

The neuro-inflammation and excitotoxicity in perinatal brain injury: The emerging role of brain mast cells

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ABSTRACT

Perinatal brain injury is a serious neurodevelopmental problem that can be occurred in preterm and term newborn infants. It is well established that neuro-inflammation is implicated in the pathophysiology of perinatal brain injury. The excitotoxicity is considered as a common molecular mechanism of perinatal brain injury. These insults are capable of leading to neuro-inflammation, but however neuro-inflammation is also able to induce the excitotoxicity in the developing brain. Thus, neuro-inflammation is both a cause and a consequence of excitotoxicity resulting in the brain damages during perinatal period. Excessive glutamate accumulation in the synaptic cleft in the brain is a prominent mechanism in the excitotoxicity while vasoactive and pro-inflammatory mediators such as histamine, prostaglandins, interleukin 1 (IL-1) β and tumor necrosis factor (TNF)- α released from brain-resident immune cells play a major role in neuro-inflammation that lead to the brain damages. Although the role of brain-resident microglial cells has been well documented in these neuro-inflammation processes, evidence for the role of brain mast cells (BMCs) has recently begun to emerge. Growing evidence indicates that brain mast cells are first responders of inflammatory insults in the developing brain and their activation is involved in induced brain injury.

We have recently demonstrated that ibotenate-induced excitotoxicity leads to the activation of brain mast cells in a model of ibotenate-induced brain injury in newborn rats. Thus, in this review we point out the current knowledge on the bidirectional role of brain mast cells in neuro-inflammation and excitotoxicity underlying perinatal brain injury.

Keywords: Perinatal brain injury, neuro-inflammation, excitotoxicity, brain mast cells, neonate.

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Introduction

Perinatal brain injury (PBI) is a condition of impaired neurological functions in newborn infants characterized by white and / or gray matter damage of the brain. Although its

incidence varies according to the defined condition, approximately 3-5 out of 1000 live births per year are born with PBI [1]. It can occur in both preterm and term newborns, and lead to long-term neurodevelopmental problems such as cerebral palsy, motor injuries, sleep disorders, hyperactivity, and cognitive and autonomic disabilities [2, 3]. Periventricular white matter damage is the most common brain lesions observed in preterm infants, however damage primarily affects gray matter in term infants [4]. The life-long

problems of surviving infants with PBI impose a huge burden on individuals, families and the country in terms of health, education expenditures and other social resources. The hypothermia approach is widely used in the treatment of PBI. Briefly, this treatment application includes starting the cooling process within 6 hours for a period of 72 hours at a target temperature of 33.5 °C [3]. However, this approach is commonly limited to its application to hypoxic-ischemic type injuries, furthermore the success rate is not high. Therefore, investigations on understanding the pathophysiology of PBI and developing novel treatment strategies are of great importance.

The etiology of PBI is multifactorial, however, the most common etiologies include some prenatal and perinatal factors such as genetic, hypoxic-ischemic encephalopathy, periventricular leukomalacia, birth asphyxia, excitotoxicity, maternal-fetal inflammation, stroke, intraventricular hemorrhage and oxidative stress [3, 4]. Inflammation and excitotoxicity from these factors have recently received considerable attention. Because, excitotoxicity is regarded as a common molecular mechanism of PBI [5], furthermore it has been suggested that neuro-inflammation associated with PBI is both a cause and a

consequence of excitotoxicity [6, 7]. Hence, there is a bidirectional interaction between inflammation and excitotoxicity in the cascade resulting in PBI. The prominent causes and common mechanism of perinatal brain injuries are given in Table 1.

Although the role of peripheral mast cells in the neuro-inflammatory disorders is well established, the role of brain mast cells (BMCs) remains unclear. However, growing evidence indicates that brain mast cells are implicated in both inflammation and excitotoxicity leading to PBI. Brain mast cells are the main source of pro-inflammatory cytokines which are released in response to excitotoxic- or the other insults, and as a result they are early responders of inflammation. Excitotoxicity is able to activate the mast cells, however mast cell activation-induced inflammation can also lead to excitotoxicity in the brain. Thus, novel pharmacological approaches targeting the stabilization of the mast cells alone or excitotoxicity in combination with mast cell stabilization may be promising for treatment of PBI in the future.

Perinatal brain injury and inflammation

Although an inflammation in physiological conditions is intended to protect the body,

Table 1. The prominent causes and common mechanism of perinatal brain injuries.

Triggering factors	Common molecular mechanism	Outcome
Hypoxic-ischemia	Excitotoxicity	Perinatal brain injury
Excitotoxicity		
Maternal/fetal inflammation		
Intraventricular hemorrhage		
Stroke		
Asphyxia		
Oxidative stress		

excessive inflammatory processes can have devastating consequences. This fine-tuning is substantially mediated by inflammatory mediators released from immune cells including microglia and mast cells in the central nervous system (CNS). Increased inflammatory responses of both maternal and fetal origin during the perinatal period can lead to various damages in the developing brain.

Excessive release of inflammatory mediators such as histamine, serotonin, prostaglandins, interleukin (IL)-1 β , IL-6, tumor necrosis factor- α (TNF- α), vascular endothelial growth factor (VEGF) and vasoactive intestinal peptide (VIP) exacerbates the development of neuro-inflammation in the brain by increasing the permeability of the blood-brain barrier [8, 9]. Changes in blood-brain barrier permeability resulting in neuro-inflammation in the CNS can be induced by both peripheral and central immune signals. Since the developing brain is more vulnerable to various insults, neuro-inflammation caused by excessive release of these mediators during the perinatal period can result in different types of perinatal brain injury. Although the inflammation plays an important role in the development of almost all types of perinatal brain injury, role of inflammation in the pathophysiology of hypoxic-ischemic and stroke-induced brain injury is well established. Hypoxic-ischemia evokes a neuro-inflammation state in the CNS characterized by activation of microglia and mast cells, as well as release of inflammatory mediators from these resident immune cells of the CNS [10, 11]. In a preclinical study was demonstrated that mast cell degranulation (activation) could directly evoke activation of microglial cells [12]. Thus, mast cell activation both directly and indirectly contributes to the neuro-inflammation by releasing inflammatory mediators such as histamine, IL-1 β , IL-6 and

TNF- α in their secretory granules, and by evoking release of such pro-inflammatory cytokines from activated microglial cells, respectively. Hypoxic-ischemia and stroke cause an inflammatory reaction in the developing brain by triggering rapid activation of resident immune cells in the brain, followed by infiltration of circulating peripheral leukocytes such as lymphocytes [13, 14]. Infiltration of peripheral immune cells into the brain further worsens the existing neuro-inflammatory condition, resulting in neuronal damages.

Although astrocytes and neurons are able to produce pro-inflammatory cytokines such as IL-1 β , IL-6 and TNF- α , the major sources of these cytokines, in particular TNF- α , during the neuro-inflammation are microglial cells and mast cells [13]. In a prospective study, it was found that cerebrospinal fluid and serum concentrations of pro-inflammatory cytokines IL-1 β , IL-6 and TNF- α were significantly elevated in infants with hypoxic-ischemic encephalopathy compared to control infants [15].

Another clinical study showed that the levels of IL-1 β , TNF- α and IL-8 in umbilical and peripheral blood were significantly increased in infants with hypoxic-ischemic encephalopathy compared to healthy infants [16]. In the same study, it was also found that elevated levels of IL-1 β in the umbilical cord blood were correlated with adverse outcomes of hypoxic-ischemic encephalopathy [16]. In addition to hypoxic-ischemic encephalopathy, it was reported that increased levels of TNF- α , IL-6 and IL-8 in the amniotic fluid, plasma and umbilical cord blood were correlated with development of cerebral palsy and periventricular leukomalacia [17-19]. Moreover, it was stated that the plasma concentrations of IL-1, IL-8, IL-9 and TNF- α were enhanced in

infants with cerebral palsy compared to controls [19, 20].

Regardless of whether it is of microbial origin, these clinical data clearly indicate that inflammation with increased pro-inflammatory cytokines plays an important role in the development of perinatal brain injuries. In addition to these previous studies, we have recently found that the plasma levels of IL-1 β , IL-6 and histamine were significantly increased in children with cerebral palsy older than two years compared to age-matched controls [21]. Our findings suggest that increased inflammatory responses are therefore involved in not only during the perinatal period but also after the perinatal period.

In addition to clinical studies, preclinical studies also revealed that inflammation with increased pro-inflammatory cytokines leads to damage to developing white matter in rodent models of perinatal brain injury. It was shown that expressions of IL-1, IL-6 and TNF- α messenger RNA were increased in the brain tissue in hypoxia-induced or hypoxia-ischemia-induced brain injury in neonatal rodents [22, 23]. Moreover, it was reported that IL-1 β and TNF- α induced white matter damage in different models of neonatal brain injury in rodents [24-26]. However, it was revealed that administration of blocking antibodies against TNF- α or IL-1 β exhibits protective effects against neonatal brain injury in rodents [22, 25, 27].

Taken together, the results of these preclinical and clinical studies provide the basis for the need to develop anti-inflammatory approaches that are capable of reducing the risks of developing brain injury in future. However, instead of blocking the receptors of inflammatory cytokines one by one, inhibiting the cascades that increase the release of these inflammatory cytokines may be more

reasonable to prevent inflammatory responses before they occur. For this, targeting the stabilization of central immune cells such as microglia and mast cells, which are the main source of inflammatory cytokines, may be a good therapeutic candidate in the future.

Perinatal brain injury and excitotoxicity

Growing preclinical and clinical studies have indicated that excitotoxicity is involved in the pathogenesis of perinatal brain injury. It was shown that glutamate levels in the cerebrospinal fluid of newborn infants with asphyxia were increased, and also there was a correlation between the severity of encephalopathy and levels of glutamate and aspartate neurotransmitters in the cerebrospinal fluid [28]. Similar results were also demonstrated in another later clinical study, however unlike the previous study, the latter study reported that high aspartate levels were correlated with the degree and short-term outcomes of hypoxic-ischemic encephalopathy [29]. Additionally, another clinical study reported that glutamate levels in the cerebrospinal fluid were significantly increased after severe traumatic brain injury in infants [30]. Thus, it has been suggested that excitotoxicity is a common mechanism of perinatal brain injuries, as perinatal insults such as cerebral hypoxia-ischemia, neuro-inflammation, stroke, hypoglycemia, trauma, and developmental and genetic vulnerabilities are able to lead to excessive glutamate accumulation in the synaptic cleft resulting in the neurotoxicity [5-7, 31, 32]. In addition, as mentioned above, perinatal inflammation with increased pro-inflammatory cytokines such as IL-1 β , IL-6 and TNF- α has well established to play a key role in the pathogenesis of perinatal brain injury. The excitotoxicity can trigger the neuro-inflammation during perinatal period as

well as neuro-inflammation is able to induce the excitotoxicity in the developing brain [6, 7, 32]. Thus, it has been suggested that there is a relationship between increased inflammatory response and glutamate excitotoxicity. It was reported that pro-inflammatory cytokine TNF- α induced glutamate release from neurons and glial cells and enhanced neuronal expression of glutamatergic receptors, thus contributing to the excitotoxicity [33, 34].

Taken together, previous studies clearly imply that factors such as hypoxic-ischemia, inflammation, stroke and trauma cause perinatal brain damage by inducing the excitotoxic pathway as a common mechanism. However, it is also important to understand the mechanisms of how the excitotoxicity causes glial and neuronal cell death underlying perinatal brain damages. For this, it is worth mentioning the normal and abnormal functioning of excitatory neurotransmission (especially via glutamate) in the CNS and its possible consequences. The amino acid neurotransmitters glutamate and aspartate are mainly excitatory neurotransmitters in the CNS, however the majority of excitatory neurotransmission in the brain is mediated by glutamate. It is considered that blood-brain barrier is impermeable to glutamate and aspartate, thus they are synthesized in the CNS [35]. Glutamate is synthesized from α -ketoglutarate (by the enzyme aminotransferase), an intermediate product in the Krebs cycle in mitochondrion, moreover it is also synthesized from glutamine (by the enzyme glutaminase) in the CNS. Then it is packaged into synaptic vesicles and released both from neurons and glial cells upon stimulation [36]. Since there are no enzymes in the extracellular space that are able to metabolise glutamate, the effects of glutamate are terminated by uptake into neurons and glia.

Then, it is degraded by the enzyme glutamate-dehydrogenase back to α -ketoglutarate in the glutamatergic neurons, or it is converted to glutamine by the enzyme glutamine synthetase mainly in astrocytes [37].

Under physiologic conditions, glutamate has critical roles in formation and elimination of synapses, as well as neuronal differentiation, migration and survival processes in the developing brain [32, 38]. To achieve these neurodevelopmental functions, glutamate concentration in the neuronal extracellular space is kept within a certain range by glutamate uptake transporters. These transporters are expressed mainly in astrocytes that are key players of glutamate clearance. However, in abnormal conditions where glutamate clearance is impaired, glutamate can be toxic to neurons due to its excessive accumulation in the synaptic cleft. Such a condition is called glutamate excitotoxicity. Glutamate excitotoxicity in the developing brain can result in cognitive and neurodegenerative disorders including perinatal brain injury. Glutamate exerts its effects on the post-synaptic brain cells through ionotropic and metabotropic G protein-coupled glutamate receptors. Glutamate possesses the three types of ionotropic receptors such as N-methyl-D-aspartate (NMDA), α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) and kainic acid. Activation of these receptors by glutamate leads to an influx of Ca^{2+} and/or Na^{+} resulting in rapid excitation of post-synaptic cells. However, activation of the metabotropic glutamate receptors produces slower responses in the post-synaptic cells than those of the ionotropic receptors because of these G protein-coupled metabotropic receptors exert their actions through intracellular second messenger systems [39, 40]. Additionally, contrary to the excitatory actions of ionotropic glutamate

receptors (NMDA, AMPA and kainic acid), activation of many of the metabotropic glutamate receptors gives rise to inhibition of Na^+ and Ca^{2+} channels in the plasma membrane of post-synaptic cells through intracellular second messenger signaling pathways [40]. Consequently, activation of the metabotropic glutamate receptors is able to enhance or suppress the excitability of the post-synaptic cells [40].

In particular, ionotropic NMDA receptor activation causes large amounts of Ca^{2+} influx that initiates intracellular signaling cascades resulting in a variety of physiological cellular responses in addition to cellular excitation [32]. Most neuronal and glial cells express glutamate receptors, indicating the critical role of glutamate in regulating neuronal and glial functions. It is well established that excitatory neurotransmission through glutamate receptors, in particular NMDA, in the brain is involved in cognitive functions such as learning and memory in the adults. Additionally, the NMDA, together with the AMPA receptor, is required for physiological processes including learning, memory and the formation of new synaptic connections during the perinatal period [30].

Contrary to their physiological functions, excessive activation of mainly ionotropic glutamate receptors produces excitotoxic effects. This excessive activation of the excitatory ionotropic glutamate receptors can be mediated by a variety of mechanisms: i) increased release of excitatory amino acids such as glutamate and aspartate from neurons to the synaptic cleft, ii) the failure in glutamate uptake in the synaptic cleft due to reduced expression and/or dysfunction of the excitatory amino acid transporters (EAATs) [32, 41], iii) an excessive increase in NMDA receptor expression and hypersensitivity of NMDA receptors to

glutamate during the perinatal period [42]. The function of glutamate transporters is impaired mostly by the decline in glucose delivery resulting from hypoxia and/or ischemia [30]. Moreover, it was reported that hypoxia-ischemia and hypoglycemia during the perinatal period lead to accumulation of extracellular glutamate by disrupting the functioning of glutamate transporters in the astrocyte-type glial cells [41]. Excessive Ca^{2+} influx into the neurons through glutamate receptors, in particular NMDA, induces intracellular signaling pathways that lead to neuronal cell death such as production of toxic oxygen- and nitrogen-free radicals, mitochondrial dysfunction and activation of apoptosis-related caspases [5, 31]. These mechanism-based explanations are also consistent with the above clinical studies reporting elevated levels of glutamate and/or aspartate in the cerebrospinal fluid of newborn infants with perinatal brain injury and their neurologic outcomes. Thus, targeting the cascades that lead to the excitotoxicity in the developing brain is of great importance in terms of developing new treatment strategies in the future.

Bidirectional role of brain mast cells in neuro-inflammation and excitotoxicity underlying perinatal brain injury

Mast cells (MCs) are multifunctional effector immune cells in both peripheral and central body structures. They originate from $\text{CD34}^+/\text{CD117}^+$ myeloid progenitor cells in the bone marrow and then mature in the tissues where they migrate [43, 44]. Activation of MCs can be induced by a broad range of endogenous and/or exogenous factors that may be immunologic or non-immunologic in nature. MCs normally mediate regulation of body functions, but excessive increase in their

number and activation has been implicated in the pathobiological events underlying various disorders. Although MCs are famous for their participation in allergic diseases, they are also involved in many neuro-inflammatory, cardiovascular, cutaneous, gastrointestinal, neurological, respiratory and systemic disorders such as inflammation, allergies, hypotension, bone pain, asthma, eczema, interstitial cystitis, irritable bowel syndrome, migraine, pulmonary hypertension, systemic mastocytosis, psoriasis, cancer, obesity, ulcers, prostatitis, brain injury, traumatic brain injury, stroke and dementia etc. [44-47]. The effects of MCs in biological and the pathobiological processes are mediated by a great variety of mediators released from their cytoplasmic granules when activated. Mediators synthesized, stored and released by MCs include a large variety of vasoactive mediators (histamine, nitric oxide, serotonin etc.), proteases (such as tryptase), cytokines (IL-1, IL-6 and TNF- α etc.), chemokines (CXCL8 and CCL2 etc.) growth factors (SCF, TGF β , β -FGF and NGF etc.), prostaglandins (such as prostaglandin D2), leukotrienes (such as leukotriene C4) and neuropeptides (substance P, vasoactive intestinal peptide, calcitonin gene-related peptide, pituitary adenylate cyclase activating polypeptide etc.) [44, 47, 48]. Mediator release from MCs is mainly based on classical exocytosis of cytoplasmic vesicles, which is called degranulation. But to a lesser extent, different pathways such as small vesicular carriers, recycling endosomes, regulated exocytosis and selective release have also been described [47, 49].

The phenotypic characteristics and mediator content of MCs may vary depending on the tissues in which they are located. Moreover, the type and amount of mediators released from MCs may vary according to the stimuli they are

exposed to in the resident tissue. This multi-effector functioning of MCs that may change according to microenvironmental circumstances in their resident tissue makes it difficult to understand how they will respond to changing microenvironmental conditions.

For instance, we have recently demonstrated that activation of MCs by the secretagogue compound 48/80 exhibited anticonvulsant effects against pentylentetrazole-induced seizures in rats [50]. In that study, it was concluded that the anticonvulsant effect of mast cell activation may be mediated by the selective release of serotonin from mast cells rather than other mediators that are capable of exacerbating the seizures. However, vast majority of studies on mast cells in the literature report that increase in mast cell activation and/or number contributes to the pathogenesis of many disorders.

Although the roles of peripheral mast cells in the pathogenesis of various disorders listed above have mainly been well established, roles of brain mast cells in the pathophysiology of these disorders remain unclear. However, growing evidence indicates that brain mast cells are involved in the pathobiology of neurodegenerative and neuro-inflammatory disorders such as brain injuries, neuropsychiatric disorders, neuro-inflammation, multiple sclerosis, Alzheimer's disease, Parkinson's disease, and autism as well as perinatal brain injury [8, 11, 45, 51-53]. In accordance with their neuro-immune functions, mast cells are located close to blood vessels and nerves in their resident tissues. Brain mast cells are commonly located around the choroid plexus, the leptomeninges and the third ventricle however, they are also present in thalamus, hypothalamus, hippocampus, pineal gland, olfactory bulb, and the dura mater [54, 55]. This strategic location, especially in the

central and peripheral nervous systems, is an important sign that mast cells modulate neuronal activity and blood-brain barrier integrity. Indeed, it has been reported that inflammatory mediators released from mast cells such as histamine, prostaglandins, IL-1 β , TNF- α , VEGF and VIP increase blood-brain barrier permeability [8, 9, 45]. Furthermore peripheral mast cells also cross into the brain through the blood-brain barrier with increased permeability and induce activation of microglia in the brain, resulting in a serious neuro-inflammatory condition [56].

Brain mast cells together with microglia are central immune players in neuro-inflammation in the developing brain and adult brain. As early responders of inflammation, mast cells rapidly degranulate in response to inflammatory or other insults, releasing their mediators into the resident environment. Then, vasoactive and pro-inflammatory mediators such as histamine, prostaglandins, VEGF, VIP, IL-1 β , IL-6 and TNF- α released from mast cells induce the migration and accumulation of the other immune cells to the site of injury by increasing vascular permeability [8, 9, 45]. Moreover, mast cells also increase their number by maturing rapidly from their progenitors during increased inflammatory conditions, aggravating existing inflammatory condition [57]. Therefore, this increased neuro-inflammatory response contributes to the development of brain damage in the developing brain during perinatal period.

It is well established that perinatal hypoxia-, hypoxic-ischemia-, or the other insults-induced inflammation result in perinatal brain injuries [3, 13, 14]. Furthermore, previous clinical studies demonstrated that increased inflammatory biomarkers in the plasma and/or the cerebrospinal fluid of newborn infants with perinatal brain injury are associated with

related-brain damage and its neurologic outcomes [15-19]. However, evidence that neuro-inflammation involving brain mast cells are implicated in perinatal brain injury has been obtained mostly from animal studies rather than clinical studies.

In experimental studies, it has been reported that increased levels of pro-inflammatory mediators, as mentioned above, in the brain lead to perinatal cerebral white matter damage by inhibiting the maturation of oligodendrocytes [26, 58]. It was reported that microglial activation plays a central role in neonatal excitotoxic lesions of the murine periventricular white matter [7]. Thus, considering that mast cells activate microglia in the brain, it can be alleged that mast cells also indirectly contribute to perinatal brain injury by activating central immune microglial cells.

In an experimental study has been shown that administration of a mast cell stabilizer cromoglycate (cromolyn sodium) reduced brain lesions due to IL-9-induced mast cell activation in a model of ibotenate-induced neonatal excitotoxic brain injury in mouse pups [52]. In a later study using a model of ibotenate-induced excitotoxic brain injury in neonatal mice, mast cell activation was shown to contribute to excitotoxic brain injury by exacerbating TGF- β 1 toxicity [53]. Furthermore, in a study, it was demonstrated that brain mast cell numbers increased significantly following hypoxia-ischemia in the model of unilateral hypoxia-ischemia in newborn rats [51]. Moreover, the same study also showed that administration of mast cell stabilizing agent cromolyn sodium restricted the brain injury [51]. In a later study using the same experimental model, it was shown that activation and number of brain mast cells and also activation of microglial cells increased in rat pups exposed to hypoxia-ischemia [11]. In the same study, it was also

demonstrated that cromolyn treatment suppressed the activation of brain mast cells and microglial cells, and reduced the number of brain mast cells and brain damage [11]. In addition to these previous studies, we have recently demonstrated for the first time histologically that ibotenate-induced excitotoxicity caused the activation of brain mast cells and increased their number in a model of ibotenate-induced excitotoxic brain

underlying perinatal brain injury is depicted in Figure 1.

Conclusion

Taken together the studies on the role of brain mast cells, inflammation and excitotoxicity in perinatal brain injury, it can be asserted that there is a bidirectional interaction between brain mast cells and excitotoxicity resulting in perinatal brain injuries. Brain mast cells may

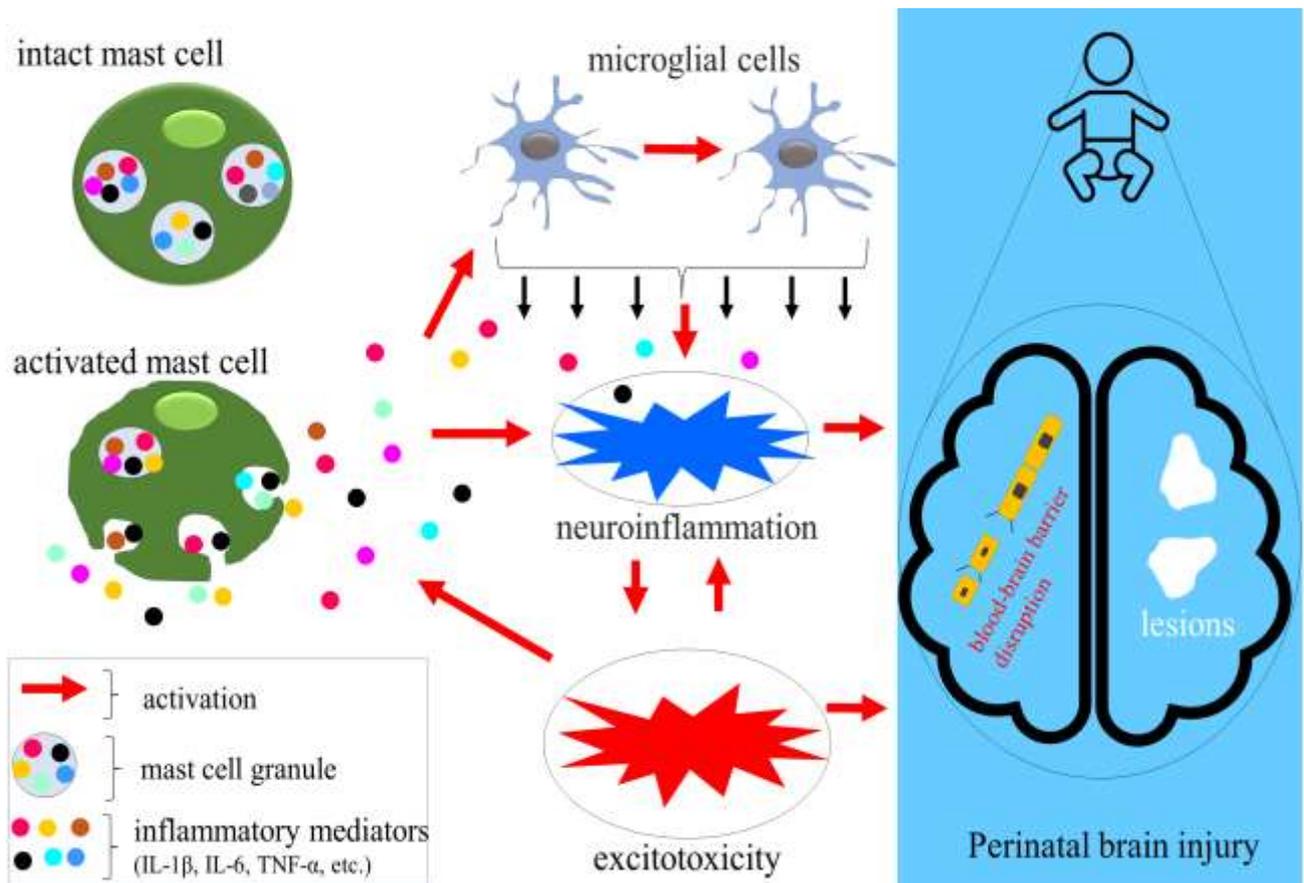


Figure 1. Schematic representation that depicts the potential bidirectional role of brain mast cells in neuroinflammation and excitotoxicity underlying perinatal brain injury.

injury in newborn rats [59, 60]. Thus, previous studies and our findings clearly indicate that the excitotoxicity following neuro-inflammation and excitotoxic-induced neuro-inflammation involving brain mast cells both contribute to perinatal brain injury. The potential bidirectional role of brain mast cells in neuroinflammation and excitotoxicity

play a central role in excitotoxicity following inflammation in the developing brain. Because, it is known that pro-inflammatory cytokines such as IL-1 β , IL-6 and TNF- α leading to the inflammation induce the excitotoxicity in the brain, resulting in exacerbation of neuro-inflammation. Both brain mast cells and microglia are major source of these pro-

inflammatory cytokines in the brain, however mast cells are also able to induce the activation of brain-resident microglial cells. Thus, brain mast cells act as a maestro in the exacerbation of neuro-inflammation and the development of inflammation-induced excitotoxicity in the developing brain. Excitotoxicity would in turn induce the activation of brain mast cells and an increase in their number during the brain development. Of course, the excitotoxicity is a common mechanism of perinatal brain injuries, however stabilization of brain mast cells emerges as an important target in breaking this vicious circle. Clinical trials with chemical mast cell stabilizers (cromolyn sodium, ketotifen fumarate) or plant-derived mast cell stabilizers (luteolin, thymoquinone) will provide a good feedback on whether mast cell stabilization will be effective in preventing perinatal brain damage in the human neonates in the future.

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References

- [1]Aslam S, Strickland T, Molloy EJ. Neonatal Encephalopathy: Need for Recognition of Multiple Etiologies for Optimal Management. *Front Pediatr.* 2019;7:142.
- [2]Larroque B, Ancel PY, Marret S, et al. Neurodevelopmental disabilities and special care of 5-year-old children born before 33 weeks of gestation (the EPIPAGE study): a longitudinal cohort study. *Lancet.* 2008;371(9615):813-20.
- [3]Novak CM, Ozen M, Burd I. Perinatal Brain Injury: Mechanisms, Prevention, and Outcomes. *Clin Perinatol.* 2018;45(2):357-75.
- [4]Degos V, Loron G, Mantz J, et al. Neuroprotective strategies for the neonatal brain. *Anesth Analg.* 2008;106(6):1670-80.
- [5]Burd I, Welling J, Kannan G, et al. Excitotoxicity as a Common Mechanism for Fetal Neuronal Injury with Hypoxia and Intrauterine Inflammation. *Adv Pharmacol.* 2016;76:85-101.
- [6]Dommergues MA, Plaisant F, Verney C, et al. Early microglial activation following neonatal excitotoxic brain damage in mice: a potential target for neuroprotection. *Neuroscience.* 2003;121(3):619-28.
- [7]Tahraoui SL, Marret S, Bodénant C, et al. Central role of microglia in neonatal excitotoxic lesions of the murine periventricular white matter. *Brain Pathol.* 2001;11(1):56-71.
- [8]Hendriksen E, van Bergeijk D, Oosting RS, et al. Mast cells in neuroinflammation and brain disorders. *Neurosci Biobehav Rev.* 2017;79:119-133.
- [9]Abbott NJ. Inflammatory mediators and modulation of blood-brain barrier permeability. *Cell Mol Neurobiol.* 2000;20(2):131-47.

- [10] Mallard C, Tremblay ME, Vexler ZS. Microglia and Neonatal Brain Injury. *Neuroscience*. 2019;405:68-76.
- [11] Jin Y, Silverman AJ, Vannucci SJ. Mast cells are early responders after hypoxia-ischemia in immature rat brain. *Stroke*. 2009;40(9):3107-12.
- [12] Dong H, Zhang X, Wang Y, et al. Suppression of Brain Mast Cells Degranulation Inhibits Microglial Activation and Central Nervous System Inflammation. *Mol Neurobiol*. 2017;54(2):997-1007.
- [13] Leavy A, Jimenez Mateos EM. Perinatal Brain Injury and Inflammation: Lessons from Experimental Murine Models. *Cells*. 2020;9(12):2640.
- [14] Li B, Concepcion K, Meng X, et al. Brain-immune interactions in perinatal hypoxic-ischemic brain injury. *Prog Neurobiol*. 2017;159:50-68.
- [15] Aly H, Khashaba MT, El-Ayouty M, et al. IL-1beta, IL-6 and TNF-alpha and outcomes of neonatal hypoxic ischemic encephalopathy. *Brain Dev*. 2006;28(3):178-82.
- [16] Liu J, Feng ZC. Increased umbilical cord plasma interleukin-1 beta levels was correlated with adverse outcomes of neonatal hypoxic-ischemic encephalopathy. *J Trop Pediatr*. 2010;56(3):178-82.
- [17] Tsukimori K, Komatsu H, Yoshimura T, et al. Increased inflammatory markers are associated with early periventricular leukomalacia. *Dev Med Child Neurol*. 2007;49(8):587-90.
- [18] Lin CY, Chang YC, Wang ST, et al. Altered inflammatory responses in preterm children with cerebral palsy. *Ann Neurol*. 2010;68(2):204-212.
- [19] Wu J, Li X. Plasma Tumor Necrosis Factor-alpha (TNF- α) Levels Correlate with Disease Severity in Spastic Diplegia, Triplegia, and Quadriplegia in Children with Cerebral Palsy. *Med Sci Monit*. 2015;21:3868-74.
- [20] Magalhães RC, Moreira JM, Lauar AO, et al. Inflammatory biomarkers in children with cerebral palsy: A systematic review. *Res Dev Disabil*. 2019;95:103508.
- [21] Demir C, Baranoglu Kilinc Y, Kilinc E. Increased Systemic Inflammatory Response With Mast Cell Activation In Elder Children With Cerebral Palsy. *Clin Exp Health Sci*. 2022 (in press).
- [22] Hagberg H, Gilland E, Bona E, et al. Enhanced expression of interleukin (IL)-1 and IL-6 messenger RNA and bioactive protein after hypoxia-ischemia in neonatal rats. *Pediatr Res*. 1996;40(4):603-609.
- [23] Quinlan S, Merino-Serrais P, Di Grande A, et al. The Anti-inflammatory Compound Candesartan Cilxetil Improves Neurological Outcomes in a Mouse Model of Neonatal Hypoxia. *Front Immunol*. 2019;10:1752.
- [24] Fan LW, Mitchell HJ, Tien LT, et al. Interleukin-1beta-induced brain injury in the neonatal rat can be ameliorated by alpha-phenyl-n-tert-butyl-nitrone. *Exp Neurol*. 2009;220(1):143-153.
- [25] Cai Z, Pang Y, Lin S, et al. Differential roles of tumor necrosis factor-alpha and interleukin-1 beta in lipopolysaccharide-induced brain injury in the neonatal rat. *Brain Res*. 2003;975(1-2):37-47.
- [26] Favrais G, van de Looij Y, Fleiss B, et al. Systemic inflammation disrupts the developmental program of white matter. *Ann Neurol*. 2011;70(4):550-65.
- [27] Sherwin C, Fern R. Acute lipopolysaccharide-mediated injury in neonatal white matter glia: role of TNF-

- alpha, IL-1beta, and calcium. *J Immunol.* 2005;175(1):155-61.
- [28] Hagberg H, Thornberg E, Blennow M, et al. Excitatory amino acids in the cerebrospinal fluid of asphyxiated infants: relationship to hypoxic-ischemic encephalopathy. *Acta Paediatr.* 1993;82(11):925-29.
- [29] Gücüyener K, Atalay Y, Aral YZ, et al. Excitatory amino acids and taurine levels in cerebrospinal fluid of hypoxic ischemic encephalopathy in newborn. *Clin Neurol Neurosurg.* 1999;101(3):171-74.
- [30] Ruppel RA, Kochanek PM, Adelson PD, et al. Excitatory amino acid concentrations in ventricular cerebrospinal fluid after severe traumatic brain injury in infants and children: the role of child abuse. *J Pediatr.* 2001;138(1):18-25.
- [31] Johnston MV. Excitotoxicity in perinatal brain injury. *Brain Pathol.* 2005;15(3):234-40.
- [32] Pregnotato S, Chakkarapani E, Isles AR, et al. Glutamate Transport and Preterm Brain Injury. *Front Physiol.* 2019;10:417.
- [33] Olmos G, Lladó J. Tumor necrosis factor alpha: a link between neuroinflammation and excitotoxicity. *Mediators Inflamm.* 2014;2014:861231.
- [34] Takeuchi H, Jin S, Wang J, et al. Tumor necrosis factor-alpha induces neurotoxicity via glutamate release from hemichannels of activated microglia in an autocrine manner. *J Biol Chem.* 2006;281(30):21362-368.
- [35] Hawkins RA. The blood-brain barrier and glutamate. *Am J Clin Nutr.* 2009;90(3):867-74.
- [36] Köles L, Kató E, Hanuska A, et al. Modulation of excitatory neurotransmission by neuronal/glia signalling molecules: interplay between purinergic and glutamatergic systems. *Purinergic Signal.* 2016;12(1):1-24.
- [37] Hertz L. The Glutamate-Glutamine (GABA) Cycle: Importance of Late Postnatal Development and Potential Reciprocal Interactions between Biosynthesis and Degradation. *Front Endocrinol (Lausanne).* 2013;4:59.
- [38] Meldrum BS. Glutamate as a neurotransmitter in the brain: review of physiology and pathology. *J Nutr.* 2000;130(4S Suppl):1007-1015.
- [39] Niswender CM, Conn PJ. Metabotropic glutamate receptors: physiology, pharmacology, and disease. *Annu Rev Pharmacol Toxicol.* 2010;50:295-322.
- [40] Purves D, Augustine GJ, Fitzpatrick D, et al. *Neuroscience.* 2nd edition. Sunderland (MA): Sinauer Associates; 2001. Glutamate Receptors. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK10802/>
- [41] Silverstein FS, Buchanan K, Johnston MV. Perinatal hypoxia-ischemia disrupts striatal high-affinity [³H]glutamate uptake into synaptosomes. *J Neurochem.* 1986;47(5):1614-19.
- [42] Chahal H, D'Souza SW, Barson AJ, et al. Modulation by magnesium of N-methyl-D-aspartate receptors in developing human brain. *Arch Dis Child Fetal Neonatal Ed.* 1998;78(2):116-120.
- [43] Kilinc E, Baranoglu Kilinc Y. Mast cell stabilizers as a supportive therapy can contribute to alleviate fatal inflammatory responses and severity of pulmonary complications in COVID-19 infection. *Anatol Clin.* 2020; 25 (supplement 1): 111-119.
- [44] Koyuncu Irmak D, Kilinc E, Tore F. Shared Fate of Meningeal Mast Cells and Sensory Neurons in Migraine. *Front Cell Neurosci.* 2019;13:136.

- [45] Kempuraj D, Selvakumar GP, Thangavel R, et al. Mast Cell Activation in Brain Injury, Stress, and Post-traumatic Stress Disorder and Alzheimer's Disease Pathogenesis. *Front Neurosci.* 2017;11:703.
- [46] Kilinc E, Ankarali S, Torun IE, et al. Receptor mechanisms mediating the anti-neuroinflammatory effects of endocannabinoid system modulation in a rat model of migraine. *Eur J Neurosci.* 2020.
- [47] Theoharides TC, Alysandratos KD, Angelidou A, et al. Mast cells and inflammation. *Biochim Biophys Acta.* 2012;1822(1):21-33.
- [48] Tore F, Tuncel N. Mast cells: target and source of neuropeptides. *Curr Pharm Des.* 2009;15(29):3433-45.
- [49] Lorentz A, Baumann A, Vitte J, et al. The SNARE Machinery in Mast Cell Secretion. *Front Immunol.* 2012;3:143.
- [50] Kilinc E, Torun IE, Cetinkaya A, et al. Mast cell activation ameliorates pentylentetrazole-induced seizures in rats: The potential role for serotonin. *Eur J Neurosci.* 2021.
- [51] Jin Y, Silverman AJ, Vannucci SJ. Mast cell stabilization limits hypoxic-ischemic brain damage in the immature rat. *Dev Neurosci.* 2007;29(4-5):373-84.
- [52] Patkai J, Mesples B, Dommergues MA, et al. Deleterious effects of IL-9-activated mast cells and neuroprotection by antihistamine drugs in the developing mouse brain. *Pediatr Res.* 2001;50(2):222-230.
- [53] Mesplès B, Fontaine RH, Lelièvre V, et al. Neuronal TGF-beta1 mediates IL-9/mast cell interaction and exacerbates excitotoxicity in newborn mice. *Neurobiol Dis.* 2005;18(1):193-205.
- [54] Traina G. Mast cells in the brain - Old cells, new target. *J Integr Neurosci.* 2017;16(s1):69-83.
- [55] Héron A, Dubayle D. A focus on mast cells and pain. *J Neuroimmunol.* 2013;264(1-2):1-7.
- [56] Dong H, Wang Y, Zhang X, et al. Stabilization of Brain Mast Cells Alleviates LPS-Induced Neuroinflammation by Inhibiting Microglia Activation. *Front Cell Neurosci.* 2019;13:191.
- [57] Bulfone-Paus S, Nilsson G, Draber P, et al. Positive and Negative Signals in Mast Cell Activation. *Trends Immunol.* 2017;38(9):657-67.
- [58] Hagberg H, Mallard C, Ferriero DM, et al. The role of inflammation in perinatal brain injury. *Nat Rev Neurol.* 2015;11(4):192-208.
- [59] Baranoğlu Kılınç Y. Yenidoğan sıçanlarda ibotenate ile oluşturulan eksitotoksik beyin hasarı üzerine kapsaisin koruyucu etkileri. *Tıpta Uzmanlık Tezi.* 2021.
- [60] Dilek M, Baranoğlu Kilinc Y, Kilinc E, et al. Activation of TRESK background potassium channels by cloxyquin exerts protective effects against excitotoxic-induced brain injury and neuroinflammation in neonatal rats. *Research Square.* 2021. <https://doi.org/10.21203/rs.3.rs-1188598/v1>.