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Breaking barriers: the role of NETosis in blood-brain barrier leakage and age-related cognitive decline

Divya Sharma, Rahul Kumar*

Department of Life Sciences, GITAM School of Sciences, GITAM (Deemed to be) University, Visakhapatnam 530045, Andhra Pradesh, India

***Correspondence:** Rahul Kumar, Department of Life Sciences, GITAM School of Sciences, GITAM (Deemed to be) University, Visakhapatnam 530045, Andhra Pradesh, India. rkumar2@gitam.edu

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Abstract

Cognitive impairment is a common age-related comorbidity with blood-brain barrier (BBB) leakage a key event. BBB leakage increases with age, but the mechanisms are still not completely understood. In the current article, we briefly discussed the role of neutrophil extracellular traps (NETs) in age-associated increase in cognitive impairment. NETosis is a process neutrophils release web-like structures called NETs composed of DNA, histones, and antimicrobial proteins. These NETs act as physical barriers to trap and kill pathogens, such as bacteria, viruses, and fungi. Excessive NETs formation has been associated with various pathological conditions such as thrombosis, cancer metastasis, inflammatory diseases, and autoimmune disorders. Recent studies further indicated that NETosis plays a key role in the BBB leakage during stroke and depletion of neutrophils can attenuate the pathology of Alzheimer's disease (AD) in murine models. In the current article, we briefly discussed the putative role of NETosis in BBB leakage and age-related cognitive impairment. It should briefly summarize the main content of the article, and it may include the background, purpose, significance, methods and conclusions of the article.

Keywords

NETosis, Alzheimer's disease, blood-brain barrier, aging, cognitive impairment, neutrophils

Introduction

An increase in the lifespan due to advancement in the healthcare system has resulted in an increase in the incidence of age associated comorbidities including neurodegenerative, cardiovascular, and metabolic diseases. Cognitive impairment has emerged as a significant health issue particularly in the elderly as it is difficult to diagnose it at an early stage. A global meta analysis based on 85 research articles revealed that the prevalence of mild cognitive impairment (MCI) increases with age; 6.5% to 34% in 50–59 years old, 5.1% to 37.5% in 60–69 years old, and 11.6% to 41% in individual ≥ 70 years old [1]. A previous study reported that around 20–40% of MCI cases can progress to dementia [2]. In addition, the majority of

available therapeutic interventions provide symptomatic relief without targeting the underlying pathological mechanisms. The condition further poses an immense economic and emotional burden on the family members as it requires prolonged nursing care. Therefore, it is imperative to understand the key mechanisms involved during the early stage and arrest the progression to the advanced stage [3].

Blood-brain barrier

There are several vascular anomalies that may play important roles in the pathophysiology of cognitive impairment, including endothelial dysfunction [4] and blood-brain barrier (BBB) leakage [5, 6]. BBB refers to the unique property of brain vasculature by which it tightly regulates the movement of molecules and cells between the brain and blood [7]. The major component of the BBB is endothelial cells, smooth muscle cells, and pericytes. A high resistance paracellular barrier is established in the cerebral vascular with the help of a tight junction that holds together the endothelial cells. The structure of tight junctions is maintained with the help of homotypic interactions between proteins such as claudins, occludins, and zona occludins [8]. Mice deficient in these proteins display significant impairment in the BBB integrity and thus promote leakage across the barrier [9].

Assessment of BBB

In postmortem brain tissues, BBB leakage can be determined by measuring the extravasation of fibrinogen into brain parenchyma from the blood [10]. In addition, the ratio of albumin in cerebrospinal fluid (CSF) to the serum albumin called as albumin quotient (Qalb), can also be used to evaluate the integrity of BBB [11]. The Qalb ratio offers an advantage as it does not require postmortem tissue and can be performed in the patients by collecting blood and CSF. However, these methods are not completely reliable as inappropriate perfusion and tissue degradation during sample processing may affect the results. However, changes in the Qalb does not specifically indicates the specific region of the brain where BBB disruption took place. Montagne et al. [12] devised a high resolution magnetic resonance imaging (MRI) method to determine the BBB leakage by calculating blood-to-brain transfer constant of gadolinium (K_{trans}).

Changes in the BBB with age and cognitive impairment

In post-mortem brain tissues from patients suffering from Alzheimer's disease (AD), Hultman et al. [10] reported an increased BBB leakage. Previous reports suggest that Qalb increases in individuals having a higher risk of developing AD [13, 14]. Montagne et al. [12] reported a linear increase with age in K_{trans} in the CA1 and dentate gyrus region of hippocampus. The same report further suggested that K_{trans} was about 60% higher in individuals suffering from MCI when compared to healthy controls with normal cognitive scores.

BBB impairment can lead to cognitive impairment and neurodegeneration as per several in vivo and clinical studies and is recognized as a major pathological event occurring during the early stages of cognitive impairment [10, 12, 13, 14]. It has been reported that BBB function can be compromised with AD [15–17]. In addition, neurodegeneration and cognitive impairment associated with high fat and fructose diet (HFF) were shown to be proceeded by impairment in BBB [18]. Various alterations at the cellular and molecular are known to take place in the endothelial cells with age that can contribute towards age-associated neurodegeneration, neuroinflammation, and cognitive impairment. P Glycoprotein 1 (PgP1) along with LDL receptor-related protein-1 (LRP1) is responsible for the clearance of drug molecules and aggregation-prone amyloid-beta (Aβ) species, a known hallmark of AD, from the brain parenchyma [15, 17]. Interestingly, the expression of PgP1 is known to decline with murine [19] as well as human aging [16]. Collectively, these reports suggest that BBB breakdown is an early event during the aging process which precede cognitive impairment. Therefore, identification of pathways and interventions that can control the progression of BBB leakage will help reduce the burden of cognitive impairment in aged individuals by allowing us to arrest it in early stage. The molecular signaling behind this BBB impairment is still a mystery and understanding of involved pathway(s) will enable us to limit age-related cognitive impairment.

Neutrophils extracellular traps and their role in aging and cognitive impairment

Release of neutrophil extracellular traps (NETs) which was first discovered by Takei et al. [20] refers to the release of NETs and involves chromatin decondensation that occurs through peptidyl arginine deiminase 4 (PAD4)-dependent deimination of nuclear histone proteins [21]. NETs consists of various components such as myeloperoxidase (MPO), citrullinated histone H3 (H3Cit), elastase, and histones. Previous literature suggests an increase in the NETosis and thus establishes its potential involvement in age related cognitive impairment. For instance, previously Kumar et al. [3] and Jylhävä et al. [22] have reported an increase in the circulating markers of NETosis such as cell free DNA and H3Cit with age. In addition, severe infiltration of H3Cit-positive neutrophils was found in elderly patients suffering from severe vasculitis [23]. Moreover, cardiac and lung fibrosis occurring in aged mice was found to be associated with increased NETosis [24]. Specifically, aging is associated with an increase in the levels of reactive oxygen species (ROS) including hydrogen peroxide [25–30]. Furthermore, in vitro, the treatment of cultured neutrophils with hydrogen peroxide can induce NETosis [31]. In our previous study, we had shown that the mice overexpressing glutathione peroxidase 1 (Gpx1, enzyme responsible for converting hydrogen peroxide to water) exhibit lower levels of circulating markers of NEtosis such as cell free DNA [3].

Taken together, this evidence suggests that aging is associated with increased NETosis, however, the cause-effect relationship still needs to be confirmed.

Suttorp et al. [32] have shown neutrophil elastase (a component of NETs) can increase the permeability of cultured pulmonary endothelial cell monolayers. Furthermore, neutrophil depletion reduces BBB breakdown and increases neovascularization following stroke [33]. In addition, depletion of neutrophils using anti-Lymphocyte antigen 6 complex locus G6D (Ly6G) can attenuate the severity of AD in transgenic models 5xFAD and 3xTg-AD murine models [34]. Together, these points suggest that the release of NETs contributes to BBB leakage and cognitive impairment in mice. The effects could be mediated through alteration in the expression of PgP1, which also changes with age. However, these claims still need to be ascertained with experimental studies. Therefore, it is highly expected that overactivation of NETosis can promote age associated BBB impairment and cognitive impairment.

Inhibitors of NETs formation

Inhibition of PAD4 either by genetic manipulation [24] or pharmacological methods results in the suppression of NETs formation [35]. Similarly, administration of deoxyribonuclease 1 (DNase 1) can reduce the burden of NETs and protect against diseases like deep vein thrombosis [3]. Owing to the expected role of NETs in age-related BBB leakage and cognitive impairment, there is a critical need for the development of novel inhibitors of NET. Although several inhibitory molecules are available, they are limited by factors such as suboptimal inhibition, limited bioavailability, and non-specificity. Although DNase 1 can degrade the NETs and formulations are available for the human usage, it doesn't treat the underlying cause of the condition [3]. GSK-484 was identified as a highly potent reversible selective inhibitor of PAD4 [35] and inhibits NETosis in several models such as ischemia-reperfusion injury, atherosclerosis, and cancer [36–38]. Although GSK-484 provided a proof-of-concept to inhibit NETs formation, this compound does not exhibit a strong potency as it requires micromolar concentrations to be effective, and therefore, it is not suitable for clinical development [39]. Recently, Martinez et al. [40] reported that tetrahydroisoquinolines (THIQs) inhibits NETosis via inhibition of neutrophil elastase at micromolar concentration. Thus, THIQs holds the potential to inhibits NETs formation, but need further derivatization to enhance the efficiency. However, till date, no systematic study has been conducted to decipher the therapeutic role of NETosis inhibition in age related cognitive impairment.

Conclusions

Taken together, all these studies indicates that NETosis activation with age can increase the BBB leakage and promotes cognitive impairment. Inhibition of NETosis using different pharmacological approaches can

prevent the onset of cognitive impairment in aged individuals via attenuation of BBB leakage and thus pave the path for the development of novel therapeutic approaches. It is noteworthy that age-related cognitive impairment places an immense financial burden on the healthcare system and therefore it is imperative to examine the underlying mechanism and develop novel therapeutic interventions.

Abbreviations

AD: Alzheimer's disease

BBB: blood-brain barrier

H3Cit: citrullinated histone H3

K_{trans}: blood-to-brain transfer constant of gadolinium

MCI: mild cognitive impairment

NETs: neutrophil extracellular traps

PAD4: peptidyl arginine deiminase 4

PgP1: P Glycoprotein 1

Qalb: albumin quotient

Declarations

Author contributions

DS: Conceptualization, Writing—original draft. RK: Writing—review & editing.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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Not applicable.

Consent to participate

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References

1. Pais R, Ruano L, Carvalho OP, Barros H. Global Cognitive Impairment Prevalence and Incidence in Community Dwelling Older Adults—A Systematic Review. Geriatrics (Basel). 2020;5:84. [DOI] [PubMed] [PMC]

2. Mitchell AJ, Shiri-Feshki M. Rate of progression of mild cognitive impairment to dementia – meta-analysis of 41 robust inception cohort studies. Acta Psychiatr Scand. 2009;119:252–65. [DOI] [PubMed]
3. Kumar R, Sonkar VK, Swamy J, Ahmed A, Sharathkumar AA, Pierce GL, et al. DNase 1 Protects From Increased Thrombin Generation and Venous Thrombosis During Aging: Cross-Sectional Study in Mice and Humans. J Am Heart Assoc. 2022;11:e021188. [DOI] [PubMed] [PMC]
4. Vendemiale G, Romano AD, Dagostino M, Matthaeis Ad, Serviddio G. Endothelial dysfunction associated with mild cognitive impairment in elderly population. Aging Clin Exp Res. 2013;25:247–55. [DOI] [PubMed]
5. Zipser BD, Johanson CE, Gonzalez L, Berzin TM, Tavares R, Hulette CM, et al. Microvascular injury and blood–brain barrier leakage in Alzheimer's disease. Neurobiol Aging. 2007;28:977–86. [DOI] [PubMed]
6. Erickson MA, Banks WA. Blood–Brain Barrier Dysfunction as a Cause and Consequence of Alzheimer's Disease. J Cereb Blood Flow Metab. 2013;33:1500–13. [DOI] [PubMed] [PMC]
7. Villabona-Rueda A, Erice C, Pardo CA, Stins MF. The Evolving Concept of the Blood Brain Barrier (BBB): From a Single Static Barrier to a Heterogeneous and Dynamic Relay Center. Front Cell Neurosci. 2019;13:405. [DOI] [PubMed] [PMC]
8. Sweeney MD, Sagare AP, Zlokovic BV. Blood–brain barrier breakdown in Alzheimer disease and other neurodegenerative disorders. Nat Rev Neurol. 2018;14:133–50. [DOI] [PubMed] [PMC]
9. Nitta T, Hata M, Gotoh S, Seo Y, Sasaki H, Hashimoto N, et al. Size-selective loosening of the blood-brain barrier in claudin-5–deficient mice. J Cell Biol. 2003;161:653–60. [DOI] [PubMed] [PMC]
10. Hultman K, Strickland S, Norris EH. The *APOE* ε4/ε4 Genotype Potentiates Vascular Fibrin(Ogen) Deposition in Amyloid-Laden Vessels in the Brains of Alzheimer's Disease Patients. J Cereb Blood Flow Metab. 2013;33:1251–8. [DOI] [PubMed] [PMC]
11. Akaishi T, Narikawa K, Suzuki Y, Mitsuzawa S, Mitsuzawa K, Kuroda H, et al. Importance of the quotient of albumin, quotient of immunoglobulin G and Reibergram in inflammatory neurological disorders with disease-specific patterns of blood–brain barrier permeability. Neurol Clin Neurosci. 2015;33:94–100. [DOI]
12. Montagne A, Barnes SR, Sweeney MD, Halliday MR, Sagare AP, Zhao Z, et al. Blood-brain barrier breakdown in the aging human hippocampus. Neuron. 2015;85:296–302. [DOI] [PubMed] [PMC]
13. Blennow K, Wallin A, Fredman P, Karlsson I, Gottfries CG, Svennerholm L. Blood-brain barrier disturbance in patients with Alzheimer's disease is related to vascular factors. Acta Neurol Scand. 1990;81:323–6. [DOI] [PubMed]
14. Bowman GL, Kaye JA, Quinn JF. Dyslipidemia and Blood-Brain Barrier Integrity in Alzheimer's Disease. Curr Gerontol Geriatr Res. 2012;2012:184042. [DOI] [PubMed] [PMC]
15. Chai AB, Leung GKF, Callaghan R, Gelissen IC. P-glycoprotein: a role in the export of amyloid-β in Alzheimer's disease? FEBS J. 2020;2874:612–25. [DOI] [PubMed]
16. Chiu C, Miller MC, Monahan R, Osgood DP, Stopa EG, Silverberg GD. P-glycoprotein expression and amyloid accumulation in human aging and Alzheimer's disease: preliminary observations. Neurobiol Aging. 2015;36:2475–82. [DOI] [PubMed]
17. Cirrito JR, Deane R, Fagan AM, Spinner ML, Parsadanian M, Finn MB, et al. P-glycoprotein deficiency at the blood-brain barrier increases amyloid-β deposition in an Alzheimer disease mouse model. J Clin Invest. 2005;115:3285–90. [DOI] [PubMed] [PMC]
18. Takechi R, Lam V, Brook E, Giles C, Fimognari N, Mooranian A, et al. Blood-Brain Barrier Dysfunction Precedes Cognitive Decline and Neurodegeneration in Diabetic Insulin Resistant Mouse Model: An Implication for Causal Link. Front Aging Neurosci. 2017;9:399. [DOI] [PubMed] [PMC]
19. Silverberg GD, Messier AA, Miller MC, Machan JT, Majmudar SS, Stopa EG, et al. Amyloid efflux transporter expression at the blood-brain barrier declines in normal aging. J Neuropathol Exp Neurol. 2010;69:1034–43. [DOI] [PubMed]

20. Takei H, Araki A, Watanabe H, Ichinose A, Sendo F. Rapid killing of human neutrophils by the potent activator phorbol 12-myristate 13-acetate (PMA) accompanied by changes different from typical apoptosis or necrosis. J Leukoc Biol. 1996;59:229–40. [DOI] [PubMed]
21. Wang Y, Li M, Stadler S, Correll S, Li P, Wang D, et al. Histone hypercitrullination mediates chromatin decondensation and neutrophil extracellular trap formation. J Cell Biol. 2009;184:205–13. [DOI] [PubMed] [PMC]
22. Jylhävä J, Nevalainen T, Marttila S, Jylhä M, Hervonen A, Hurme M. Characterization of the role of distinct plasma cell-free DNA species in age-associated inflammation and frailty. Aging Cell. 2013;12:388–97. [DOI] [PubMed]
23. Matsuda Y, Itabashi M, Tachibana Y, Sugihara T, Sakashita Y, Matsubara T, et al. Citrullinated histone H3 expression in anti-neutrophil cytoplasmic antibody-associated vasculitis in older Japanese autopsy patients. Geriatr Gerontol Int. 2019;19:259–64. [DOI] [PubMed]
24. Martinod K, Witsch T, Erpenbeck L, Savchenko A, Hayashi H, Cherpokova D, et al. Peptidylarginine deiminase 4 promotes age-related organ fibrosis. J Exp Med. 2017;214:439–58. [DOI] [PubMed] [PMC]
25. Sohal RS, Sohal BH. Hydrogen peroxide release by mitochondria increases during aging. Mech Ageing Dev. 1991;57:187–202. [DOI] [PubMed]
26. Sullivan-Gunn MJ, Lewandowski PA. Elevated hydrogen peroxide and decreased catalase and glutathione peroxidase protection are associated with aging sarcopenia. BMC Geriatr. 2013;13:104. [DOI] [PubMed] [PMC]
27. Ahn B, Ranjit R, Kneis P, Xu H, Piekarz KM, Freeman WM, et al. Scavenging mitochondrial hydrogen peroxide by peroxiredoxin 3 overexpression attenuates contractile dysfunction and muscle atrophy in a murine model of accelerated sarcopenia. Aging Cell. 2022;21:e13569. [DOI] [PubMed] [PMC]
28. Du L, Chen E, Wu T, Ruan Y, Wu S. Resveratrol attenuates hydrogen peroxide-induced aging through upregulation of autophagy in human umbilical vein endothelial cells. Drug Des Devel Ther. 2019;13:747–55. [DOI] [PubMed] [PMC]
29. Cavazzoni M, Barogi S, Baracca A, Castelli GP, Lenaz G. The effect of aging and an oxidative stress on peroxide levels and the mitochondrial membrane potential in isolated rat hepatocytes. FEBS Lett. 1999;449:53–6. [DOI] [PubMed]
30. Lisanti MP, Martinez-Outschoorn UE, Lin Z, Pavlides S, Whitaker-Menezes D, Pestell RG, et al. Hydrogen peroxide fuels aging, inflammation, cancer metabolism and metastasis: the seed and soil also needs “fertilizer”. Cell Cycle. 2011;10:2440–9. [DOI] [PubMed] [PMC]
31. Metzler KD, Goosmann C, Lubojemska A, Zychlinsky A, Papayannopoulos V. A myeloperoxidase-containing complex regulates neutrophil elastase release and actin dynamics during NETosis. Cell Rep. 2014;8:883–96. [DOI] [PubMed] [PMC]
32. Suttorp N, Nolte A, Wilke A, Drenckhahn D. Human neutrophil elastase increases permeability of cultured pulmonary endothelial cell monolayers. Int J Microcirc Clin Exp. 1993;13:187–203. [PubMed]
33. Kang L, Yu H, Yang X, Zhu Y, Bai X, Wang R, et al. Neutrophil extracellular traps released by neutrophils impair revascularization and vascular remodeling after stroke. Nat Commun. 2020;11:2488. [DOI] [PubMed] [PMC]
34. Zenaro E, Pietronigro E, Bianca VD, Piacentino G, Marongiu L, Budui S, et al. Neutrophils promote Alzheimer's disease–like pathology and cognitive decline via LFA-1 integrin. Nat Med. 2015;21:880–6. [DOI] [PubMed]
35. Lewis HD, Liddle J, Coote JE, Atkinson SJ, Barker MD, Bax BD, et al. Inhibition of PAD4 activity is sufficient to disrupt mouse and human NET formation. Nat Chem Biol. 2015;11:189–91. [DOI] [PubMed] [PMC]
36. Cedervall J, Dragomir A, Saupe F, Zhang Y, Ärnlöv J, Larsson E, et al. Pharmacological targeting of peptidylarginine deiminase 4 prevents cancer-associated kidney injury in mice. Oncoimmunology. 2017;6:e1320009. [DOI] [PubMed] [PMC]

37. Zeng H, Fu X, Cai J, Sun C, Yu M, Peng Y, et al. Neutrophil Extracellular Traps may be a Potential Target for Treating Early Brain Injury in Subarachnoid Hemorrhage. Transl Stroke Res. 2022;13:112–31. [DOI] [PubMed]
38. Molinaro R, Yu M, Sausen G, Bichsel CA, Corbo C, Folco EJ, et al. Targeted delivery of protein arginine deiminase-4 inhibitors to limit arterial intimal NETosis and preserve endothelial integrity. Cardiovasc Res. 2021;117:2652–63. [DOI] [PubMed] [PMC]
39. Li M, Lin C, Deng H, Strnad J, Bernabei L, Vogl DT, et al. A Novel Peptidylarginine Deiminase 4 (PAD4) Inhibitor BMS-P5 Blocks Formation of Neutrophil Extracellular Traps and Delays Progression of Multiple Myeloma. Mol Cancer Ther. 2020;19:1530–8. [DOI] [PubMed] [PMC]
40. Martinez NE, Zimmermann TJ, Goosmann C, Alexander T, Hedberg C, Ziegler S, et al. Tetrahydroisoquinolines: New Inhibitors of Neutrophil Extracellular Trap (NET) Formation. Chembiochem. 2017;18:888–93. [DOI] [PubMed]