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ORIGINAL ARTICLE

Extracellular ferritin contributes to neuronal injury in an in vitro model of ischemic stroke

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Abstract

Previous clinical and experimental studies have shown that neurological decline and poor functional outcome after acute ischemic stroke in humans are associated with high ferritin levels in serum and cerebrospinal fluid (CSF) within 24 h of ischemic stroke onset. The aim of the present study was to find out if and how high extracellular ferritin concentrations can increase the excitotoxicity effect in a neuronal cortical culture model of stroke. Extracellular ferritin (100 ng/ml) significantly increased the excitotoxic effect caused by excessive exogenous glutamate (50 μM and 100 μM) by leading to an increase in lipid peroxidation, a reduction in mitochondrial membrane potential, and a decrease in neuron viability. Extracellular apoferritin (100 ng/ml), the iron-free form of the protein, does not increase the excitotoxicity of glutamate, which proves that iron was responsible for the neurotoxic effect of the exogenous ferritin. We present evidence that extracellular ferritin iron exacerbates the neurotoxic effect induced by glutamate excitotoxicity and that the effect of ferritin iron is dependent of glutamate excitotoxicity. Our results support the idea that body iron overload is involved in the severity of the brain damage caused by stroke and reveal the need to control systemic iron homeostasis.

Keywords Apoferritin · Excitotoxicity · Ferritin · Glutamate · Iron · Stroke

Introduction

Stroke is the second most common cause of death and the leading cause of acquired disability in adults' worldwide [12]. There are two major types of stroke: ischemic and hemorrhagic. Ischemic strokes caused by the interruption of the blood flow (ischemia) due to thrombosis or embolism account for the majority of strokes. Less frequently (<15%), strokes are caused by hemorrhage or cardiac arrest. The pathophysiological mechanisms involved in cerebral ischemia are multifactorial and complex [5, 20, 24]. Among the mechanisms involved in stroke, excitotoxicity is considered a primary intracellular event that induces neuronal death [20]. Neuronal excitotoxicity generally refers to the injury and death of neurons that arise from prolonged exposure to glutamate, as occurs in a stroke [20]. Overactivation of glutamate receptors impairs cellular calcium homeostasis, and activates oxidative stress and programmed cell death [8].

Deregulation of brain iron homeostasis may act as a mediator of neuronal damage in important neurodegenerative diseases including stroke [3, 19]. Ferritin is an iron-binding protein whose main function is the sequestration of excess iron to prevent the presence of iron in free form and the increase in the production of reactive oxygen species (ROS) [6]. Despite this, ferritin also has the ability to release iron from its core and, under certain conditions, can act as a major source of free iron [21, 22].

In previous clinical study, we found that high plasma and CSF ferritin concentrations within the first 24 h from the onset of ischemic stroke were associated with early neurologic deterioration [10]. Subsequent experimental studies in rats fed with an iron-rich diet, which lead to increase the body iron stores and the levels of serum ferritin, confirm that increased

Key points Extracellular ferritin increases glutamate excitotoxicity in neuronal cultures.
Patients at risk of cerebral stroke should take care of systemic iron level.

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body iron stores contribute to stroke progression by enhancing the cytotoxic mechanisms in cerebral ischemia [7]. In this study, the progression of stroke was not associated with a greater amount of iron in brain tissue. These findings suggest that ferritin iron, involved in the acceleration of neurological deterioration in acute cerebral infarction, would be of systemic origin. Research on the role of iron in ischemic diseases has focused on the relationship between raised ferritin stores, atherosclerosis, and risk of ischemic stroke [26, 28].

The aim of the present study is to analyze whether extracellular ferritin-iron can contribute to increasing the excitotoxic effect that occurs in strokes. We evaluated the effects of extracellular ferritin on lipid peroxidation, mitochondrial dysfunction, and neuronal viability by exposing primary neuronal cortical cultures to excessive exogenous glutamate to model ischemic stroke.

Methods

Cell culture

A primary culture of cortical neurons was used in this study. Cortical neurons were chosen because the cortical area is one of the areas affected by a stroke. Moreover, the neurons in this area contain a large number of glutamate receptors, so it is prone to excitotoxicity. Primary neurons were isolated from Wistar rats (Harlan, Spain) on embryonic day 17. All experimental procedures were carried out following the guidelines of the Committee for the Care of Research Animals of the University of Barcelona, in accordance with the directive of the Council of the European Community (2010/63 y 86/609/EEC) on animal experimentation. The experimental protocol was approved by the local University Committee (CEEA-UB, Comitè Ètic d'Experimentació Animal de la Universitat de Barcelona) and by the Catalan Government (Generalitat de Catalunya, Departament de Territori I Sostenibilitat) with the approval number #9431.

The cells were cultured for 9 days in Neurobasal ® growth medium for neurons containing penicillin/streptomycin, enriched with GlutaMAX ® and B27 ® (Antioxidants) and supplemented with 1/5 of Neurobasal Medium plus GlutaMAX ® and B27 ® previously conditioned in astrocyte cultures, in culture incubator at 37 °C in a humidified atmosphere containing 5% CO2/95% air (pH 7.2). To halt the proliferation of non-neuronal cells, 3 days after plating, half the culture medium was replaced with medium containing cytosine arabinoside (AraC) (final concentration: 10 μM). On day 5 after plating, half of the medium was replaced with Neurobasal ® medium containing 2 mM glutamine and 2% B27 (AO+), and the neurons were analyzed at day 9. Approximately 98% of the cells were Map2-positive.

On the twelfth day in culture, the medium was replaced with conditioned Neurobasal medium containing 2 mM glutamine and 2% B-27.

To test the neurotoxic effect of glutamate, doses of 50 and 100 μM of glutamate were applied and cells incubated for 1 h at 37 °C. After this incubation, the medium containing glutamate was removed and replaced by Neurobasal medium. Cells were further incubated for 3, 24, and 48 h at 37 °C. Ferritin (100 ng/ml) and apoferritin (100 ng/ml) were added when indicated, and maintained for 3, 24, and 48 h at 37 °C in a CO_2 incubator, as is summarized in Fig. 1 Neuron viability was analyzed over time of incubation.

Assay of glutamate and ferritin neurotoxicity

Cytotoxicity was quantified by measuring the activity of the lactate dehydrogenase (LDH, Sigma St Louis, MO, USA) released, using the method described by Dringen et al [13]. The data presented are the means ± S.E.M. for five independent experiments.

Lipid peroxidation

Lipid peroxidation was evaluated by the formation of thiobarbituric acid reactive substances (TBARS), in accordance with the method described by Uchiyama and Mihara [25], with minor modifications [9]. The cells were incubated in Neurobasal medium with glutamate and ferritin at the indicated concentrations. At 48 h, cells were collected and sonicated over ice. An aliquot of the cell lysate was added to the reaction mixture and heated at 95 °C for 60 min. The formation of TBARS was spectrophotometrically measured at 540 nm and the protein concentration of cell lysates was determined using a protein assay (Bio-Rad, Hercules, CA, USA). Lipid peroxidation was calculated as nanomoles of TBARS per milligram of protein and converted to relative units of the control. Data represent mean ± SEM of three wells derived from five independently prepared neuronal cultures.

Mitochondrial permeability transition potential

Mitochondrial membrane potential ($\Delta\Psi_m$) was performed using 5,5′,6,6′-tetrachloro-1,1′3,3′- tetraethylbenzamidazolcarboncyanine (JC-1, Invitrogen, Carlsbad, CA, USA). JC-1 is a cationic dye whose mitochondrial uptake is directly related to the magnitude of the $\Delta\Psi_m$. In healthy cells, the dye stains the mitochondria bright red. Cationic fluorescent probes (JC-1) are an invaluable tool for monitoring mitochondrial function in intact cells during excitotoxic injury [27]. The negative charge established by the intact $\Delta\Psi$m allows the lipophilic dye to enter the mitochondrial matrix, where it accumulates. In apoptotic cells, the $\Delta\Psi$m collapses and the JC-1 cannot accumulate within the mitochondria. In these cells, JC-1 remains in the

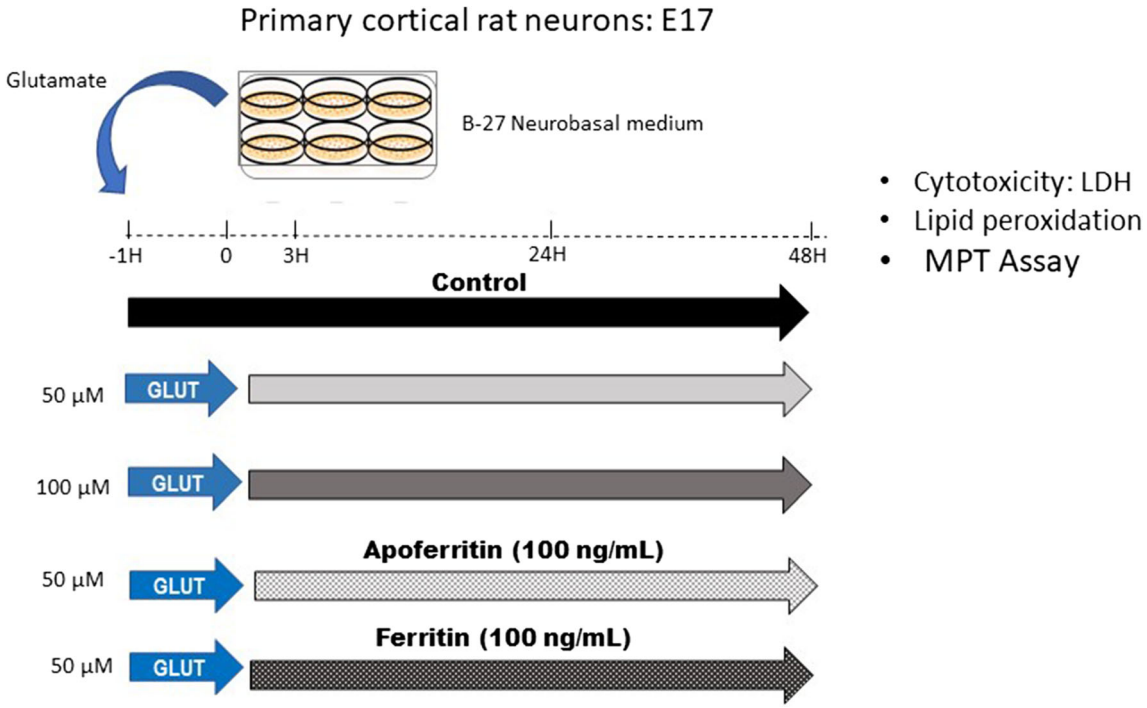

Fig. 1 Schematic representation of experimental design. For more details please refer to the "Methods" section

cytoplasm in a green fluorescent monomeric form. The greater the mitochondrial uptake, the greater the concentration of JC-1 aggregate forms that have a red fluorescent emission signal, as opposed to the JC-1 monomer that emits green fluorescence [23]. Several studies have demonstrated a linear relationship between the red and green ratio of JC-1 fluorescence and membrane potential over a physiological range.

Briefly, 0.5 ml of cell suspension was transferred into a sterile centrifuge tube and centrifuged at 400 *g* for 5 min at room temperature. The supernatant was removed and the cells were resuspended in JC-1 reagent. After 15 min of incubation at 37 °C in a 5% CO_2 incubator, the pellet was removed, resuspended in 2 ml assay buffer, centrifuged, and the supernatant removed. The remaining pellet was resuspended in 0.3 ml of assay buffer and immediately observed in a fluorescence microscope using a dual band-pass filter to detect fluorescein. In live non-apoptotic cells, mitochondria appear red following aggregation of the JC-1 reagent. Loss of mitochondrial membrane potential is indicated by a decrease in the 590/530 fluorescence intensity ratio. The data presented are means ± S.E.M for five independent experiments.

Statistics

Results were expressed as mean ± S.E.M. Data were evaluated by analysis of variance (ANOVA) followed by a post hoc Tukey's multiple comparisons test using GraphPad InStat statistical software. Differences were considered significant when $P \leq 0.05$.

Results

LDH release due to glutamate-induced cell death increased with extracellular ferritin

Incubation with glutamate (50 µM and 100 µM) caused the release of LDH in a dose- and time-dependent manner (Fig. 2a). As shown in Fig. 2b, the neurotoxic effect of glutamate (50 µM) is greater when ferritin (100 ng/ml) but not apoferritin (100 ng/ml) is present in the extracellular medium, suggesting the neurotoxic effect of ferritin is due to iron and further showing that apoferritin inhibits the neurotoxic effect of glutamate. In previous studies, (data not shown) we found that neuron cells incubated with 100 ng ferritin-iron/ml for 3 h, 24 h, or 48 h released LDH at similar rates to control neurons indicating that the neurotoxic effects described do not derive simply from extracellular ferritin-iron.

Extracellular ferritin increased the lipid peroxidation and the loss of mitochondrial membrane potential caused by excitotoxicity

Excitotoxicity is known to cause an increase in the formation of ROS that can damage the structural molecules of membrane cells. Here, we assessed the formation of lipid peroxidation. The results show that exogenous glutamate significantly increase lipid peroxidation. When extracellular ferritin was added to the glutamate group, the oxidation of lipids was significantly enhanced (Fig. 3a).

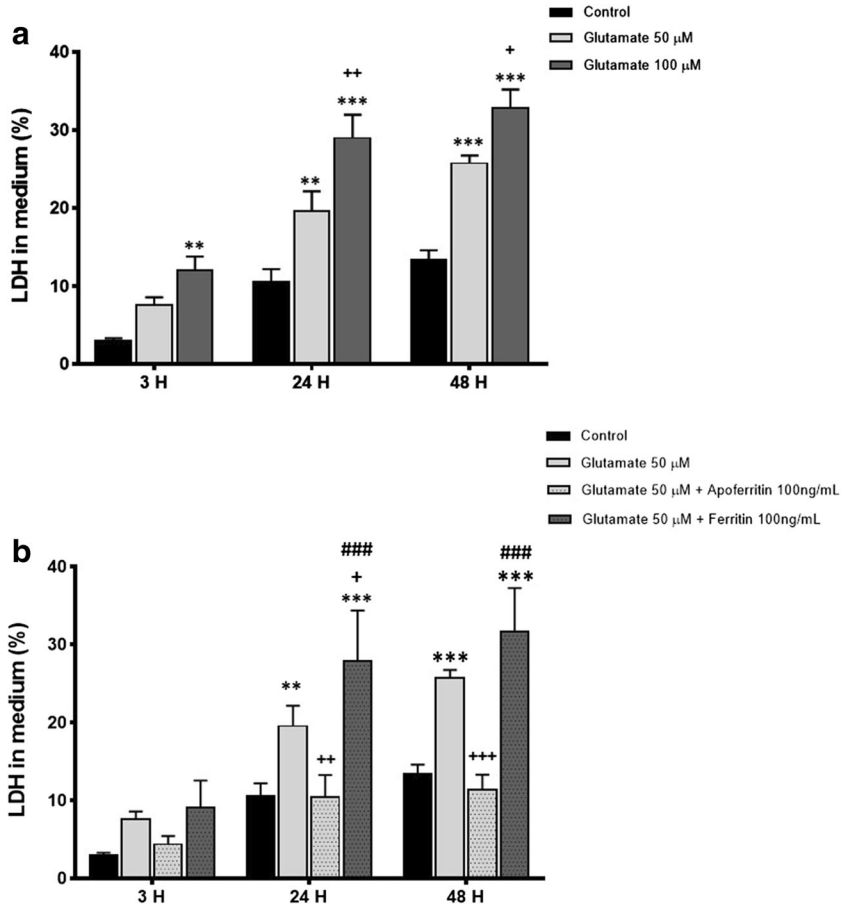

Fig. 2 The effect of iron and glutamate on the viability of primary culture rat neurons. **a** Cells were incubated with glutamate at the indicated concentrations for 1 h. After this incubation, the medium containing glutamate was removed and replaced by Neurobasal medium **b** Cells were incubated with glutamate (1 h). After this incubation, the medium containing glutamate was removed and replaced by Neurobasal medium. Ferritin (100 ng/ml) and apoferritin (100 ng/ml) were added when indicated, and maintained for 3, 24, and 48 h at 37 °C in a CO_2 incubator. Cell viability was determined by lactate dehydrogenase (LDH) assay at 3, 24, and 48 h. The data represent mean ± SEM of three wells derived from five independently prepared neuronal cultures. Significant differences from control values: $^{**}P < 0.01$ and $^{***}P < 0.001$. Significant differences from glutamate 50 μM values: $^{+}P < 0.05$, $^{++}P < 0.01$, and $^{+++}P < 0.001$. Significant differences from glutamate 50 μM + apoferritin values: $^{\#\#\#}P < 0.001$

We also assessed the mitochondrial membrane potential ($\Delta\Psi_m$) as an indicator of possible mitochondrial function impairment using the membrane-permeant JC-1 dye. The results show that glutamate (50 μM) in the extracellular medium leads to a reduction in the red/green ratio (Fig. 3b), which indicates a loss of $\Delta\Psi_m$. This reduction in $\Delta\Psi_m$ was greater in the presence of extracellular ferritin (100 ng/ml). The loss of $\Delta\Psi_m$ is an indicator of mitochondrial dysfunction and a hallmark of apoptosis [16].

Discussion

In this study, we provide the first evidence that neuronal damage caused by the presence of excess extracellular glutamate (excitotoxicity) in cultured cortical neurons is aggravated by the presence of ferritin in the extracellular medium. We provide evidence that this neurotoxic effect of ferritin is attributable to the presence of stored iron in the ferritin molecule, since apoferritin, the iron-free form of the protein, does not increase the excitotoxicity of glutamate. It is striking that apoferritin not only does not contribute to increasing the excitotoxic effect of glutamate but also eliminates it, showing a protective action. After more than 80 years of research, ferritin is known for its role in iron homeostasis as a molecule that stores iron in a safe and bioavailable form and in protecting against the oxidative damage of iron [2]. Recent data shows that ferritin is a multifunctional protein [15] and we can expect to find new functions of ferritin in non-iron-dependent processes in the near future [1, 17].

Excitotoxicity is considered a primary intracellular event that induces neuronal death in stroke [20]. It is well known that excitotoxic mechanisms primarily cause free radical generation and mitochondrial dysfunction [18]. We found that excessive amounts of extracellular glutamate caused an increase in lipid peroxidation levels, mitochondrial membrane

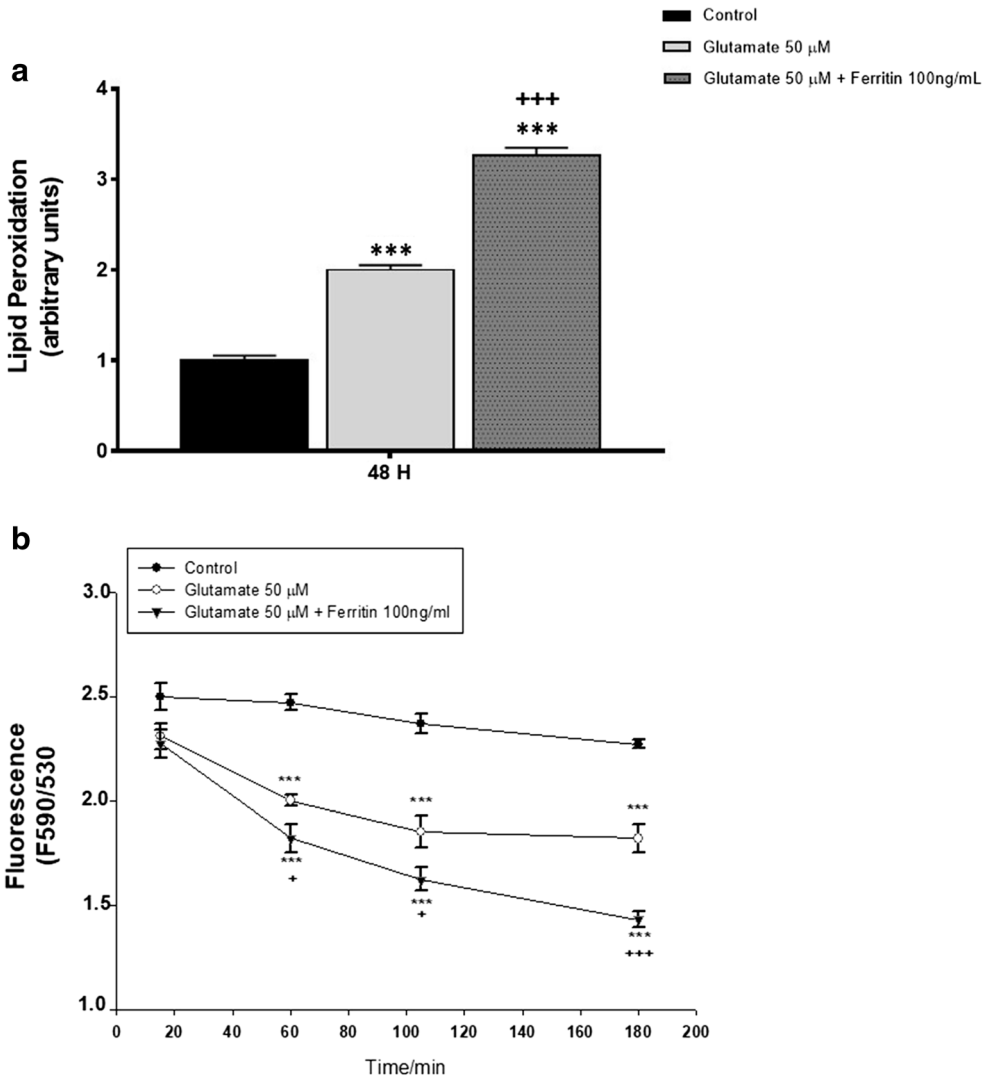

Fig. 3 Extracellular ferritin increases lipid peroxidation and the loss of mitochondrial membrane potential caused by excitotoxicity. **a** Lipid peroxidation was determined by thiobarbituric acid reaction substances (TBARS) assay at 48 h. **b** Mitochondrial membrane depolarization was determined by adding JC-1 at 48 h. Data represent mean ± SEM of three wells derived from five independently prepared neuronal cultures. Significant differences from control values: $^{***}P < 0.001$. Significant differences from glutamate 50 μM values: $^{+}P < 0.05$, $^{+++}P < 0.001$

potential loss, and a reduction in neuronal viability. Our results are consistent with those obtained in other studies on the neurotoxic effect of excessive glutamate levels in cortical neuronal culture [27]. Both excessive free radicals and mitochondrial dysfunction have been described as events involved in the activation of the apoptotic cascade that occurs in the ischemic penumbra. [4].

Several lines of research indicate that oxidative stress is a main mediator of neurological injury after cerebral ischemia [8]. In particular, clinical [10], experimental [5], and epidemiological [26] studies show that poor outcomes after stroke are associated with increased ferritin content in plasma and CSF. Iron release from ferritin is facilitated by the presence of reactive oxygen and nitrogen species [21, 22] all of which increased in the ischemic region during a stroke [6, 26].

Initially, it was thought that increased brain injury associated with iron content after stroke was due to increased iron levels in the brain because of alterations in brain iron homeostasis [11]. CSF ferritin levels were interpreted to indicate the iron content of brain tissue and, similarly, plasma ferritin levels were thought to be directly proportional to the body's iron stores and could be used to assess systemic iron overload in the absence of inflammation, cancer, and infectious diseases [29]. However, experimental studies using rats fed an iron-enriched diet to induce systemic iron overload confirm that a worsened outcome after stroke was associated with the ferritin level in serum and not with iron in the brain tissue [7]. Although iron accumulation in the brain has been associated with many neurodegenerative disorders [30], it should be noted that the increased neurotoxic effect in stroke is due to an iron overload in systemic tissue [7].

These results raise the question of how an iron overload situation in the systemic tissue can cause higher levels of brain damage in stroke. When a stroke occurs, the integrity of the blood-brain barrier (BBB) is lost in the infarct area [14]. This facilitates the entrance to the ischemic brain area of the accumulated ferritin in the microvasculature and in the plasma. Once ferritin is in the ischemic zone, factors caused by

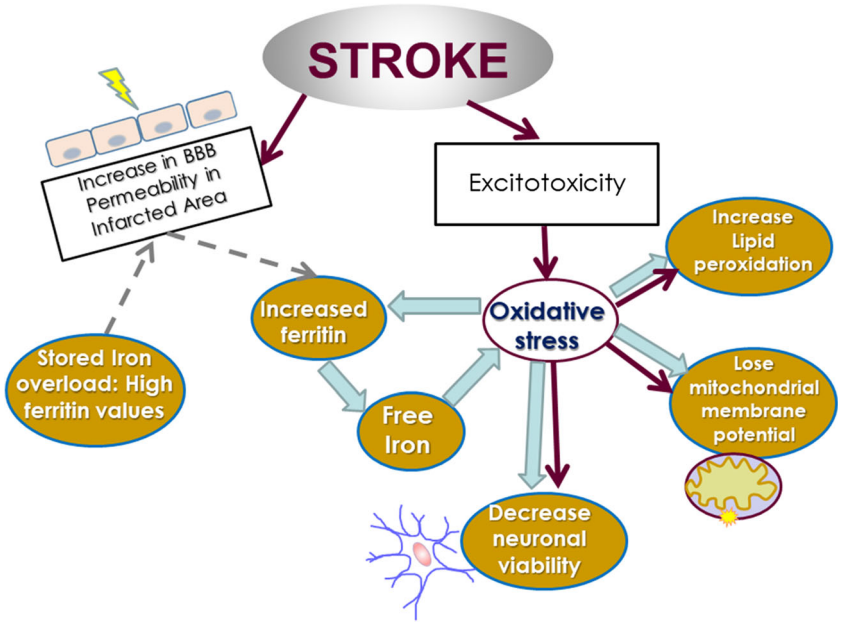

Fig. 4 Proposed model for the role of body ferritin stores in stroke. When stroke occurs, a disruption of the blood-brain barrier (BBB) can allow serum ferritin to enter the ischemic zone, where reactive oxygen species (ROS) produced by excitotoxicity can release iron from ferritin, exacerbating the cascade of events that lead to neuronal death in the penumbra zone. Purple arrows: mechanisms activated by stroke. Arrows in blue: expanded effects due to ferritin in the interstitial fluid

excessive glutamate in the extracellular medium, such as ROS and acidic pH, can release iron from ferritin [14]. This free iron may increase oxidative damage in the area of the ischemic penumbra, contributing to the neurological decline and, finally, causing a greater neurological injury in stroke than would occur without alterations in iron metabolism.

Possible mechanisms of ferritin action were summarized in Fig. 4. The results suggest that blood-brain barrier disruption in the ischemic penumbra enables plasma ferritin to enter the interstitial fluid, and factors caused by excessive glutamate in the extracellular medium, such as superoxide ($O_2^{\bullet-}$), nitric oxide ($NO^{\bullet}$), and acidic pH, can release iron from ferritin. Under these conditions, ferritin can release and increase the amount of free iron. This free iron can increase free radical production in the ischemic penumbra area, which causes greater oxidative stress, contributes to neurological decline in ischemic stroke, and finally, causes greater neurological injury in stroke than in normal body iron metabolism.

Conclusions

We have demonstrated that extracellular ferritin increases the excitotoxicity of glutamate in a primary culture of cortical neurons. Our results support that body iron overload is involved in the severity of brain damage caused by stroke and point to take care of systemic iron levels in patients at risk of cerebral stroke.

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Author contribution The authors declare that all data were generated in-house and that no paper mill was used.

Declarations

Informed consent All experimental procedures (approval number #9431) were carried out following the guidelines of the Committee for the Care of Research Animals of the University of Barcelona, in accordance with European directive (2010/63 and 86/609/EEC).

Conflict of interest The authors declare no competing interests.

References

1. Arosio P, Levi S (2010) Cytosolic and mitochondrial ferritins in the regulation of cellular iron homeostasis and oxidative damage. Biochim Biophys Acta 1800(8):783–792. https://doi.org/10.1016/j.bbagen.2010.02.005
2. Arosio P, Ingrassia R, Cavadini P (2009) Ferritins: a family of molecules for iron storage, antioxidation and more. Biochim Biophys Acta 1790(7):589–599. https://doi.org/10.1016/j.bbagen.2008.09.004
3. Bou-Abdallah F (2010) The iron redox and hydrolysis chemistry of the ferritins. Biochim Biophys Acta 1800(8):719–731. https://doi.org/10.1016/j.bbagen.2010.03.021
4. Broughton BRS, Reutens DC, Sobey CG (2009) Apoptotic mechanisms after cerebral ischemia. Stroke 40(5):e331–e339. https://doi.org/10.1161/STROKEAHA.108.531632
5. Brouns R, De Deyn PP (2009) The complexity of neurobiological processes in acute ischemic stroke. Clin Neurol Neurosurg 111(6):483–495. https://doi.org/10.1016/j.clineuro.2009.04.001
6. Carbonell T, Rama R (2007) Iron, oxidative stress and early neurological deterioration in ischemic stroke. Curr Med Chem 14:857–874. https://doi.org/10.2174/092986707780363014
7. Castellanos M, Puig N, Carbonell T, Castillo J, Martinez JM, Rama R, Dávalos A (2002) Iron intake increases infarct volume after permanent middle cerebral artery occlusion in rats. Brain Res 952:1–6. https://doi.org/10.1016/S0006-8993(02)03179-7
8. Chung J-W, Ryu W-S, Kim BJ, Yoon B-W (2015) Elevated calcium after acute ischemic stroke: association with a poor short-term

outcome and long-term mortality. J Stroke 17(1):54–59. https://doi.org/10.5853/jos.2015.17.1.54
9. Coimbra-Costa D, Alva N, Duran M, Carbonell T, Rama R (2017) Oxidative stress and apoptosis after acute respiratory hypoxia and reoxygenation in rat brain. Redox Biol 12:216–225. https://doi.org/10.1016/j.redox.2017.02.014
10. Dávalos A, Castillo J, Marrugat J, Fernandez-Real JM, Armengou A, Cacabelos P, Rama R (2000) Body iron stores and early neurologic deterioration in acute cerebral infarction. Neurology 54(8):1568–1574. https://doi.org/10.1212/wnl.54.8.1568
11. Ding H, Yan CZ, Shi H, Zhao YS, Chang SY, Yu P, Wu WS, Zhao CY, Chang YZ, Duan XL (2011) Hepcidin is involved in iron regulation in the ischemic brain. PLoS One 6(9):e25324. https://doi.org/10.1371/journal.pone.0025324
12. Donnan GA, Fisher M, Macleod MDS (2008) Stroke. Lancet. 371(9624):1612–1623. https://doi.org/10.1016/S0140-6736(08)60694-7.12
13. Dringen R, Kussmaul L, Hamprecht B (1998) Detoxification of exogenous hydrogen peroxide and organic hydroperoxides by cultured astroglial cells assessed by microtiter plate assay. Brain Res Brain Res Protocol 2(3):223–228. https://doi.org/10.1016/s1385-299x(97)00047-0
14. Fraser PA (2011) The role of free radical generation in increasing cerebrovascular permeability. Free Radic Biol Med 51(5):967–977. https://doi.org/10.1016/j.freeradbiomed.2011.06.003
15. Hagen WR, Hagedoorn PL, Honarmand Ebrahimi K (2017) The workings of ferritin: a crossroad of opinions. Metallomics. 9(6):595–605. https://doi.org/10.1039/c7mt00124j
16. Körper S, Nolte F, Rojewski MT, Thiel E, Schrezenmeier H (2003) The K+channel openers diazoxide and NS1619 induce depolarization of mitochondria and have differential effects on cell Ca2+in CD34+cell line KG-1a. Exp Hematol 31(9):815–823. https://doi.org/10.1016/S0301-472X(03)00199-1
17. Krisanova N, Sivko R, Kasatkina L, Borysov A, Borisova T (2014) Excitotoxic potential of exogenous ferritin and apoferritin: changes in ambient level of glutamate and synaptic vesicle acidification in brain nerve terminals. Mol Cell Neurosci 58:95–104. https://doi.org/10.1016/j.mcn.2013.12.002
18. MacDougall G, Anderton RS, Mastaglia FL, Knuckey NW, Meloni BP (2019) Mitochondria and neuroprotection in stroke: cationic arginine-rich peptides (CARPs) as a novel class of mitochondria-targeted neuroprotective therapeutics. Neurobiol Dis 121:17–33. https://doi.org/10.1016/j.nbd.2018.09.010
19. Morris G, Berk M, Carvalho AF, Maes M, Walker AJ, Puri BK (2018) Why should neuroscientists worry about iron? The emerging role of ferroptosis in the pathophysiology of neuroprogressive diseases. Behav Brain Res 341:154–175. https://doi.org/10.1016/j.bbr.2017.12.036
20. Moskowitz MA, Lo EH, Iadecola C (2010) The science of stroke: mechanisms in search of treatments. Neuron. 67(2):181–198. https://doi.org/10.1016/j.neuron.2010.07.002
21. Reif DW (1992) Ferritin as a source of iron for oxidative damage. Free Radic Biol Med 12:417–427. https://doi.org/10.1016/0891-5849(92)90091-t
22. Reif DW, Simmons RD (1990) Nitric oxide mediates iron release from ferritin. Arch Biochem Biophys 283:537–541. https://doi.org/10.1016/0003-9861(90)90680-w
23. Smiley ST, Mottola-Hartshorn C, Smith TW, Reers M, Chen A, Chen LB, Lin M, Steele GD (2006) Intracellular heterogeneity in mitochondrial membrane potentials revealed by a J-aggregate-forming lipophilic cation JC-1. Proc Natl Acad Sci 88(9):3671–3675. https://doi.org/10.1073/pnas.88.9.3671
24. Tymianski M (2011) Emerging mechanisms of disrupted cellular signaling in brain ischemia. Nat Neurosci 14(11):1369–1373. https://doi.org/10.1038/nn.2951
25. Uchiyama M, Mihara M (1978) Determination of malondialdehide precursor in tissues by thiobarbituric acid test. Anal Biochem 86:271–278. https://doi.org/10.1016/0003-2697(78)90342-1
26. Van der A DL, Grobbee DE, Roest M, JJM M, Voorbij HA, Van Der Schouw YT (2005) Serum ferritin is a risk factor for stroke in postmenopausal women. Stroke 36:1637–1641. https://doi.org/10.1161/01.STR.0000173172.82880.72
27. Ward MW, Rego AC, Frenguelli BG, Nicholls DG (2000) Mitochondrial membrane potential and glutamate excitotoxicity in cultured cerebellar granule cells. J Neurosci 20(19):7208–7219. https://doi.org/10.1523/JNEUROSCI.20-19-07208.2000
28. Wolff B, Volzke H, Ludemann J, Robinson D, Vogelgesang D, Staudt A, Kessler JC, Dahm JB, John U, Felix SB (2004) Association between high serumferritin levels and carotid atherosclerosis in the Study of Health in Pomerania (SHIP). Stroke. 35:453–457. https://doi.org/10.1161/01.STR.0000114875.31599.1C
29. Worwood M (1987) The diagnostic value of serum ferritin determinations for assessing iron status. Haematologia 20:229–235
30. Zecca L, Youdim MB, Riederer P, Connor JR, Crichton R (2004) Iron, brain ageing, and neurodegeneratives disorders. Nat Rev Neurosci 5:863–873. https://doi.org/10.1038/nrn1537