A role for lipids as agents to alleviate stroke damage: the neuroprotective effect of 2-hydroxy arachidonic acid

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Cerebrovascular accident (CVA) or stroke is one of the world's leading causes of death and permanent disability. The high social and medical costs associated with this pathology mean there is an urgent need to find effective therapies. Occlusion of the middle cerebral artery (MCAO), mainly by clots, is the origin of most CVAs in humans. The vessel occlusion (ischemic stroke) presents as a region with a significant reduction in blood flow, known as the ischemic core. This is surrounded by a region called the penumbra, where the blood flow is only partially reduced and neurons can still survive if they are able to recover their homeostatic balance (Durukan and Tatlisumak, 2007). Both the ischemic core and penumbra can be reproduced in experimental transient MCAO (tMCAO), a focal cerebral ischemia model widely used to analyze the neuroprotective role of molecules with the potential to become therapeutic drugs.

Inflammation and oxidative stress are considered promising pathways in the search for useful targets to reduce stroke-induced damage and to be useful in a clinical context. In this regard, some anti-inflammatory drugs have exhibited neuroprotective effects in different animal models of ischemia (Candelario-Jalil and Fiebich, 2008). Putative therapies for stroke based on decreasing reactive oxygen species (ROS) and reactive nitrogen species (RNS), either by inhibiting their formation or by increasing the cell antioxidant power are also being studied, and some of them have reached advanced phases of clinical trials (Shirley et al., 2014).

A role for lipid membranes in stroke: The previous idea of lipids as passive constituents supporting the membrane protein components has drastically changed. Nowadays, it is well known that membrane lipids play crucial functions by participating directly as messengers or regulators of signal transduction (Adibhatla et al., 2006; Tucker and Honn, 2013). In this regard, evidence of deregulated lipid metabolism in many neurological disorders, including neurodegenerative diseases, supports the key role of lipids in brain homeostasis. In particular, the role of altered lipid profiles in damage progression after stroke has been proposed since the late 1990s (Adibhatla et al., 2006).

Stroke-induced energy failure results in harmful cytosolic Ca^{2+} levels, which activates several phospholipases (PLA2, PtdIns-PLC, PtdCho-PLC and PtdCho-PLD) that promote the release of free fatty acids (FFA) from plasma membranes. The released FFAs can either increase the ischemic damage, such as omega-6 arachidonic acid (AA), or promote prosurvival responses, such as omega-3 docosahexaenoic acid (DHA). In this regard, neuroprotectin D1 (NPD1), a metabolite derived from DHA, presents pro-survival properties by downregulating pro-apoptotic signals and inflammation, and upregulating pro-survival genes (Belayev et al., 2011).

Two main peaks in FFA release, at 1 and 24 hours of reperfusion, have been described in the tMCAO model (Adibhatla et al., 2006). The first peak is mainly associated with the Ca^{2+}-dependent cPLA2 activation after ischemia, while the

second peak is associated with sPLA2 IIA activation. The sPLA2 activation is induced by proinflammatory stimuli on the reactive astroglia in the penumbra (Adibhatla et al., 2006; Hoda et al., 2009). The first FFA release peak occurs very early after a stroke, which complicates its pharmacological control. However, the delay in the second peak of FFA release provides more time and, therefore, more opportunities to modulate the different mechanisms that can alleviate the effect of stroke. In this regard, a neuroprotective effect has been demonstrated by using inhibitors of PLA2, such as 7,7-dimethyleicosadienoic acid (DEDA) or quinacrine, in the rat tMCAO model. The treatment with DEDA reduced the FFA release and the transcript levels of sPLA2 after 24 hours of reperfusion (Hoda et al., 2009). The role of PLAs in the neuroprotective effect is also supported by the effects of 2-hydroxy arachidonic acid (2OAA) on both cPLA2 and sPLA2 transcriptional levels (Ugidos et al., 2017). These results support a role of PLAs in stroke that not only relies on their activity but also occurs at the transcriptional level.

Crosstalk between oxidative stress and FFA released from membranes: AA is the most commonly released FFA associated with oxidative stress. It is modified by different enzymes, such as cyclooxygenases (COX) and lipoxygenases (LOX), generating metabolites (prostaglandins, thromboxames and leukotrienes), which are proinflammatory eicosanoids. Both COX and LOX activities result in the insertion of oxygen into AA, a process that releases highly reactive intermediates including superoxide radicals ($O^{2-\cdot}$), hydroxyl radicals ($\cdot OH$) or toxic aldehydes such as malonaldialdehyde (MDA) or 4-hydroxynonenal (HNE) (Nanda et al., 2007). These reactive species all contribute to a huge pool of ROS and RNS that exceed the cell antioxidant capacity and exacerbate the neuronal damage (Adibhatla et al., 2006; Nanda et al., 2007). ROS and RNS ignite the peroxidation of polyunsaturated fatty acids (PUFAs) in a process called lipid peroxidation, which unbalances the cell homeostasis, impairs the cell signaling from plasma membranes and leads to the uncoupling of the mitochondrial membrane potential. The subsequent mitochondrial impairment results in a positive feedback that, in turn, increases ROS in the cell (Adibhatla et al., 2006). Neurons are highly vuluerable to ROS after stroke because they present reduced levels of glutathione (GSH), which forms the primary endogenous antioxidant defense in the brain by reducing H_2O_2 and therefore decreasing the lipid peroxidation (Adibhatla et al., 2006).

Modulating COX-1 and COX-2 as targets for alleviating stroke: COX-1 and COX-2 inhibitors have been widely tested in different ischemia models as putative therapies to alleviate stroke damage. In this regard, the pharmaceutical industry has focused on developing selective COX-2 inhibitors to prevent the deleterious gastrointestinal effects caused by the COX-1 blocking (Choi et al., 2009). Some of these selective COX-2 inhibitors have exhibited a neuroprotective effect, such as NS-398 or nimesulide (Candelario and Fiebich, 2008). However, the treatment with some selective COX-2 inhibitors, such as rofenacoxib and etoricoxib, has resulted in the enhancement of cardiac arrest and stroke, especially in older patients. These effects have been associated with an imbalance in blocking COX-2 and COX-1. In this regard, selective COX-2 inhibitors block the constitutive endothelial COX-2, leading to dysfunctions in endothelium-dependent contractions. On the other hand, COX-1 promotes platelet activation, which would not be prevented by highly selective COX-2 blockers. Thus, the combined effect on endothelium contraction promoted by selective anti-COX-2 agents and the platelet activation, which would not be inhibited by COX-2 blockers, would facilitate thrombotic activity (Candelario-Jalil and Fiebich, 2008; Grosser et al., 2010). Moreover, the crucial role of microglia in brain inflammation and the constitutive expression of COX-1 in these cells have led to a new perspective in the use of COX-1 inhibitors in acute and chronic neurological disorders (Candelario-Jalil and Fiebich, 2008; Choi et al., 2009). In fact, COX-1 inhibition in transient global ischemia reduces neuronal injury and oxidative stress and contributes to the neuroprotective effect (Choi et al., 2009). Nevertheless, the deleterious effect of COX-1 in the organism should be taken into account when administering proper doses to minimize peripheral damage. Thus, the use of highly selective COX-2 inhibitors should be reconsidered for stroke and other neuropathologies associated with inflammation (Candelario-Jalil and Fiebich, 2008; Choi et al., 2009).

Neuroprotective effect of 2-hydroxy arachidonic acid, a novel rationally designed lipid: The rationally designed lipid 2OAA is a derivative of AA, which competitively inhibits both isoforms COX-1 and COX-2, and has been reported to decrease proinflammatory cytokine levels in the serum of LPS-treated mice (López et al., 2013). The lipid character of this molecule allows it to cross the blood-brain barrier (BBB) without the need for carrier molecules. A recent study reports the neuroprotective effect of 2OAA in a rat model of tMCAO (Ugidos et al., 2017). This study reports that intragastric administration 1 hour after reperfusion of 1 g/kg of 2OAA results in an approximate 50% reduction of infarct volume, while lower doses of 2OAA provide poor or non-neuroprotective effects in tMCAO. The study also indicates that a 1 g/kg dosage of 2OAA for more than 2 days results in deleterious effects which can be prevented by

decreasing the dose after the second day.

These effects of 2OAA illustrate the importance of properly controlling the inhibition of COX-1 and COX-2 and of early treatment. Thus, a quick blocking COX-1 and COX-2 with 2OAA confers neuroprotection detectable after 24 hours of reperfusion (Ugidos et al., 2017) suggesting the attenuation or delay in the first inflammation steps. In contrast, other lipid treatments administered at similar times, such as DHA or eicosapentaenoic acid (EPA), act later, at the resolution of the inflammation and their neuroprotective effect cannot be detected until several days after a stroke (Belayev et al., 2011).

There are few data comparing the anti-inflammatory effect of 2OAA with other agents, thus, in LPS-induced endotoxemia in mice, the treatment with 2OAA decreases tumor necrosis factor alpha (TNF-α) more greatly than the non-specific non-steroidal anti-inflammatories (NSAIDs) ibuprofen (López et al., 2013). Moreover, 2OAA can cross the BBB by simple diffusion, in contrast to other NSAIDs that use transporters whose efficiencies depend on microenviromental conditions (Novakova et al., 2014). Thus, 2OAA has certain advantages over other NSAIDs, which makes it a promising drug for the treatment of various disorders such as stroke (López et al., 2013; Ugidos et al., 2017). On the other hand, results of treatment with 2OAA are similar to those of the steroidal anti-inflammatory cortisone without the detrimental steroid effects (López et al., 2013).

Regarding the neuroprotective effect of 2OAA, this may initiate at the membrane level, decreasing the expression of PLA2 resulting in a subsequent decrease in FFA release, ultimately modifying the oxidative state of the cell. Ugidos et al. (2017) explored the effects of 2OAA in decreasing the transcription of antioxidant enzymes regulated by the nuclear factor (erythroid-derived 2)-like 2 (Nrf-2) transcription factor, such as superoxide dismutase (SOD), NAD(P)H quinone dehydrogenase 1 (NQO1), glutamate-cysteine ligase modifier subunit (GCLM) and heme oxygenase 1 (HMOX1). The authors conclude that the reduction of FFA release and the PLA2 transcriptional reduction as a consequence of 2OAA treatment lead to a decrease in oxidative stress. Therefore, the authors hypothesize that this agent attenuates the ROS and RNS generation rather than increases the antioxidant response, at least under their study conditions (**Figure 1**).

In conclusion, the strong neuroprotective effect resulting from post-ischemic treatment with 2OAA after tMCAO illustrates the possibilities of using rationally designed lipids as putative agents against stroke (Ugidos et al., 2017). The authors hypothesize that membrane lipid regulation could play a role in cell homeostasis. The effect of 2OAA also reveals the importance of a controlled balance in COX-1 and COX-2 enzyme inhibition. Since these isoforms play crucial and different roles in the response to ischemic damage, the inhibition of both COX-1 and COX-2 could result in more effective neuroprotection than the use of a selective COX-2 blocker. The strong neuroprotective effect resulting from post-ischemic treatment with 2OAA after tMCAO, inhibiting both COX-1 and COX-2 enzymes (Ugidos et al., 2017) supports the claims of using balanced COX-1 and COX-2 inhibitors as putative anti-stroke therapies, showing advantages over other NSAIDs (López et al., 2013).

The use of rationally designed lipids appears to be a promising new form of therapy for stroke, one of the main advantages of which is the ability to cross the BBB. In the case of 2OAA, a more accurate control of doses and time of treatment are required to enhance and prolong its neuroprotective effect, preventing peripheral detrimental damage in long treatments.

Footnotes

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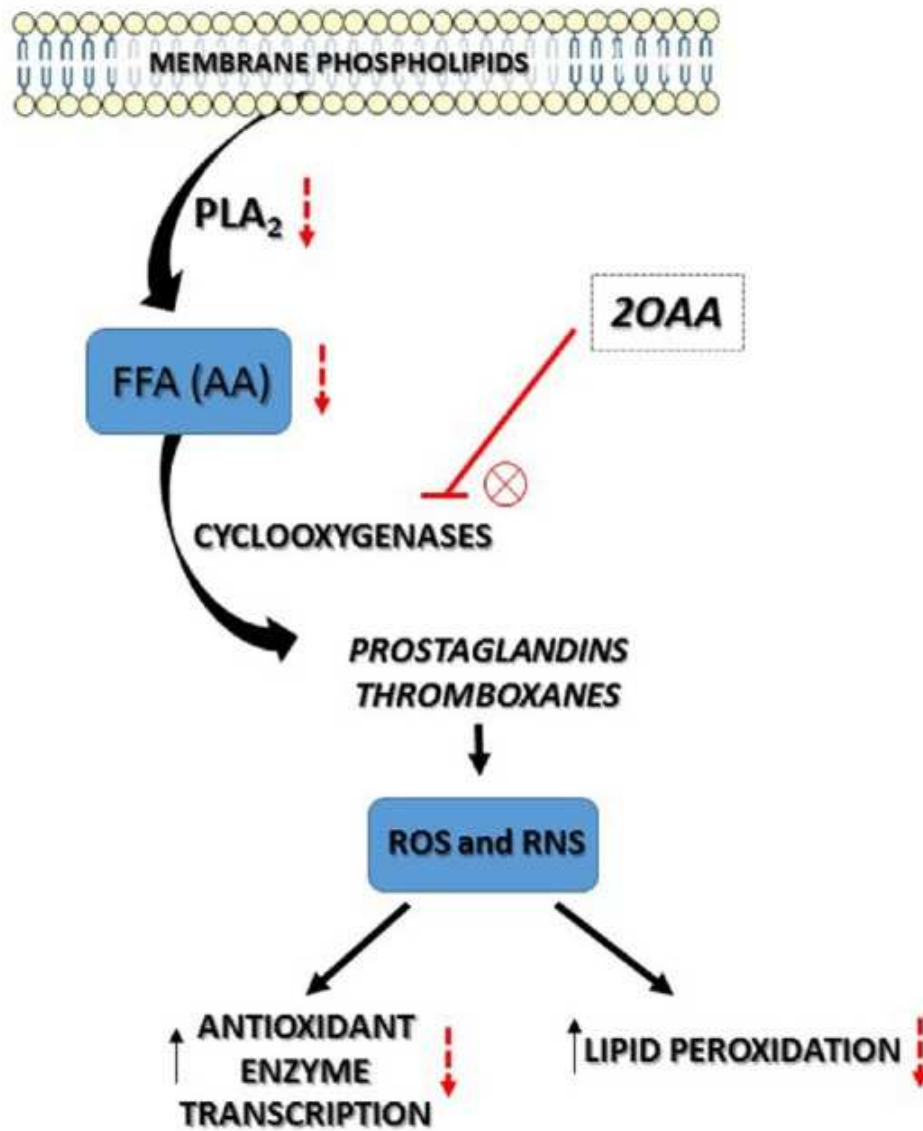
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Figures and Tables

Figure 1

Effect of 2OAA treatment after transient middle cerebral artery occlusion.

In black, lipid cascade after stroke. The continuous red line shows the 2OAA effect blocking cyclooxygenases. The discontinuous red arrows show the indirect effects of 2OAA at different steps in the pathway 2OAA: 2-Hydroxy arachidonic acid; AA: arachidonic acid; FFA: free fatty acids; PLA2: phospholypases A2; RNS: reactive nitrogen species; ROS: reactive oxygen species.