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From Plugging the Dam to Fueling the Firing: Platelets Breach the Barrier to Seize the Brain

Platelets Promote Epileptic Seizures by Modulating Brain Serotonin Level, Enhancing Neuronal Electric Activity, and Contributing to Neuroinflammation and Oxidative Stress

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The drugs currently available for treating epilepsy are only partially effective in managing this condition. Therefore, it is crucial to investigate new pathways that induce and promote epilepsy development. Previously, we found that platelets interact with neuronal glycolipids and actively secrete pro-inflammatory mediators during central nervous system (CNS) pathological conditions such as neuroinflammation and traumatic brain injury (TBI). These factors increase the permeability of the blood–brain barrier (BBB), which may create a predisposition to epileptic seizures. In this study, we demonstrated that platelets substantially enhanced epileptic seizures in a mouse model of pentylenetetrazole (PTZ)-induced seizures. We found that platelets actively secreted serotonin, contributed to increased BBB permeability, and were present in the CNS parenchyma during epileptic seizures. Furthermore, platelets directly stimulated neuronal electric activity and induced the expression of specific genes related to early neuronal response, neuroinflammation, and oxidative phosphorylation, leading to oxidative stress in neurons. The intracranial injection of physiological numbers of platelets that mimicked TBI-associated bleeding was sufficient to induce severe seizures, which resembled conventional PTZ-induced epileptic activity. These findings highlight a conceptually new role of platelets in the development of epileptic seizures and indicate a potential new therapeutic approach targeting platelets to prevent and treat epilepsy.

Commentary

The blood–brain barrier (BBB), a unique barrier that limits the movement of ions, molecules, and cells between the blood and the brain, can be breached by brain insults such as trauma and during seizures. This is believed to contribute to epileptogenesis.[1,2] Naturally, how the brain reacts to blood constituents during this unusual exposure is of considerable interest.[1,3,4] There is a growing body of literature on how certain blood components including heme from red blood cells and serum albumin can contribute to development of epilepsy following brain insults.[5,6] Heme and albumin can contribute to epileptogenesis by triggering secondary processes including activation of immune responses, altering glial function, and augmenting neuronal excitability.[5,6] Similarly, the role of vascular immune cells such as macrophages and T-cells in epileptogenesis has been investigated.[3] However, the role of platelets, the second most numerous cell type in the blood, has received little attention in the context of a compromised BBB and seizures. Platelets, small anucleate blood cells once considered mere cell fragments which aggregated to form platelet plugs for hemostasis, are now recognized to play roles in inflammation, angiogenesis, and tissue repair.[7] Platelets contain secretory granules with several bioactive molecules including neurotransmitters such as serotonin (5-HT), inflammatory molecules, platelet activating factor, and growth factors, which they can release upon activation. Interestingly, platelets undergo degranulation and release transmitters in response to certain gangliosides enriched in lipid rafts in neuronal membranes which promotes synaptic plasticity after brain injury.[8] However, whether platelets play a traditional role by stopping BBB leaks, have noncanonical roles in inflammation and tissue repair, or augment neuropathology during seizures is not known. Kopeikina and colleagues[9] bring to bear a diverse set of tools to directly address the multifaceted role of platelets in BBB permeability and network excitability during seizures.

The study leads off with the demonstration that mice depleted of serum platelets exhibit delayed and less severe acute seizures in response to pentylenetetrazole (PTZ) injection followed by a systematic investigation of the logical interpretation that platelets enter the brain during seizures and promote excitability. Contrary to expectations based on the traditional role of platelets, mice depleted of serum platelets had lower

BBB permeability following PTZ-induced seizures than mice with normal platelets, indicating that platelets augment BBB permeability during seizures. Unfortunately, although PTZ injection was associated with behavioral seizures, the authors defined any 10-second period with over 20 to 30 high amplitude spiking (HAS) events (events crossing a 20-μV threshold above baseline) as an electrographic seizure, regardless of the evolution and termination of activity, making it difficult to assess whether the "seizures" represented ictal activity. Nevertheless, immunostaining confirmed that platelets, normally restricted to the vasculature, were present in the brain parenchyma where they associated with neuronal lipid rafts following PTZ-induced seizures. Consistent with in vitro studies identifying that neuronal lipid rafts promote 5-HT release from platelets, PTZ-induced seizures increased 5-HT levels, measured using liquid chromatography and mass spectrometry, in brain tissue obtained within minutes after seizures. However, increase in 5-HT in response to PTZ-seizures was significantly blunted in platelet-depleted mice. Moreover, both mice treated with a drug to deplete 5-HT and transgenic mice with deletions of a ganglioside enriched in neuronal lipid rafts showed reduced electrographic HAS events, suggesting that the interaction of platelets with lipid rafts and subsequent release of 5-HT are essential for platelet-mediated enhancement of excitability. Furthermore, they demonstrate that direct intracranial injection of either platelets, platelet-rich plasma, or 5-HT could trigger HAS even in the absence of PTZ. The ability of platelets to directly increase network activity was further confirmed using multielectrode array recordings in acute brain slices. In control studies, HAS activity was absent following injection of platelet poor plasma and reduced in platelet-depleted mice, suggesting that while injected platelets are sufficient to induce neuronal hyperactivity, circulating platelets also reach the brain and contribute to network excitability. In an elegantly designed set of experiments, using adoptive transfer of platelets from unmanipulated or 5-HT-depleted donor mice into unmanipulated or platelet-depleted acceptor mice, they demonstrate that platelet-derived, and not brain-derived, 5-HT contributes to enhancement of PTZ-induced seizures. Although the study focused on the role of platelets on excitability, whole transcriptome RNA sequencing analysis and subsequent validation in unmanipulated and platelet-depleted mice revealed that exposure to platelets increases markers of activity, inflammation, and oxidative stress that merit further analysis.

Overall, the study provides novel insights into the potential role for platelets in seizures. The data support a cascade of events by which platelets entering the brain through compromised BBB undergo degranulation and release 5-HT on contact with neuronal lipid rafts which further permeabilizes BBB and increases acute neural activity, setting up a vicious cycle of enhanced platelet entry and excitability. They further propose that the cascade of platelet activation and excitability contribute to changes in oxidative phosphorylation and release of inflammatory cytokines which could contribute to epileptogenesis. This curious cycle of platelet-derived 5-HT and neuronal activity has interesting parallels to the itch-scratch cycle, wherein scratching excites pain fibers leading to feedback release of 5HT on spinal neurons which further promotes the sensation of itch, while suppressing pain, resulting in vicious escalation of itch.[10] The demonstration that platelets, unlike albumin,[6] promote acute seizures is interesting and whether this could contribute to epileptogenesis needs careful consideration. Regrettably, the authors conflate chemically evoked acute increase in excitability with electrographic seizures and epileptogenesis. While their results indicate that platelets increase excitability and may be pro-convulsant, the role of platelets in spontaneous recurrent seizures and epileptogenesis remain open questions. Moreover, the relation of HAS activity to seizures is tenuous as electroencephalographs were recorded using cerebellar surface electrodes and were not correlated with behavioral seizures. Regardless, the results introduce platelets as a new player in the compromised BBB and raise questions about the long-term consequences of platelet-derived increases in 5-HT, inflammatory cytokines, and reactive oxygen species in epileptogenesis. The duration of platelet-mediated effects following a seizure and whether platelets lead to chronic changes in neuronal activity, synaptic plasticity, and network reorganization will need further analysis in established models of epileptogenesis and by long-term follow-up of animals with intracerebral platelet injections. Moreover, the notion that platelets contribute solely to pathological changes may be simplistic, as the interaction between platelets and neuronal lipid rafts can aid vascular integrity following central nervous system injury,[8] and platelets have been proposed to support immune responses, angiogenesis, and tissue repair.[7] Thus, as with inflammatory responses, the location and timing of platelets in the brain may determine their role in repair versus epileptogenesis.

In summary, Kopeikina et al,[9] demonstrate that the role of platelets in BBB compromise during injury and seizures can no longer be ignored. While the study focused on mechanisms and consequences of platelet-derived 5-HT, the RNA sequencing data identify a wide array of pathways from inflammation to oxidative stress which merit further studies. Further validation of the role of platelets in promoting seizures in models of epilepsy could reveal whether mechanisms identified here are viable targets to limit intractable status epilepticus.

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