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Review article

How is the formation of microthrombi after traumatic brain injury linked to inflammation?

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ABSTRACT

Traumatic brain injury (TBI) is characterized by mechanical disruption of brain tissue due to an external force and by subsequent secondary injury. Secondary brain injury events include inflammatory responses and the activation of coagulation resulting in microthrombi formation in the brain vasculature. Recent research suggests that these mechanisms do not work independently. There is strong evidence that FXII and platelet activation connects both, inflammation and the formation of microthrombi. This review summarizes the current knowledge on posttraumatic microthrombus formation and its link to inflammation.

1. Introduction

Traumatic brain injury (TBI) is the result of an outside force causing mechanical disruption of brain tissue triggering secondary pathomechanisms, which collectively exacerbate the primary injury. It often has devastating neurological effects and is the leading cause of disability in young adults. The resulting disabilities are numerous and of varying severity. Survivors of severe TBI commonly suffer from motor and cognitive disabilities, and mood disorders (Maas et al. 2017). In addition, TBI can lead to seizures and increases the risk of developing dementia and Alzheimer's disease (Maas et al. 2017). Not only patients with severe TBI, but also patients with mild or moderate TBI are at risk of developing long-lasting disabling conditions. Most patients sustain brain injuries in motor vehicle and sports accidents or due to interpersonal conflicts. In 2013, 2.8 million TBI-related Emergency Department visits and 56.000 TBI-related deaths were registered in the US (Taylor et al. 2017). The total medical costs in 2013 were estimated to range from $63.4 to $79.1 billion dollars (Ma et al. 2014). Despite this high socioeconomic relevance, causal treatment options are lacking and several clinical studies with initially promising active substances failed to prove their efficacy (Ker and Blackhall 2008; Nichol et al. 2015; Skolnick et al. 2014). This is mostly attributed to the highly heterogeneous clinical manifestations, causes and severity of injury and the complex set of pathomechanisms.

It is well accepted that inflammation is an important aspect of secondary injury mechanisms following TBI and can have detrimental as well as beneficial effects on the outcome (for a comprehensive overview see Jassam et al. 2017). The inflammatory response is propagated by biochemical events involving the local vascular system, the immune system, and various cells within the injured tissue. As it became evident in recent years that inflammation and thrombotic processes are tightly interrelated (Ekdahl et al. 2016), research should increasingly focus on the formation of microthrombi following TBI. This review summarizes the current knowledge of microthrombus formation and its link to inflammation in the traumatized brain. Due to limited data, the focus rests on the role of factor XII (FXII) and platelet-activating mechanisms.

Abbreviations: CCL2, chemokine (C−C motif) ligand 2; FXI, factor XI; FXII, factor XII; GP, glycoprotein; ICAM, intercellular adhesion molecule; PAF, platelet activating factor; TBI, traumatic brain injury

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2. Posttraumatic microthrombi formation

Thrombus formation at sites of endothelial disruption is a dynamic process that requires a defined series of molecular events including the action of platelets and activation of plasma coagulation factors. The first step of thrombus formation involves sequential interactions of platelet surface receptors with macromolecules of the exposed subendothelial matrix. For the initial recruitment of flowing platelets at high shear rates, the platelet receptor glycoprotein (GP)Ib is essential as explained in section 4. The initial platelet adhesion facilitates the binding and activation of other platelets resulting in the formation of reversible platelet aggregates, which are subsequently stabilized by a fibrin network (Varga-Szabo et al. 2008). Endothelial disruption also triggers activation of the plasma coagulation system, which acts in concert with activated platelets. Both, the tissue factor-driven extrinsic and the factor XII (FXII)-driven intrinsic pathway of plasma coagulation result in the formation of thrombin. This in turn enhances platelet activation and converts fibrinogen into fibrin, thereby contributing to thrombus growth.

Immediately after experimental TBI platelets are activated and form aggregates in the cerebral microvasculature, resulting in a decrease in peri-contusional blood flow (Dietrich et al. 1996; Schwarzmaier et al. 2010, 2016). The number of microthrombi increases within the first 3 days and then declines at 8 and 15 days after TBI (Lu et al. 2004; Stein et al. 2002; Schwarzmaier et al. 2016). Importantly, posttraumatic microthrombi formation seems to be independent of the severity (Stein et al. 2002) and nature (focal or diffuse pattern) (Hopp et al. 2016) of the primary injury. Similar to the situation in experimental TBI (Table 1), microthrombi formation is increasingly recognized as a common pathological feature in clinical TBI as well (Table 2). It is hypothesized that these thrombi might result in an ischemia-like injury of remote brain areas, leading to a worsening of the prognosis (Dietrich et al. 1996; Hopp et al. 2016; Laroche et al. 2012; Maegele 2013; Stein et al. 2002).

3. The role of factor XII

Activation of the contact-kinin system at an early stage after TBI leads to an increase of vascular permeability and consecutively to the development of brain edema (Kunz et al. 2013). Inhibition of the contact-kinin system on the level of the kinin receptors is protective after TBI by diminishing blood-brain barrier damage and inflammatory processes like astroglia activation and immune cell infiltration. Nevertheless, there exists a controversy about which kinin receptor (B1R or B2R) is the essential one involved in the pathomechanisms after injury (for a comprehensive review see Albert-Weissenberger et al. 2014a). Blocking the function of plasma kallikrein and activated FXII by administration of C1-esterase inhibitor resulted in reduced contusion volumes, less behavioral deficits, a reduction of cell migration to sites of inflammation inflammatory processes and cytokine release (Albert-Weissenberger et al. 2014b; Longhi et al. 2009; Longhi et al. 2008). The vascular permeability, which facilitates immune cell trafficking and contributes to brain edema was reduced after administration of C1-esterase inhibitor. Furthermore, administration of C1-esterase inhibitor diminished the formation of microthrombi as a significantly smaller amount of fibrin(ogen) and less vessel occlusion were found in the traumatic hemisphere of treated mice (Albert-Weissenberger et al. 2014b). These effects of C1-esterase inhibitor might be attributable to the inhibition of activated FXII.

Two most prominent functions of FXII are triggering the contact-kinin system as well as the intrinsic pathway of blood coagulation. Using FXII-deficient mice we found that posttraumatic bradykinin formation, which is the major product of the contact-kinin system exerting its effects via the kinin receptors, is strongly dependent on FXII (Hopp et al. 2017). Subsequently, brain edema formation, immune cell invasion, and cytokine release at the site of injury was diminished in FXII-

Table 1
Experimental TBI studies on microvascular thrombus formation and intravascular coagulation (CBF: Cerebral Blood Flow; H&E: Hematoxylin and Eosin stain; IHC: Immunohistochemistry).

	TBI model	Method of thrombus detection	Assessed points of time	Result
Mice				
Schwarzmaier et al. 2010	Controlled Cortical Impact	Intravital microscopy	0.5 h, 1 h, 1.5 h, 2 h	Microthrombi formation within first 2 h following injury, impairment of CBF in traumatic penumbra
Hopp et al. 2016	Weight drop, Cold lesion model	CBF, H&E, IHC (GPIb), H&E, IHC (GPIb)	7d, 1d, 3d	Decrease of CBF until day 7, platelet aggregates in perilesional areas in focal and diffuse trauma
Schwarzmaier et al. 2016	Controlled Cortical Impact	Intravital microscopy, H&E	2-3 h, 24 h	Intravascular microthrombi formed 2–3 h post-trauma; higher numbers of microthrombi were found 48 h after injury induction (occlusion of > 60% of all cerebral vessels)
Rats				
Hekmatpanah and Hekmatpanah 1985	Weight drop	H&E, toluidine blue staining	1 h, 3 h	Intravascular clots are part of microvascular obstruction
Dietrich et al. 1996	Fluid percussion	Autoradiographic study, platelet labeling: [^{111}In], local CBF: [^{14}C]-iodoantipyrine	0.5 h	Focal sites of platelet accumulations, but reduction of CBF in the whole injured hemisphere
Stein et al. 2002	Fluid percussion	IHC (antithrombin III)	1 h, 2 h, 24 h, 48 h	Intravascular thrombi occurred 1 h post-trauma, more widespread after 48 h
Lu et al. 2004	Controlled Cortical Impact	IHC (platelets)	1 h, 4 h, 1d, 3d, 8d, 15d	Increase of intravascular coagulation until day 3 also in non-injured areas
Ploplis et al. 2014	Controlled Cortical Impact	IHC (P-selectin, TF, fibrin[ogen])	15 min, 30 min	Local activation of blood coagulation
Pigs				
Stein et al. 2002	Axial-plane rotational acceleration head injury	IHC (antithrombin III)	8 h, 3d, 7d	Diffuse intravascular coagulation in scattered clusters

Table 2
Overview of studies on microvascular thrombus formation in human brain tissue following TBI (H&E: Hematoxylin and Eosin stain; IHC: Immunohistochemistry; PTAH: Phosphotungstic acid hematoxylin).

	Source of brain tissue	Method of thrombus detection	Time after injury	Result
Humans				
Huber et al. 1993	Autopsy	PTAH	1 h to ≥14d	Number of microthrombi increased towards day 9 post-trauma and decreased afterwards
Lafuente and Cervos-Navarro 1999	Autopsy	H&E, PTAH, Mallory-Heidenhain staining	0 h to 3wk	Enhanced presence of microthrombi in contused brains in both hemispheres, increase up to day 9
Stein et al. 2002	Decompression surgery	IHC (antithrombin III)	1 h to 9d	Microvascular thrombi visible until day 9 post-injury
Stein et al. 2004	Autopsy	IHC (antithrombin III)	up to 2d	Degree of intravascular microthrombosis is linked to neuronal death
Hopp et al. 2016	Decompression surgery and autopsy	IHC (GPIb)	N/A	Microvascular platelet aggregates in brain tissue as common pathological feature

deficient mice. Targeting activated FXII by a specific inhibitor administered once 1 h after brain injury had a similar effect (Hopp et al. 2017). In addition, microthrombi formation in the traumatized brain is strongly dependent on FXII or more precisely on FXII activation. It is important to note that FXII-deficiency as well as inhibition of activated FXII improved the functional outcome or diminished neuronal cell death but had no effect on bleeding (Hopp et al. 2016).

Activated FXII accelerates the activation of factor XI (FXI) followed by successive activation of factor IX and factor X, which finally results in thrombin generation. Selective inhibition of FXII-dependent FXI activation in a controlled cortical impact model in mice did neither significantly reduce microthrombi formation in the cerebral vessels nor the brain lesion volume (Schwarzmaier et al. 2016). Similarly, in our hands brain lesion volume, neuronal cell death, and brain inflammation were not significantly affected by FXI-deficiency in mice after focal TBI. The number of microthrombi, however, was reduced (unpublished data). Deficiency of FXI in mice significantly increased posttraumatic intracranial bleeding when compared to control mice (Hopp et al. 2016).

4. The role of platelets

One of the best-known actions of platelets is their function in hemostasis and thrombosis. Platelets, however, are also inflammatory effector cells, which are able to modulate innate immune responses as well as adaptive immunity (for a comprehensive overview seeRondina et al. 2013).

At high shear rates, the platelet receptor GPIb as a part of the GPIb-IX-V complex mediates the initial binding of platelets to the subendothelial matrix as a first step of thrombus formation. As inhibiting GPIb was recently shown to have antithrombotic and anti-inflammatory effects (Schuhmann et al. 2017), we studied its impact on focal TBI by administering 100 μg of a GPIb antigen-binding fragment (p0p/B Fab, Bergmeier et al. 2000) intravenously 1 h after injury induction. GPIb Fab-treatment was not associated with hemorrhages so far, neither in mouse models (Kleinschnitz et al. 2007; Kraft et al. 2015), nor in primates (Cauwenberghs et al. 2000). As readouts, we assessed brain lesion volume, neuronal cell death, immune cell infiltration, and microthrombus formation (methods as described in Hopp et al. 2016, 2017). In GPIb Fab-treated mice, vessel occlusion was reduced by 20%. Most interestingly, we observed less neuronal cell death and a highly significant decrease of immune cell infiltration in the damaged brain area 24 h after injury induction in the GPIb Fab-treated group (Fig. 1). As the subunit GPIbα hosts a binding site for FXIIa (Berndt et al. 2001), we next analyzed whether GPIb blockade influences bradykinin levels or, as a result of bradykinin release, mitigates blood-brain barrier damage (methods as described in Hopp et al. 2017). In control mice, plasma bradykinin levels increased within 2 h and blood-brain barrier instability was detectable 24 h after injury induction. No significant change in these parameters was found in GPIb Fab-treated mice (Fig. 2). Therefore, we suppose that platelet-induced inflammation in our experimental setup is not attributable to an interference with the contact-kinin system, but to other signaling cascades.

As a result of their activation, platelets secrete several cytokines that initiate and enhance pro-coagulant and pro-inflammatory cascades (Joseph et al. 2002). Two cytokines which are known to be upregulated in patients with TBI are interleukin-1β and tumor necrosis factor-α (Ross et al. 1994; Winter et al. 2002). Both interact with platelet activating factor (PAF) and exhibit pro-coagulant functions in this way. Conversely, PAF induces the synthesis of tumor necrosis factor-α and is involved in neutrophil extravasation (Joseph et al. 2002). The adhesion of platelets leads to an activation of NF-κB and the following release of chemokine (C−C motif) ligand 2 (CCL2) and intercellular adhesion molecule (ICAM)-1 (Gawaz et al. 1998). This is in line with studies, where interleukin-1β, ICAM-1 and CCL2 are secreted into the brain parenchyma following TBI (Hopp et al. 2017; Morganti-Kossmann et al.

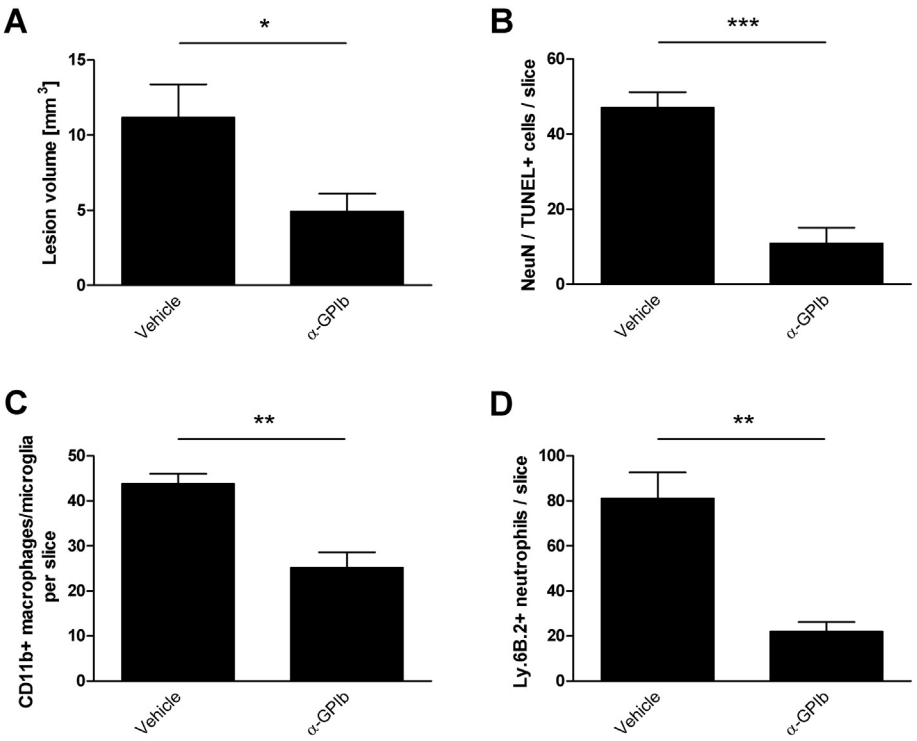

Fig. 1. (A) Lesion volumetry shows less necrotic brain tissue in GPIb-treated mice in comparison with control mice 24 h after injury ($n = 7$ per group, $^{*}P < 0.05$, unpaired, two-tailed Student's *t*-test). (B) The number of TUNEL-positive neurons per brain slice in the injured hemisphere is significantly reduced in GPIb-treated mice 24 h after injury when compared with control mice ($n = 4$ per group, $^{***}p < 0.001$, unpaired, two-tailed Student's t-test). (C) CD11b-positive macrophages and activated microglia were quantified 24 h after brain trauma. Treatment with GPIb led to a diminished infiltration of macrophages into the lesioned brain tissue (n = 4 per group, $^{**}P < 0.01$, unpaired, two-tailed Student's t-test). (D) Neutrophils were quantified in the lesioned hemispheres 24 h after brain trauma. GPIb-treated mice showed less neutrophil infiltration into the brain tissue in comparison to control mice (n = 4 per group, **P < 0.01, unpaired, two-tailed Student's t-test).

2007). The above-described cytokine release, as well as immune cell infiltration, is also (but not exclusively) dependent on FXII activation.

5. Conclusion

The original view that thrombosis and inflammation are independent pathomechanisms is challenged by several studies showing that immune cells and platelets, as well as their respective signaling cascades interact on several levels. Due to the close relationship between thrombotic and inflammatory processes, no decisive distinction between the categories is possible. This intricate link between thrombosis and inflammation and its implications for the pathology of brain disorders was thoroughly discussed in ischemic stroke studies (as reviewed in De Meyer et al. 2016). *Despite an obviously different etiology*, the events initiated after ischemic or traumatic brain insult share common pathophysiologies; the most prominent among them being the initiation of the FXII-driven plasma kallikrein-kinin system (summarized in Albert-Weissenberger et al., 2013). Thus, findings from stroke research are at least partly transferable to TBI pathophysiology.

Currently, we can draw the following assumptions from the available data: First, there is profound evidence that FXII links the pathological formation of microthrombi following TBI with the contact-kinin system, which triggers inflammation. Second, platelet surface proteins like GPIb are not only essential for inducing microthrombi formation, but are also involved in mediating inflammatory processes. Therefore, there is a need for novel treatment strategies, which do not focus on anti-inflammation alone, but which target inflammation and thrombosis synergistically following TBI.

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Availability of data and materials

The datasets analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval

All experiments were approved by institutional and regulatory

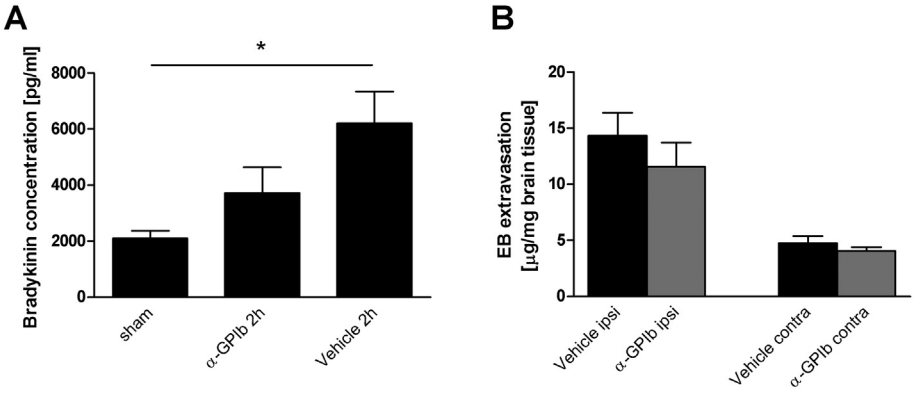

Fig. 2. (A) Determination of bradykinin plasma level by ELISA 2 h after injury induction reveals an increase 2 h post-injury in control mice in comparison to sham-operated mice, but no reduction of bradykinin levels in GPIb-treated animals in comparison to control mice (n = 4 per group, *P < 0.05, 1-way ANOVA with followed Bonferroni multiple comparison test). (B) Fluorometric measurement of Evans Blue extravasation into the brain parenchyma shows no improvement of blood-brain-barrier stability in GPIb-treated animals 24 h post-injury ($n = 6$–7 per group, $P > 0.05$, unpaired, two-tailed Student's t-test). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

authorities and were conducted in accordance with the EU Directive 2010/63/EU, the United States Public Health Service's Policy on Humane Care and Use of Laboratory Animals, and the ARRIVE criteria Consent for publication.

Competing interests

The authors declare that they have no competing interests.

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