precisely directed to optimise all possible processes for recovering cells from the ischaemic penumbra. The neurovascular unit in the ischaemic penumbra is dynamically changing, switching off and on the pathways that could lead to cell death or survival. Free radicals are an important part of those pathways and can play a role in either cell loss or recovery. Even though results of preclinical studies are optimistic, the effects of antioxidant supplements have not been clinically validated in cerebral ischaemia. Moreover, there are increasing data showing warning signs. "Preventive" antioxidant supplements could decrease the endogenous antioxidant defence that is needed at the

precise moment of cerebral ischaemia. Antioxidant treatment (dietary or supplemental) should be carefully managed, depending on the basal conditions and the endogenous antioxidant defence of every person. There is some hope for applying RIsP in cerebral ischaemia, since it seems to have beneficial effects on the clinical physiological and pathological conditions associated with oxidative stress. Preclinical studies, even with their disadvantages, help to elucidate the pathophysiology of cerebral ischaemia and thereby the design of future treatments.

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