1521-0081/74/2/439–461$35.00
PHARMACOLOGICAL REVIEWS

dx.doi.org/10.1124/pharmrev.121.000418
Pharmacol Rev 74:439–461, April 2022

ASSOCIATE EDITOR: ERIC BARKER

Therapeutic Opportunities and Delivery Strategies for Brain Revascularization in Stroke, Neurodegeneration, and Aging

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This work was supported by the University of the Basque Country (UPV/EHU) [Grant ESPDOC19/47] (postdoctoral fellowship to I.V.B.); and the Basque Country Government (Consolidated Groups) [Grant IT907-16].

No author has an actual or perceived conflict of interest with the contents of this article.

dx.doi.org/10.1124/pharmrev.121.000418.

***Abstract*——Central nervous system (CNS) diseases, especially acute ischemic events and neurodegenerative disorders, constitute a public health problem with no effective treatments to allow a persistent solution. Failed therapies targeting neuronal recovery have revealed the multifactorial and intricate pathophysiology underlying such CNS disorders as ischemic stroke, Alzheimer's disease, amyotrophic lateral sclerosis, vascular Parkisonism, vascular dementia, and aging, in which cerebral microvasculature impairment seems to play a key role. In fact, a reduction in vessel density and cerebral blood flow occurs in these scenarios, contributing to neuronal dysfunction and leading to loss of cognitive function. In this review, we provide an overview of healthy brain microvasculature structure and function in health and the effect of the aforementioned cerebral CNS diseases. We discuss the emerging new therapeutic opportunities, and their delivery approaches, aimed at recovering brain vascularization in this context.**

***Significance Statement*——The lack of effective treatments, mainly focused on neuron recovery, has prompted the search of other therapies to treat cerebral central nervous system diseases. The disruption and degeneration of cerebral microvasculature has been evidenced in neurodegenerative diseases, stroke, and aging, constituting a potential target for restoring vascularization, neuronal functioning, and cognitive capacities by the development of therapeutic pro-angiogenic strategies.**

I. Introduction

Neurologic disorders are the second leading cause of death and the principal cause of disability in the world (GBD 2015 Neurologic Disorders Collaborator Group, 2017). Increasing life expectancy and population growth worldwide imply that more and more people are reaching ages in which neurologic disorders are more prevalent. The rising incidence and prevalence of these related central nervous system (CNS) diseases have an important socioeconomic impact, so it becomes a real problem not only for patients and families but also for the economy and healthcare systems (Harper, 2014; Wimo et al., 2020). There are no curative pharmacological treatments able to attain a complete neurovascular recovery in CNS diseases; they can only slow down the neurologic degenerative processes. This scenario underscores the difficulty of current pharmacological drugs to target and efficiently act in the brain. One of the main obstacles that lacks the success of such therapies is the blood-brain-barrier (BBB), along with other factors that must be taken into consideration, such as the presence of other extracellular and intracellular barriers and the complexity of the neurovascular network with interactions at several levels. For this reason, huge research efforts are being conducted to find and develop novel therapeutic strategies for CNS diseases (Niu et al., 2019; Poovaiah et al., 2018; Teleanu et al., 2019).

A few years ago, neuroscientists considered the brain as a dichotomized organ comprised of brain cells and cerebral blood vessels, with no relationship among these two entities. Nowadays however, the scientific community is aware of the close connection established and required between neuronal and vascular CNS cells for correct brain functioning. The brain is one of the most highly perfused organs in the body; in fact, nearly every neuron has its own capillary (Zlokovic, 2005), highlighting the pivotal relationship between the neuronal and vascular systems, called the neurovascular network. The neurovascular network in CNS is responsible for supplying the 20% of the cardiac output carrying oxygen and nutrients to the brain (Iadecola, 2013) and thus contributing to a healthy neurologic function. That is why lack of this supply, caused by vessel damage or degeneration, could have a major role in the pathogenesis of CNS diseases. Consequently, it is not surprising that the cognitive impairment that occurs in many CNS diseases could be related to cerebrovascular disruptions, mainly at the microvasculature level, and cerebral blood flow reduction, as in the case of ischemic stroke, amyotrophic lateral sclerosis (ALS), Alzheimer's disease (AD), vascular Parkisonism (VP), vascular dementia (VaD) and aging, which will be described in depth in section III, Vascular Disorders in Brain CNS Diseases.

This review provides an overview of the cellular and molecular mechanisms needed to manage the

ABBREVIATIONS: AAV, adeno-associated virus; Aβ, amyloid-β; AD, Alzheimer's disease; ALS, amyotrophic lateral sclerosis; ANGPTL4, angiopoietin-like 4; AV, adenovirus; BBB, blood-brain barrier; BMSC, bone marrow stromal cells; CNS, central nervous system; EC, endothelial cell; ECM, extracellular matrix; FGF, fibroblast growth factor; HIF-1α, hypoxia inducible factor-1α; IL, interleukin; JAM, junctional adhesion molecules; MCAO, middle cerebral artery occlusion; miR, microRNA; NMN, nicotinamide mononucleotide; NRP1, neuropilin-1; PDGF, platelet-derived growth factor; PEG, polyethylene glycol; PLGA, poly(lactic-co-glycolic acid); PlGF, placental growth factor; PPS, poly(propylene sulfide); RO, retro-orbital; ROS, reactive oxygen species; VaD, vascular dementia; VEGF, vascular endothelial growth factor; VP, vascular Parkisonism; ZO, zonula occludens.

cerebral microvasculature, as well as an up-to-date perspective of CNS diseases related to cerebral microvasculature damage or deterioration, as is the case of ischemic stroke, Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS), vascular Parkisonism (VP), vascular dementia (VaD) and aging, all of which are associated with cognitive impairment. In particular, we describe evidence for microvasculature regeneration as a form of neurologic and cognitive function improvement by looking at progress in the identification of potential therapeutic pro-angiogenic factors and focusing on the nanotechnological approaches, advanced opportunities, and the administration strategies employed.

II. Mechanisms Regulating Brain Microvasculature

The vascular system of CNS originates during embryonic development by angiogenesis as blood vessels on the leptomeningeal surface grow in toward the parenchyma (Marin-Padilla, 1985), and, in this way, CNS vessels and microvasculature are exclusively formed by angiogenic processes from the perineural vascular plexus (Flamme et al., 1997). Angiogenesis involves the creation of new blood vessels by sprouting or diverging from preexisting vessels, giving rise to the CNS microvasculature network. Both cellular and molecular mechanisms are involved in maintaining a suitable brain microvasculature, in which the cellular scaffold and the molecular exchange are intimately linked. Additionally, epigenetics, gene switching, cytokines, and extracellular matrix (ECM) molecules are also involved in maintaining correct neurovascular unit performance, not only in physiologic conditions but also under a pathologic stimulus.

A. Cellular Mechanisms

Physical and biologic characteristics of the cerebrovasculature are provided by endothelial cells (ECs), pericytes, smooth muscle cells also known as mural cells, or Rouget cells and astroglial foot processes (Giannoni et al., 2018; Sweeney et al., 2019), which together with neurons constitute the neurovascular unit (Iadecola, 2017; McConnell et al., 2017) (Fig. 1). This concept of neurovascular unit emerged in 2001, during the Stroke Progress Review Group meeting of the National Institute of Neurologic Disorders and Stroke (https://www.nia.nih.gov/health/vascular-contributions-cognitive-impairment-and-dementia) to reinforce the close connection between a correct CNS function and its microvasculature, achieved by effective paracrine regulations.

Within the neurovascular unit we find the cerebral endothelium, known as the BBB, which is composed of a monolayer of ECs with more special features than the ECs present in other tissues. In particular, CNS ECs are held together by tight junctions (Kniesel and Wolburg, 2000; Luissint et al., 2012a) regulating the homeostatic movement of ions, molecules, and cells between the blood and the CNS (Daneman and Prat, 2015; Serlin et al., 2015) (Fig. 1). Integral membrane proteins are responsible for these roles in tight junction complexes, namely occludins, claudins, and junctional adhesion molecules (JAM), which interact with cytoplasmic scaffolding proteins, such as actin cytoskeleton, zonula occludens (ZO) proteins, and other associated proteins (Luissint et al., 2012b; Vorbrodt and Dobrogowska, 2003). In fact, it has been described that ZO-1, one of the tight junction adaptor proteins, regulates CNS angiogenic potential and EC migration, as well as BBB formation (Tornavaca et al., 2015). JAM family proteins, which are present in tight junctions as mentioned above, have also been linked to angiogenesis, EC migration, and crosstalk with bFGF and $\alpha V\beta 3$ integrin signaling (Cooke et al., 2006; Peddibhotla et al., 2013).

All these features make BBB a dynamic interface; more specifically, this meticulously attuned and specialized system is able to: 1) keep the brain separated and protected from the compounds present in peripheral blood circulation, 2) selectively transport necessary components for the brain, 3) detect changes in blood flow and transmit this information to brain, 4) metabolize substances present in brain and blood, and 5) carry out the clearance of own neurotoxic compounds or xenobiotics generated in brain (Huber et al., 2001) (Fig. 1).

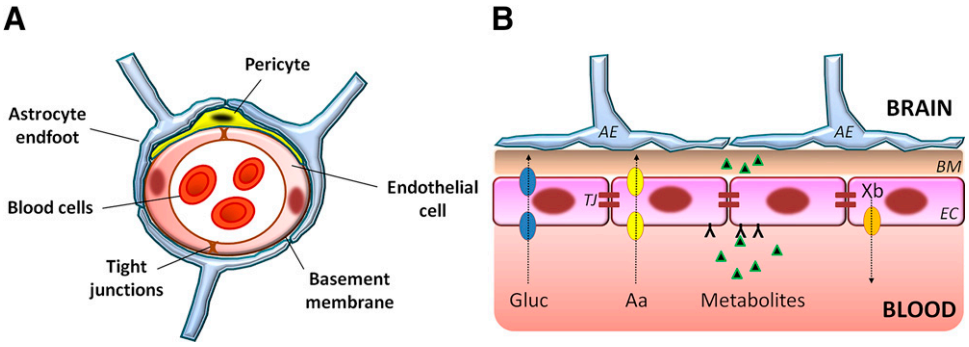

Fig. 1. Cellular components of central nervous system microvasculature and microenvironment integrity are essential for a correct neurovascular function. (A) Schematic representation of the neurovascular unit. (B) Blood-brain barrier exchange function. Gluc, glucose; Aa, aminoacids; Xb, xenobiotics; *AE*, astrocyte endfoot; *TJ*, tight junction; *EC*, endothelial cell; *BM*, basement membrane.

However, under pathophysiological conditions, the BBB disrupts, altering the biologic function of this key barrier. Changes in BBB permeability can trigger microglial activation and infiltration of immune cells into the brain, alterations in CNS homeostasis, and variable damage to the nearby brain tissue (Thurgur and Pinteaux, 2019), which could ultimately lead to the damage and reduction of brain microvasculature, as happens in many CNS diseases.

B. Molecular Mechanisms

1. Growth Factors. Contrary to the developing brain, where pro-angiogenic processes are upregulated, angiogenesis in the adult brain is physiologically downregulated. This fact was evidenced in 1985 by a study that analyzed brain capillary proliferation in post-natal rats by measuring [^{3}H] thymidine incorporation (Robertson et al., 1985). In this work, it was demonstrated that only 0.3% of ECs incorporated this substance. Hence, vessel growth in CNS is tightly regulated by pro-angiogenic, for stimulating, and anti-angiogenic, for inhibiting, factors (Chu LH et al., 2012; Harrigan, 2003; Vallon et al., 2014) (Fig. 2). In physiologic conditions, vascular quiescence is preserved in CNS due to angiogenic inhibitors, while the balance changes in favor of pro-angiogenic factors promoting angiogenesis in pathophysiological conditions such as in ischemic stroke or brain hypoxia that occurs in neurodegenerative CNS diseases (Harrigan, 2003; Vallon et al., 2014). Hence, understanding the molecular mechanisms involved in managing brain microvasculature would reveal potential therapeutic candidates.

Most of the factors promoting angiogenesis consist in growth factors (Fig. 2, Fig. 3) and molecules of the ECM (Martino et al., 2015). Among them, the most relevant is the vascular endothelial growth factor (VEGF), followed by the fibroblast growth factor (FGF) (Harrigan, 2003; Logsdon et al., 2014; Mackenzie and Ruhrberg, 2012; Mancuso et al., 2008; Rosenstein et al., 2010). In particular, for the VEGF family, comprised of VEGF-A to VEGF-F and the placental growth factor (PlGF), although some evidence shows angiogenic roles of VEGF-C and PlGF (Freitas-Andrade et al., 2012; Gaál et al., 2013), VEGF-A is the main central actor in angiogenesis. Meanwhile, for the FGF family, grouped into 6 subfamilies, the acidic FGF (aFGF) and the basic FGF (bFGF) FGF1 subfamily members, are involved in the maintenance of CNS vasculature integrity in adults and healing after brain injury (Dordoe et al., 2021). In addition, VEGF-A and bFGF interact synergistically in boosting angiogenesis (Harrigan, 2003) (Fig. 3). Another key factor that promotes angiogenesis is the hypoxia-inducible protein complex (HIF-1) and specifically HIF-1α transcription factor, which is expressed in hypoxic conditions and enhances VEGF-A gene expression, inducing blood vessel growth (Hoeben et al., 2004) (Fig. 3). Also, the transforming growth factor β1 seems to play a role in CNS revascularization (Du et al., 2020; Li et al., 2010a). In this sense, a study showed that mice subjected to cortical freeze lesion presented high mRNA and protein levels of these growth factors, leading to neovascularization by 20 days post-injury (Penkowa et al., 2000).

All these growth factors bind to their specific receptors (Fig. 3), triggering the cascade of angiogenic signaling processes. In particular, VEGF family members

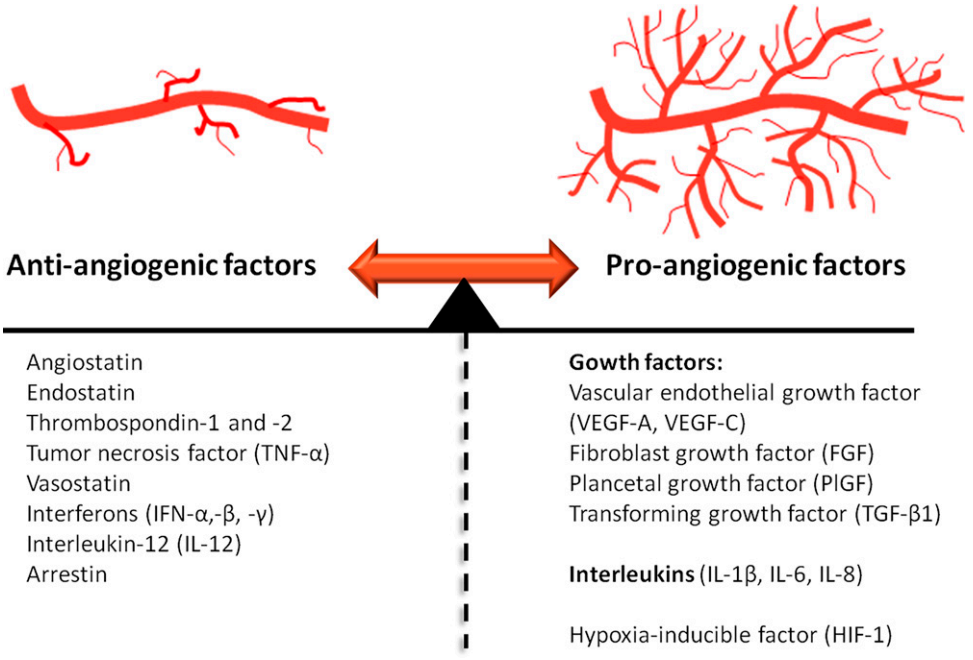

Fig. 2. Angiogenesis is governed by a dynamic balance of stimulators and inhibitors. In normal brain, angiogenesis is tightly downregulated but ischemic or hypoxic situations in central nervous system lead to an alteration of the physiologic anti- versus pro-angiogenic balance to try to compensate for vessel damage or degeneration. The most potent pro-angiogenic factor is vascular endothelial growth factor, followed by fibroblast growth factor. Pro-angiogenic factors also include transforming growth factor-β1, placental growth factor and interleukins, while notable among anti-angiogenic factors are angiostatin, endostatin and thrombospondins-1 and -2 (Harrigan, 2003; Lawler and Lawler, 2012). Identifying the signals that regulate central nervous system vascularization in health and disease offers new insights for therapeutic strategies against central nervous system diseases.

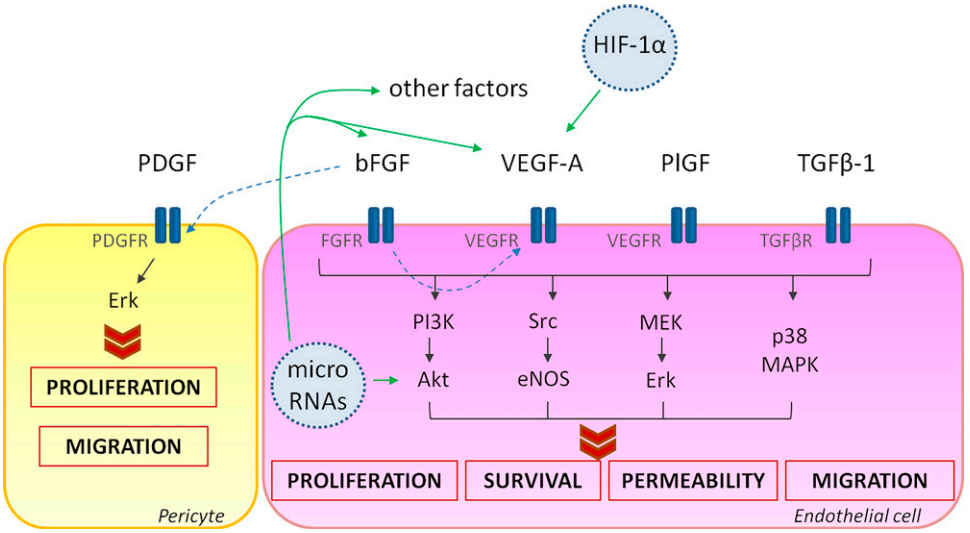

Fig. 3. Overview of the main factors and signaling pathways involved in brain angiogenesis. Schematic representation of the main growth factors and their respective receptors, that trigger the cascade of intracellular signals that induce cell proliferation, survival, permeability, and migration. Especially in hypoxic situations, these pathways, as well as hypoxia-inducible factors (such as HIF-1α and some microRNAs), are stimulated and promote angiogenesis. As shown, some of these factors can interact with each other, leading to synergistic angiogenic effects.

bind to tyrosine kinase receptors VEGFR1-R3, where VEGFR2 is the main receptor in ECs and mediates mitogenesis and vascular permeability regulation. Even though VEGFR1 is involved in the first stages of vasculature development by promoting EC division, and despite having high affinity for VEGF-A, it has low kinase activity; hence, VEGF-A angiogenic effects are predominantly addressed through its highly homologous VEGFR2 (Abhinand et al., 2016). Meanwhile, VEGF-B and PlGF bind to VEGFR1 and VEGF-C and -D to VEGFR3 (Simons et al., 2016). Additionally, some isoforms of VEGF-A and PlGF have the ability to bind to the co-receptor neuropilin1 (NRP1), which enhances their binding affinity to VEGFR2 (Herzog et al., 2011). Regarding bFGF, this growth factor exerts its angiogenic effects through FGFR1 and, in turn, the signaling cascade can activate *Vegfr2* gene expression via the adapter protein FRS2α and making ECs more sensitive to VEGF-A stimuli (Simons et al., 2016) (Fig. 3). This fact explains the synergistic angiogenic effect of bFGF and VEGF-A mentioned above. Additionally, it has been described that bFGF can upregulate PDGFR expression in CNS pericytes (Fig. 3) after ischemic stroke, which could contribute to both neuroprotection and angiogenesis (Nakamura et al., 2016).

2. Extracellular Matrix Signals and Interactions. The ECM is essential to provide signals for reaching an adequate vascular cell survival, proliferation, migration, differentiation, and maturation to establish a functional vascular network where nascent vessels had matured into durable, stable, non-leaky and functional vessels. During maturation, the new vessel is surrounded by pericytes, which inhibit EC proliferation (Folkman and D'Amore, 1996), but if vessel stabilization does not occur, then the immature vessel undergoes apoptosis (Benjamin et al., 1998). There is evidence that ECM-integrin interactions play an important role in regulating vessel maturation in the CNS (Milner and Campbell, 2002). In this sense, it has been shown in developing mice that CNS blood vessel maturation was accompanied by an increased expression of β1 integrin along with α4 and α5 integrins in the early development, and with α1 and α6 in the late development and in adulthood (Milner and Campbell, 2002). Under pathophysiological conditions, integrins αVβ3 are expressed in the activated microvessels of CNS ECs and have essential functions in the angiogenesis activation that occurs in cerebral ischemia (Abumiya et al., 1999). In the opposite scenario, an antagonism of integrin αV has been employed in experimental models to reduce brain glioma size by the inhibition of angiogenesis (MacDonald et al., 2001).

Also, metalloproteins, which are ECM proteins able to activate growth factors, surface factors, and adhesion molecules, seem to play key roles in maintaining CNS vasculature integrity. In this sense, the use of a metallothionein knockout model has revealed important functions for metallothioneins, a family of cysteine-rich metalloproteins (Penkowa et al., 2000). As a consequence of the lack of these metalloproteins, the levels of trophic factors VEGF, FGF, and transforming growth factor β1 decreased, which hampered CNS recovery as late as 90 days post-lesion, inflicted by a focal cerebral cryoinjury, due to a reduced angiogenesis and late regeneration.

3. MicroRNAs. Interestingly, vascularization of CNS is also regulated epigenetically by microRNAs (miRs). These small non-coding RNA molecules of 22–24 nucleotides in length play a relevant role in a wide variety of biologic processes through the post-transcriptional modulation of genes. Recent studies have indicated the key function of miRs in the regulation of angiogenesis (Suárez and Sessa, 2009) (Fig. 3). Some of them, especially the miR-9 and miR-30 families, are mainly involved in the development of CNS vasculature (Cho et al., 2019; Madelaine et al., 2017).

In the cases in which cerebral microvasculature is compromised, miR-15a/16-1 cluster (Sun P et al., 2020), miR-124 (Li et al., 2019a), miR-126 (Nammian et al., 2020; Qu et al., 2019), and miR-210 (Zeng LL et al., 2016) seem to be potential candidates for brain revascularization, as will be discussed below.

III. Vascular Disorders in Brain CNS Diseases

Until only a few years ago, research on the design of effective therapies for CNS diseases had been focused mainly on neurons, what has been referred to as the neurocentric view. Although placing great emphasis on neurons has provided deep knowledge about their impaired cell biology during chronic neurodegenerative situations, it fails to address the underlying disease pathology and, in consequence, this strategy has not given rise to disease-modifying therapeutics. The explanation is that non-neuronal cells are also involved in the complex pathogenesis of CNS diseases and play key roles in the neurodegenerative process.

Brain microvasculature has gained attention in recent years in many CNS diseases, such as ischemic stroke, AD, ALS, VP, VaD, and aging (Fig. 4), since it is a pivotal element to maintain or improve the neurovascular network and, therefore, brain functioning. In these CNS diseases, the neurovascular unit (Fig. 2) is damaged, mainly by the injury of ECs, affecting the vascular network and, consequently, the neurovascular coupling due to the deficit in signaling, in neuronal stem cell proliferation/migration, as well as the lack of oxygen and nutrients normally provided by ECs and BBB exchange (Hatakeyama et al., 2020) (Fig. 2). All this leads to a cascade of events (Fig. 4) that contribute to the loss of cognitive function or other basic brain functions. The insults for such injury include BBB disruption, altering not only the homeostatic movement of ions, nutrients, and cells between the blood and the CNS but also the clearance of xenobiotic or neurotoxic components. The impairment of clearance pathways can also foment the accumulation of unwanted injurious molecules prompting proteinopathies. Disruption of the neurovascular unit can also provoke a reduction in cerebral blood flow, which may lead to a diminished brain oxygenation and hence hypoxic microenvironment. On the other hand, less trophic factors are produced and released in response to the insult, which consequently diminishes the chemotactic signals for EC migration and proliferation, contributing to a higher vulnerability and susceptibility of neurons and glial cells to the disease. Additionally, the damaged neurovascular unit can ultimately degenerate, leading to a decrease of the vascular network which would aggravate all the aforementioned issues.

All of the stated CNS diseases (Fig. 4) present a decline in microvasculature and reduced cerebral blood flow. Additionally, this blood flow typically diminishes at a rate of 0.5% per year during aging; thus, in the elderly, a 20% flow reduction is normally observed (Leenders et al., 1990; Zou et al., 2009). This fact is yet an aggravating factor for neurodegenerative diseases in which the chronic brain hypoperfusion is added to the aging-related decline of cerebral blood flow.

A. Acute Events

Among stroke cases, 80% comprise ischemic stroke, hemorrhagic stroke is responsible of 15% and 5% are of unknown etiology (Beal, 2010). It is particularly a matter of concern that epidemiologic studies report an increasing incidence and proportion of young adult patients with stroke (Ekker et al., 2018). Control of hypertension, along with a moderate or low salt intake, is the major determinant of the long-term declines in cerebrovascular diseases (hemorrhagic and ischemic stroke) mortality (Levi et al., 2009; Petersen et al., 2019). Also, endogenous levels of other substances, such as the anti-oxidant ascorbic acid, also known as vitamin C, have been studied in ischemic stroke models since evidence supports that vitamin C deficiency could be a risk factor for stroke (Chen et al., 2013; Sánchez-Moreno et al., 2004; Yokoyama et al., 2000) as it functions as an inhibitor of the reactive oxygen species (ROS) produced in response to ischemic pathophysiology.

In ischemic stroke, the cerebral artery occlusion provokes a decrease in blood flow and an ischemic environment which leads to brain dysfunction. In this process, the brain tissues are not affected equally, and hence, the cellular response is different in the ischemic core and in the penumbra region (Bandera et al., 2006). In the ischemic core, a severe ischemia occurs, in which necrosis and irreversible cell damage take place, while in the penumbra region, there are metabolically active cells that can be recoverable if reperfusion or increased collateral flow can be provided to sustain the low-flow area. If not, it will evolve/expand into the final injury/infarction as well. Therefore, the penumbra is in the spotlight for therapeutic applications (Baron, 1999; Jung et al., 2013). Angiogenesis and tissue remodeling is highly active following stroke, particularly in penumbral regions. Angiogenesis promotes the delivery of blood flow and cell metabolism recovery to ischemic tissue and is positively correlated with the survival rate of ischemic stroke patients (Arenillas et al., 2007). Therefore, it is reasonable to assume that pro-angiogenic therapies would be beneficial for the improvement or recovery of a correct neurovascular and brain functioning. Currently, the treatment is limited to the intravenous administration of the thrombolytic recombinant tissue plasminogen activator (rt-PA), but this has a narrow therapeutic window (IST-3 collaborative group et al., 2012), within the 6 hours after

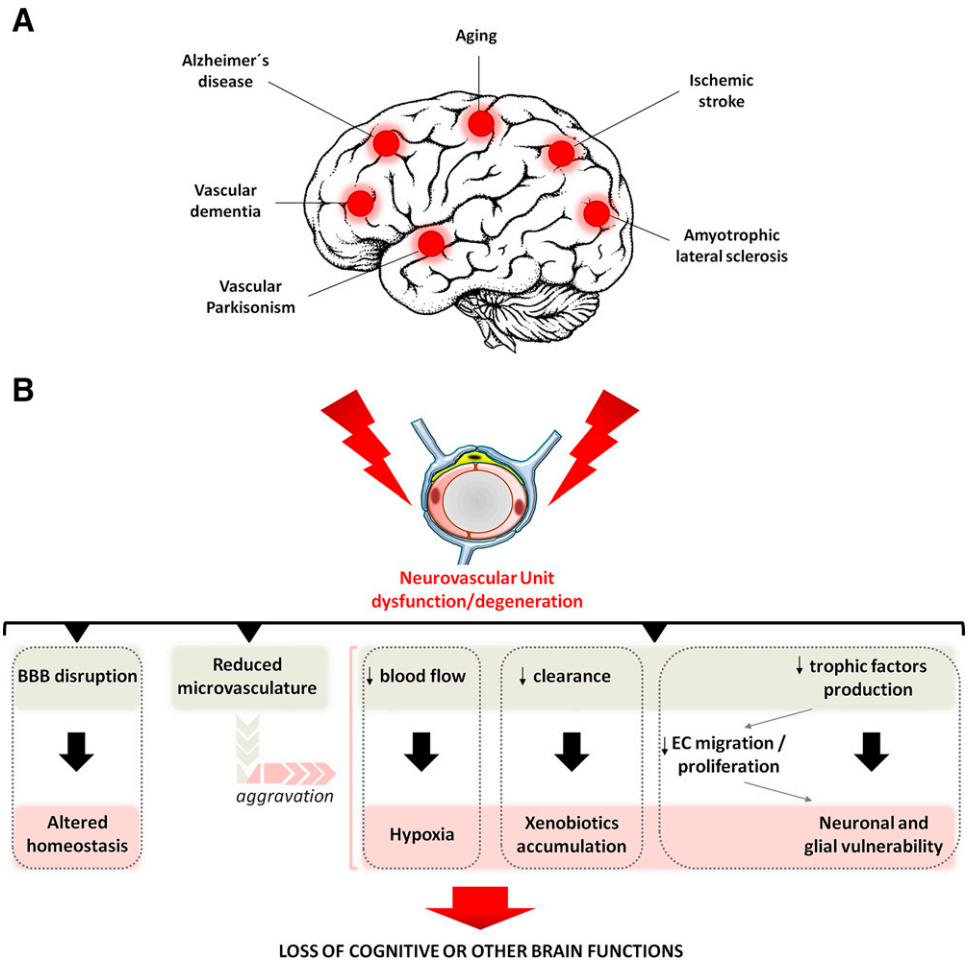

Fig. 4. Brain microvasculature damage/decrease is involved in many central nervous diseases. (A) Main central nervous diseases in which a reduction in brain microvasculature has been described. (B) Cascade of events that occur in the illustrated diseases due to the damage/degeneration of the neurovascular unit. BBB, blood-brain-barrier; EC, endothelial cell.

onset of stroke, and injurious effects, such as intracranial hemorrhage and neurotoxicity (Fan et al., 2016; Kaur et al., 2004). Interventional surgery for mechanical endovascular thrombectomy is another treatment option in the case of acute ischemic stroke due to large-vessel occlusion. In particular, blood clot retrievers, combined or not with rt-PA, have demonstrated improved functional outcomes, achieving reperfusion, and reduced healthcare costs (Campbell et al., 2017; Saver et al., 2015; Zhu et al., 2019). However, additional efforts aimed at longer-term recovery could be facilitated by inducing angiogenesis into penumbra, which is additionally supported by clinical observations demonstrating a strong correlation between neovascularization and functional recovery, suggesting that newly formed vessels contributed to behavioral improvements after ischemic stroke (Gunsilius et al., 2001).

B. Neurodegenerative Diseases

Chronic brain hypoperfusion is present in AD, ALS, VP, VaD and aging, and may be ahead of the appearance of clinical symptoms or neurodegenerative signs, so it is quite feasible that the pathogenesis of these neurodegenerative diseases could depend on the formation of vascular pathology and/or loss of microvasculature (de la Torre, 2017).

1. Alzheimer's Disease. AD is the most common cause of dementia. The greatest risk factors for late-onset Alzheimer's are older age, up to 65 years of age, (Hebert et al., 2010), genetics (Farrer et al., 1997) and having a family history of Alzheimer's (Green et al., 2002; Lautenschlager et al., 1996; Mayeux et al., 1991). Pathologically, it is characterized by the deposition of extracellular protein aggregates of amyloid-β (Aβ), forming senile plaques, and intracellular neurofibrillary tangles containing phosphorylated tau in the brain parenchyma. Aβ also accumulates in the vessels as cerebral amyloid angiopathy (CAA). However, the understanding of the pathophysiology of AD is constantly changing, and many impaired factors seem to play a role in the disease process (Anand et al., 2014). In fact, although many clinical trials have been carried out through immunotherapy by employing monoclonal antibodies against the presumably main

cause of AD, the Aβ plaques, all of them have failed (Huang et al., 2020). This strengthens the concept of a multifactorial pathophysiology in AD. There is also a genetic predisposition to AD such as inheriting the APOE4 gene. In particular, the APOE4 ε4 allele is present in around 14% of the general population and in 40–60% of all cases of AD as one -showing 3.5-fold higher risk of developing AD, or two copies, increasing the risk by 8–15-fold (Holtzman et al., 2012; Michaelson, 2014).

Clinically, AD manifests itself by a gradual decline of cognitive function. Currently available treatments for AD dementia are aimed at treating the symptoms by manipulating the levels of two neurotransmitters, acetylcholine and glutamate, but do not alter the course of the disease. They consist of four cholinesterase inhibitors, tacrine, donepezil, rivastigmine, and galantamine, and a N-methyl-d-aspartate (NMDA) receptor antagonist, memantine, which are approved by the US Food and Drug Administration (Cummings et al., 2014). Vascular risk factors, as well as aging, stroke atherosclerosis and diabetes mellitus enhance the risk to of developing AD (Gorelick, 2004), which together may result in a decreased response to vasodilators and reduced cerebrovascular blood flow, leading to local hypoxia (Aliev, 2011).

The role of microvasculature damage/dysfunction in AD is increasingly widespread (Bersini et al., 2020; Liesz, 2019; Zlokovic, 2005). The deposition of Aβ in the cerebrovasculature causes a decline in cerebral perfusion and promotes ischemic damage, accompanied by vascular and neuronal degeneration and subsequent cognitive decline (Di Marco et al., 2015; Roth et al., 2005). Additional evidence suggests that the chronic cerebral hypoperfusion and Aβ accumulation can boost an impaired angiogenesis that results in dysfunctional microvessels (Biron et al., 2011). Paradoxically, in this scenario, several pro-angiogenic factors are released by brain microvessels in AD (Grammas, 2011), VEGF, IL-1β, IL-6, IL-8, TNF, TGFβ, MCP1, thrombin, angiopoietin 2, αVβ3, and αVβ5 integrins, and hypoxia inducible factor-1α (HIF-1α); however, no increase in functional vascularization occurs. In fact, some studies report vascular regression and decreased microvasculature density in AD brain (Brown and Thore, 2011). The explanation is that this endogenous VEGF binds directly to Aβ peptides, which would result in a local deficiency of accessible VEGF and, consequently, a cerebrovascular degeneration and reduced neuroprotection (Patel et al., 2010; Yang, S. P. et al., 2004). In this regard, current advances in brain vascular vessel imaging techniques, such as micro-optical sectioning tomography, have allowed scientists to precisely visualize and quantify the reduction of the cerebral vascular network, in terms of vessel mean diameter and volume fraction, in AD mouse models compared with wild-type mouse models (Zhang X et al., 2019).

Some homeobox genes are involved in both physiologic and pathologic scenarios related to vascular remodeling in the adult. In particular, the homeobox gene MEOX2 regulates vascular differentiation (Gorski and Walsh, 2003). In this sense, it has been described that the low expression of the MEOX2 gene (Table 1) found in AD brain is associated with aberrant angiogenesis and vessel regression, ultimately resulting in reductions in brain capillary density and cerebral blood flow (Wu et al., 2005).

2. Amyotrophic Lateral Sclerosis. ALS is a neurodegenerative disorder characterized by progressive motor neuron degeneration in the brain and spinal cord, provoking muscle atrophy, paralysis, and early death since the diagnosis (Haverkamp et al., 1995; Rowland and Shneider, 2001). Most ALS cases are sporadic, and only around 15% of cases have a genetic basis, with more than 20 genes associated with the disease, such as hexanucleotide expansions in chromosome 9 open reading frame 72 (C9orf72), mutations in superoxide dismutase 1 (SOD1), TAR DNA-binding protein 43 (TARDBP), fused in sarcoma (FUS) and TANK-binding kinase 1 (TBK1) (van Es et al., 2017). To date, there is no cure or effective treatment of ALS, and only multidisciplinary care, nutritional and respiratory support, and symptom management are conducted.

Compelling evidence demonstrates the existence of CNS microvascular pathology and neurovascular unit impairment in ALS (Garbuzova-Davis et al., 2011) revealed by the reduced capillary blood flow in spinal cord of ALS mice (Garbuzova-Davis et al., 2007; Zhong Z et al., 2008) and brain of ALS patients (Rule et al., 2010; Waldemar et al., 1992). This would provoke vascular hypoperfusion/dysregulation which occurs before motor neuron degeneration or brain atrophy. Additionally, a significant decrease of circulating ECs has been found in peripheral blood of ALS patients at different disease stages, highlighting an impaired endothelization throughout the course of the illness (Garbuzova-Davis et al., 2010). The microvascular involvement greatly influences the understanding of ALS pathogenesis, demanding reconsiderations in therapeutic approaches and highlighting new treatment strategies to recover or maintain the neurovascular unit function and thus, promote neuron survival and correct CNS functioning (Vangilder et al., 2011).

3. Vascular Parkisonism. Vascular changes in the brain are the hallmarks of VP (Korczyn, 2015), which accounts for 3–5% of all patients with parkinsonism (Jellinger, 2003). Although the exact changes in blood vessels have not yet been described in detail, these changes are normally ischemic and affect areas of the brain that are relevant to parkinsonism, including

TABLE 1
Potential candidates for revascularization in brain CNS diseases where cerebral microvasculature is impaired

Target	Name	Function	CNS disease	Ref.
VEGF	Vascular endothelial growth factor	Cooperates with Angiopoietin-1 to mediate angiogenesis and vessel maturation in ischemic brain. Binds to Aβ peptides present in AD brain microvasculature, which might result in local deficiency of accessible VEGF, leading to cerebrovascular degeneration and reduced neuroprotection. Preliminary evidence suggests a relationship between vasculature, hypoxia and motor neuron survival in ALS.	Focal cerebral ischemia, Alzheimer's disease, Amyotrophic lateral sclerosis and aging	(Evans et al., 2013; Patel et al., 2010; Zhang et al., 2000; Zhang et al., 2002)
PlGF	Placental growth factor	Contributes to neuroprotection, angiogenesis, vessel growth and maturation, maintaining vessel permeability. Synergistic angiogenic role with VEGF-A in hypoxic conditions.	Cerebral ischemia	(Carmeliet et al., 2001; Freitas-Andrade et al., 2012)
ANGPTL4	Angiopoietin-like 4	Potent inducer of cerebral neovascularization under hypoxic conditions. May also serve as diagnostic biomarker in patients with clinically assessed vascular cognitive impairment.	Alzheimer's disease, aging and ischemic stroke	(Bersini et al., 2020; Bouleti et al., 2013; Chakraborty et al., 2018)
bFGF	Basic fibroblast growth factor	Maintains the integrity of cerebral microvasculature. Angiogenic and neuroprotective effects under hypoxia, promotes the proliferation and migration of pericytes via its interaction with PDGF-BB. Interacts with VEGF-A synergistically in promoting angiogenesis.	Cerebral ischemia, ischemic stroke	(Dordoe et al., 2021; Harrigan, 2003; Lyons et al., 1991; Nakamura et al., 2016)
$\alpha v\beta 3$	Integrin $\alpha v\beta 3$	Expressed in the activated microvessels of CNS endothelial cells in response to hypoxia. Essential function in the angiogenesis activation.	Cerebral ischemia	(Abumiya et al., 1999)
NAD+	Nicotinamide adenine dinucleotide	Protects the integrity of cerebral microvasculature by controlling endothelial cells cellular metabolism, energy production and survival.	Aging	(Csiszar et al., 2019)
MEOX2	MEOX2 gene (also known as GAX)	Promotes the angiogenic response of CNS endothelial cells to hypoxia, suppresses apoptosis and increases Aβ clearance efflux.	Alzheimer's disease	(Wu et al., 2005)
miR- 15a/16-1	microRNA- 15a/16-1 cluster	Controls VEGF and FGF expression regulating angiogenesis and cerebral blood flow.	Cerebral ischemia	(Sun P et al., 2020; Yin et al., 2012)
miR-30a	microRNA-30a	Controls BBB damage, infarct volume and neurovascular deficit caused by zinc accumulation in microvessels under ischemia, targeting the ZnT4 zinc transporter.	Ischemic stroke	(Wang P et al., 2020)
miR-124	microRNA-124	Regulates cerebromicrovascular impairment, including the decline in microvascular density and reduced angiogenesis.	Alzheimer's disease	(Li et al., 2019a)
miR-126	microRNA-126	Regulates angiogenesis and neurogenesis by the proliferation and migration of endothelial cells.	Focal cerebral ischemia	(Qu et al., 2019)
miR-210	microRNA-210	Acute ischemic stroke patients with higher circulating blood miR-210 show better clinical outcomes. Circulating blood miR-210 level associates with brain miR-210 level in a mouse model of ischemia, representing	Ischemic stroke	(Zeng L et al., 2011; Zeng L et al., 2014)

(*continued*)

TABLE 1—*Continued*

Target	Name	Function	CNS disease	Ref.
		a biomarker of brain injury and repair. Promotes vascular endothelial cell migration and tube formation under hypoxia, and associates with local increased levels of VEGF.		

subcortical white matter, basal ganglia, thalamus, and upper brainstem (Foltynie et al., 2002). Chronic ischemic changes that affect the subcortical white matter might result in gait disorders and cognitive impairment (Vale et al., 2012) that are not improved by dopaminergic medication (Benítez-Rivero et al., 2013). In fact, the pathologic vascular features of VP together with the unresponsiveness to treatment with dopaminergic drugs, distinguish this condition from idiopathic Parkinsońs disease. Patients with VP have vascular risk factors, such as hypertension, dyslipidemia, and diabetes mellitus. Hence, modification of the VP disease course could be possible through a reduction of vascular risk factors or revascularization strategies.

4. Vascular Dementia. As stated in the National Institute on Aging (http://www.nia.nih.gov/health/vascular-contributions-cognitive-impairment-and-dementia), is called a cerebrovascular disease when the damage or pathology of brain microvasculature—also known as small vessel disease—leads to brain tissue injury—due to a decline of blood flow, oxygen or nutrients—contributing to cognitive impairment and dementia (Román, 2004). VaD terminology refers to people with dementia whose brain shows evidence of cerebrovascular disease. VaD is the second most common type of dementia after AD, contributing to nearly 17% of all dementias (Kapasi et al., 2017; O'Brien and Thomas, 2015). The location, number, and size of the brain injuries determine the severity of the dysfunction. In particular, it has been found that cortical microvascular pathology contributes to dementia through pyramidal cell loss (Kril et al., 2002). Therefore, restoration of cerebral microvasculature could potentially alleviate neuronal cell loss and improve cognitive function.

5. Aging. Aging constitutes the main risk factor for most neurodegenerative diseases, increasing their prevalence with increasing age (Hou et al., 2019). This fact could be directly or indirectly associated with the endothelial dysfunction (Bersini et al., 2020; Ungvari et al., 2013; Ungvari et al., 2020), decline in cerebral blood flow, around 20% flow reduction, (Leenders et al., 1990; Zou et al., 2009) and BBB leakage (Verheggen et al., 2020) that occurs with aging. Additionally, scientific consensus exists on the critical role of microvasculature contributions to cognitive impairment in elderly patients (Tarantini et al., 2017).

At the molecular level, age has been shown to impair angiogenesis through various pathways, including lower endothelial nitric oxide synthase and HIF-1α activity and decreased availability of VEGF (Lähteenvuo and Rosenzweig, 2012). Additionally, nicotinamide adenine dinucleotide (NAD+) deficiency has been associated with aging as a mediator of the endothelial dysfunction (Csiszar et al., 2019; Gomes et al., 2013).

IV. New Opportunities for Brain Revascularization

As explained before, angiogenesis is switched-on under the aforementioned brain hypoxic situations, in which the endogenous pro-angiogenic signals are not enough to achieve sufficient functional vessels to restore brain vascularization. Therefore, pro-angiogenic therapies seem to be a logical solution to this challenge. It is known that boosting brain angiogenesis implies an increase in the number of endothelial cells that can restore the performance of the neurovascular unit and cerebral blood flow. Hence, the extracellular regulatory signals secreted by the generated microvasculature, facilitate the proliferation and migration of neural stem cells as well as nutrients and oxygen supply, promoting neurologic recovery and cognitive function improvement (Hatakeyama et al., 2020).

Among the pro-angiogenic factors employed to achieve brain revascularization, VEGF-A is the most potent and widely employed candidate due to its angiogenic and neuroprotective effects (Korpisalo and Yla-Herttuala, 2010; Lange et al., 2016; Shim and Madsen, 2018). A relevant feature of VEGF function, linked to its therapeutic implications, is its interaction with ECM, which commands its localization in tissues and controls the outcome of the angiogenic process (Martino et al., 2015). Basically, there are three VEGF isoforms with diverse affinity for ECM (Ferrara, 2010), depending on the alternative mRNA splicing of the VEGF-A transcript, comprised of 121, 165, and 189 residues in humans or 120, 164, and 188 in rodents, respectively (Tischer et al., 1991). Among them, $VEGF_{164/165}$ is the isoform of choice for therapeutic delivery since it is the only isoform able to promote physiologic vascular networks in the absence of the other isoforms, by generating intermediate gradients of concentration around cells with balanced matrix affinity (Ruhrberg et al., 2002). Additionally, as stated before, VEGF-A exerts synergistic effects in angiogenesis when combined with bFGF (Harrigan,

2003) (Fig. 3). In this sense, evidence shows that bFGF is able to induce neuroprotective and angiogenic effects in hypoxic situations by promoting the proliferation and migration of pericytes via its interaction with PDGF-BB (Nakamura et al., 2016) (Fig. 3).

For its part, the platelet-derived growth factor (PDGF) (Fig. 3) and its receptor PDGFR-β signaling have been studied in knockdown mouse models. They seem to play a key role in BBB restoration after cerebral ischemia, in part via the regulation of TGF-β signaling (Shen J et al., 2019), through the induction of pericyte recruitment, migration, and proliferation (Shen J et al., 2012). This occurs by the close coordination between ECs and pericytes, allowing the maturation of the emergent new vessels. In fact, the lack of this coordinated signaling occurs in ischemic stroke and neurodegenerative diseases, leading to brain microvasculature impairment and loss of cognitive function (Uemura et al., 2020).

Hence, identifying the specific signals involved in the microvasculature disruption in CNS highlights potential therapeutic angiogenic candidates for recovering the neurovascular network in stroke, neurodegeneration, and aging. Some of the potential candidates for such angiogenic strategy are summarized in Table 1.

A. Therapeutic Factors

1. Growth Factors. As mentioned before, the most potent and widely employed pro-angiogenic factor to achieve brain revascularization is VEGF-A (Table 1) due to its angiogenic and neuroprotective effects (Lange et al., 2016; Shim and Madsen, 2018). It has been shown that systemic or intracerebral early administration of soluble VEGF after stroke increased BBB opening, intensified edema, and promoted disorganized and immature vasculature, while its antagonist reduced the injury (Ma et al., 2012). Importantly, a work by Zhang et al. provided insights into the adequate time point for VEGF therapy, where intravenous VEGF-A administration 48 hours after MCAO increased microvessel density and improved cerebral microvascular perfusion in the ischemic penumbra, while administration at early stage, 1 hour after MCAO increased BBB leakage (Zhang ZG et al., 2000). This provided a starting point for subsequent studies regarding VEGF administration in ischemic stroke animal models, promoting angiogenesis, neurogenesis, reduced infarct size, and improved behavior outcomes (Chu K et al., 2005; Sun Y et al., 2003; Yang JP et al., 2009a).

It is assumed that other ischemia-induced factors are involved in controlling vascular integrity. Among them, angiopoietin-like protein 4 (ANGPTL4) (Table 1) is a secreted vascular growth factor induced by hypoxia in vascular cells in AD, aging, and stroke (Bersini et al., 2020; Bouleti et al., 2013; Chakraborty et al., 2018). It has been found that single intravenous injection in the tail vein of recombinant human ANGPTL4 1 hour after MCAO in a mouse model of ischemic stroke, led to a reduction in infarct size and improved behavior performance (Bouleti et al., 2013). In this manner, ANGPTL4 seems to counteract the loss of vascular integrity, and consequently diminishes vascular leakage and cerebral edema. Similarly, bFGF (Table 1) intracerebral administration in animal models has evidenced some potential to induce angiogenesis in physiologic conditions (Puumala et al., 1990), but overall, in brain ischemic situations (Lyons et al., 1991) (Table 2), denoting the influence of hypoxia on the angiogenic effect of bFGF.

2. Bioactive Substances. Apart from vascular derived growth factors, bioactive substances, such as ligustilide and astragaloside IV, promote angiogenesis and recovery from ischemic stroke. In particular, it has been recently described that ligustilide, a bioactive substance isolated from Ligusticum chuanxiong, increases cerebral vessel number and neuroprotection after MCAO, and that the VEGF-endothelial nitric oxide synthase signaling pathway (Fig. 3) is involved in this process (Ren et al., 2020). Also, some studies have found that astragaloside IV, a triterpenoid saponin bioactive compound of Astragalus mongholicus with many beneficial effects in the brain, stimulates angiogenesis, including EC proliferation and migration, by increasing the expression of miRNA-210, which induces the activation of the HIF-VEGF-Notch signaling pathway (Fig. 3) (Liang et al., 2020). Other substances, such as the anti-oxidant ascorbic acid, have been studied in ischemic stroke models, since evidence supports that vitamin C deficiency could be a risk factor for stroke (Chen et al., 2013; Sánchez-Moreno et al., 2004; Yokoyama et al., 2000). Preclinical studies have shown that systemic administration of vitamin C after ischemic stroke, is able to maintain the BBB integrity and ECs tight junctions, protecting from the ischemic damage (Chang et al., 2020). In particular, this study showed that vitamin C reduced brain infarction volume and edema and also prevented neuronal damage; however, these outcomes were not because of angiogenesis. Even therapies for NAD+ repletion (Table 1) seem to protect the integrity of the cerebral microvasculature, improving cognitive performance in aging (Csiszar et al., 2019). As aforementioned, age-related NAD+ depletion has been associated with endothelial dysfunction. In this regard, restoring the NAD+ levels seems to protect the integrity of the cerebral microvasculature, improving cognitive performance in aged mice. In fact, the stimulation of cerebromicrovascular ECs isolated from aged F344xBN rats with nicotinamide mononucleotide (NMN), has evidenced an improvement in the angio-genic capacity, which could counteract, in part, the adverse effects of aging (Kiss et al., 2019). Some of the approaches for NAD+ repletion, aimed at the prevention of vascular

TABLE 2
Administration routes of pro-angiogenic therapies aimed at promoting brain revascularization in preclinical trials

Route	Procedure	Vector	Therapeutic factor	Dose	Duration of treatment	CNS disease	Ref.
Systemic	Tail vein injection/ infusion	-	rhANGPTL4	40 μg/kg	Single dose	IS	(Bouleti et al., 2013)
		-	$rhVEGF_{165}$	1 mg/kg	5 μl/min, 4 hours	IS	(Zhang ZG et al., 2000)
		-			$rhVEGF_{165}$human neural stem cells	50 μg/ kg500 μl	1 μg/kg, 1hour10^4 cells/ μl, 5min
		IS	(Chu K et al., 2005)				
	AV transfected human mesenchymal stem cells	*PlGF*	10^7 cells	Single dose	IS	(Liu H et al., 2006)	
	Retro-orbital sinus	Fusogenic liposomes	Resveratrol	2 mg/kg/day	4 days	Ag	(Wiedenhoeft et al., 2019)
	Intraperito-neal	-	$rhVEGF_{165}$	8 μg/kg/day	3 days	AD	(Wang P et al., 2011)
-		Vitamin C	2000 mg/kg	Single dose	IS	(Chang et al., 2020)	
Intracerebral	Diffusion from cortex	PLGA nanospheres	$rhVEGF_{165}$	1 μg/mouse	Single dose	AD	(Herran et al., 2013a)
		Encapsulated BHK-VEGF cells	$hVEGF_{165}$	20–30 microcapsules	Single dose	AD	(Spuch et al., 2010)
		Encapsulated BHK-VEGF cells	$hVEGF_{165}$	1microcapsule (10^6 cells); 10-20 ng VEGF released/day	Single dose	IS	(Yano et al., 2005)
	Osmotic mini-pumps	-	$rhVEGF_{165}$	10 μg/ml	1 μl/hour, 3 days	IS	(Sun Y et al., 2003)
		IgG antibody	Anti-Nogo-A	7 mg/ml	7 days	IS	(Rust, et al., 2019b)
	Local injection	AAV	h*Vegf*$_{165}$	5 μl (5x10^9 TU/ ml)	0.2 μl/min, 15 min	IS	(Shen F et al., 2006)
		AAVH9	h*Vegf*$_{165}$	5 μl (2x10^9 TU/ ml)	0.2 μl/min, 15 min	IS	(Shen F et al., 2008)
		AAV	*Sestrin2*	10 μl (10^{10} TU/ ml)	0.5 μl/min, 20 min	IS	(Li Y et al., 2020)
		AAV	*PlGF*	3x10^9 AAV particles	2 μl × 4 injections	IS	(Gaál et al., 2013)
		AV	h*bFGF*	50 μl (1x10^8 TU)	3.3 μl/min, 15 min	IS	(Watanabe et al., 2004)
		Lentivirus	miR-210	2.2 μl (2x10^9 TU/ ml)	0.2 μl/min, 15 min	N, IS	(Zeng L et al., 2014; Zeng LL et al., 2016)
		Lentivirus	miR-126	2 μl (5.6x10^8 TU/ ml)	0.2 μl/min, 10 min	IS	(Qu et al., 2019)
		Lentivirus	miR-124	0.2 μl (10^8 TU/ ml)	Single dose	AD	(Li AD et al., 2019)
		Niosomes	h*Vegf*$_{165}$	5 μl	Single dose	N	(Gallego et al., 2020)
		PLGA microparticle	hNSC$rhVEGF_{165}$	20 μg/μl (10^4 cells)	Single dose	IS	(Bible et al., 2012)
		hNSC	h*Vegf*$_{165}$	2 μl (2x10^5 cells)	Single dose	IS	(Lee et al., 2007)
		HSV-1 transfected BMSC cells	h*bFGF*	10 μl (1x10^6 cells)	Single dose	IS	(Ikeda et al., 2005)
		HA hydrogel	$rhVEGF_{165}$	6 μl	Single dose	IS	(Ju et al., 2014; Nih et al., 2016)
		PEG-PPS hydrogel	iPS-NPC cells	-	Single dose	N	(Zhang J et al., 2011)
Intranasal	Neuro-olfactory pathway	-	$rhVEGF_{165}$	100 μl/day(200 μg/ml)	10 μl at a time for 18.5min, at intervals of 2min, alternating the nostrils. 3 days	IS	(Yang et al., 2009a)
		RVG29-PEG-PLGA nanoparticles	miR-124	2 drops containing miR-124 at 5 μg/ml	Every 2 days after the modeling during a week	IS	(Hao et al., 2020)

AAV, adeno-associated virus; AD, Alzheimer's disease; Ag, aging; ANGPTL4, angiopoietin-like 4; AV, adenovirus; BHK, baby hamster kidney cells; BMSC, bone marrow stromal cells; CNS, central nervous system; HA, hyaluronic acid; HSV-1, herpes simplex virus type 1; iPS-NPC, human induced pluripotent stem-neural progenitor cells; IS, ischemic stroke; hMSCs human mesenchymal stem cells; hNSC, human neural stem cell; N, normal; NSC, neural stem cells; PEG, poly(ethylene glycol); PLGA, poly(-lactic-co-glycolic acid); PPS, poly(propylene sulfide); rh, recombinant human; TU, transducing units; VEGF, vascular endothelial growth factor.

cognitive impairment, consist in the intraperitoneal administration of the poly(ADP-ribose) polymerase (PARP-1) inhibitor, named as PJ-34 (Tarantini et al., 2019), or of NMN in drinking water (Mills et al., 2016), among others reviewed by Csiszar et al. (Csiszar et al., 2019).

3. Considerations. The dosage and the spatio-temporal control of the factor delivery to avoid aberrant vessel formation, BBB leakage, gliomas, and other potential undesirable side effects (Manoonkitiwongsa et al., 2004; Ozawa et al., 2004; Uccelli et al., 2019; von Degenfeld et al., 2006), must be taken into account to achieve better clinical results. Accordingly, it is paradoxical that VEGF levels are usually upregulated after brain insults and how this endogenous molecule, far from being therapeutic, promotes vessel leakage, increases BBB permeability and produces aberrant angiogenesis with vessel regression, in neurodegenerative diseases overall (Dvorak et al., 1995; Manoonkitiwongsa et al., 2004). It has been shown that systemic or intracerebral early administration of soluble VEGF after stroke increased BBB opening, intensified edema, and promoted disorganized and immature vasculature, while its antagonist reduced the injury (Ma et al., 2012). In this sense, the dose of VEGF seems to play a crucial role not only in achieving therapeutic effects, but also in maintaining a correct balance between angiogenesis and brain tissue integrity. In particular, in contrast to non-angiogenic doses of $VEGF_{165}$, the administration of angiogenic doses of this growth factor in rat brain through invasive techniques augmented macrophage density, the typical cell marker of inflammation, in the cortical tissue of ischemic brains (Manoonkitiwongsa et al., 2006). Hence, the authors propose that VEGF monotherapy may not be enough to achieve a therapeutic effect. Accordingly, it has been suggested that a preferable and more complete approach would be the implementation of a cocktail of angiogenic factors rather than VEGF alone. In this sense, a strategy that combines growth factors for mature vessel formation would include VEGF, for promoting angiogenesis, and angiopoietin-1 (ANG-1), for inducing vessel maturation (Greenberg, 2015; Liu J, 2015; Valable et al., 2005; Zhang ZG et al., 2002).

Even though this strategy, consisting of the direct administration of the potential therapeutic factor has been widely explored, in ischemic stroke scenarios by systemic administration overall (Table 2), the approach has critical limitations due to rapid factor degradation and low stability in vivo (Rust et al., 2019a). Moreover, systemic circulation of these factors could affect other organs and, even in the target organ, the sudden local increase of the exogenous substance levels could create an important imbalance. For this reason, nanotechnology based approaches have been proposed in the last decades to achieve a more sustained and safer delivery of the angiogenic factors, as explained below.

B. Nanotechnology Platforms

Nanotherapies are gaining momentum as a feasible medical option for the treatment of CNS diseases. Nanotechnology platforms, advanced therapies and biomaterials, constitute promising tools for an efficient and safe delivery of therapeutic factors. This approach enables the design and development of nanocarriers that allow the controlled and sustained release of drugs and gene-related products. This approach, protects therapeutic factors from degradation, improves biodistribution, and allows targeting cells/tissues. In fact, there are numerous papers reflecting the high efforts made in this promising field to achieve safe and effective drug or gene therapies targeting CNS (Agrawal et al., 2020; Lombardo et al., 2020; Mulvihill et al., 2020; Teixeira et al., 2020).

Different nanotechnology-based approaches for boosting angiogenesis have been employed, especially in wound healing repair, vectoring pro-angiogenic factors, and in anti-cancer therapies, vectoring anti-angiogenic factors. However, until now, few works have developed systems vectoring pro-angiogenic factors to promote revascularization in brain CNS diseases. Some of these nanotechnology strategies employed as drug, gene and even cell delivery systems focused on achieving therapeutic brain angiogenesis (Fig. 5, Table 2), are discussed below.

1. Nanocarriers. Currently, a nanocarrier-based clinical trial is ongoing for CNS revascularization in ALS patients by the intracerebroventricular administration of a solution containing recombinant human VEGF165 using an implanted drug delivery system consisting of an implanted catheter and a SynchroMed II Pump drug infusion system, known as sNN0029 (NCT01384162) (https://www.clinicaltrials.gov/ct2/show/NCT01384162). The phase I of this trial revealed that this therapy was safe and well tolerated by ALS patients.

a. Promoting angiogenesis. Regarding experimental studies, drug delivery of VEGF for angiogenic purposes has been carried out employing polymeric poly(lactic-co-glycolic acid) (PLGA) nanospheres in the amyloid precursor protein/presenilin-1 transgenic mouse model of AD (Herrán et al., 2013a) (Table 2). PLGA nanoparticles are colloidal systems combined with the biodegradable and biocompatible PLGA polymer, which has been approved by the US Food and Drug Administration for several therapeutic applications. In the aforementioned work, 3 months after VEGF loaded nanosphere implantation, the vascular density in the cerebral cortex was augmented by 51% compared with control mice group, accompanied by a reduction in β-amyloid deposits, and improvement in memory deficit. This

approach using VEGF-PLGA nanospheres has also been employed also in Parkinsońs disease with neuroregenerative results (Herrán et al., 2013b), showing ability to cross the BBB efficiently (Meng et al., 2020). However, the mechanism of VEGF-PLGA nanoparticles for crossing the BBB remains unclear and would require nanoparticle-targeting track, as well as an analysis of the potential side-effects of this therapy in other organs.

b. Facilitating the function of remaining microvasculature. In the case of aging, rather than promoting angiogenesis, some researchers have focused their attention on the recovery of the endothelial function. In this sense, it has been observed that treatment with fusogenic liposomes loaded with resveratrol, a plant-derived polyphenolic stilbene, rescued endothelial function and neurovascular coupling responses in aged mice in vitro in cerebromicrovascular ECs (Csiszar et al., 2015) and in vivo after retro-orbital (RO) injection (Wiedenhoeft et al., 2019) (Table 2). On the one hand, resveratrol had previously been implemented for endothelial dysfunction recovery by modulating nitric oxide metabolism and protecting against ROS through the activation of Nrf2 antioxidant defense mechanism in EC and vascular smooth muscle cell cultures (Li et al., 2019b). In fact, treatment of aged rodents with resveratrol in food, 200 mg/kg/d for 10 days, exerts protective effects on the cerebral microcirculation and cognitive function (Oomen et al., 2009; Toth et al., 2014). On the other hand, the use of fusogenic liposomes, consisting of the neutral lipid DOPE, the positively charged lipid DOTAP, and the aromatic resveratrol, significantly enhanced the drug uptake into endothelial cells through their fusion with cell membranes, overcoming the limited endocytic uptake, often below 1%, of conventional liposomes (Csiszar et al., 2015; Wiedenhoeft et al., 2019) (Table 2). Other liposome-associated limitations include low stability, quick metabolic degradation of phospholipids, fast systemic clearance, poor sustained release, and moderate efficiency for the entrapment of lipophilic drugs (Wong et al., 2012). Some of these problems have been overcome by surface coating the liposomes with polyethylene glycol (PEG) to extend the liposomes circulating time.

2. Gene Therapy Systems. Therapies based on gene therapy approaches, divided into viral and non-viral vectors, have emerged in recent years as potential tools for the treatment of many congenital, acquired and age-related diseases (Ingusci et al., 2019). Gene therapy strategies cover the chance of developing gene editing, the translation of the gene into endogenous protein/factor of interest, provides the possibility of achieving a stable or inducible expression of the therapeutic gene, as well as a specific expression in target cells.

Clinical trials regarding *Vegf* gene administration are currently ongoing and one of them, known as Neovasculgen (https://www.clinicaltrials.gov/ct2/show/NCT02538705) (pl-vegf165; NCT02538705), has been commercialized since 2011. It consists of the gene transfer of the $Vegf_{165}$ gene embedded in a DNA plasmid vector (pCMV-vegf165) for therapeutic angiogenesis in ulcers related to diabetic foot syndrome by intramuscular injections. Particularly, it increases the number of functioning capillaries in ischemic tissues, but it can even be applied in combination with surgical revascularization to improve the long-term results of reconstructive operations. Neovasculgen applicability to CNS disorders is currently being tested in clinical trials for other angiogenic therapies, such as for peripheral nerve injury (NCT02352649) by performing intraneural injections (https://www.clinicaltrials.gov/ct2/show/NCT02352649).

a. Pretreatment with viral vectors to protect the vasculature. In the ischemic stroke scenario, it has been shown that intracranial administration of recombinant adeno-associated virus (AAV)-mediated *Vegf* transfer reduces infarct volume by 55% in mouse transient MCAO models (Shen F et al., 2006) and improves focal cerebral blood flow restoration (Shen F et al., 2008) (Table 2). However, these strategies were administered five days prior to the ischemic process, which would constitute more a preventive therapy than a potential treatment post-injury. Along the same line, a recent work has found an angiogenic role for sestrin2, a stress-inducible protein that maintains homeostasis, helps in cellular repair, and eliminates toxic metabolites as a result of various insults, including hypoxia. In particular, intracranial injection of adenoviruses and AAVs vectoring the *Sestrin2* gene two weeks before the insult promoted angiogenesis, reduced the infarct volume and brain edema, improving the neurologic function (Li Y et al., 2020) (Table 2).

b. Treatment with viral vectors to promote revascularization. After ischemic stroke, intracerebral administration of adenoviral (AV) vectors expressing bFGF 2 hours after the onset of MCAO can reduce the infarct area by 44.3%, leading to neurologic improvement (Watanabe et al., 2004) (Table 2). It has also been reported that this same strategy, but comparing the potential therapeutic effects of AV encoding the different VEGF family members, promoted the creation of mature microvessels without significant side effects in the case of PlGF, while VEGF-A and VEGF-C promoted angiogenesis accompanied by vessel leakage, and VEGF-B did not induce angiogenesis (Gaál et al., 2013). Hence, the authors proposed PlGF as a potential candidate for therapeutic brain revascularization (Table 2), although no behavioral evaluation was assessed in this study.

In addition to gene delivery, epigenetic approaches have been developed to promote cerebral angiogenesis using viral vectors and microRNAs. Most of them have been applied in ischemic stroke animal models

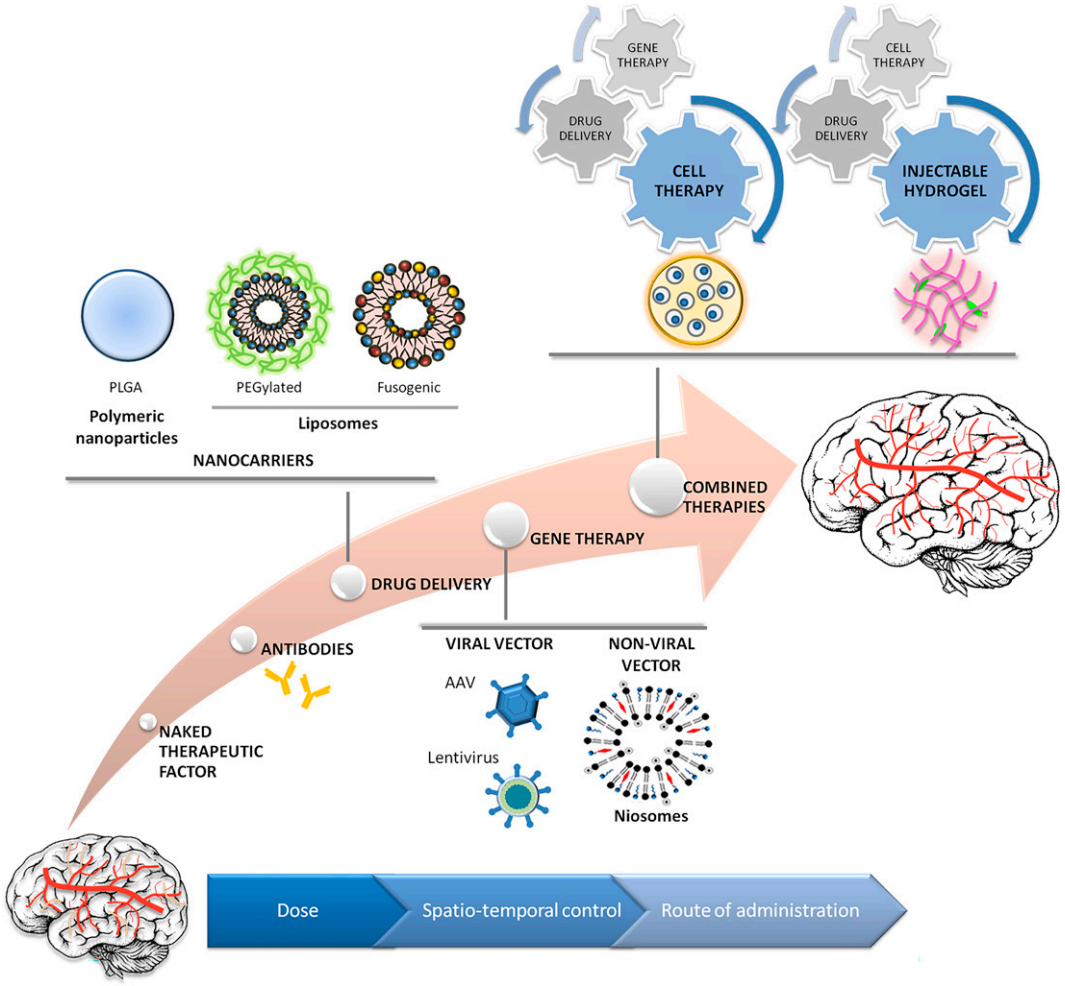

Fig. 5. Therapeutic opportunities employed for achieving brain angiogenesis and neurovascular network recovery. The design of the therapeutic strategy can embrace from the simplest form—with no vector—to the most complex approaches combining various technologic systems. During its implementation, relevant issues that should be considered include dosage of the therapeutic agent, concentration and number of doses, time point of administration, duration of the treatment, and the route of administration.

employing lentivirus vectors, since they can transfect neurons effectively. For instance, angiogenesis was achieved with lentiviral-mediated overexpression of miR-210 in normal mouse brain (Zeng L et al., 2014) and in ischemic mouse models by upregulating brain-derived neurotrophic factor (Zeng LL et al., 2016) (Table 2). Also, lentiviral-mediated overexpression of miR-126 promoted angiogenesis by activating AKT and ERK signaling pathways (Qu et al., 2019) (Fig. 3) (Table 2) or by regulating EC response to VEGF through repressing Sprouty-related EVH1 domain-containing protein 1 and phosphoinositol-3 kinase regulatory subunit 2 (PIK3R2/p85-b) (Nammian et al., 2020). MiR-15a/16-1 cluster endothelium-targeted knockdown models in vitro by lentivirus and in vivo by endothelial cell selective miR-15a/16-1 conditional knockout-enhanced post-stroke angiogenesis and cognitive recovery by upregulating the protein expression of VEGF-A, bFGF, and their receptors VEGFR2 and FGFR1 (Sun P et al., 2020) (Fig. 3). In a transgenic amyloid precursor protein/presenilin-1 animal model of AD, lentivirus-mediated overexpression of miR-124 promoted angiogenesis and vascular integrity at the hippocampus and cerebral cortex levels by regulating the classic complement of the innate immune system C1ql3 (Li et al., 2019a) (Table 2).

c. Treatment with non-viral vectors to promote revascularization. Even though viral vectors are a good option for efficient gene delivery to CNS, they present many inconveniences related mainly to the relatively small packaging capacity, elevated costs and safety, immunogenicity, mutagenicity, risk of oncogenesis, and persistence of viral vectors in the brain (Kariyawasam et al., 2020; Mingozzi and High, 2013; Provost et al., 2005). Additionally, pre-clinical and clinical trials with viral vectors have evidenced severe symptoms associated with the high and uncontrolled expression of exogenous *Vegf* (Martino et al., 2015). In this regard, non-viral vector-based gene therapy strategies are gaining interest due to their safety profile, high packing capacity, low

cost, and high-scale production potential (Foldvari et al., 2016; Jayant et al., 2016). Additionally, non-viral approaches based on lipid nanoparticles allow a sustained release of the cargo that would also facilitate the desirable transient but prolonged *Vegf* expression needed for at least 4 weeks to allow newly induced vessels to stabilize and persist (Ozawa et al., 2004; Tafuro et al., 2009).

Among lipid nanoparticles, niosomes have been employed for in situ gene therapy purposes in CNS (Al Qtaish et al., 2020). Interestingly, brain angiogenesis has been achieved in vivo by the intracerebral administration of niosomes vectoring the $Vegf_{165}$ gene at the cortex level (Gallego et al., 2020) (Table 2). In this work, niosomes were able to efficiently deliver the therapeutic gene, achieved VEGF release to the extracellular medium, and promoted angiogenesis in mouse brain. In fact, vascular density 1 week after intracerebral injection increased 76% in an extensive area in the vicinity of the injection point, compared with controls. Also, polymeric nanoparticles represent an encouraging strategy, as is the case of microRNA-124-loaded RVG29-PEG-PLGA nanoparticles, which have been reported to prevent the ischemic brain injury, contributing to the recovery of neurologic function (Hao et al., 2020) (Table 2).

3. Other Approaches and Combined Systems. To deliver physiologic amounts of the angiogenic factor to the brain tissue in a continuous and localized manner, cell encapsulation provides an interesting approach. In this way, a polymer matrix surrounded by a semi-permeable membrane that encapsulates the engineered cells preserves them against immune cell-mediated and antibody-mediated rejection. Additionally, this membrane regulates the bidirectional diffusion, allowing a controlled and continuous delivery of the therapeutic factor (Gurruchaga et al., 2015; Orive et al., 2003). Even, dual gene therapy and cell therapy strategies, with or without encapsulation, can be implemented. The combination of the three systems, gene therapy, cell therapy, and cell encapsulation matrix, with angiogenic outcome has been applied to an animal model of AD (Spuch et al., 2010). In particular, fibroblast cells transfected to produce human VEGF were encapsulated in an alginate solution and coated with poly-L-lysine plus another alginate layer. These authors found that implantation of VEGF-secreting microcapsules onto the brain for 3 months induced angiogenesis, which contributed to an enhancement of Aβ clearance and behavioral improvement in amyloid precursor protein/presenilin-1 mice, proposing VEGF microcapsule implementation as part of the treatment in AD patients (Spuch et al., 2010) (Table 2). Another example of this multi-combinational approach has been implemented for brain angiogenesis after stroke by combining gene therapy to develop stable genetically engineered cells and cell encapsulation in sterile capsules made of polysulfone hollow fibers (Yano et al., 2005) (Table 2). Here, authors first transfected cells with a plasmid encoding for $VEGF_{165}$ and established a VEGF-secreting cell line; then, these encapsulated cells and the capsules containing VEGF-secreting cells were locally administered into the brain parenchyma, achieving a continuous intracerebral release of $VEGF_{165}$. This design exerted neuroprotective and angiogenic effects on focal cerebral ischemia and led to significant functional recovery in rats after stroke. A similar approach, but with no encapsulation, has been also developed in stroke animal models, which involves transplanting into the brain the multipotent human neural stem cells overexpressing VEGF after gene transfection and selection with puromycin (Lee et al., 2007) (Table 2). Similarly, a therapeutic strategy combining viral gene therapy and cell therapy after ischemic stroke has been performed with bFGF gene-transferred bone marrow stromal cells (BMSCs) by the herpes simplex virus type 1 vector (Ikeda et al., 2005) (Table 2). With this approach, BMSCs are intended to secrete several growth factors and have the potential to differentiate into neurons and/or glial cells. Additionally, the enhanced bFGF release from these transduced BMSCs, can induce angiogenesis in hypoxic environments. Thus, intracerebral implantation of bFGF gene-transferred BMSCs 24 hours after MCAO achieved infarct volume reduction, accompanied by a significant brain function recovery 14 days after MCAO. In this same line, the intravenous infusion of human mesenchymal stem cells alone or previously transduced with adenovirus encoding PlGF, 3 hours after MCAO in rats, has evidenced the higher angiogenic effect of this cell therapy when combined with the enhanced expression of PlGF (Liu, H. et al., 2006) (Table 2). Specifically, 7 days after MCAO, the infarct size volume was reduced, angiogenesis was induced without cerebral edema, and this was accompanied by an improvement in behavioral performance.

Another dual strategy used is the combination of cell therapy with a drug delivery system. In this regard, the intracerebral implantation of human neural stem cells attached on the surface of VEGF-releasing PLGA microparticles after stroke, attracted host ECs to invade this neuroscaffolding, and allowed the development of a vascular network (Bible et al., 2012) (Table 2).

The design and development of hydrogels for brain therapy purposes represents an attractive therapeutic opportunity. They can be considered as scaffolds designed to match the mechanical properties of the brain by modulating the crosslinking density and to serve not only as local drug delivery systems (Nih et al., 2016) but also for tissue regeneration and cell transplantation purposes. In this line, some authors have proposed to employ a therapeutic angiogenic material delivering VEGF directly to the stroke cavity

to stimulate angiogenesis and repair brain tissue after stroke (Nih et al., 2018) (Table 2). To this end, they developed a biomaterial consisting of a hyaluronic acid hydrogel containing VEGF and injected it directly within the stroke cavity. The stroke cavity is the resulting damaged brain tissue after stroke which is deficient in extracellular matrix and that becomes a fibrotic scar devoid of neural tissue over time (Fitch et al., 1999). However, it represents a potential transplant space since it can accept a large volume of injection without affecting the unaffected brain tissue (Moshayedi et al., 2016; Nih et al., 2016; Zhong, J. et al., 2010). In this way, the injectable angiogenic biomaterial induced the formation of a vascular and neuronal structure that led to behavioral improvement (Nih et al., 2018). Similarly, a more complex combined approach using HA hydrogel and PLGA microspheres containing VEGF for angiogenesis and ANG-1 for vessel maturation has shown encouraging results in an MCAO mouse model of brain ischemia (Ju et al., 2014) (Table 2). Two weeks after MCAO, the HA-PLGA composite was implanted in the ischemic area, leading to in situ angiogenesis from the sixth week after MCAO and implantation, accompanied with a trend toward better behavior performance. On the other hand, cell transplantation has been carried out, employing an injectable hydrogel scaffold formed by PEG and poly(-propylene sulfide (PPS) (Zhang J et al., 2011) (Table 2). This 3D cell matrix, including human induced pluripotent stem-neural progenitor cells (iPS-NPCs) injected into the brain of naive mice, allowed for angiogenesis even without the addition of pro-angiogenic factors, enhancing stem cell survival. Interestingly, this matrix admits the incorporation of further desired signals, such as proteins, growth factors, and DNA.

Other authors have identified anti-Nogo-A antibodies as potential pro-angiogenic therapy for CNS ischemia that follows stroke, promoting a mature vascular network that, in turn, protects from vascular leakage (Rust et al., 2019b) (Table 2). The neurite outgrowth inhibitor Nogo-A is a membrane protein present in oligodendrocytes and some neurons that limits vascular growth in development and after CNS ischemia. In contrast, its neutralization enhances vascular repair in the ischemic border zone leading to functional outcome (Joly et al., 2018; Walchli et al., 2013).

V. Administration Routes for Targeting Brain Revascularization

Choosing the correct delivery strategy for the administration of the therapeutic systems is critical to ensure the effectiveness, safety, and functionality of the treatment. Each delivery route for the administration of therapies to reach the brain and promote new vessel formation, neurovascular network enhancement and cognitive/behavioral improvement in brain CNS diseases, involves specific advantages and drawbacks (Fig. 6). Compelling evidence at the preclinical level shows that systemic and intracerebral routes are the most researched. In this regard, emerging administration strategies along with the implementation of nanotechnology-based systems (Fig. 5) are critical factors that will define the efficacy of the angiogenic therapy for the recovery of the neurovascular network (Table 2).

A. Systemic Administration

The route of choice for systemic administration is usually tail vein injection, where the administered factor is normally not associated with any vector to avoid potential interactions with biologic components present in blood flow. In fact, many of the therapies for brain revascularization or microvasculature protection discussed in this review have employed this approach by single injection or infusion with a minipump (Bouleti et al., 2013; Chu K et al., 2005; Zhang ZG et al., 2000). However, the RO sinus route in experimental animals has emerged as an alternative systemic route, which seems to be easier, faster, presents fewer complications, and reports lower distress to the animal compared with lateral tail vein injection (Wang F et al., 2015; Yardeni et al., 2011). Regarding the field of cerebral microvasculature impairment, fusogenic liposomes vectoring resveratrol for brain vasculature protection in aged mice have been administered by this RO route (Csiszar et al., 2015; Wiedenhoeft et al., 2019). Also, intraperitoneal administration of VEGF in the AD PDGF-hAPP^{V717I} transgenic mouse has been carried out with angiogenic outcome in the hippocampus (Wang P et al., 2011), as well as ascorbic acid parenteral administration 1 hour after MCAO, reducing brain infarction volume (Chang et al., 2020). However, regardless of the systemic administration route, the challenge of affecting other organs still persists together with the therapeutic agent degradation and the difficulty in crossing the BBB (Rust et al., 2019a).

B. Intracerebral Administration

A large number of works have employed intracerebral administration to implement new opportunities for brain angiogenesis by administering naked factors, nanocarriers, gene therapy, cell therapy, biomaterials, antibodies, or systems combining these approaches (Table 2). The methods for intracerebral administration are the following:

Diffusion from cerebral cortex into brain: this approach has been carried out in the amyloid precursor protein/presenilin-1mouse model of AD after brain craniotomy. 1) VEGF-loaded PLGA nanospheres have been dropped directly onto the surface of the cerebral cortex, enhancing the cerebral vascular density by 51% (Herrán et al., 2013a). 2) Microcapsules of VEGF-producing cells have been placed in the craniotomy site, resulting in a patch of alginate (Spuch

et al., 2010) or polysulfone (Yano et al., 2005) microcapsules overlying the cerebral cortex. Two weeks after their implantation, vascular density augmented gradually during 3 months, accompanied by Aβ clearance and alleviation of the behavioral impairment (Spuch et al., 2010).

Osmotic mini-pumps: these have been employed to infuse the angiogenic factor, 1 day following ischemia, introducing a catheter by the intracerebroventricular route (Rust et al., 2019b; Sun Y et al., 2003).

Intracerebral injection: specially employed for gene therapy, cell transplantation and biomaterials as injectable hydrogels. 1) Viral gene delivery by local injection of AAV vectoring *Vegf* (Shen F et al., 2006) or *Sestrin2* (Li Y et al., 2020) has been carried out in stroke animal models prior to the insult to prevent neurovascular impairment. Also, viral delivery of *PlGF* (Gaal et al., 2013) and *hbFGF* (Watanabe et al., 2004), after MCAO, has been performed with AAV and AV, respectively. 2) Non-viral *Vegf* delivery into the cerebral cortex has been conducted by employing niosomes (Gallego et al., 2020), which enhanced angiogenesis by 55% in normal adult mouse brain, not only at the injection point but also in the vicinity. 3) Epigenetic regulation to achieve an angiogenic response has been developed employing lentiviral-mediated overexpression of miRs injected into the desired area in normal adult mouse brain, with miR-210 (Zeng L et al., 2014), in stroke animal models, with miR-126 (Qu et al., 2019) or miR-210 (Zeng LL et al., 2016), and in AD animal models, with miR-124 (Li et al., 2019a). 4) VEGF (Bible et al., 2012; Lee et al., 2007) and bFGF (Ikeda et al., 2005) secreting cells have been injected into brain parenchyma in stroke mouse models. 5) Injectable or hyaluronic acid hydrogels containing VEGF are usually placed directly within the stroke cavity 2 weeks after MCAO (Ju et al., 2014; Nih et al., 2016), although 3D cell matrix of PEG and PPS hydrogels containing iPS-NPCs have also been injected into the brain of naive mice promoting angiogenesis and stem cell survival without the addition of pro-angiogenic factors (Zhang J et al., 2011).

C. Intranasal Administration

Many agents and gene vectors have been administered by intranasal administration in a wide number of disease models, some of them related to CNS diseases (Aly and Waszczak, 2015). The intranasal region of administration is also a pivotal item to take into account, since it influences the drug absorption to the brain or to the systemic circulation, in which case would cause side effects in other organs, especially in the lungs. In this sense, the olfactory area, posterior and upper region, is the proper position for drug absorption to target the therapeutic factor to the brain (Dhuria et al., 2010). Focusing on revascularization strategies for brain CNS diseases, it has been observed that the pro-angiogenic VEGF rapidly enters the brain by this route. In a first-step, work comparing intranasal versus intravenous administration of human recombinant VEGF-165 labeled with a radioisotope, it was found that within 30 minutes post-administration, significantly more VEGF was localized in many cerebral sites, including the cerebral cortex, following intranasal administration (Yang et al., 2009b). Although less VEGF was accumulated in peripheral tissues in the nose-brain pathway compared with the systemic pathway, the lungs accumulated the higher levels of the growth factor. In this regard, VEGF overexpression in

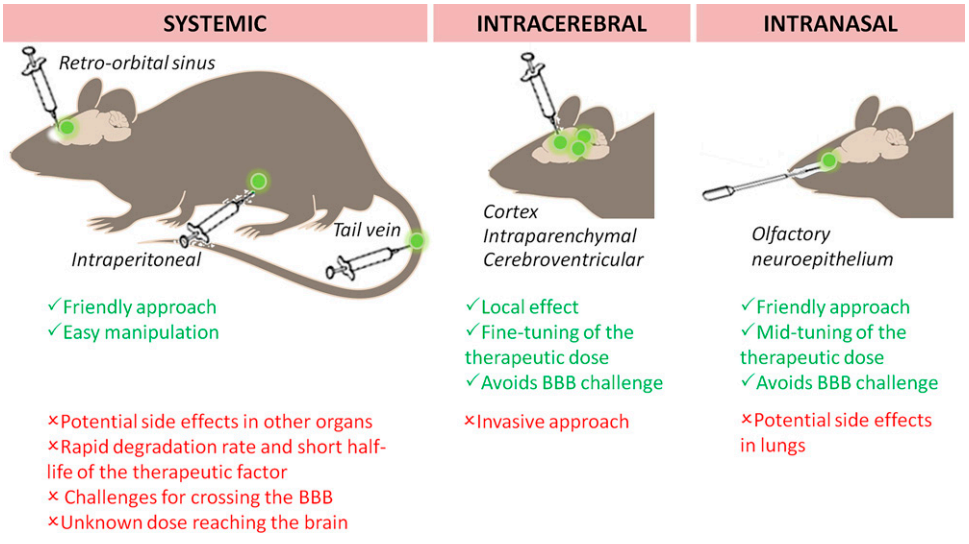

Fig. 6. Features of the potential routes used for the administration of angiogenic factors to brain. The goal of such delivery strategies is to promote angiogenesis, neurovascular network enhancement, and cognitive/behavioral improvement in brain central nervous system diseases, by surpassing the blood-brain-barrier and other biologic barriers. The main routes employed in animal models for therapies focused on brain central nervous system diseases are the systemic intravenous delivery and the intracerebral administration. However, it is now known that the olfactory neuroepithelium provides a non-invasive route of entry into central nervous system, bypassing the blood-brain-barrier.

the lungs can occur following intranasal delivery, which might increase pulmonary vascular permeability and cause pulmonary edema, as observed in mice after administration of an adenoviral vector expressing VEGF-165 through the respiratory tract (Kaner et al., 2000). This fact evidences once more the importance of dosage for achieving therapeutic effects in the target tissue without provoking side effects either in the brain or in peripheral organs. Addressing dose tuning, intranasal therapeutic VEGF dosage has been assessed in a rat model of ischemic stroke, 3 days after MCAO (Yang et al., 2009a) (Table 2). Although in this work authors did not evaluate VEGF accumulation and potential side effects in peripheral organs, with especial significance for lungs, they found an intranasal dosage-dependent VEGF effectiveness on reducing infarct volume and neurologic function, enhancing angiogenesis and improving behavioral recovery.

An interesting approach for intranasal delivery of VEGF to avoid direct contact and potential side effects of the factor on its way to brain, would be nanocarrier-mediated drug delivery or non-viral gene therapy. In particular, non-viral RVG29-PEG-PLGA nanoparticles loaded with miR-124 have been administered intranasally, reducing the ischemic brain injury (Hao et al., 2020) (Table 2).

VI. Conclusions and Future Directions

Although CNS diseases, and in particular the neurodegenerative ones, are an increasing reality in our society, there are still no curative pharmacological treatments able to achieve a complete neurovascular recovery. It is becoming increasingly evident that brain microvasculature impairment and deficiency is a key feature, not only in the development of neurodegenerative diseases, but also in aging. Until a few years ago, scientific research sought therapies focused on neurologic recovery. However, it is now known that vessel integrity is crucial for a correct functioning of the neurovascular unit, promoting neuron survival and preserving cognitive capacities. Thus, new therapeutic approaches must be reconsidered to improve the impaired microvasculature rather than target the affected neurons.

In this review, many brain revascularization strategies have been discussed, showing the great efforts made by the scientific community in this field. Although the majority of brain revascularization approaches are still in the experimental phase, expectations to a translational clinical application is in the process of becoming a reality. For this purpose, several nanotechnology platforms based on nanocarriers, gene therapy, cell therapy and biomaterials, among others, have been developed and have been discussed in detail in the present work.

Currently, some of these approaches are limited to intracerebral administration due to the nature of the vector and/or to avoid potential undesired side-effects in other organs. Although it is possible to reach the brain non-invasively in experimental animals, future translation to clinical practice would require the selective action of the therapeutic factor in the brain to avoid its distribution to other tissues. Taking into account the existence of the selective BBB, strategies to overcome this barrier, to deliver the therapeutic cargo by non-invasive routes of administration, are being developed. Actually, pre-clinically developed non-invasive strategies for brain revascularization require multiple administration doses at high concentrations, augmenting the non-specific systemic absorption and potential damage of other organs. In this sense, the nose-to-brain route of administration seems to be a promising strategy for regular clinical practice, since it represents a less invasive approach able to reach the brain with more precision than systemic administration. In this regard, to minimize the mucociliary clearance of the therapeutic factor in the intranasal vestibular region, optimized delivery devices are being developed. These systems include specially designed intranasal devices, mucoadhesive systems and surface-engineered nanocarriers, among others (Agrawal et al., 2018; Wang Z et al., 2019). Hence, the rise of device manufacturers will enable the benefits of the nose-to-brain delivery route to become translated into approved products for clinical practice. In addition to the development of nanotechnology platforms to recover brain microvasculature and cognitive function, powerful and sensitive imaging system platforms should be implemented. In this regard, cutting-edge techniques are being developed to visualize and analyze the entire brain vascular network to discriminate in detail the decline or increase after a therapeutic angiogenic treatment of microvasculature in the diseases discussed in this review. At the preclinical level, this is made possible by the combination of tissue-clearing methods to render the brain completely transparent (Silvestri et al., 2016) with advanced microscopic techniques, such as micro-optical sectioning tomography, which enables whole-brain imaging at the submicron level (Li et al., 2010b), or light-sheet fluorescence microscopy, employing a fluorescent blood vessel lumen staining for whole-brain vasculature reconstruction at the capillary level (Di Giovanna et al., 2018). At the clinical level, such a detailed vessel analysis it is still not possible; instead, current imaging systems are able to analyze cerebral blood flow. However, it constitutes a promising field in combination with potential pro-angiogenic therapies.

Acknowledgments

The authors wish to thank the ICTS "NANBIOSIS" and the Drug Formulation Unit (U10) of the CIBER in Bioengineering,

Biomaterials, and Nanomedicine (CIBER-BBN) and Charlotte A. White for the English revision.

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