Biomimetic Strategies to Treat Traumatic Brain Injury by Leveraging Fibrinogen

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Abstract

There were over 27 million new cases of traumatic brain injuries (TBIs) in 2016 across the globe. TBIs are often part of complicated trauma scenarios and may not be diagnosed initially as primary clinical focus is on stabilizing the patient. Interventions used to stabilize trauma patients may inadvertently impact the outcomes of TBIs. Recently, there has been a strong interest in the trauma community towards administrating fibrinogen-containing solutions intravenously to help stabilize trauma patients. While this interventional shift may benefit general trauma scenarios, fibrinogen is associated with potentially deleterious effects for TBIs. Here, we deconstruct what components of fibrinogen may be beneficial as well as, potentially harmful, following TBI and extrapolate this to biomimetic approaches to treat bleeding and trauma that may, also, lead to better outcomes following TBI.

Traumatic brain injury and breakdown of the blood break barrier

Traumatic brain injury (TBI) is one of the most common neurologic disorders and a leading cause of disability affecting patient function and quality of life (*1, 2*). Most recent global estimates of the incidence and prevalence of TBI indicate that in 2016 alone there were over 27 million new cases of TBI with an age-standardized prevalence of TBI at 369 per 100,000 population (increase by 8.4% from 1990 to 2016) (*2*). Most concerning is that to date, despite promising pre-clinical data (*3*), no new pharmacological strategies have demonstrated improved patient outcomes in a phase III clinical trial. A TBI is defined as a mechanical insult that initiates immediate cellular death (i.e. primary injury) and stimulates a broad range of complex deleterious signaling cascades (i.e. secondary injury). In particular, the traumatic insult initiates

the breakdown of the blood-brain barrier (BBB) and the influx of plasma proteins and cytokines into the parenchyma that contributes to activation of both local and systemic inflammatory players.

The BBB disruption after TBI may lead to extravasation of blood components into the brain parenchyma. Studies in different TBI animal models have demonstrated acute and delayed BBB disruption followed by restoration as evidenced by extravasation of standard molecules, such as endogenous serum immunoglobulins (IgG) (*4, 5*) and/or intravenously injected small molecule tracers, including Evans Blue and horseradish peroxidase (HRP) (*4, 5*). For example, previous studies with focal injury models (controlled cortical impact; CCI) established the BBB disruption as indicated by the extravasation of HRP (*6*) or Evans Blue (*7, 8*) post-injury. Specifically, the BBB was compromised immediately after injury and remained significantly permeable for 5-7 days post-injury within the injury penumbra (with a second peak at ~3 days) (*6, 8*). Similarly, Schmidt et. al. demonstrated that BBB disruption displayed regional differences following diffuse midline fluid percussion injury (FPI) with prominent HRP leakage in the cerebral cortex (proximal to injury hub) and corpus callosum (*4*). Collectively, these seminal studies support the notion that TBI disrupts the BBB resulting in the extravasation of blood constituents into the normally impermeable brain parenchymal space. Moreover, our recent studies identify a size-dependent BBB permeability within the injury zone that enables nanotherapeutic delivery after TBI (*9, 10*).

Leveraging the positive effects of fibrinogen in trauma while avoiding the off-target effects in the CNS

There has been a push in trauma research to move from infusing with saline to infusion of plasma or plasma products (*11-14*). Plasma or plasma products reduce vascular permeability in a number of trauma models and improves clinical outcomes including survival (*15-17*). However, there are safety challenges with plasma, including lot to lot variability and immune concerns, promoting the study of specific plasma components following trauma. Recent trauma studies including clinical trials have shown the presence of fibrinogen, either in plasma or as a single therapeutic component, reduces the need for transfusions and increases survival (*18-23*).

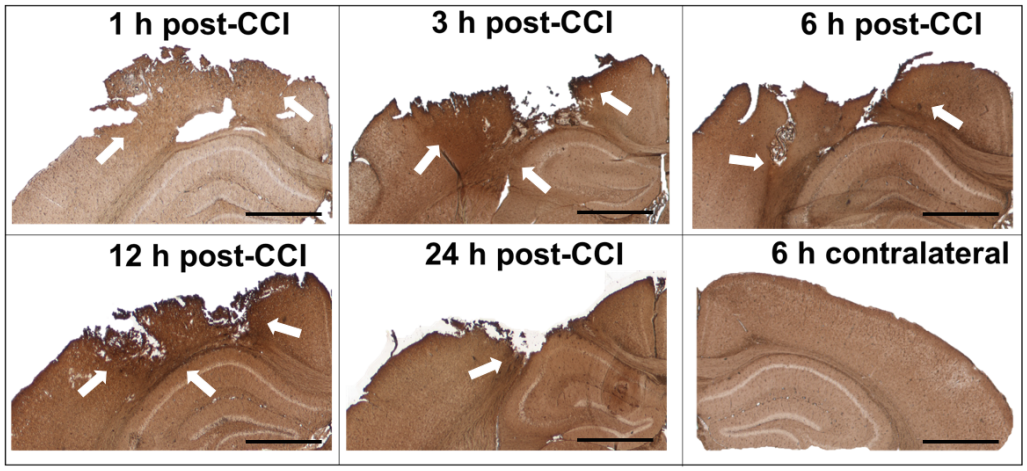

Figure 1: Fibrin(ogen) deposition post-TBI. Representative time-course immunohistochemistry images with anti-fibrin(ogen) antibody in mouse brain tissue sections following CCI (HRP secondary antibody followed by DAB chromogenic substrate). Regions of positive fibrin(ogen) were observed in the brain parenchyma out to 24hrs post-injury as indicated with the dark brown stain highlighted with white arrows are regions of positive fibrin(ogen) compared to control contralateral tissue. Scale bars = 1mm.

Fibrinogen is a glycoprotein found in plasma that is critical for hemostasis but is also linked to important roles in reducing endothelial dysfunction and vascular permeability (*24*). Fibrinogen is thought to be critical in trauma and may reduce edema, yet in the TBI literature, fibrinogen is implicated in worse outcomes including neurodegeneration (*25, 26*). The disruption of the BBB following TBI provides ample opportunity for fibrinogen to extravasate into the brain parenchyma. The representative immunostain images in Figure 1 show the prominent presence of fibrin(ogen) up to 24hr post-injury in a mouse focal TBI model (controlled cortical impact; CCI; **Figure 1**).

The disconnect between the trauma and TBI disciplines is critical to understand because there could be unintended negative impacts to infusing fibrinogen-containing compounds. Additionally, by understanding the disconnect between the trauma and TBI communities, we may be able to develop new, more effective therapies that are suitable for trauma broadly and CNS trauma, in particular.

What is the source of the differences between the trauma and CNS literature?

While the source of the differences between the trauma and CNS literature is multifactorial, a major contributor may come down to the complex structure of fibrinogen. Fibrinogen is composed of two identical chains connected by disulfide bonds. Each half consists of three polypeptide chains (alpha, beta, and gamma) (*27, 28*). Each of these chains, and in fact their subunits, play important roles in the permeabilization and stabilization of vessels as well as inflammation and neural injury. The alpha, beta, and gamma chains are joined in the central domain of fibrinogen, which is found within the "E" region of the molecule (**Figure 2)**. The "E" region is flanked on either side by the "D" regions.

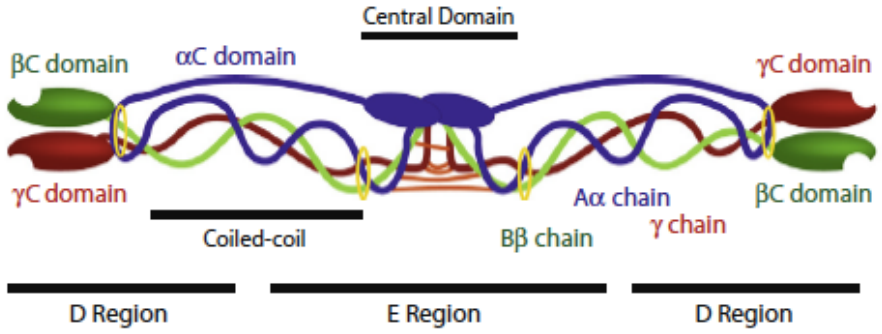

Figure 2: Fibrinogen structure. Aα chains are shown in blue, Bβ chains are shown in green and γ chains are shown in red. Interchain disulfide bridges connecting the six polypeptide chains in the central domain are shown in orange and disulfide rings stabilizing the coiled-coil regions are shown in yellow ((*28*))

The "E" and "D" regions correspond to degradation products resulting from proteolytic cleaving by plasmin. D-dimers also result from cleavage of polymerized fibrin. Following activation of the coagulation cascade, soluble fibrinogen is converted to an insoluble fibrin matrix via proteolytic cleavage by thrombin of the fibrinopeptides within the central domain. Release of the

fibrinopeptides exposes active fibrin knob sites which facilitate polymerization via interaction with corresponding fibrin hole sites on the beta and gamma chains within the D regions on adjacent fibrin(ogen) molecules. The various proteolytic fragments, including the E fragments, D fragments, and fibrinopeptides are known to influence inflammation and tissue repair outcomes.

Endothelial permeabilization and fibrinogen

The gamma chain of fibrinogen is an important player in disruption of tight junctions and transport. A peptide from the gamma chain of fibrinogen, known as γ3 corresponding to amino acids 117-133 of the gamma chain, reportedly promotes intracellular transport of nanoparticles across endothelial cells (*29*). Fibrinogen has been shown to impact endothelial cell tight junctions through ICAM signaling which strongly suggests that the gamma chain triggers the permeability (*30*). The same group noted that it appeared to be fibrinogen and not fibrin that was involved in the vascular permeability (*30*).

However, the gamma chain is not alone in promoting permeabilization. Work that has dissected the role of the different peptides involved in the fibrinogen structure has shown that the beta polypeptide chain is responsible for endothelial permeabilization (*27, 31*). However, a subunit of the beta chain also stabilizes tight junctions of endothelial cells (*32, 33*). This subunit, the fibrinopeptide Bbeta15-42, reduces vascular leakage and stabilizes endothelial cell junctions *in vivo* (*34*). This peptide is known as FX06 and has been studied in clinical trials (*35-37*).

Finally, the alpha chain interacts with the Sdc1 and promotes tight junction formation following trauma (*24*). So, parts of fibrinogen promote permeabilization of vessels, and parts promote stabilization and reduce leakiness. Depending on the state of coagulation and ratio of fibrinogen to fibrin polymerization, different parts of the chains will be more available for permeabilization (*27*). This complexity may explain, in part, some of the differences between the observations in the trauma field and those regarding CNS injury.

Inflammation and fibrinogen

At sites of BBB disruption, fibrinogen converts to fibrin which releases reactive oxygen species via triggered M1-like activation of macrophages and microglia (*38, 39*). While fibrinogen is not proinflammatory, fibrin is (*38*). The cleavage of fibrinogen exposes part of the gamma chain that can bind with the CD11b/CD18 integrin on a number of cells and triggers the M1 phenotype in macrophages and microglia even in the absence of an injury (*39*). In this particular study, fibrinogen from plasma or recombinant fibrinogen trigged inflammation and demyelination, but a fibrinogen derived from transgenic mice that produce a mutated gamma chain that does not bind the CD11b/CD18 receptor showed far less inflammation and demyelination. The exciting thing is that when the binding region to CD11b/CD18 is either absent or blocked with an inhibitor, the inflammation and demyelination are not observed (*39*). In fact, most of the neuroinflammatory responses to fibrin are associated with the activation of the CD11b/CD18 receptor, and inhibitors of this interaction significantly reduce inflammation and degeneration associated with a range of disease and injury models including TBI, Alzheimer's disease, and multiple sclerosis (MS) (*40*). Additionally, Schachtrup et al. recently demonstrated that fibrinogen is a courier for latent transforming growth factor-β protein (TGF-β) that ultimately stimulated reactive astrogliosis via the active TGF-β pathway (*41*). Thus, if one is able to engineer a version of fibrinogen without the reactive portions of the gamma chain, one may be able to leverage the positive impacts of fibrinogen without the inflammatory component.

Fibrinogen and neural inhibition

Fibrinogen inhibits neurite outgrowth (*42*) via interaction with beta3 receptor on neurons, and it inhibits remyelination by oligodendrocytes (*43*). However, using ANCROD which cleaves only fibrinopeptide A (*44*) from fibrinogen leads to remyelination in the same injury model (*43*). It is possible that fibrinogen has negative impacts on CNS tissue beyond the issues of permeability and inflammation. However, one of the challenges in the literature is that some treat fibrin and fibrinogen interchangeably (*45*) when they have very different structures, roles, and impacts on tissue in general and the CNS in particular. This is, in part, a function of groups using antibodies that react with both fibrin and fibrinogen in the brain (*46*). By developing materials that allow us to probe the peptides associated with the fibrinogen chains, we will be able to determine the impact of the different peptides on both the vascular and neural components of the CNS.

Synthetic Mimics of Fibrinogen for Trauma

Much of the work utilizing biomimetic strategies involving fibrinogen look at using the whole molecule as a template for building new tissue models (*24, 47, 48*). There is great logic in this approach. Fibrinogen presents a number of binding site for cells to attach, and fibrin(ogen) plays important roles in signaling events for tissue repair (*48, 49*). While there are fewer biomimetics of fibrinogen for hemostats, those that exist are quite exciting.

One of the best recent reviews of intravenous hemostats was by Dr. Pun's group (*50*); we summarize a few examples here. Fibrinogen coated albumin particles reduced bleeding in thrombocytopenic rabbits (*51*) but were large (3.5-4.5 um in diameter) leading to accumulation in the capillary beds of the lungs (*52*) motivating development of smaller particles. In recent years, fibrinogen-coated nanoparticles have shown promise in thrombocytopenic models (*53*). Interestingly, fibrinogen-based nanoparticles have also shown interest for delivering drugs to tumors since there are receptors in many cancers for components of fibrinogen (*54, 55*) and for delivery of growth factors to promote dermal wound healing (*56*).

Since the different regions of fibrinogen may have either beneficial or adverse impacts in TBI, it is exciting that a number of biomimetics have looked at using peptides drawn from fibrinogen. Liposomes carrying the fibrinogen γ chain dodecapeptide (HHLGGAKQAGDV), located on amino acids 400-411 of the γ chain, reduced bleeding in thrombocytopenic rats and rabbits compared with saline (*57, 58*). A variant of these particles also delivers adenosine diphosphate (ADP) which activates more platelets and has a rapid hemostatic response (*59, 60*). While these molecules are exciting, the presence of the dodecapeptide from the γ chain might be less than ideal for TBI since it may promote breakdown of the BBB. Liposomes with multiple targeting ligands for the glycoprotein IIb/IIIa receptor as well as von Willibrand factor have been pursued as well (*61*). Using longer peptides and more peptides increases the complexity of the design and may exhibit off-target effects.

For TBI, it seems wise to avoid peptides associated with permeabilizing vessels such as those associated with the γ chain and parts of the β chain. One approach is to focus on the RGD peptide from the α-chain. While RGD is ubiquitous throughout the body, the GPIIa/IIIb receptor on activated platelets has a tremendously high affinity for the RGD peptide and rapidly binds upon platelet activation (*62-65*). Polyester nanoparticle functionalized with RGD have been shown to

reduce bleeding in a number of models (*66, 67*) and are associated with better neurological outcomes following blast-induced brain injury (*68*). Ultimately, pursuing an approach that leverages the potential of fibrinogen but excludes the potentially problematic regions may be wise.

However, perhaps the wiser approach is to avoid the issues surrounding fibrinogen-mimics and look at alternative synthetic hemostats. Hydrogel particles with antibody fragments have shown promise in models of bleeding (*69*). These particles are highly deformable, and over time deform fibrin matrices to mimic the platelet response of clot retraction.

Conclusions and outlooks

Fibrinogen looks incredibly promising for treating trauma, but with TBI being involved in a large fraction of traumas, there are concerns about the potential for fibrinogen to exacerbate neurological outcomes. Bioconjugate approaches may be able to address the shortcomings of broad fibrinogen strategies by maximizing the benefits of specific molecular subunits while mitigating the risks. It is essential to understand the complexities and intricacies of trauma, coagulation, and neural injury to then apply that understanding to develop new fibrinogen-mimetic bioconjugate systems appropriate for all trauma scenarios.

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