

● PERSPECTIVE

Targeting microthrombosis and neuroinflammation with vepoloxamer for therapeutic neuroprotection after traumatic brain injury

Traumatic brain injury (TBI): Despite improved supportive and rehabilitative care of TBI patients, TBI remains a leading cause of death and disability worldwide. To date, no effective pharmacological treatments are available for TBI. The mechanisms underlying brain damage and repair following TBI are complex and not completely understood. Coagulopathy after TBI is frequent and an independent prognostic factor for unfavorable outcome and prognosis (Stein and Smith, 2004). It may be amenable to treatment, and effective management of coagulopathy may protect from secondary injury and poor outcomes. Although the main challenge for TBI management is to address the risk of hypocoagulopathy with prolonged bleeding and progression of hemorrhagic lesions, the risk of hypercoagulopathy with an increased microthrombosis formation warrants investigation to reduce neurological deficits after TBI.

Cerebral microthrombosis in TBI: Among many secondary injury events that occur after primary injury in TBI, posttraumatic intravascular thrombosis has been suggested as an important factor of secondary ischemia after TBI (Lu et al., 2004; Stein et al., 2004). There is a strong link between intravascular microthrombosis and neuronal death after brain trauma in humans that may have important implications for new therapeutic approaches (Stein and Smith, 2004). Although much attention has been paid to treat deep vein thrombosis (DVT) after TBI, less effort has been made to treat microthrombosis in the injured brain. This may result from the fact that DVT is rigorously screened and DVT if left without treatment is lethal while cerebral microthrombosis is not easily detected and therefore largely ignored. Our previous study demonstrates that thrombosis occurs in the lesion boundary zone and in the hippocampus, starting at 1–4 hours, peaking at 1–3 days, and then declining at 8–15 days in rats after TBI induced by controlled cortical impact (CCI) (Lu et al., 2004). A recent study shows that the most dramatic observation in the microcirculation of the TBI penumbra is the formation of microthrombi in up to 80% of all investigated venules, and up to 40% of all investigated arterioles at 1–2 hours in mice after CCI-TBI (Schwarzmaier et al., 2010, 2013). The microthrombi occur as early as 30 minutes after trauma, and reach a maximum level between 60 and 90 minutes after injury in this mouse CCI-TBI model (Schwarzmaier et al., 2013). Not all of these thrombi occlude the affected vessel immediately. However, as time passes, the microthrombi tend to grow and completely occlude venules and arterioles (Schwarzmaier et al., 2013). These preclinical studies reveal that microthrombosis formation in the cerebral microcirculation occurs within 1 hour after trauma, and persists for up to 2 weeks. Microthrombi may be responsible for the perfusion deficits observed in the traumatic penumbra, and for the secondary expansion of the contusion seen following TBI.

Vepoloxamer: Vepoloxamer is a purified form of Poloxamer 188, an amphiphilic polyethylene-polypropylene-polyethylene triblock copolymer, where impurities associated with renal dysfunction have been removed (Emanuele and Balasubramaniam, 2014). Poloxamer 188 is reported to seal membranes and restore plasma membrane integrity in cells damaged by mechanical and electrical injury and in experimental models of muscular dystrophy, heart failure and neurodegenerative disorders (Moloughney and

Weisleder, 2012). Our recent study indicates that early (2 hours post injury) intravenous administration of vepoloxamer promotes functional recovery including sensorimotor function and cognitive function after TBI induced by controlled cortical impact (Zhang et al., 2018). However, to date, there is a paucity of information about vepoloxamer for treatment of TBI and the mechanisms underlying its beneficial effects. We tested the hypothesis that vepoloxamer provides neuroprotection by targeting cerebral microthrombi and neuroinflammation to reduce brain damage, and subsequently improves functional recovery after TBI. Our data demonstrate that compared to saline controls vepoloxamer normalizes the bleeding time, reduces brain hemorrhage and cerebral microthrombosis formation, reduces cortical lesion volume, and reduces activation of microglia/macrophages and astrogliosis in many brain regions including injured cortex, corpus callosum and hippocampus in rats with CCI (Zhang et al., 2018) (Figure 1).

Loss of plasma membrane integrity is a feature of acute cellular injury/death *in vitro* and *in vivo*. Plasmalemma-resealing agents are protective in acute central nervous system injury models, but their ability to reseal cell membranes *in vivo* has not been fully investigated. These agents may provide neuroprotection by blocking megachannel opening, which may be related to their membrane sealing action, and warrant further investigation for treatment of TBI and ischemic stroke (Yildirim et al., 2015). Poloxamer 188 protects neurons against cerebral ischemia/reperfusion injury, and the protection involves multi-mechanisms in addition to the membrane resealing (Gu et al., 2013). Classic membrane-resealing agents such as poloxamer P188, and the newly discovered Kollidon VA64, exert protective effects in central nervous system injury paradigms by mechanisms other than or in addition to maintaining permeable cell membranes sealed (Miller et al., 2014). For example, the effect of Kollidon VA64 on TBI is found to be mainly mediated by reducing blood brain barrier damage, tissue loss, and brain edema, instead of rescuing the membrane-interrupted neurons (Mbye et al., 2012).

Conclusions and future perspectives: For decades, the primary approach and goal of therapy for TBI has focused on early neuroprotective interventions alone designed to reduce neural cell loss and the lesion volume. However, these efforts have failed to demonstrate efficacy in clinical trials of TBI. Many reasons exist for the lack of clinical efficacy including the heterogeneity of human TBI and the lack of methodological agreement between preclinical and clinical studies. Cerebral microthrombosis is a universal response to TBI and would therefore seem to be one of the critical secondary adverse events after brain trauma. Microthrombi not only lead to ischemia and cell death but also prevent therapeutic drugs from entering into the affected brain and therefore constrain the efficacy of therapeutic drugs, which may be one of important factors ignored during preclinical and clinical trials. Microthrombosis is an under-recognized, yet important contrib-

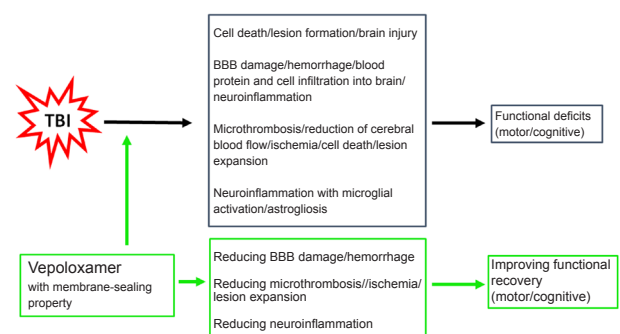


Figure 1 Neuroprotection of vepoloxamer treatment for traumatic brain injury (TBI).

BBB: Blood-brain barrier.

utor to the secondary brain damage that occurs after TBI (Stein et al., 2004; Schwarzmaier et al., 2010), and would therefore seem to be one of the important secondary events after brain trauma to bear in mind when designing treatment strategies. Alternatively, however, microthrombosis formation may also have the useful pathophysiological function of sealing damaged microvessels, thereby preventing bleeding, or in cases of less severe damage, leakage of plasma fluid into the extracellular space (*i.e.*, brain edema formation). Thus, therapeutic interventions aimed at interfering with the formation of microthrombi may have both advantages and disadvantages, and will therefore need thorough pre-clinical testing before clinical use is considered.

The mechanisms underlying posttraumatic ischemia remain elusive. A previous study indicates that the immediate posttraumatic decrease in peri-contusional blood flow in mice after TBI may not be caused by arteriolar vasoconstriction, but by platelet activation and the subsequent formation of thrombi in the cerebral microcirculation (Schwarzmaier et al., 2010). As the platelet dysfunction observed stems from an isolated TBI and occurs almost immediately after injury in both rats and humans (Castellino et al., 2014), it is likely that the platelet dysfunction is caused by substances liberated from the injured/activated endothelial cells (ECs) or from brain cells secondary to mechanical disruption of the blood-brain barrier (BBB). One candidate substance is von Willebrand factor (vWF) derived from injured/activated ECs. vWF is widely used as a marker for endothelial injury, and plasma vWF levels correlate inversely with clinical outcome of severe TBI (De Oliveira et al., 2007). vWF can induce microthrombosis formation (Dhanesha et al., 2016). Our data demonstrate that the level of vWF in plasma increases starting at 1 hour, progressively increases at 4 hours and 1 day, peaks at 3 days after TBI, and then declines and returns to the sham control level at 15 days after TBI (Lu et al., 2004). Immunohistochemical staining shows that vWF is involved in the formation of the delayed thrombotic formation in the vessels of lesion boundary zone and the ipsilateral hippocampal CA3 region. These temporal profiles of vWF changes match the temporal profile of platelet activating factor and delayed cerebral microthrombosis (Lu et al., 2004), suggesting that vWF plays an important role in the delayed thrombosis formation. So far, the mechanisms of post-trauma microthrombosis formation are poorly understood. To date, there is a paucity of information about Vepoloxamer for treatment of TBI and the mechanisms underlying beneficial effects. We speculate that TBI induces the BBB damage and release of endothelial-derived vWF, which leads to platelet aggregation and subsequent cerebral microthrombosis-induced secondary injury while Vepoloxamer treatment may preserve microvascular integrity, reduce the BBB damage and endothelial-derived vWF release and subsequently reduce cerebral microthrombosis and increase cerebral perfusion which leads to reduction in neuroinflammation and brain injury as well as improvements in functional recovery after TBI. Further investigation is warranted to test this hypothesis.

Vepoloxamer as a novel agent to reduce intravascular microthrombosis formation and neuroinflammation may be promising for treatment of TBI. Further investigation of the optimal dose and therapeutic window of vepoloxamer treatment for TBI and the molecular mechanisms underlying its therapeutic effects is warranted. Given the negative impact of coagulopathy on TBI outcome, chemoprophylaxis should be deferred until coagulopathy has been corrected and not recurred. Therefore, it is important to determine which TBI patients should be treated with vepoloxamer and when they should be treated. Laboratory testing for coagulopathy must be completed prior to the initiation of any antithrombotic treatment in TBI patients.

Ye Xiong^{*}, Li Zhang, Zheng Gang Zhang, Asim Mahmood, Michael Chopp

Department of Neurosurgery, Henry Ford Hospital, Detroit, MI, USA

(Xiong Y, Mahmood A)

Department of Neurology, Henry Ford Hospital, Detroit, MI, USA

(Zhang L, Zhang ZG, Chopp M)

Department of Physics, Oakland University, Rochester, MI, USA

(Chopp M)

*Correspondence to: Ye Xiong, M.D., Ph.D., yxiong1@hfhs.org.

orcid: 0000-0001-9770-6031 (Ye Xiong)

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References

- Castellino FJ, Chapman MP, Donahue DL, Thomas S, Moore EE, Wohlaer MV, Fritz B, Yount R, Ploplis V, Davis P, Evans E, Walsh M (2014) Traumatic brain injury causes platelet adenosine diphosphate and arachidonic acid receptor inhibition independent of hemorrhagic shock in humans and rats. *J Trauma Acute Care Surg* 76:1169-1176.
- De Oliveira CO, Reimer AG, Da Rocha AB, Grivicich I, Schneider RF, Roisenberg I, Regner A, Simon D (2007) Plasma von Willebrand factor levels correlate with clinical outcome of severe traumatic brain injury. *J Neurotrauma* 24:1331-1338.
- Dhanesha N, Prakash P, Doddapattar P, Khanna I, Pollpeter MJ, Nayak MK, Staber JM, Chauhan AK (2016) Endothelial cell-derived von Willebrand factor is the major determinant that mediates von willebrand factor-dependent acute ischemic stroke by promoting postischemic thrombo-inflammation. *Arterioscler Thromb Vasc Biol* 36:1829-1837.
- Emanuele M, Balasubramaniam B (2014) Differential effects of commercial-grade and purified poloxamer 188 on renal function. *Drugs R D* 14:73-83.
- Gu JH, Ge JB, Li M, Xu HD, Wu F, Qin ZH (2013) Poloxamer 188 protects neurons against ischemia/reperfusion injury through preserving integrity of cell membranes and blood brain barrier. *PLoS One* 8:e61641.
- Lu D, Mahmood A, Goussev A, Qu C, Zhang ZG, Chopp M (2004) Delayed thrombosis after traumatic brain injury in rats. *J Neurotrauma* 21:1756-1766.
- Mbye LH, Keles E, Tao L, Zhang J, Chung J, Larvie M, Koppula R, Lo EH, Whalen MJ (2012) Kollidon VA64, a membrane-resealing agent, reduces histopathology and improves functional outcome after controlled cortical impact in mice. *J Cereb Blood Flow Metab* 32:515-524.
- Miller BF, Keles E, Tien L, Zhang J, Kaplan D, Lo EH, Whalen MJ (2014) The pharmacokinetics and pharmacodynamics of Kollidon VA64 dissociate its protective effects from membrane resealing after controlled cortical impact in mice. *J Cereb Blood Flow Metab* 34:1347-1353.
- Moloughney JG, Weisleder N (2012) Poloxamer 188 (p188) as a membrane resealing reagent in biomedical applications. *Recent Pat Biotechnol* 6:200-211.
- Schwarzmaier SM, Kim SW, Trabold R, Plesnila N (2010) Temporal profile of thrombogenesis in the cerebral microcirculation after traumatic brain injury in mice. *J Neurotrauma* 27:121-130.
- Schwarzmaier SM, Zimmermann R, McGarry NB, Trabold R, Kim SW, Plesnila N (2013) In vivo temporal and spatial profile of leukocyte adhesion and migration after experimental traumatic brain injury in mice. *J Neuroinflammation* 10:32.
- Stein SC, Smith DH (2004) Coagulopathy in traumatic brain injury. *Neurocrit Care* 1:479-488.
- Stein SC, Graham DI, Chen XH, Smith DH (2004) Association between intravascular microthrombosis and cerebral ischemia in traumatic brain injury. *Neurosurgery* 54:687-691.
- Yildirim T, Eylen A, Lule S, Erdener SE, Vural A, Karatas H, Ozveren MF, Dalkara T, Gursoy-Ozdemir Y (2015) Poloxamer-188 and citicoline provide neuronal membrane integrity and protect membrane stability in cortical spreading depression. *Int J Neurosci* 125:941-946.
- Zhang Y, Chopp M, Emanuele M, Zhang L, Zhang ZG, Lu M, Zhang T, Mahmood A, Xiong Y (2018) Treatment of traumatic brain injury with Vepoloxamer (purified Poloxamer 188). *J Neurotrauma* 35:661-670.