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New Perspectives in Neuroprotection for Ischemic Stroke

María Pérez-Mato [a], Esteban López-Arias [a], Ana Bugallo-Casal [a], Clara Correa-Paz [a], Susana Arias [b], Manuel Rodríguez-Yáñez [b], María Santamaría-Cadavid [b], Francisco Campos [a,c,*]

[a] *Translational Stroke Laboratory Group (TREAT), Clinical Neurosciences Research Laboratory (LINC), Health Research Institute of Santiago de Compostela (IDIS), 15706 Santiago de Compostela, Spain*

[b] *Stroke Unit, Department of Neurology, Hospital Clínico Universitario, 15706 Santiago de Compostela, Spain*

[c] *Universidade de Santiago de Compostela, 15782 Santiago de Compostela, Spain*

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ABSTRACT

The constant failure of new neuroprotective therapies for ischemic stroke has partially halted the search for new therapies in recent years, mainly because of the high investment risk required to develop a new treatment for a complex pathology, such as stroke, with a narrow intervention window and associated comorbidities. However, owing to recent progress in understanding the stroke pathophysiology, improvement in patient care in stroke units, development of new imaging techniques, search for new biomarkers for early diagnosis, and increasingly widespread use of mechanical recanalization therapies, new opportunities have opened for the study of neuroprotection. This review summarizes the main protective agents currently in use, some of which are already in the clinical evaluation phase. It also includes an analysis of how recanalization therapies, new imaging techniques, and biomarkers have improved their efficacy.

INTRODUCTION

Stroke is defined as "a neurological deficit attributed to acute focal injury to the central nervous system (CNS) from a vascular cause, including cerebral infarction, intracerebral hemorrhage, and subarachnoid hemorrhage" and is a leading cause of disability and death worldwide, according to the American Stroke Association/American Heart Association (Sacco et al., 2013). Of the 15 million people worldwide who experience stroke annually, 5 million die and 5 million are left with various disabilities (World Health Organization data). In men and women younger than 55 years, the incidence remains similar, but it is higher in men between the ages of 55 and 75 years (Collaborators GBDLRoS et al., 2018; Collaborators GBDN, 2019). Improvements in the management of patients with stroke in primary care and stroke units, particularly in developed countries, have contributed to a reduction in mortality in recent years. However, the incidence does not follow the same trend owing to factors such as demographic changes and increased prevalence of hypertension, obesity, and other comorbidities (Ovbiagele and Nguyen-Huynh, 2011).

Stroke can be divided into two main types: hemorrhagic and ischemic. In hemorrhagic stroke, the rupture of a blood vessel causes intracerebral hemorrhage owing to the extravasation of blood into the brain parenchyma, whereas in ischemic stroke, the occlusion of a blood vessel by a thrombus causes a decrease in cerebral blood flow (Amarenco et al., 2009). From a therapeutic point of view, in intracerebral hemorrhage, control of hemorrhage and reduction of intracranial pressure are the main therapeutic strategies to prevent neuronal damage (Hemphill et al., 2015). In ischemic stroke, reperfusion strategies (pharmacological thrombolysis and mechanical thrombectomy [MT]) are the therapies aimed at restoring cerebral blood flow (CBF). Pharmacological therapy, based on the use of recombinant tissue plasminogen activator (rtPA, also known as alteplase) or its new derivative tenecteplase (TNK), is the only drug treatment for acute ischemic stroke. These thrombolytics convert plasminogen into plasmin, which breaks down the fibrin network constituting the thrombus. This treatment has been in use since 1995, and it is effective in improving clinical outcomes, reducing disability, and achieving successful recanalization in approximately 30% of patients. However, rtPA and

Abbreviations: ASo, antisense oligonucleotides; CBF, cerebral blood flow; CNS, central nervous system; CT, computed tomography; FGF21, fibroblast growth factor 21; GOT, glutamate oxaloacetate transaminase; HT, hemorrhagic transformation; LVO, large vessel occlusion; MRI, magnetic resonance imaging; MT, mechanical thrombectomy; NA1, nerinetide; NMDA, N-methyl-d-aspartate; NMDAR, NMDA glutamate receptor; PET, positron emission tomography; rtPA, recombinant tissue plasminogen activator; RBM3, RNA-binding motif protein 3; S1P, sphingosine 1-phosphate; SH, systemic hypothermia; SPAN, Stroke Preclinical Assessment Network; TNK, tenecteplase; TLR, toll-like receptor.

* Corresponding author.
E-mail addresses: francisco.campos.perez@sergas.es, francisco.campos@rai.usc.es (F. Campos).

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TNK have many drawbacks that limit their use, such as a short half-life (a few min), which implies transient effects, neurotoxic side effects owing to the modulation of *N*-methyl-D-aspartate (NMDA) glutamate receptors, risk of hemorrhagic transformation (HT), and proinflammatory effects (Thiebaut et al., 2018). These limitations result in a short therapeutic window of 4.5–6 h after stroke onset, which subsequently reduces the number of patients who can benefit from this therapy (Tomsick et al., 2010). MT, either alone or combined with pharmacological thrombolysis, has improved recanalization rates (up to 80%) and decreased the risk of hemorrhagic transformations (HTs), with a gap between the increased recanalization rate and improved neurological functions. However, this mechanical strategy is useful only when thrombi are located in large vessels and requires specialized hospital facilities (Gauberti et al., 2021; Laredo et al., 2022).

Given that the number of stroke candidates for recanalization treatments for ischemic stroke is limited, there is an urgent need to identify alternative neuroprotective (or cytoprotective) agents that can be used in a larger number of patients. It is generally accepted that after cerebral vessel occlusion, the ischemic region can be divided into two regions: an internal core region characterized by necrotic cell death within the first few hours after stroke and a potentially salvageable region surrounding the ischemic core, defined as the penumbra. Therefore, it represents a potential therapeutic target for reducing the effects of ischemic injury, as long as the changes resulting from the ischemic cascade do not cause irreversible damage leading to tissue death. Considerable research has been devoted to the development of novel therapies to protect the brain from damage after stroke, with particular attention paid to the penumbral region. Several neuroprotective treatments, including glutamate antagonists, calcium blockers, oxidative stress-free radicals, and immunomodulators, have shown great potential in animal models of stroke. However, almost all of these treatments have failed to provide protection in human trials (Savitz et al., 2017).

Many arguments can explain why neuroprotective drugs have not worked in patients with stroke, such as translational experimental limitations or clinical issues, including proper patient selection in clinical trials and a narrow therapeutic window (Sutherland et al., 2012). All these preclinical studies focused on the search for new and effective protective agents that are crucial in defining and characterizing the molecular events that occur after ischemic onset. However, the persistent failure in the search for effective neuroprotective therapies has reduced the enthusiasm of pharmaceutical companies, mainly owing to the high risk of inversion. One of the best examples is AstraZeneca's development of XY059, a free radical scavenger (Antonic et al., 2018). Disodium 2, 4-sulphophenyl-*N*-*tert*-butylnitrone (known as NXY-059) is a free radical scavenger that reduces the infarct volume in rodent and primate stroke models. The translational promise of these publications, together with the evidence of safety in control and acute stroke populations, led the company to conduct two randomized, placebo-controlled phase III trials of NXY-059. Although the first study showed a weak positive signal in the primary and secondary analyses, the second, larger study (3306 vs. 1722 patients) was neutral, providing no evidence of the efficacy of NXY-059 in patients with stroke (Antonic et al., 2018).

If we extend these analyses to other studies conducted in the early 1990s, approximately 500 clinical trials were conducted to test more than 200 new potential neuroprotective treatments for ischemic stroke. However, as of 2018, only nine of these treatments have successfully progressed to phase III trials (Shi et al., 2018).

Recent advances in the understanding of stroke pathophysiology, improvement of patient care in stroke units, development of new imaging modalities, search for new biomarkers for early diagnosis, and increasing use of mechanical recanalization therapies have opened up new opportunities for the study of new protective therapies (Chamorro et al., 2021). This review provides an overview of current and promising approaches in the field of neuroprotective stroke treatment (Chamorro et al., 2016, 2021; Savitz et al., 2017; Shi et al., 2018; Paul and Candelario-Jalil, 2021; Fisher and Savitz, 2022; Ghozy et al., 2022; Haupt et al., 2023; Liu et al., 2023) and analyzes how new advances in the field of imaging, biomarkers, and recanalization therapies have contributed to improving the efficacy and utility of protective agents (Kidwell et al., 2013; Rocha and Jovin, 2017; Shi et al., 2018; Driga et al., 2021; Fisher and Savitz, 2022).

EXPERIMENTAL PROCEDURES

The present review was performed by searching the MEDLINE database using the PubMed search engine without applying any restrictions related to the year of publication, country, or category of publication (e.g., original article or review). Only articles published in English were included. The search criteria used were keywords related to the protective therapies included in this manuscript, which, in the authors' opinion, have the greatest impact in Europe in the field of stroke and have reached the clinical analysis stage. In addition, a manual search was performed to include information on clinical trials in patients with stroke whose results have been published in conferences or congresses but not in journals detected by PubMed.

NEUROPROTECTION AGAINST NEURONAL EXCITOTOXICITY

Glutamate is one of the most important excitatory neurotransmitters in the CNS. Owing to its high excitatory effect on neuronal cells, its release must be highly controlled to avoid an excitotoxic response in neuronal tissue. In contrast to other neurotransmitters, such as dopamine and acetylcholine, which use an enzymatic system to control the neuronal response in the synaptic cleft, the glutamatergic system uses faster and more efficient glutamate transporters to prevent excessive activation of receptors by continuously removing glutamate from the extracellular fluid (Zhou and Danbolt, 2014). The concentration of extracellular glutamate is tightly regulated by high-energy sodium-dependent transporters (also known as excitatory amino acid transporters) located mainly on astrocytes, which bind to and remove neurotransmitters for processing and recycling (Danbolt, 2001). Glutamate excitotoxicity occurs when the homeostatic balance of the neurotransmitter is disrupted and glutamate levels increase in the extracellular fluid (Danbolt, 2001). Failure of the nutrient supply after ischemia causes neuronal depolarization, leading to a massive release of glutamate into the extracellular space. In addition, because glutamate uptake is a highly energy-dependent process, the restriction of energy after ischemia causes a drastic disruption of glutamate transporters, enhancing the excitotoxic effect and triggering neuronal death (Castillo et al., 2016). Real-time analysis of glutamate in the ischemic region indicates that glutamate release occurs immediately (in min) in the core region; therefore, the therapeutic interventional option to block its effects has a very narrow timeframe. Conversely, in the penumbral region, residual blood flow allows the maintenance of glutamate uptake systems and ionic pumps. However, the spread of glutamate from the ischemic core to its periphery accelerates the progression of damage in the still salvageable penumbra region (Ramos-Cabrer et al., 2011). Therefore, early control of glutamate excitotoxicity is an ambitious therapeutic strategy to block the downstream domino ischemic cascade; however, the narrow interventional timeframe limits the clinical applications of drugs designed to block this excitotoxic process (Castillo et al., 2016). We could define this as the “uncertainty principle of glutamate,” that is, we cannot get protection and time with perfect accuracy.

Decades ago, clinical studies reported that glutamate plays a critical role in neuronal damage following ischemic stroke (Castillo et al., 1995; Castillo et al., 1996; Castillo et al., 1997; Castillo et al., 1999). The prevention of glutamate-mediated toxicity, mainly based on the use of selective glutamate antagonists, has been a central ther-

apeutic target in numerous studies; however, many of these have shown toxic side effects or failed to show efficacy when evaluated in clinical trials. Despite these disappointing results, the critical involvement of glutamate-mediated toxicity in functional outcomes after acute ischemic stroke has not been ignored, and recent studies in the last years have expanded beyond the use of classical glutamate antagonists to control glutamate-related downstream signals. Examples of these new strategic therapies include drugs, such as nerinetide (NA1) and blood glutamate scavengers.

Nerinetide

NA1 is a 20-amino acid peptide that prevents attachment of postsynaptic density 95 (PDS-95) protein to the NMDA glutamate receptor (NMDAR) subunit. Elevated glutamate levels in the extracellular space lead to NMDAR activation. This results in an influx of calcium, ultimately leading to cellular damage in ischemic infarcts. Therefore, blocking these receptors has been proposed as a potential treatment strategy for ischemic stroke. However, the use of NMDA antagonists in ischemic stroke has been shown to induce side effects in healthy neurons and has a very narrow timeframe for inducing positive effects. NA1 acts by inducing selective inhibition of the PDS-95 protein and reducing neuronal toxicity, and more importantly, it does not block the normal synaptic activity of NMDARs. Intervention studies performed before endovascular aneurysm surgery have shown that pretreatment with NA1 decreases the frequency of iatrogenic stroke (ENACT trial) (Hill et al., 2012). This compound has also been tested in macaques, and the results showed positive behavioral findings and magnetic resonance imaging (MRI) results after ischemic stroke (Cook et al., 2012). These previous preclinical findings led to a multicenter, randomized clinical trial with more than 1000 participants using NA1 as a potential neuroprotective agent in combination with rtPA recanalization therapy (ESCAPE-NA1) (Hill et al., 2020). Unfortunately, the administration of NA1 did not improve neurological outcomes in patients who have undergone thrombolysis compared with the placebo group. A secondary sub-analysis showed that NA1 is susceptible to proteolytic cleavage when administered after rtPA and therefore loses its effect in patients receiving thrombolytic therapy (Mayor-Nunez et al., 2021). Two new trials of NA1, ESCAPE-NEXT and FRONTIER, were developed to test NA1 in patients who have not undergonethrombolysis and for pre-hospital administration in patients with suspected stroke, respectively (Ghozy et al., 2022; Haupt et al., 2023).

Both were recently concluded; the EXCAPE-NEXT trial showed neutral results, whereas the FRONTIER trial showed some benefits in patients who were treated in a pre-hospital setting (ambulance) very early after stroke onset, reflecting the importance of blocking the excitotoxic cascade very early after stroke onset (results presented in the 2023 World Stroke Congress in Toronto, Canada).

Blood/brain glutamate scavengers

Blood/brain glutamate grabbing or scavenging processes are emerging as novel protective strategies to reduce the excitotoxic effects of excess extracellular glutamate that accumulates in the brain after ischemic injury (Leibowitz et al., 2012; Castillo et al., 2016; Kaplan-Arabaci et al., 2022). The blood/brain glutamate grabbing mechanism is based on the reduction of blood glutamate concentration, which leads to a larger natural glutamate gradient between the brain and blood, thus facilitating the efflux of extracellular glutamate into the blood. Therefore, the manipulation of this mechanism may have a neuroprotective effect after a stroke. The main advantage of this novel therapeutic strategy is that it occurs in the blood circulation and therefore does not interfere with normal brain neurophysiology, as has been described for other drug treatments (such as glutamate antagonists) (Castillo et al., 2016). This new approach has been validated in several ischemic animal models by independent laboratories (Rink et al., 2011; Boyko et al., 2012a, 2012b; Rink et al., 2017) and has also been successfully tested in other pathological models associated with increased glutamate levels in the brain, such as traumatic brain injury (Zhang et al., 2019), glioma (Ruban et al., 2012), Alzheimer disease (Zhang et al., 2017), and amyotrophic lateral sclerosis (Ruban et al., 2015; Rogers et al., 2023), with successful results.

The first interventional clinical trial designed to demonstrate the beneficial efficacy of blood glutamate scavenging in stroke used dialysis to filter the blood and remove excess glutamate from patients. Peritoneal dialysis in rodent models of ischemia resulted in a decrease in blood glutamate levels, with a corresponding increase in glutamate in the dialysis solution, followed by a significant reduction in infarct size (Godino Mdel et al., 2013). However, the use of peritoneal dialysis (EudraCt Number: 2012-000791-42) or hemodialysis (NCT04297345) in patients with stroke had to be discontinued because of the side effects of this invasive procedure.

To demonstrate the clinical efficacy of blood glutamate scavenging therapy in patients with stroke, the glutamate scavenging properties of riboflavin (vitamin B2) were tested in a proof-of-concept phase IIb clinical trial (NCT02446977) with 25 control (placebo) and 25 riboflavin (20 mg)-treated patients with stroke (da Silva-Candal et al., 2018). This analysis confirmed a reduction in blood glutamate levels in the treated patients and a trend toward functional recovery compared with the placebo group. Despite the promising clinical results observed with riboflavin, the main limitation of this clinical approach is the highly water-soluble nature of the vitamin, reflected in its rapid blood clearance, which requires continuous administration of high doses to achieve significant results.

One of the most explored and effective pharmacological strategies for reducing blood glutamate levels is the exogenous administration of glutamate oxaloacetate transaminase (GOT) enzyme, also known as aspartate aminotransferase. GOT plays a critical role in the regulation of blood glutamate levels by catalyzing the reversible conversion of oxaloacetate and glutamate to aspartate and α-ketoglutarate, respectively. Thus, the administration of the purified recombinant form of human GOT (rGOT) in ischemic animal models leads to the metabolism and reduction of glutamate in the blood and lowering of glutamate in the cerebral parenchyma, which is associated with a reduction of the ischemic lesion and sensorimotor recovery (Perez-Mato et al., 2014). In addition, through bioconjugation with polyethylene glycol, it was possible to increase the half-life of rGOT in the blood after administration; a single administration of this new formulation had a protective effect similar to that of rGOT administered four times (Zhang et al., 2019). The inhibition of blood GOT activity also results in higher brain glutamate levels and greater damage (Dopico-Lopez et al., 2021). These experimental findings are also consistent with two retrospective studies with more than 400 patients with ischemic stroke, indicating that endogenous blood GOT activity levels greater than 17 U/L at hospital admission were associated with lower blood glutamate concentrations and good outcomes 3 months after stroke, supporting the clinical use of rGOT as a therapy (Campos et al., 2011a, 2011b).

The therapeutic mechanisms postulated for the protective effect of rGOT are mainly related to the metabolism and reduction of blood glutamate; however, other studies have also reported that rGOT metabolizes brain glutamate for use as an energy substrate in anaerobic conditions, such as ischemia (Rink et al., 2011; Rink et al., 2017). This hypothesis is supported by the fact that GOT overexpression in the brain reduces glutamate levels and prevents ATP loss under ischemic conditions. Recent studies have reported the ability of GOT to rescue the activity of mitochondria affected by ischemia and protect against energy failure (Xu et al., 2020).

The administration of an endogenous serum enzyme, such as GOT, as a new protective treatment against ischemia is an interesting strategy because the administration of rGOT is unlikely to induce toxic effects in humans, as the levels of this enzyme vary in healthy individ-

uals (7–45 U/L) and have been shown to increase more than 10-fold in patients with liver damage. Further human studies are required to demonstrate the potential therapeutic effects of this enzyme.

NEUROPROTECTION AGAINST STRESS OXIDATIVE DAMAGE

Oxidative and nitrosative stress are critical players in the process of ischemic injury. Both processes are triggered after excitotoxic ischemic injury and occur as a consequence of an increase in secondary messenger systems coupled with enzymatic generation of free radicals (e.g., via cyclooxygenase or nitric oxide synthases) (Chamorro et al., 2016). Such mechanisms are particularly exacerbated after delayed recanalization, producing large amounts of oxidative and nitrosative free radicals, including superoxide (O_2^-), hydrogen peroxide (H_2O_2), nitric oxide (NO), hypochlorous acid (HClO), and peroxynitrite ($ONOO^-$) in the CNS and peripheral systems, activating adhesion molecules, and promoting inflammatory and immune cell infiltration in ischemic regions (Chen et al., 2020).

NXY-059

Neutralization of oxidative and nitrosative stress has been explored as an ambitious therapeutic strategy because the ischemic brain is highly susceptible to oxidative damage owing to its high oxygen consumption, high iron and unsaturated lipid content, and relatively low endogenous antioxidant capacity. Oxidative and nitrosative radicals are mainly generated in the ischemic penumbra, and preventing the effects of these compounds has been shown to be an effective measure to limit the expansion of ischemic lesions in preclinical stroke models (Chamorro et al., 2016). Peroxynitrite, generated from the interaction between nitric oxide and superoxide, has particular therapeutic value because it promotes multiple cell-related stress processes such as lipid peroxidation, mitochondrial and DNA damage, protein nitration, and oxidation or depletion of antioxidant reserves (Leira et al., 2023). One of the widely known peroxynitrite scavengers tested was NXY-059. NXY-059 initially showed positive results in rodent models of stroke and promising protective evidence in the clinical trial SAINT I but not in the large trial SAINT II (Chamorro et al., 2016).

Uric acid

After NXY-059, new oxidative and nitrosative free radical scavengers have emerged such as uric acid. Uric acid is the final oxidation product of purine catabolism in humans and accounts for up to two-thirds of the total antioxidant capacity of the plasma (Chamorro et al., 2016, 2021; Leira et al., 2023). In preclinical approaches, its protective efficacy has been widely reported to prevent glutamate-induced cell death *in vitro*, whereas in transient or permanent ischemia in rodents, it suppresses oxidative and nitrosative radicals and peroxynitrite-mediated damage, reduces infarct volume, and improves functional outcomes (Romanos et al., 2007; Onetti et al., 2015).

New trends aimed at improving the reproducibility of preclinical data and demonstrating the translational value of new candidates for the treatment of acute ischemic stroke recommend the development of multi-laboratory preclinical trials by independent laboratories before moving to clinical assays. With this aim, a Stroke Preclinical Assessment Network (SPAN) tested six treatment candidates (including uric acid) in four stages in different rodent models of ischemic stroke (Lyden et al., 2023). In this analysis, uric acid supplementation was the only treatment that showed benefits in male and female animals, young mice, young rats, aging mice, obese mice, and spontaneously hypertensive rats (Leira et al., 2023), supporting its potential applicability in humans.

The efficacy of uric acid treatment has been widely assessed in several clinical trials, such as the phase IIb/III URICOICTUS trial, which confirmed the safety of a combination of uric acid and alteplase (started within 4–5 h of symptom onset) in patients with acute ischemic stroke. The trial did not demonstrate the efficacy of uric acid on the primary outcome (modified Rankin score at the 90-day follow-up) (Chamorro et al., 2014). However, uric acid reduced the incidence of early clinical worsening, and more patients treated with uric acid achieved full independence at follow-up than those who received placebo. Uric acid has also been shown to reduce infarct growth and improve functional outcomes in predefined patient subgroups such as women or patients with pretreatment hyperglycemia or early recanalization (Amaro et al., 2015; Llull et al., 2015). Because free radical agents are released after recanalization and produce large amounts of free radicals, future directions suggest combining uric acid with MT to reduce oxidative stress and improve microcirculatory hypoperfusion (Leira et al., 2023). Conclusive clinical trials of uric acid treatment are required to validate the clinical protective effects of this promising free radical scavenger.

Edaravone

Another antioxidant agent that has shown great affinity as a chelator of hydroxyl radicals with clear benefits in experimental models of ischemia is edaravone (also known as MCI-186) (Nishi et al., 1989; Oishi et al., 1989), which is manufactured by Mitsubishi Tanabe Pharma Corporation (Osaka, Japan). Favorable preclinical data led to this compound to be tested in clinical trials, with satisfactory results, allowing its approval in countries such as Japan, China, and India (Kobayashi et al., 2019). In Japan, nearly half of the patients with ischemic stroke receive edaravone for acute treatment (Kobayashi et al., 2019). Becasuse edaravone is known to have potent antioxidant effects, Japanese authorities have also validated its clinical application in combination with rtPA (Kimura et al., 2012). Although the data available thus far have confirmed its safety in Asian countries, its application is not yet widely accepted for treating ischemic stroke in Western countries. A recent meta-analysis of randomized controlled clinical trials conducted to date with this compound showed that although the available clinical data are limited, edaravone appears to improve neurological impairment, with a survival benefit at the 3-month follow-up, regardless of the mean age and treatment course (Chen et al., 2021). However, larger studies are required to determine the benefits of edaravone in patients before it can be approved in Western countries.

INFLAMMATION

The inflammatory response following acute ischemic stroke is one of the most complex processes mediating the progression of infarct lesions. This complexity is caused by various factors, including local and circulating immune cells and cytokines (inflammatory and immunomodulatory factors). It is well known that the region of ischemic brain injury releases several damage-associated molecules, such as heat shock proteins, ATP, S100 proteins, heparan sulfate, DNA, and RNA, which subsequently trigger an inflammatory response and infiltration of immune cells into the brain parenchyma, according to the temporal pattern of chemoattractant and adhesion molecules (Eltzschig and Eckle, 2011; Chamorro et al., 2016, 2021). After an ischemic event, the first immune cells to respond are brain-intrinsic microglia, which release mediators that further attract neutrophils, monocytes, and lymphocytes (Eltzschig and Eckle, 2011; Kolaczkowska and Kubes, 2013). Once in the brain parenchyma, the immune cells trigger a cascade of secondary events that contribute to inflammatory responses, brain injury, and subsequent neurological deficits.

The first wave of immune/inflammatory response is essential to remove dead cells and cell debris; however, exacerbated inflammatory response may contribute to the development of injury through the release of highly oxidant and cytotoxic compounds (Chamorro et al.,

2016, 2021; Brea, 2023). Therefore, therapeutic interventions must be properly designed to act at a specific time point after stroke and modulate the balance between inflammatory and immunomodulatory factors to promote protective and restorative processes (Ceulemans et al., 2010). Rapid advances in our understanding of the inflammatory response after stroke, much of which have focused on describing patterns of infiltrating immune cells and the release of inflammatory molecules, have opened the door to new protective therapies with highly selective activity on key players associated only with the beneficial effect of the immune response. We review some of the most relevant pharmaceutical approaches used to modulate the inflammatory response after a stroke that has achieved to clinical analysis, including fingolimod, natalizumab, interleukin (IL)-1Ra, and the novel ApTOLL agent.

Fingolimod

Fingolimod (also known as FTY720) is a high-affinity sphingosine 1-phosphate (S1P) receptor agonist that blocks lymphocyte release from lymph nodes, thereby limiting lymphocyte infiltration into the brain and inhibiting local activation of microglia and macrophages. The active (phosphorylated) form of fingolimod binds to lymphocyte S1P receptors and causes their internalization and degradation, resulting in lymphopenia (Naseh et al., 2021). Lymphocytes play important roles in the pathogenesis of stroke and tissue injury. They function as modulators of leukocyte and platelet adhesion following an ischemic stroke. Activated T lymphocytes appear as early as 24 h after reperfusion in ischemic brain tissue, and they produce inflammatory cytokines such as IL-17 and interferon γ in the site of injury. In particular, lymphocytes $CD4^+$ helper, $CD8^+$ cytotoxic, and γδT cells play damaging roles in experimental stroke. Thus, the deficiency of $CD4^+$ helper/$CD8^+$ cytotoxic T cells leads to a decreased number of adherent leukocytes and lymphocytes, resulting in smaller ischemic infarct sizes and amelioration of neurological outcomes following ischemic damage in mice. Rodent models of brain ischemia have shown that lymphopenia caused by fingolimod treatment is associated with smaller infarct size, neurological deficits, and edema (Wei et al., 2011; Liu et al., 2013). In a mouse model of thromboembolic stroke, in which the beneficial effect of rtPA-induced reperfusion and HT associated with delayed administration was similar to that occurring in humans, fingolimod was observed to reduce the infarct size and risk of hemorrhage of the rtPA thrombolytic drug (Campos et al., 2013; Naseh et al., 2021). However, in the recent multi-laboratory preclinical trial, SPAN, fingolimod did not show effective results, contrary to the results observed with uric acid (Lyden et al., 2023).

As fingolimod is an oral drug approved for the treatment of different types of sclerosis, it can be easily repositioned for testing in stroke, saving critical steps in the development of the first safety phase I study. With this aim in mind, an open-label pilot study tested the efficacy of oral fingolimod in improving functional outcomes after stroke (Fu et al., 2014). When administered within 72 h of stroke onset, the treatment limited secondary tissue injury, achieving less microvascular permeability, improving neurological deficits, and promoting recovery; in addition, it did not cause severe adverse effects (Fu et al., 2014). Furthermore, when administered in combination with rtPA, within 4.5 h of stroke onset, there were fewer circulating lymphocytes, smaller lesion volumes, less hemorrhage, and better neurological outcomes than with alteplase alone (Zhu et al., 2015; Chamorro et al., 2016, 2021). However, despite these promising results, the protective effect of fingolimod in stroke still needs to be tested in double-blind studies, and further extensive work is needed before it can be approved as a treatment for stroke (Cao et al., 2023).

Natalizumab

Natalizumab is a humanized CD49d antibody that blocks the α4 integrin, thereby reducing the entry of leukocytes into the CNS. It has been approved as an intravenous drug for multiple sclerosis. In the field of stroke, some preclinical studies have tested the efficacy of natalizumab in rodent models, with controversial results thus far: one study showed positive results, whereas the other, despite reducing T-cell and neutrophil infiltration of the CNS, did not show efficacy in protecting mice from functional deficits after stroke (Liesz et al., 2011). Another multicenter preclinical study in mice showed a reduction in infarct size in models of mild ischemia, but not in large infarcts (Llovera et al., 2015). The ACTION trial, designed to test the effect of natalizumab in stroke within 9 or 24 h, did not show any effect on infarct volume growth (the primary study endpoint), but improvements in several prespecified secondary and tertiary endpoints of functional outcome at 30 and 90 days were observed, compared with placebo (Elkind et al., 2020). Currently, as far as we know, there are no new clinical analyses on the effect of natalizumab in patients with stroke.

Interleukin 1Ra

The proinflammatory cytokine IL-1 is closely related to the immune response after an ischemic event, and an increase in its levels is associated with greater ischemic damage, as observed in preclinical models. The close relationship between IL-1 levels and poor prognosis has led to the development of antagonists against the IL-1 receptor (IL-1Ra), which has high efficacy in blocking the response to this cytokine (Smith et al., 2018). Subcutaneous or intravenous administration in experimental models has shown satisfactory results in terms of long-term functional recovery (Maysami et al., 2016). A subsequent meta-analysis of existing preclinical data of 1283 animals validated the efficacy of IL-1Ra treatment in terms of reduction of infarct volume and supported its subsequent translation to the clinic (McCann et al., 2016). Its intravenous administration in patients with acute stroke in a phase II placebo-controlled study demonstrated safety and high efficacy in reducing inflammatory marker levels (Emsley et al., 2005). IL-1Ra has been approved for subcutaneous treatment of rheumatoid arthritis, making it easy to administer. A subsequent phase II study, SCIL-STROKE (Subcutaneous Interleukin-1 Receptor-Antagonist in Ischemic Stroke), analyzed the effect of IL-1Ra administered subcutaneously after acute ischemic stroke and demonstrated the efficacy of the treatment to significantly reduce inflammatory markers associated with a worse outcome after stroke, but no significant effect related to functional improvement in the modified Rankin scale was observed; thus, further studies are still required to demonstrate its clinical benefit in acute stroke (Smith et al., 2018).

ApTOLL

ApTOLL is an unmodified single-stranded DNA aptamer designed to antagonize toll-like receptor 4 (TLR4) and has a high specificity for blocking the inflammatory response produced after different insults, such as acute ischemic stroke and acute myocardial infarction.

Toll-like receptors are a family of highly conserved innate immune receptors that recognize highly preserved structures in pathogens, called pathogen-associated molecular patterns. However, these immune receptors also recognize damage-associated molecular patterns from endogenous molecules released as a result of tissue injury such as cerebral ischemic stroke. The level of activation of TLRs mediated by tissular endogenous ligands can also be similar to that caused by ligands from infectious agents, in a way that promotes the recruitment of several adaptive proteins to activate nuclear factor-κB, which induces the expression of proinflammatory genes, inflammatory cytokines, and adhesion molecules and activation of adaptive immunity. Up to 14 types of TLRs have been described; however, one of the most frequently associated with the inflammatory response after stroke is TLR4, which is widely correlated with poor outcomes. This association has been well demonstrated in TLR4 knockout mice, which

showed smaller infarct sizes and improved neurological test scores than wild-type mice (Caso et al., 2007; Brea et al., 2011), whereas upregulation correlated with ischemic stroke severity (Yang et al., 2008).

Based on the critical role of TLR4 in the immune response after stroke and its correlation with a poor prognosis, ApTOLL was designed to specifically modulate the immune response through this receptor, thereby preventing the first wave of the inflammatory cascade from occurring after stroke onset. Experimental models of ischemic stroke have shown outstanding neuroprotective effects, reducing brain damage by up to 65% within an effective time window of 12 h (Caso et al., 2007). To evaluate the clinical effects of ApTOLL in patients with stroke, a first-in-human study was conducted to assess the safety and pharmacokinetics of ApTOLL in healthy individuals (Hernandez-Jimenez et al., 2022). The data derived from this study showed an excellent safety profile in healthy humans. A subsequent assessment of the safety and efficacy of ApTOLL in combination with MT in patients with ischemic stroke (APRIL study) showed that 0.2 mg/kg ApTOLL administered within 6 h of onset in combination with MT was safe and associated with a potentially meaningful clinical effect, reducing mortality and disability at 90 days compared with placebo. These preliminary results have led to the development of larger pivotal trials to verify the proposed effects of this immunosuppressant (Hernandez-Jimenez et al., 2023a, 2023b).

NON-PHARMACOLOGICAL PROTECTIVE APPROACHES

Neuroprotection against stroke is usually associated with the use of pharmaceutical drugs designed to selectively interfere with or block key molecular targets in the ischemic cascade to reduce the progression of brain injury. However, in the long search for effective protective therapies against ischemic stroke, alternative non-pharmaceutical approaches have emerged with varying degrees of success. Here, we report three examples of clinically tested non-pharmaceutical approaches: hypothermia, ischemic preconditioning, and oxygen supplementation.

Hypothermia

Hypothermia is one of the most effective neuroprotective therapies reported at the preclinical level for cerebral ischemia (Campos et al., 2012). However, although effective in pediatric units (e.g., for fetal hypoxia), its clinical translation (body or systemic hypothermia [SH]) in adult patients with stroke is hampered by side effects such as shivering, hypotension, arrhythmia, and increased risk of pneumonia, which usually require sedation or anesthesia (Geurts et al., 2017). Non-invasive focal hypothermia applied locally (over the skull) to the cerebral ischemic region decreases the side effects of cooling stress in awake animals while retaining benefits similar to those of SH. Undoubtedly, the clinical translation of this approach has critical limitations because the human skull effectively insulates the brain, requiring the use of prolonged skin-damaging cold to achieve target intracerebral temperatures (Vieites-Prado et al., 2016).

Despite the challenges that involve the use of hypothermia as an alternative therapy, analysis of the physiological mechanisms underlying its therapeutic effects has provided new molecular protective candidates for reducing ischemic lesions (Han et al., 2012). For example, although hypothermia downregulates global protein synthesis and cell metabolism, it induces the upregulation of other known cold shock proteins (CSPs). The two main CSPs identified in mammals are cold-inducible RNA-binding protein (CIRP) and RNA-binding motif protein 3 (RBM3). Although CIRP is detrimental to enhancing the inflammatory response, interest in RBM3 has significantly increased because of its critical role in the protective effects of hypothermia (Zhu et al., 2016).

The expression of RBM3 has been well investigated in stroke models subjected to systemic and focal brain hypothermia and in patients with stroke subjected to body therapeutic cooling in the phase III EuroHYP-1 trial. Preclinical data from ischemic animals subjected to systemic and focal hypothermia confirmed an increase in brain RBM3 expression, which was selectively higher in cooled hemispheres of animals undergoing focal brain hypothermia, confirming the direct effect of hypothermia on RBM3 expression. Consistent with these experimental findings, blood samples from patients with stroke who underwent hypothermic treatment in the EuroHYP-1 trial showed an association between RBM3 and cooling. These results suggest that the pharmacological induction of RBM3 could be a potential means of neuroprotection against stroke in the absence of hypothermia. However, a drug or agonist that directly targets RBM3 expression or activity has not yet been developed to test this hypothesis (Avila-Gomez et al., 2020).

In the absence of a specific agonist, the upstream induction of RBM3, mediated by tropomyosin receptor kinase B (TrkB), has been evaluated as an alternative neurobiological mechanism of therapeutic hypothermia. TrkB is a transmembrane receptor for brain-derived neurotrophic factor, which is involved in neural development, proliferation, and survival and is also associated with increased expression of RBM3 mediated by cooling. TrkB agonism has been reported to induce RBM3 without exposure to cold, thereby preventing neurodegenerative damage. These data clearly demonstrate that pharmacological activation of RBM3 can be used therapeutically without inducing hypothermia (Peretti et al., 2021).

Clinical studies have also reported that fibroblast growth factor 21 (FGF21) is an obesity- and temperature-related hormone that upregulates the expression of RBM3. Clinical retrospective studies have shown that higher concentrations of FGF21 on admission and RBM3 at 72 h were associated with good outcomes but were inversely related to the maximum temperature during the first 24 h after stroke (Avila-Gomez et al., 2022). This association between FGF21 and RMB3 led to the postulation that FGF21 is an inducer of RBM3 expression and a protective drug against stroke. This growth factor has already been used as a recombinant treatment under different experimental pathological conditions (Dordoe et al., 2021); however, the main limitation of the use of recombinant FGF21 to induce RBM3 expression is the side effects observed in clinical trials with patients with stroke (Dordoe et al., 2021). New genetic strategies based on the use of antisense oligonucleotides (ASO) have been demonstrated to manipulate RBM3 levels independent of cooling. Indeed, this treatment led to remarkable neuroprotection in animal models of Alzheimer disease, with the prevention of neuronal loss and spongiosis despite high levels of disease-associated prion proteins (Preussner et al., 2023). These results in mice support the possibility that RBM3-inducing ASOs also deliver neuroprotection in stroke pathology.

Ischemic preconditioning

Ischemic preconditioning was first described in the field of cardiology and refers to the ability of a tissue to better resist ischemic damage when previously subjected to short periods of ischemic lesions. This phenomenon was later translated to experimental cerebral ischemia, in which under controlled transient ischemia in the carotid and middle cerebral arteries, injury was significantly associated with smaller cerebral infarct sizes (Stagliano et al., 1999). Although this intervention presented low clinical applicability, it has allowed us to explain or justify why patients with recent transient ischemic attacks (TIA) prior to cerebral infarction have better clinical and radiological evolution than those who have not experienced TIA (Sol et al., 2022).

Thus, after the first description of ischemic preconditioning, a new and more practical approach consisting of remote preconditioning by transient and mild ischemia of the femoral artery was suggested. The first demonstration of this phenomenon was performed in coronary

ischemia by inducing repetitive inflation and deflation of a blood pressure cuff on the limb. Remote ischemic preconditioning before hospital admission safely was observed to increase myocardial salvage (Botker et al., 2010; Hess et al., 2015).

Remote conditioning, applied before, during, or after ischemia, represents a new paradigm of neuroprotection with multiple mechanisms of action mediated by the neurohumoral pathways. Local ischemia induces the release of humoral factors such as adenosine, bradykinin, and opioids, which activate afferent nerves or are released into the bloodstream. The vagus nerve is also activated, which is inhibited by parasympathetic inflammatory processes induced by the liver and spleen. Simultaneously, it activates the sphenopalatine ganglion and other parasympathetic pathways responsible for increasing cerebral blood flow (Botker et al., 2010; Purroy et al., 2020a, 2020b; Torres-Querol et al., 2021).

Several trials have tested the effect of remote preconditioning in the acute phase of stroke, usually by applying brief episodes of transient ischemia to a limb, demonstrating its ease of application and safety (An et al., 2020; Pico et al., 2020; Purroy et al., 2020a, 2020b; Poalelungi et al., 2021; Landman et al., 2023), whether applied to patients with ischemic stroke or to those with hemorrhagic strokes (Hougaard et al., 2014). However, none have demonstrated clinical benefits, probably owing to methodological limitations (number of cycles or location of cycles) and insufficient sample size. New modifications to this therapy have been optimizing by testing remote ischemic postconditioning; for example, patients with minor stroke in the RICAMIS study demonstrated a better evolution in those who received a remote ischemic postconditioning strategy twice daily for 10–14 days (Chen et al., 2022). This approach seems to be more favorable for evaluating and optimizing the clinical application of ischemic preconditioning.

Oxygen supplementation

In clinical practice, the occurrence of hypoxia during the acute phase of stroke is common and is associated with a worse prognosis, neurological deterioration, and higher mortality rate. The importance of this parameter is evidenced by the fact that monitoring and controlling oxygen saturation is associated with improved outcomes (Bravata et al., 2010). Based on the fact that decreased oxygen delivery is one of the main causes of ischemic damage, hypoxia control has long been postulated as a way to protect tissue at risk of cell death (Ronning and Guldvog, 1999). However, oxygen delivery presents some risks, such as vasoconstriction, toxicity, and even airway infection, when applied to patients with stroke. At least three studies have tested the use of high-flow (45 L/min) therapeutic oxygen for less than 12 h without observing improvements in patients (Singhal et al., 2005; Padma et al., 2010; Wu et al., 2012). A larger trial (n = 550) using low-dose supplemental oxygen (3 L/min for 24 h) did not show any benefit either; however, early neurological recovery improved when low-dose oxygen was administered for 72 h (Ronning and Guldvog, 1999).

One of the most comprehensive studies designed to analyze the therapeutic contribution of oxygen is the Stroke Oxygen Study. Its main objective was to determine whether low-dose oxygen therapy during the first 3 days after an acute stroke improved prognosis compared with usual care (oxygen only when needed). Since oxygen can restrict mobility and interfere with daytime activities, it is administered only at night, when hypoxia is more likely (Roffe et al., 2017). A study of 148 treated patients vs. 141 controls showed that oxygen supplementation initiated within 24 h of hospital admission for acute stroke produced a slight improvement in patient recovery, but the benefit of this therapy remains inconclusive until a larger, longer-term study is performed.

NEW ADVANCES IN THE IMPROVEMENT OF NEUROPROTECTIVE THERAPIES

As previously reported, hundreds of new protective agents or therapies have been tested to reduce the progression of neuronal damage after ischemic events, with frustrating results to date. Several explanations for this persistent failure have been proposed. One of the most widely accepted reasons is the appropriate selection of patients included in the trials. Patients with stroke usually have multiple comorbidities; the time after stroke onset is often unknown, the use of recanalization therapies is often not been well considered, and salvageable penumbra regions have not been evaluated. In addition, for safety reasons, many clinical trials of neuroprotection in patients with stroke must start after hospital arrival, which significantly reduces the efficacy of the drugs, as the they are designed to interfere with the initial pathways of the ischemic cascade (Grupke et al., 2015). The increasing use of MT, combined with the analysis of new biomarkers for early diagnosis and improvements in high-resolution imaging, has contributed significantly to the selection of candidates according to drug efficacy design. The relevance of recanalization therapy, imaging, and biomarkers in improving neuroprotection is described below (Fig. 1).

Recanalization therapies

Since most clinical trials were designed to compare a placebo with a treated group, the number of enrolled participants who also received thrombolysis was very low; therefore, the possibility of reperfusion before or after treatment administration is unlikely. Historical failure in the search for an effective protective drug has led to the assumption that the lack of recanalization prevents the drug from effectively reaching the lesion region, thus impeding its effect (Fisher and Savitz, 2022) (Fig. 2). Additionally, many neuroprotectants have been studied in preclinical models of transient ischemia, but without adequate reperfusion in patients with stroke (Fisher and Savitz, 2022). Based on this previous evidence, in the “recanalization era,” new clinical trials have started to design the efficacy analysis of new neuroprotective agents in combination with reperfusion therapies (thrombolysis, thrombectomy, or both), as a first step toward drug analysis.

However, clinical analyses of neuroprotective agents in combination with recanalization therapies have revealed some limitations (mainly in combination with rtPA therapy) that should be considered when designing future clinical trials. One of these limitations was the possible interaction between the drug under study and rtPA. Since rtPA is a serine protease that cleaves peptide bonds in proteins, it may degrade new compounds or annulate their activity. A recent example is the clinical analysis of NA1 in the phase III ESCAPE-NA1 trial. In this study, patients in the NA1 group who underwent rtPA thrombolysis showed no significant benefit. However, in patients who did not receive rtPA, 59.3% of patients who received NA1 had a favorable outcome (Hill et al., 2020). A subsequent analysis confirmed an interaction between NA1 and rtPA, avoiding the therapeutic effect of this new compound (Mayor-Nunez et al., 2021). These data reflect the importance of evaluating the compatibility of new thrombolytic drugs during the preclinical phase, before moving to human validation.

The second consideration is proper selection of the functional independence value to detect the additional benefits of new protective drugs when combined with recanalization therapies. For instance, in the case of rtPA, there is 33% estimated partial or complete arterial recanalization after early administration; however, this rate has improved significantly with MT (varying between 63.7% and 95%, mainly in strokes with large vessel occlusion [LVO], depending on the study reviewed) (Moreu et al., 2023). Although successful recanalization is not always associated with favorable outcomes (defined as futile reperfusion), many patients (with successful reperfusion) usually are asymptomatic and functionally independent. In the latter case, the

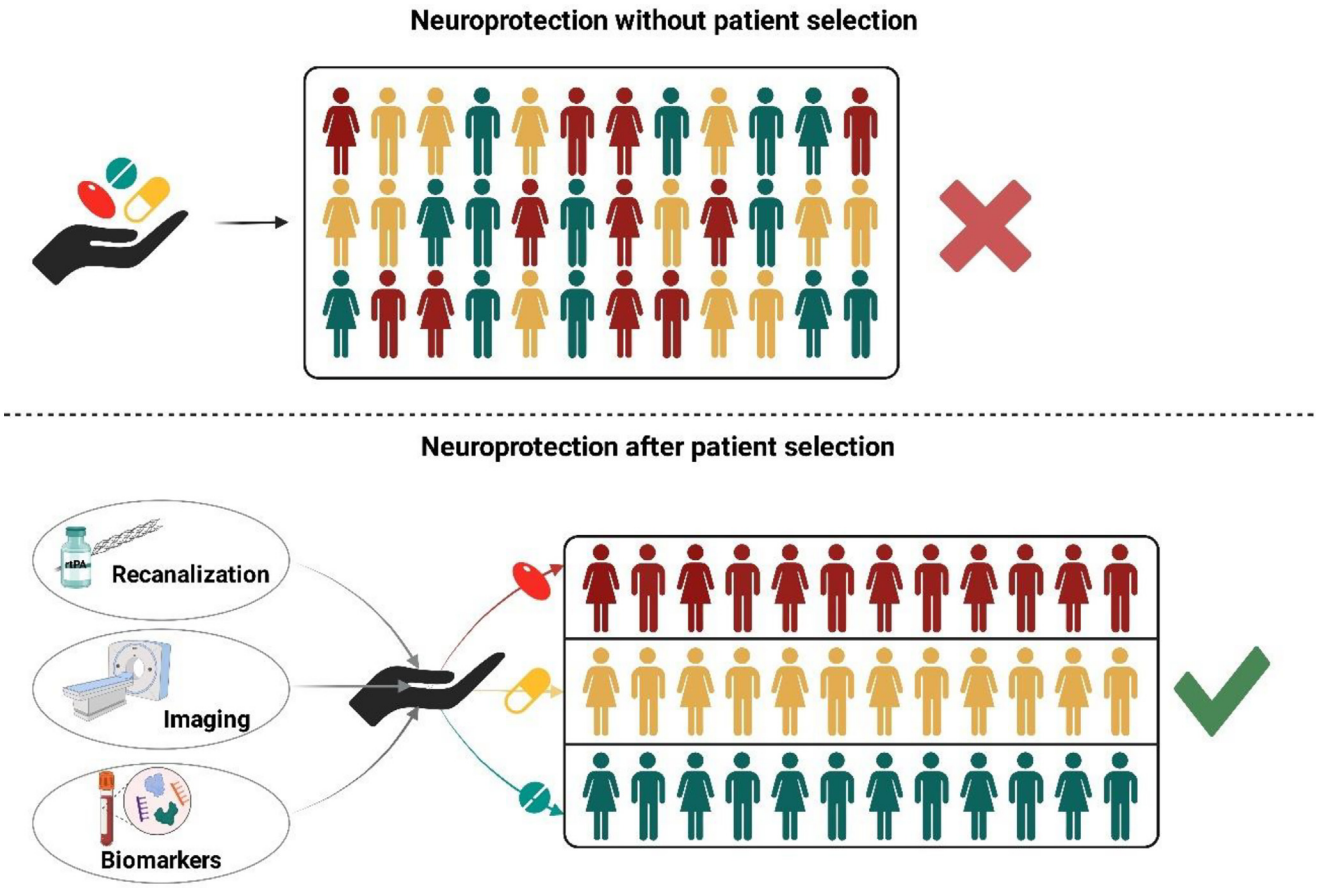

Fig. 1. Schematic representation of the contribution of new recanalization therapies, improvements in high-resolution imaging and biomarkers for early diagnosis. Appropriate segmentation of patients allows selection of candidates according to drug efficacy design. Images created with BioRender.com.

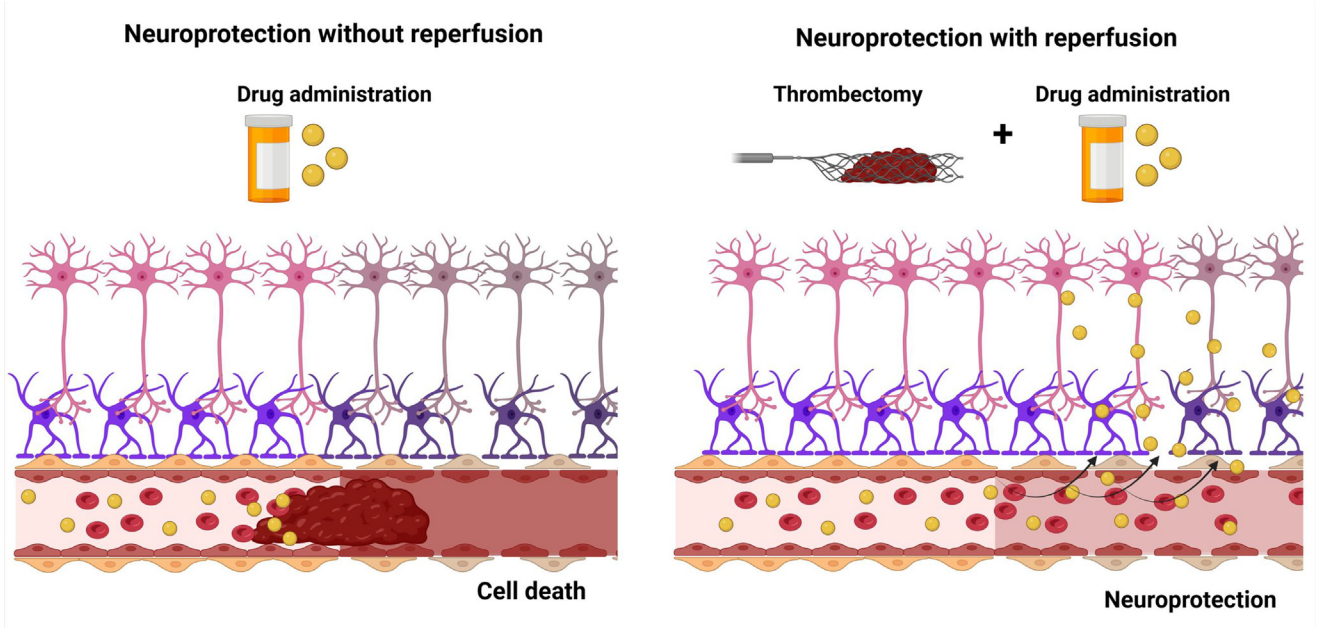

Fig. 2. Schematic representation of protective therapies with and without recanalization. Lack of recanalization prevents the drug from effectively reaching the lesion region, resulting in failure or reduced efficacy of the therapy. Images created with BioRender.com.

additional benefit of a new protective drug could be nullified or masked by reperfusion interventions. An example of this situation was the clinical analysis of the protective drug citicoline used in patients who undergone thrombolysis treated with rtPA. Citicoline, or CDP-choline, is a drug that combines neurovascular protection and repair effects. It has been used to treat acute ischemic stroke and other neuro-

logical disorders and has an excellent safety profile. In 2002, a formal meta-analysis of trials of CDP-choline in acute and subacute stroke suggested a beneficial and substantial treatment effect, with absolute reductions of 10%–12% in the rates of long-term death and disability. However, a new trial (ICTUS trial) on citicoline did not demonstrate effectivity in the treatment of moderate to severe acute ischemic stroke (Davalos et al., 2012). Posterior analyses reported that the inclusion of patients treated very early with rtPA in a multidisciplinary stroke unit could mask the protective benefits of citicoline (Secades et al., 2016).

However, the efficacy and use of MT have increased significantly in recent years, particularly in stroke cases where thrombi are located in accessible LVOs. Laboratory and clinical imaging studies have shown that early after LVO, the core of ischemia is still reduced, whereas the surrounding infarcted tissue corresponds to a salvageable penumbra, an ideal condition for protection analyses. Therefore, patients with LVO awaiting thrombectomy appear to be excellent candidates for trials, as the first step in testing the protective effects of new agents. (Shi et al., 2018; Fisher and Savitz, 2022).

Imaging approaches

Early reperfusion after stroke aims to prevent expansion of the core region into the penumbral area and subsequently reduce the neurological sequelae of the injury. The growth of the ischemic core varies between patients and depends on collateral flow capacity and parenchymal ischemic tolerance. Based on this premise, patients with reduced collateral flow capacity and parenchymal ischemic tolerance can be classified as “fast progressors,” with worse clinical outcomes, larger infarcts, and an increased risk of malignant cerebral edema and HT. In contrast, patients with superior collateral flow and ischemic tolerance, defined as “slow progressors,” tend to exhibit a smaller ischemic core several days after stroke onset. These patients typically experience superior clinical outcomes in response to reperfusion therapy (Rocha and Jovin, 2017; Shi et al., 2018) (Fig. 3).

Therefore, in fast progressors, administration of reperfusion therapy and/or protective drugs as early as possible (first hours after stroke onset) is crucial, whereas slow progressors can receive significant benefits even with delayed reperfusion (and protective drugs), as demonstrated in the DAWN and DEFUSE-3 trials (Shi et al., 2018). The speed of infarct progression is a critical parameter in selecting optimal candidates, defining the range of interventions, and selecting the most convenient protective therapy. In this regard, the use and development of neuroimaging techniques based on multiparametric computed tomography (CT) and MRI are powerful tools to measure the collateral blood flow capacity and speed of infarct progression and improve the selection of patients for trial enrollment (Goyal et al., 2022).

The combination of positron emission tomography (PET) with radiolabeled O_2 was initially the gold standard (at least in experimental practice) for visualizing collateral blood flow and penumbra progression. As this imaging technique is not feasible in the acute clinical setting, MRI perfusion- and diffusion-weighted imaging mismatch and CT perfusion have been proposed as the most accessible methods for identifying the penumbra in patients with acute ischemic stroke (Chalet et al., 2022). In the literature, multiple novel methods based on the use of PET, CT, and MRI techniques to define the penumbral region and measure ischemic progression seem to focus on the development of automated image analysis with the support of machine learning or artificial intelligence-related algorithms to assist physicians in obtaining more accurate and standardized interpretations of brain images, which may improve stroke management and patient selection for appropriate treatment (Mokli et al., 2019). The rapid processing of PerfusIon and Diffusion software (iSchemaView) is now one of the most common tools used to identify the ischemic penumbra in many ischemic stroke clinical studies (Zhang et al., 2022).

Biomarker utility

For safety reasons, many clinical trials of neuroprotection against stroke require initiation after hospital admission and neuroimaging diagnostics, which significantly reduces the intervention time and efficacy of the drugs (Grupke et al., 2015). Early treatment of patients is crucial to maximize the benefits after stroke; therefore, pre-hospital

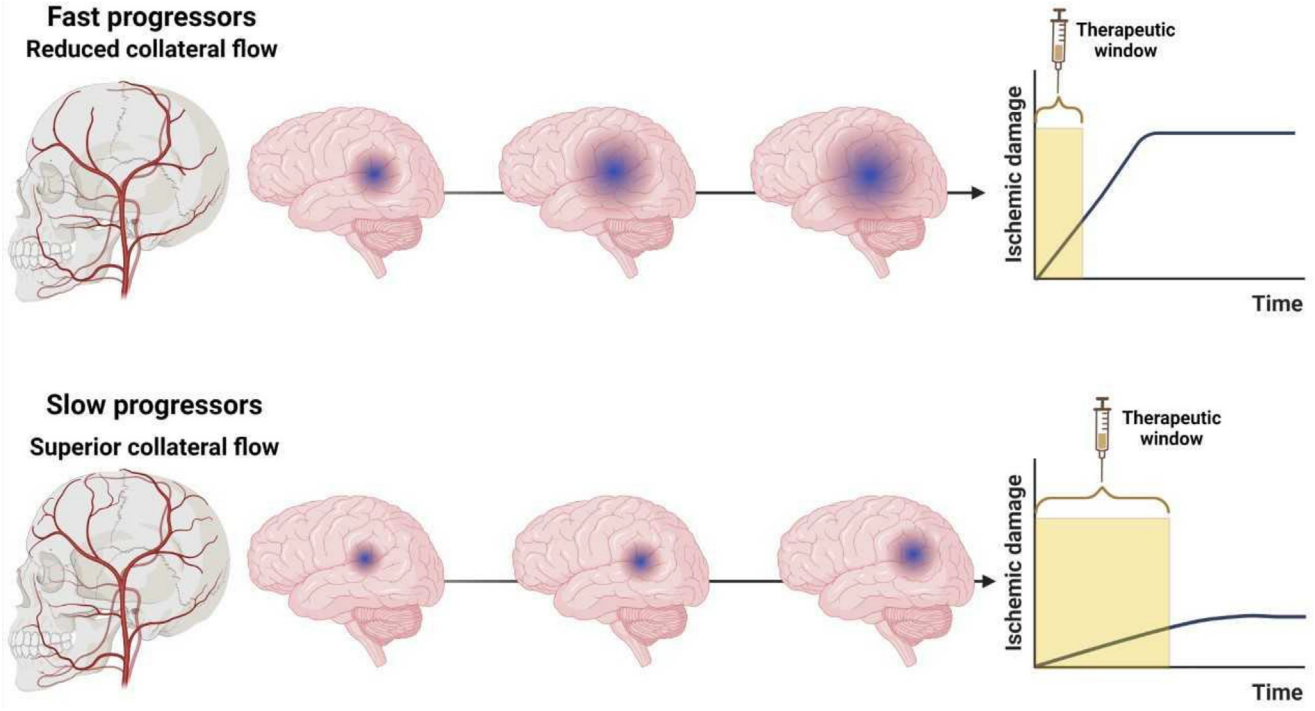

Fig. 3. Schematic representation of the growth of ischemic injury in fast and slow progressors. In fast progressors, the core grows rapidly and the penumbra diminishes with increasing time from stroke onset, thereby reducing the time for medical intervention. In slow progressors, the ischemic core grows very slowly, allowing the use of protective drugs in a longer therapeutic window. Images created with BioRender.com.

administration of protective agents would increase their efficacy (Fisher and Savitz, 2022).

This ambitious idea requires alternative strategies for an early diagnosis before neuroimaging analysis. With the development of effective neuroprotectors, the search for reliable biomarkers has become a pending task for ischemic stroke diagnosis. A stroke biomarker has to distinguish, with high specificity and sensitivity, a hemorrhagic stroke from an ischemic stroke and even detect stroke mimics; they should also be good prognostic predictors, indicating, for instance, the risk of HT (Dagonnier et al., 2021). Some of the most common biomarkers explored for stroke diagnosis are the following: glial protein S100B, which is highly specific to nervous tissue and used for stroke diagnosis; glial fibrillary acidic protein, which is specific to astrocytes and an option used to differentiate hemorrhage and ischemic stroke; serum concentration of neuron-specific enolase, which is related to infarct size and stroke symptom severity; matrix metalloproteinase 9, which is used to evaluate the risk of HT; cellular fibronectin, which is associated with vascular damage and risk of secondary bleeding; circulating non-coding RNAs (miRNAs, lncRNAs and circRNAs), which predict hematoma growth in patients with stroke hemorrhage (Dagonnier et al., 2021). With the aim of increasing the sensitivity and specificity of biomarkers, and considering the molecular complexity of the ischemic cascade, considerable effort has been invested in the simultaneous study of multiple molecules (Montaner et al., 2011; Dagonnier et al., 2021). More recent approaches use the metabolomic and lipidomic profiles of patients with suspected stroke for diagnosis, to determine the presence of intracranial occlusion, and to evaluate penumbra-at-risk tissue.

The current clinical trial (BIOFAST 1, NCT04612218) (Parody-Rua et al., 2023) aimed to evaluate a point-of-care device to validate a biomarker panel differentiating ischemic and hemorrhagic stroke at the pre-hospital setting using a blood sample and to validate a second biomarker panel for the early identification of patients with LVOs who are candidates for MT. This study also combined machine learning techniques to generate predictive models of hemorrhagic versus ischemic stroke and predictive LVO scales.

Although the search for diagnostic biomarkers has mainly focused on discriminating between hemorrhagic and ischemic stroke or for the proper selection of candidates for thrombolysis or thrombectomy, the use of stroke biomarkers to accelerate diagnosis will undoubtedly help in the future to anticipate the use of protective treatments for patients before hospital arrival.

Conclusion

Although the accumulation of failed results in the clinical translation of a neuroprotective treatment for stroke has reduced expectations in last years, the improvement and increasingly widespread use of recanalization therapies have allowed optimism to resurface in neuroprotection, with some promising results reported for ApTOLL or uric acid. The combination of protective drugs after successful reperfusion appears to be critical for exerting a direct protective effect on the ischemic penumbra. This has led to the consideration of patients with LVO stroke who have undergone thrombectomy as excellent candidates for trials, as the first step in testing the protective effects of new agents. This represents a smart approach, and successful results in this direction could lead to advances in stroke management. Unfortunately, recanalization therapies cannot be used in more than 50% of cases (in the best of cases), and successful reperfusion occurs in no more than 50% of treated patients (in the best of cases); therefore, future directions need to find a way to extend the use of protective drugs beyond recanalization. The use of protection as soon as possible in pre-hospital settings for patients suspected of having a stroke seems to be ideal for extending the administration of protective agents to a large number of patients.

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