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REVIEW ARTICLE

Implications of age-related changes in the blood-brain barrier for ischemic stroke and new treatment strategies

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Abstract

Ischemic strokes are prevalent across all age groups. Recent research has highlighted the importance of better understanding ischemia-induced damage of the blood-brain barrier (BBB) because it is related to both the severity of ischemic injury and neurological outcomes. The influence of advancing age on the structure and function of the BBB and the potential influence of these changes on ischemic stroke injury have received little consideration to date. Therefore, the present review outlines how ischemic injury influences the structure and function of the BBB at the anatomical, cellular, and molecular levels, and how these changes differ between adult and elderly populations with and without age-related comorbid diseases. This review further discusses how age-dependent changes and features of the BBB, and the corresponding alterations in response to ischemia, can affect the efficacy and delivery of current and future treatment options. Current research efforts are underway to develop prospective stroke treatment strategies that target the restoration of BBB functionality. This review also discusses the importance of considering the unique properties and characteristics of the BBB in elderly individuals for developing new stroke treatment strategies.

Keywords: Ischemic stroke; Blood-brain barrier; Inflammation; Drug delivery; Diabetes; Alzheimer's disease

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1. Introduction

Ischemic strokes can occur at any point in life, independent of age. Nevertheless, the blood-brain barrier (BBB) changes with age[1], and changes in BBB integrity and functionality may significantly affect the brain. With a growing line of evidence focusing on the effect of BBB integrity on ischemic injury and recovery[2-4], it is of increasing importance that age-dependent characteristics of the BBB be considered. In a healthy state, the BBB acts as a physical and enzymatic barrier that protects the neuronal environment from blood-borne toxins, inflammation, and potential fluctuations in ion and water homeostasis to maintain neuronal health and activity[5]. This is accomplished by a single-cell layer of specialized endothelial cells connected together through tight junctional complexes made of occludin, zona occludens (ZO), and claudin proteins. This microvascular barrier of the brain limits paracellular and transcellular movement from the blood into the brain[5].

Shortly after an ischemic stroke, the BBB undergoes significant damage[6]. As a result of ischemic injury to neural tissue, oxidative stressors accumulate and induce rapid degradation and functional breakdown of tight junctions, thereby opening the BBB. This enables inflammatory chemokines to enter the brain and cause disruptions in both ion and water homeostasis[7]. These localized actions on the BBB cause further immune cell activation and infiltration in response to ischemic injury, leading to the release of inflammatory mediators and other molecules that further degrade endothelial tight junction complexes[7]. The loss of BBB integrity leaves the brain susceptible to greater inflammation and edema, which may worsen ischemic injury and contribute to neuronal loss far beyond acute injury[3,7]. Interestingly, due to variations in cellular and molecular mechanisms, the BBB in elderly patients undergoes far more extensive damage following ischemic injury than that observed in the younger adult brain; that is, the BBB additionally deteriorates only in patients with age-associated health conditions[8,9]. Exacerbated degradation of the BBB leaves brains in elderly patients vulnerable to greater ischemic injury and poorer prognosis.

The current review outlines the cellular and molecular mechanisms of BBB damage following stroke with an emphasis on how age influences the nature of these degradative processes. Furthermore, the BBB influences the delivery and efficacy of ischemic stroke treatment. Thus, the influence of age-dependent BBB degradative mechanisms on treatments and how the BBB itself may be a key therapeutic target to restore or maintain BBB function and integrity in the elderly population are discussed.

2. Age and ischemic injury

Ischemic stroke, although much more commonly studied in young-adult-to-middle-aged animal models and human populations, is prevalent across all age groups. The incidence of stroke in the elderly population is higher than in the young-adult population, and the presentation and clinical outcomes of stroke are highly age-dependent. Importantly, aging populations are vulnerable to ischemic injury and may receive significantly worse prognoses.

2.1. Common etiologies and risk factors

The incidence of stroke increases with age, and thus younger adults are generally at a lower relative risk. It is estimated that 10 – 20% of ischemic events occur in those aged 18 – 50 years[10]. In addition, compared with other age groups, young-to-middle-aged adults differ quite significantly in both the etiology and presentation of ischemic stroke. For instance, compared with elderly patients, young-adult patients presenting with ischemic stroke are more likely to be male and experience a stroke resulting from cervical vessel dissection[11-13]. Furthermore, clinical data demonstrate that in comparison to elderly patients over the age of 80 years, younger adults are more likely to present with hyperlipidemia and obesity and have a history of smoking, drinking, or heavy drug use, all of which are considered to act as major risk factors for stroke in this age group[11,12,14,15]. Aside from the aforementioned hyperlipidemia and obesity, comorbid disease is much less common in younger ischemic patients[11,12,15].

Ischemic stroke is much more prevalent in the elderly population than in young or middle-aged adults. Specifically, 80% of ischemic stroke occurs in the elderly (over the age of 65 years), with 25% of these cases attributed to those over the age of 80 years[16]. Furthermore, these cases occur in a larger proportion of female patients, in contrast to what is observed in younger patients[12,14]. Older patients (over the age of 50 years) tend to have more physiologically based risk factors for ischemic stroke, including numerous vascular-related comorbidities that can influence the basal structure and function of the BBB. Older patients more frequently report hypertension, cardiac failure, coronary heart disease, cardiac arrhythmia, and atrial fibrillation in addition to high cholesterol[12,14].

2.2. Prognosis

Unlike elderly populations, young adults have a relatively good prognosis following ischemic injury. Young ischemic patients aged ≤50 years had a 100% survival rate during 1-, 3-, and 6-month follow-ups, and this survival rate only decreased to 96.8% by the 3-year follow-up[11]. Moreover, recurrent stroke only occurs in 2% of those under the age of 50 years[11]. With increasing age, the incidence of ischemic stroke increases and clinical outcomes worsen. Ischemic tissue surrounding the infarct following injury is much more likely to convert into an infarction as age increases in both men and women[17,18]. In comparison to younger patients, those aged >50 years exhibit significantly lower post-stroke cognitive function and higher rates of disability and social impairments[12], which may be attributed to more severe injury. Middle cerebral artery occlusion (MCAO) animal models using 18-month-old Sprague-Dawley rats (roughly equivalent to humans over the age of 45 years)[19] exhibited infarctions that were up to twofold greater than those observed in 3-month-old rats (roughly equivalent to young adults)[19], following stroke[9]. In addition, unlike younger rats, and aged rats experienced more severe behavioral deficits and did not significantly recover after a 2-week period[9]. A brief overview of the differing etiologies and prognoses of adult and elderly ischemic stroke patients is illustrated in Figure 1.

Age-related comorbidities may further worsen prognoses in elderly patients. Diabetes and Alzheimer's

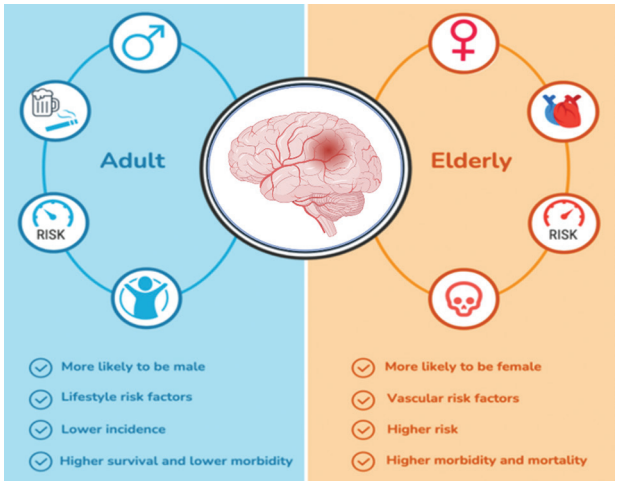

Figure 1. Risk factors and prognoses following stroke in adult and elderly patients. A brief comparison of the commonly reported risk factors of ischemic stroke in adult and elderly patients and later prognosis and survival. The illustration was created with BioRender.com.

disease (AD) significantly worsen the clinical outcome of stroke in both humans and animal models[20,21]. The incidence of stroke increases in AD patients[22], along with the increased risk of a worse ischemic outcome. Dementia patients experience a two- to three-fold increased risk of death following ischemic stroke compared with non-dementia patients, with the risk of death increasing with the severity of dementia[23]. Mouse models of AD further demonstrate a significant increase in ischemic damage compared with non-AD controls, with amyloid precursor protein overexpression resulting in significantly larger cerebral infarcts and greater reductions in cerebral blood flow[20,24]. Diabetic mouse models also demonstrate greater ischemic injury and the development of more severe neurological deficits post-stroke[21,25], potentially linked to greater inflammatory responses[21,26]. Overall, the observed vulnerability of the aged brain is due to a variety of age-related physiological factors that may contribute to a weak BBB, including inflammatory mechanisms and a naturally aging neurovascular unit.

3. BBB injury following ischemic stroke

Opening of the BBB after ischemia is observed in all age groups but may vary in timing and degree of opening, which is largely thought to correlate with the degree of injury severity and prognosis. Elevations in BBB permeability following injury leave the brain susceptible to blood-borne toxins, greater immune cell infiltration, inflammatory mediators, and water influx, which can further worsen neuronal damage and brain edema. Several key cellular and molecular events correspond to BBB injury following stroke, and these vary in degree and form between adult and elderly patients (Figure 2). Maintaining BBB integrity clearly may diminish neuronal damage and thus lead to better clinical outcomes after ischemic stroke. Therefore, understanding the characteristics and underlying mechanisms of BBB injury in different age populations is crucial.

3.1. BBB structure and function following ischemic stroke

Adults experience significant opening of the BBB shortly after ischemic stroke, which contributes to ischemic injury. Despite previous assumptions of a biphasic opening of the BBB following ischemia[27], more recent data support a continuous opening of the BBB that can last few days to few weeks post-injury. For instance, adult Wistar rats that underwent MCAO experienced continuous BBB opening with the use of small and large tracers that were observable beginning 25 min post-injury and lasting up to 4 weeks[28]. Similarly, in humans, a continuously disrupted BBB was observed in 33% of adult patients in the first week following stroke[2], which began around 12.9 h post-ischemia[2] and was reported to last up to 90.7 h[29]. Notably, early BBB disruption is associated with poorer clinical outcomes that are significantly correlated to hemorrhagic transformation, stroke severity, and long-term neurological scores assessed up to 90 days post-injury[2-4]. Thus, any age-related variations in the timing and degree of BBB injury following stroke may considerably affect both acute and long-term outcomes.

Elderly patients are more susceptible to ischemia-induced BBB damage than younger adult patients; this is also worsened by comorbid conditions prevalent in elderly patients. Compared with a younger BBB, an aged BBB exhibits numerous qualities that make it more susceptible to ischemic injury and result in a more severe prognosis. This includes a large age-dependent reduction in barrier function, which is observed in both aged animal models[9,30] and humans[1]. Consequently, the aged BBB undergoes earlier and more severe disruption after injury, which precedes a large portion of neuronal damage[9,31]. In fact, aged rats experience up to a two-fold greater increase in BBB permeability after ischemia compared with younger adults[9]. Diabetes further exacerbates ischemic BBB opening, contributing to edema and poorer clinical outcomes[32]. Diabetes significantly affects basal BBB integrity, as evident in diabetic mouse models exhibiting a 2.4-fold greater BBB permeability than non-diabetic controls[33]. This translates to significantly greater BBB leakage after ischemic stroke[34]. Similarly, the elevated susceptibility to ischemic damage associated with AD is thought to be a result of vascular dysfunction, which is significantly associated with BBB disruption. Cerebral amyloid angiopathy (CAA), which is observed universally

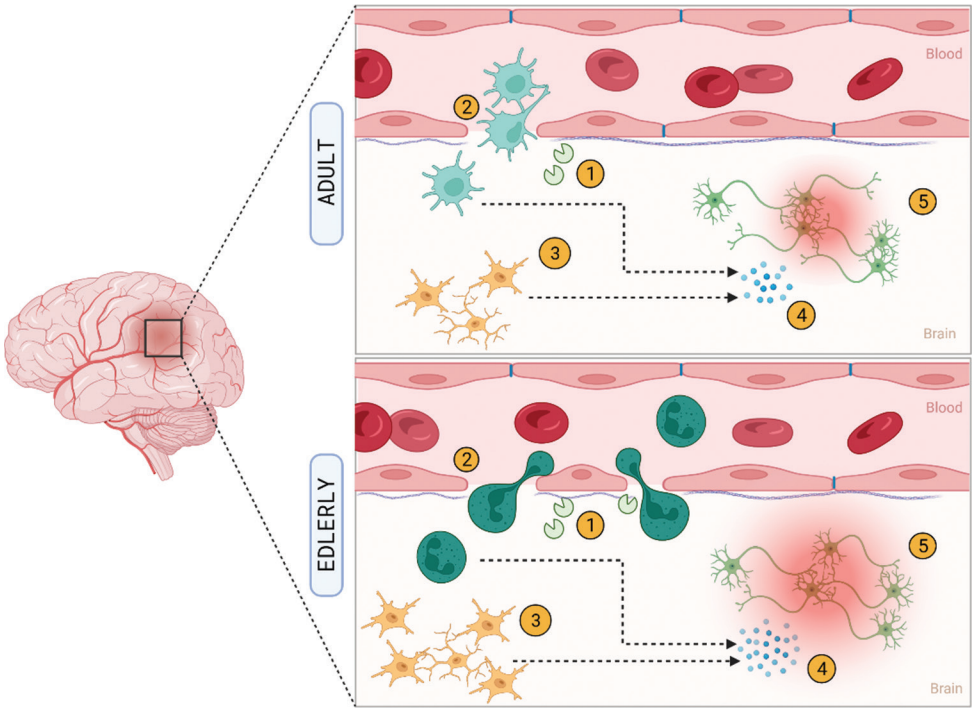

Figure 2. An overview of the cellular and molecular events contributing to BBB damage and consequential neuronal injury in ischemic adult (top) and elderly (bottom) stroke patients. Compared with ischemic stroke in the adult brain, in elderly patients, it is accompanied by greater tight junction loss and increased BBB permeability due to amplified matrix metalloproteinase (MMP) enzymatic activity (1). This leads to greater immune cell infiltration (2), which is characterized by a more neutrophilic response in the elderly brain compared with a monocytic response in the adult brain. Infiltrating immune cells coalesce with activated resident microglial cells (3), which are activated to a greater extent in the elderly brain, producing a myriad of reactive oxygen species (ROS) and inflammatory cytokines (4). This ultimately contributes to neuronal injury (5), which is amplified in the elderly compared with the adult brain. The illustration was created with BioRender.com.

in AD patients and progresses with age[35], has been suggested to be the driver of the observed heightened vulnerability to injury and larger infarct volumes. CAA exerts profoundly deleterious effects on cerebrovascular function and is associated with significantly greater elevations in BBB permeability, up to 1.5-fold greater than that observed in the absence of CAA[35-37]. Taking the above into consideration, the elderly BBB can be inferred to be susceptible to greater ischemic damage both under physiological and pathological conditions.

3.2. Cellular mechanisms of BBB degradation

The large-scale changes to BBB function in response to ischemia are attributed to a variety of cellular transformations within the BBB and its supporting cells, including endothelial cell degeneration and immune cell activation and infiltration. Notably, these cellular mechanisms of BBB degradation vary with age.

In general, oxidative stress within the brain due to acute ischemic events leads to damaged endothelial cells and loss of tight junctions. This reduction in barrier integrity increases paracellular transport of water and disrupts ion homeostasis, contributing to cerebral edema. In comparison, aging is associated with significantly enhanced endothelial degradation and tight junction disruption in the BBB after ischemic stroke. Aging is naturally associated with a loss of tight junction proteins in the BBB, with older subjects expressing significantly less occludin-1 and ZO-1 compared with those of younger age[30]. This phenomenon is only worsened by ischemic events beyond that observed in younger patients. Older mice experience a significantly greater loss of tight junction proteins compared with younger adults following ischemia, which is apparent up to 3 days post-injury[38]. Moreover, those with additional health conditions can experience an even more dramatic ischemia-induced loss of tight junctions. High cholesterol induces greater reductions in tight junction protein expression, specifically occludin-1, after ischemic injury[39]. Diabetes is also associated with a 1.3-fold greater reduction in occludin, ZO-1, and claudin-5 expression following stroke[32-34]. Furthermore, AD-associated CAA is linked to endothelial cell degradation[36,37] and subsequent reductions in tight junction protein expression within the BBB. Compared with that in controls, the expression of claudin-1 and -5 in CAA patients is reduced by 84% and 43%, respectively; this is compounded by the already

greater loss of tight junctions following ischemia in the aging population[37]. Therefore, endothelial barrier degradation following ischemia clearly varies with age and appears to occur to a greater extent in the elderly and to an even greater extent in the presence of pre-existing conditions.

Ischemia in adults also activates resident microglia immune cells and increases the degree of infiltrating leukocytes, including macrophages, which contribute to greater destructive inflammatory responses in the brain, including BBB degradation and resulting hemorrhagic transformation[40]. While aging is similarly associated with an elevated immune response after ischemia that further disrupts BBB integrity and in turn worsens clinical outcome[9], the cellular profile of this inflammatory response is quite distinct from the young adult response. In mice subjected to MCAO injury, older mice express more $CD68^+$ cells in the peri-infarct region up to 14 days post-ischemia than younger mice, indicating greater macrophage and microglia activation[38]. The disproportionately large microglial response is thought to be a key contributing factor to exacerbated ischemic BBB injury in aged models[41]. In addition to an intensified resident immune response, aged mice demonstrate a strong neutrophilic response to ischemia that appears to be one of the more differentiating features of ischemic events in this age group, as younger adults are subject to a stronger monocytic response[41]. The neutrophilic response to ischemic stroke in the aging population correlates with more severe ischemic injury outcomes and hemorrhagic transformation, according to both animal and human data[41]. In addition, recent evidence suggests that a disproportionately elevated level of neutrophils correlates with greater cognitive impairment post-stroke, affecting both memory and visuospatial abilities up to 3 months post-injury[42]. Subjects with diabetes also experience this strong neutrophilic reaction, which may be compounded by age, demonstrating earlier, and larger neutrophil infiltration in cortical and subcortical regions of the ipsilateral hemisphere 4 and 24 h post-stroke, which is strongly associated with greater infarct volume[34,43]. Although it is yet to be elucidated, AD patients may also be at a further disadvantage because of their natural predisposition for greater neutrophil reactivity[44]. However, the role this plays in ischemia and the consequences on the BBB remain to be explored.

3.3. Molecular mechanisms of BBB damage

Activation of the cellular mechanisms of BBB degradation is accompanied by the release of numerous molecular factors that can disassemble tight junction complexes and increase BBB permeability. These molecular mechanisms contribute to the age-dependent changes within the BBB that influence the degree of ischemic insult and clinical outcome. The key molecular players in stroke-induced BBB degradation are MMPs, inflammatory cytokines, and ROS. Comparable to cellular changes, the molecular mechanisms described below differ in their characteristics and the degree to which they contribute to BBB degradation after stroke.

As reviewed by Lakhan *et al.*, MMPs, specifically MMP-2 and MMP-9 secreted by resident and infiltrating inflammatory cells, are largely thought to regulate BBB integrity after stroke by degrading tight junction and basal lamina proteins[45]. MMP-induced BBB degradation contributes to further leukocyte infiltration, edema, and hemorrhage. MMP-2 and MMP-9 were both elevated in adult ischemic stroke patients on admission[46]. MMP elevation is correlated with both injury severity and prognosis[46,47]. MMP-9 expression in response to stroke is two-fold greater in elderly patients than that in younger adults[41]. MMP-2 further contributes to ischemia-induced BBB breakdown, and the activities of both MMP-9 and MMP-2 were aggravated in aged-related comorbidities. Patients with diabetes exhibit significantly greater and earlier MMP-2 activity and a seven-fold increase in MMP-9 activity after stroke, as compared with those without diabetes[33,34,48]. The observed elevation in MMP-9 activity after stroke in the context of diabetes is thought to be the result of both a greater neutrophilic response, which is already elevated in older patients, and reductions in MMP-9 inhibition[34,43]. In addition, AD-associated CAA was also associated with significant elevations in MMP-2 and MMP-9 expression, with a significant 2.4-fold increase in MMP-9 activity compared with controls[37]. Therefore, while MMPs play a role in both adult and elderly BBB degradation, the timing and degree to which they do so vary across age groups with and without additional medical conditions.

Certain pro-inflammatory cytokines, namely, tumor necrosis factor alpha (TNF-α), interleukin (IL)-6, and IL-1b, are thought to contribute to ischemic injury by degrading the BBB and are associated with poorer clinical outcomes[49-51]. After stroke, microglial, endothelial, and infiltrating immune cells release such cytokines, which wreak havoc on the integrity of the BBB, beginning hours after injury and lasting days post-stroke[50,51]. Patients with higher TNF-α and IL-6 levels had significantly lower neurological scores, both acutely and 30 days after stroke[49], which correlated to significantly elevated BBB permeability through reductions in claudin-5, occludin, and ZO-1 expression as a consequence of these inflammatory cytokines[52]. Aging is associated with elevated inflammatory responses to ischemia, correlating

with severe BBB disruption and subsequent injury. Aging is naturally associated with elevated inflammatory mediators, such as IL-6 and TNF-α, resulting in a natural leakiness of the BBB via the loss of tight junctions[30]. This is more exacerbated during ischemic events in the elderly compared to younger adults. Compared with 2-month-old adult mice, aged (12-month-old) mice that started to experience senescence[53] had significantly higher IL-1b and IL-6 expression after ischemia, correlating to more severe and longer-lasting behavioral deficits and infarction due to greater BBB leakage[38]. Thus, similar to MMPs, inflammatory cytokines play a greater role in vulnerable elderly brains (with greater damage inflicted on the BBB post-stroke) than in the brains of younger adults.

Finally, oxidative stress caused by ROS and other free radicals released by reactive and infiltrating immune cells plays an important role in ischemic injury progression. It contributes to the loss of tight junctions and promotes MMP-9 degradation of the BBB, ultimately leading to a loss of barrier integrity[50]. Microglia and neutrophils have been shown to produce more ROS in response to ischemia in elderly compared to younger adult brains[41]. This may be further observed under age-associated comorbid conditions, such as hyperglycemia, which is associated with greater oxidative stress, superoxide production, BBB degradation, and increased MMP-9 activity after stroke[48].

4. The BBB in ischemic treatment and the influence of age

Not only can the BBB influence the timing of administration and effectiveness of ischemic stroke treatments and strategies but also it is itself considered to be an increasingly attractive target for future therapeutic development. As outlined throughout the current review, the degree of ischemic injury and later recovery depend on the degradation of the BBB. Thus, maintaining the BBB after stroke may be the key for achieving better long-term clinical outcomes. However, due to the above-mentioned age-dependent mechanisms of BBB degradation after stroke, considering these cellular and molecular variations is crucial for developing new potential therapeutics.

4.1. Drug delivery and efficacy

The time frame for drug delivery and treatment efficacy are largely dictated by post-ischemia BBB dynamics and age-specific BBB characteristics and comorbidities. At present, tissue plasminogen activator (tPA) is the only therapy for ischemic stroke in the mature brain approved by the U.S. Food and Drug Administration. However, tPA has a short time frame of delivery of <3 h, largely due to the increased risk of hemorrhagic transformation because of delayed tPA-induced BBB degradation. This is especially relevant in older patients who are already at a greater risk of hemorrhage after treatment[31]. Along these lines, the elderly BBB undergoes greater damage following tPA treatment, which is correlated to the greater likelihood of hematoma formation compared with the young brain[31] and can limit the potential benefits of tPA treatment. tPA-induced damage to the BBB and the corresponding risk of hematoma and death are suspected to be linked to the neutrophilic response that the elderly brain experiences during ischemia[41,54]. The elderly neutrophilic response is associated with increased MMP-9, which is a predictor of hemorrhagic transformation and death following tPA administration[55]. Furthermore, the efficacy of tPA treatment may be reduced in elderly patients with comorbid health conditions. For example, diabetes significantly reduces tPA efficacy in infarction reduction, which is associated with increased hemispheric swelling and hemorrhage due to greater BBB degradation[56], conceivably through even greater microglial proliferation and increased MMP-9 activity.

Appreciating the different timelines of BBB disruption following ischemic injury is critical for the development and administration of new potential neuroprotective drugs in both adult and elderly populations[57]. Most research is conducted using adult rodent models, even though, as discussed, BBB opening can occur to a greater extent and at a much earlier time in elderly patients compared with young and middle-aged adults. Thus, unlike with younger patients, for elderly patients (with a higher level of BBB permeability), it may be useful to develop potential neuroprotectants with a longer time frame for delivery and larger composition. To this end, understanding the timing, extent, and mechanisms of BBB degradation in vulnerable patients with and without comorbid conditions could facilitate the development of new and more effective treatment options that take advantage of the timelines of BBB permeability in different age groups.

4.2. Targeting the BBB in ischemic stroke treatment

BBB degradation is associated with more severe ischemic injury, subsequent edema, and hemorrhage, and therefore protecting the BBB from further damage following ischemia has been a goal to improve clinical prognosis for all age groups[58,59]. Such treatments are largely suggested to target the inflammatory cytokine and MMP proteolytic mechanisms underlying BBB degradation, as discussed in the current review. In addition, targeting the BBB may allow effective treatment to be delivered during a more clinically relevant time frame and may widen the time frame for delivery of current therapeutics[60,61]. However, the above-mentioned age-specific characteristics of the BBB under ischemic conditions must be taken into consideration.

As discussed, older patients experience significantly increased MMP-9 activity following ischemic stroke compared with younger patients. Because MMP-9 activity plays a crucial role in BBB degradation (which leads to poorer clinical outcomes), it is pertinent that future therapies aim to reduce this proteolytic activity. The mechanisms of MMP-9 inhibition in the context of ischemic stroke treatment are reviewed by Chaturvedi and Kaczmarek[59]. Altogether, MMP-9 inhibition seems to be a viable target for adult and especially elderly stroke patients, given their comparably robust MMP-9 response to ischemia. However, MMP-9 has also been linked to critical angiogenic mechanisms[62] and neurological recovery in the late stages of stroke[63]. Thus, targeting MMP-9 activity would require a highly controlled mechanism and time frame of inhibitory action because a long-term therapeutic strategy to reduce MMP-9 activity may interfere with the beneficial actions and effects of this enzyme during the later recovery stages after stroke.

Inhibition of inflammatory processes successfully mitigates injury severity in older patients. Older stroke patients are subject to greater inflammatory BBB damage after stroke, and thus drugs that restrict stroke-induced inflammation seem to be a viable avenue to use in this cohort of patients. Inhibiting inflammation in the aged brain significantly diminishes stroke volume and improves neurological function by reducing TNF-α- and MMP-9-mediated BBB degradation and corresponding inhibition of neutrophil infiltration[64]. Because the aging population experiences a strong neutrophilic response to ischemia, which largely contributes to BBB degradation and poor clinical outcomes, a drug that could inhibit neutrophil infiltration and reduce systemic neutrophil levels[42] would be ideal for improving acute and chronic stroke outcomes in aging patients.

In fact, the discrepancy in the cellular profile of the immune response after ischemia between adult and elderly brains may provide key insight into the optimal treatment of post-stroke patients by taking into consideration age-dependent disease characteristics. The strong neutrophilic response in elderly stroke patients, as reviewed, contributes to both greater ischemic injury in terms of infarct volumes as well as secondary injury due to BBB damage, and has further been linked to worsened cognitive impairment months after injury[42,65]. Interestingly, this inflammatory event may be one of the easier targets to address, as it begins systemically rather than beyond the BBB. Furthermore, extensive knowledge has accumulated regarding the subsets of neutrophils that respond to injury, essentially classifying the helpful from the harmful based on extracellular proteins[65]. Using this knowledge, efforts should be made to identify clinically relevant methods that may disarm the damaging response of the hyperinflammatory neutrophils in systemic circulation before entry into the brain. This would reduce MMP, ROS, and inflammatory cytokine loads, while still allowing anti-inflammatory subsets to permeate the BBB. Thus, leveraging the heterogeneity of the nucleophilic response and avoiding global suppression of critical immune function may effectively support ischemic injury recovery and suppress inflammatory destruction of the BBB.

Additional therapeutic avenues that maintain BBB integrity and can be delivered in conjunction with current treatment options to improve clinical outcomes are also under investigation. Adjuvant therapeutics in conjunction with tPA treatment that stabilize the BBB not only reduce injury severity and risk of hemorrhagic transformation but also increase the time frame for treatment in elderly patients. Delivery of tPA outside the recommended 3-h time frame after initial injury may increase the likelihood of hemorrhagic transformation because of tPA-induced MMP-9 activation[55]. Because, as previously discussed, elderly patients are at a greater risk of hemorrhagic transformation after tPA treatment, providing an MMP-9 inhibitor in conjunction with tPA may improve clinical outcomes in this population. Treatment with minocycline (an MMP inhibitor) in combination with tPA significantly reduces 24-h infarct size and ameliorates hemorrhagic transformation, allowing tPA treatment to successfully delay ensuing ischemic injury as a direct consequence of MMP-9 inhibition[66]. However, how MMP-9 inhibition affects later stages of recovery and angiogenic events still needs to be elucidated.

Neural stem cell transplantation in conjunction with tPA treatment has also been shown to reduce infarct volume in the aged brain after stroke through reduction of pro-inflammatory cytokines (e.g., TNF-α and IL-6) and MMP-9, thereby reducing BBB damage and maintaining tight junctions, while allowing a longer time frame of successful tPA treatment (6 h)[67]. Stem cell administration in animal models of ischemic stroke has further been associated with improved neurological function as a result of reductions in post-stroke ischemic injury[68,69].

Neutrophil activity inhibits the efficacy of tPA treatment through tPA-induced formation of neutrophil extracellular traps, which largely contributes to an increased risk of hemorrhage[70]. Thus, efforts should be made to identify the means to selectively inhibit inflammatory neutrophil activity in conjunction with tPA treatment. Knecht *et al.* further outline specific pharmacological and non-drug interventions that target molecules contributing to BBB degradation in ischemic stroke; these may represent potential adjuvant therapies to tPA, increase the time frame

of delivery, and improve clinical outcomes[71]. Accordingly, elderly ischemic stroke patients would greatly benefit from therapeutics that reduce the inflammatory degradation of the BBB and boost the efficacy of current treatment options (such as tPA) by supporting BBB integrity.

5. Conclusions

Ischemic stroke is prevalent in all age groups. Ischemic stroke contributes to severe BBB degradation, which is associated with worse prognoses, edema, and hemorrhagic transformation. Age not only influences the features of ischemic stroke but also how the BBB is affected after injury. This can be explained by age-specific cellular and molecular mechanisms of BBB degradation, such as increases in the loss of tight junctions, immune cell infiltration, neutrophilic responses, MMP-9 activity, release of inflammatory cytokines, and oxidative stress. Elderly populations are at a greater risk of more substantial BBB damage after ischemic injury due to ischemia-induced cellular and molecular mechanisms of BBB degradation that occur to a greater extent than that observed in younger adults. Such degradation is exacerbated by age-related comorbid health conditions, such as diabetes and AD. As such, with the BBB mediating the delivery and efficacy of ischemic stroke treatment, new stroke therapies that may be more beneficial to vulnerable patient populations, with special emphasis on selectively inhibiting the neutrophilic response, are required. Current research is highlighting the potential of BBB maintenance following ischemic stroke as a therapeutic avenue. Therefore, age-dependent post-stroke BBB characteristics should be taken into consideration to better improve current and future ischemic stroke therapeutics.

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Conflict of interest

The authors declare no conflict of interests.

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