Brain, Behavior, & Immunity - Health 38 (2024) 100762

Contents lists available at ScienceDirect

Brain, Behavior, & Immunity - Health

journal homepage: www.editorialmanager.com/bbih/default.aspx

Effects of poloxamer 188 on traumatic brain injury

Renqing Wu [a,1], Roopa Koduri [a,1], Michael Cho [b], Nagham Alatrash [a], Vanessa Nomellini [a,*]

[a] Division of Burn, Trauma, Acute, and Critical Care Surgery, Department of Surgery, UT Southwestern Medical Center, Dallas, TX, USA
[b] Department of Bioengineering, UT Arlington, Arlington, TX, USA

ARTICLE INFO

Keywords:
Poloxamer 188
P188
Vepoloxamer
Traumatic brain injury
TBI

ABSTRACT

Traumatic Brain Injury (TBI) is a major cause of severe disability and death, resulting in significant health care and economic burden. Poloxamer 188, a synthetic tri-block copolymer approved by the FDA, has been studied for its potential effects on traumatic brain injury (TBI). The neuroprotective abilities of P188 have attracted significant attention. This systematic review aims to compile evidence of P188's effect on the treatment of TBI. A comprehensive literature search was conducted using PubMed, SCOPUS, and Google Scholar databases, which yielded 20 articles that satisfied the inclusion criteria. These articles have shown direct protective effects of P188 on brain tissue following TBI, including restitution of the increase cell membrane permeability, attenuation of neuronal necrosis and apoptosis, improvement of mitochondrial viability, reduction in axonal disruption, and restoration of the blood brain barrier. In animals, P188 has been shown to improve sensorimotor functions, as well as spatial learning and memory.

1. Introduction

1.1. Overview of traumatic brain injury

Traumatic brain injury (TBI) has an estimated incidence of 27–69 million and a prevalence of 55.5 million, making it one of the major causes of mortality and disability worldwide. It resulted in 8.1 million years of life lived with disability globally in 2016 (2019; Dewan et al., 2018). In the US, an average of 1.4 million cases of TBI occur each year, with approximately 1.11–2.87 million TBI-related emergency department visits, 223,135 to 235,000 hospitalizations, and 50,000 to 69,473 deaths according to the Centers for Disease Control and Prevention (CDC) (Centers for Disease Control and PreventionN.C.f.I.P.a.C., 2023; (Langlois et al., 2006). Amongst survivors living with TBI, an estimated 3.17 to 5.3 million people are expected to have long-term impairments (Thurman et al., 1999; Zaloshnja et al., 2008). Approximately $406 billion in lifetime health care cost is attributable to nonfatal TBI, with approximately $80 billion spent on medical treatment, and $326 billion for lost productivity (Corso et al., 2006).

TBI occurs when an external traumatic force induces structural or physiologic disruptions of the brain. TBI can be penetrating, where the traumatic force pierces the skull, or due to blunt force, where the disruptions of the brain occur with or without damage to the skull (Mckee and Daneshvar, 2015). Suicide is the leading cause of TBI-related death, followed by motor vehicle collisions (Daugherty et al., 2019; Miller et al., 2020). TBI can be classified as mild, moderate, or severe based on the Glasgow Coma Scale (GCS) (Teasdale and Jennett, 1974). Mild TBI consists of a GCS of 13–15 and is often characterized by concussion with full neurologic recovery. Moderate TBI consists of a GCS of 9–13, while severe TBI consists of a GCS of 3–8. Irrespective of the grade, all patients with TBI can experience long-term physical, emotional, and cognitive damages (Langlois et al., 2006).

The injuries that occur in TBI can be characterized as primary and secondary (see Table 1 for the various injury models and cell types evaluated). The primary injuries in TBI involve direct damage by the external force to neurons, glia, and the blood supply, while the secondary injuries include ischemia and reperfusion injuries, local and systemic inflammation, edema leading to increased intracranial pressure, excitotoxicity, mitochondrial dysfunction, and neuronal degeneration (Kaur and Sharma, 2018; Mckee and Daneshvar, 2015). Primary injuries can be focal, such as brain contusions; or diffused, such as diffuse axonal injury (DAI) (Kaur and Sharma, 2018). Damage to the blood supply can lead to the formation of focal or diffuse intraparenchymal, subarachnoid, subdural or epidural bleeds (Kaur and Sharma, 2018). The pathophysiology of secondary injuries includes complex pathways consisting of multidimensional cascades, the full

* Corresponding author.
E-mail address: vanessa.nomellini@utsouthwestern.edu (V. Nomellini).
[1] Co-first authors.

https://doi.org/10.1016/j.bbih.2024.100762
Received 28 December 2023; Received in revised form 15 March 2024; Accepted 28 March 2024
Available online 31 March 2024

Table 1
In vitro and In vivo brain injury models based on mechanism.

Type of cells/animal	Injury Model	References
Sprague-Dawley rats	Striatal quinolinic acid induced excitotoxicity	Curry et al. (2004)
Mouse brain microvascular endothelial cells	Shock wave generated micro-cavitation	Inyang et al. (2020)
Isolated primary murine neurons	Hypoxia/reoxygenation (H/R) with and without compression	Meyer and Riess (2021)
C57BL/6 mouse primary brain microvascular endothelial cells	Hypoxia/reoxygenation (H/R) with and without compression	Lotze and Riess (2021)
Murine hippocampal (HT22) cells	Cerebral ischemia/reperfusion injury	Gu et al. (2013)
Male ICR mice	Transient Middle Cerebral Artery Occlusion	
Primary cortical neuronal tissue	Shear stress by in vitro cell shearing device (VCSD)	Luo et al. (2013)
Embryonic chick forebrain neurons	Fluid-shear stress injury	Kilinc et al. (2008)
Fetal rat hippocampal neurons, fetal Purkinje neurons, and bovine adrenal chromaffin cells	NMDA induced excitotoxity and oxidative stress via the Fenton reaction	Marks et al. (2001)
Mouse brain cortical & hippocampal dentate gyrus neurons	Cortical spreading depression	Yıldırım et al. (2015)
Male Wistar rats	Controlled cortical impact	Zhang et al. (2018)
Pheochromocytoma (PC2) cells	Shear stress by controlled cell shearing device (CCSD)	Serbest et al. (2005)
Mouse astrocytes C8-D1A	Shock wave generated micro-cavitation	Kanagaraj et al. (2018)
Male CD1 mice	Controlled cortical impact	Bao et al. (2012)
Pheochromocytoma (PC-12) cells	Scratch injury	Bao et al. (2016)
Adult male CD1 mice	Controlled cortical impact	
Rat forebrain neurons	Oxidative stress induced by cardiac arrest secondary to asphyxiation in vivo or H2O2 exposure in vitro	Pille and Riess (2021)
Male ICR mice	Collagenase induced intracerebral hemorrhage	Wang et al. (2015)
Mouse astrocytes C8-D1A	Shock wave generated micro-cavitation	Chen et al. (2019)

extent of which are still under investigation (Cornelius et al., 2013; Crupi et al., 2020; Gottlieb, 2011; Jha et al., 2019; Kalogeris et al., 2012).

Clinical heterogeneity of injuries sustained by patients, the vast differences between the sterile environment in which injuries are induced in the laboratories and in real life scenarios, and the lack of standardized quantifiable outcome metrics all make TBI research challenging. Despite showing great promise in pre-clinical stages, various therapies, such as hypothermia, magnesium, nimodipine, and progesterone for TBI have failed in the clinical trial stages (Clifton et al., 2011; Langham et al., 1996; Winn et al., 2007; Wright et al., 2014). Consortiums, such as Operation Brain Trauma Therapy (OBTT), have been formed to identify promising therapies in various stages of pre-clinical testing. It has given priority to testing therapies that are already approved by the Food and Drug Administration and with pre-clinical data specific to TBI (Kochanek et al., 2015). However, a substantial gap is still present between the current research and the clinical use of any therapeutic agents.

1.2. Poloxamer 188 (P188)

P188 is a synthetic tri-block copolymer, consisting of a central hydrophobic chain of polyoxypropylene and two hydrophilic chains of polyoxyethylene. It has a molecular weight of 8400 Da and an Hydrophile-Lipophile Balance (HLB) value of 29 (Chen et al., 2022; Moloughney and Weisleder, 2012). In humans, P188 has a half-life of 18 h, has been demonstrated to be safe when given for up to 72 h, and is ultimately excreted by the kidney (Patricia et al., 1997). Its surfactant properties make it very useful in cosmetic, pharmaceutical, and industrial realms, such as food additives, organ perfusates, chemotherapeutics, emulsion formulation, dispersants, and anti-biofouling coatings (Bodratti and Alexandridis, 2018; Curry et al., 2004; Inyang et al., 2020).

P188 was approved by the FDA as a therapeutic reagent to reduce viscosity in the blood before transfusions. Though it showed great promise in pre-clinical investigations in its ability to decrease red blood cell aggregation and blood viscosity in sickle cell patients, P188 did not show the same effects in clinical trials (Sandor et al., 2016). A recent phase III, multicenter randomized controlled trial of P188 for the treatment of vaso-occlusive crisis in patients with sickle cell disease found that it did not shorten the time to the last dose of parenteral opioids (Casella et al., 2021). Aside from sickle cell disease, P188 has been tested in various pre-clinical models of cardiac and neurological disorders. Studies have demonstrated the cardioprotective effect of P188 in various ischemia reperfusion (IR) injury models, as well as its ability to enhance the sealing of cardiac myocytes (Maskarinec et al., 2002; Yasuda et al., 2005). Furthermore, P188 was able to reduce cardiac injury and preserve mitochondrial function in a porcine model of ST-segment elevation myocardial infarction (STEMI) (Bartos et al., 2016; Zargari et al., 2023).

Several studies have examined the effect of P188 on central nervous system disorders. It was found to have neuroprotective properties on endothelial cells, neurons, and glial cells in in vitro studies as well as animal models of stroke, Parkinson's Disease, Amyotrophic Lateral Sclerosis, and TBI (Chen et al., 2022). When examining neural transplantation of fetal dopaminergic cells as a potential therapy for Parkinson's Disease, P188 treatment increased survival of dopaminergic cells and enhanced striatal reinnervation in Parkinsonian rats that received intrastriatal transplants of said dopaminergic cells (Quinn et al., 2008). P188 was also found to rescue the disruption in membrane impermeability, leakage of cell content and cell death caused by amyloid oligomer toxicity, which can be seen in a variety of amyloid-related neurodegenerative diseases (Mina et al., 2009). To better understand P188's potential as a therapeutic agent for TBI, we explore and examine the available literature in this systematic review.

2. Literature search

To understand the effects of P188 on in vitro and in vivo models of traumatic brain injury, a comprehensive literature search was conducted using PubMed, SCOPUS, and Google Scholar databases to identify studies related to traumatic brain injury and P188 from January 2000 to July 2023. Sixteen articles from PubMed and a total of 2560 articles from SCOPUS and Google Scholar were identified. Only full-text articles published in English were included in the search. All research that notably examined P188's effects on traumatic brain injury utilizing various in vivo and in vitro models were included in this review. Given that P188 has not been approved by the FDA for the treatment of TBI, all research included in this review are pre-clinical studies. Studies that examined neurological conditions other than traumatic brain injury, including P188 and its role in nanoparticles or hydrogel delivery systems, and studies where P188 was used in combination with other pharmacological agents were excluded. After applying the inclusion and exclusion criteria, 34 articles were considered. Duplicates were removed, resulting in a final selection of 20 studies (Fig. 1).

3. P188 and TBI

3.1. Injuries in TBI

The initial insult in traumatic brain injury leads to primary injuries, including neuron and glial necrosis, as well as diffuse axonal injuries due

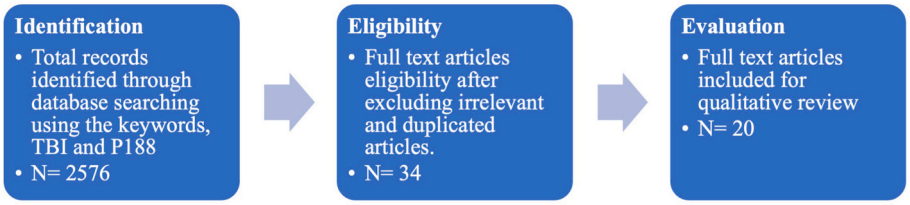

Fig. 1. Flow-chart describing literature search.

to the shearing and tearing mechanical forces (Ng and Lee, 2019). But there is also a cascade of secondary damages that can occur. These secondary injuries include more extensive cell death via apoptosis and/or necrosis due to activation of cell death receptors and excitotoxicity respectively, mitochondrial dysfunction, damage to the blood brain-barrier (BBB), and hypoxia-reperfusion injuries, often associated with hemorrhage and subsequent vasospasm (Kaur and Sharma, 2018). Though the precise mechanism of action of P188 has yet to be elucidated, many groups have shown the various ways in which P188 provides neuroprotection as shown in Table 2 and demonstrated schematically in Fig. 2. We will explore the effects of P188 on each specific injury that occurs in TBI.

3. 1. 1. Membrane permeability

An increase in cell membrane permeability can be seen in mechanical, chemical, and hypoxic injury models (Gu et al., 2013; Inyang et al., 2020; Kilinc et al., 2008; Luo et al., 2013; Meyer and Riess, 2021; Wang et al., 2015). P188 can attenuate this and often can restore membrane permeability comparable to that of uninjured cells. For example, P188, at 100 μM–300 μM, can reduce the rate of lactate dehydrogenase (LDH) released from injured pheochromocytoma cells (PC2), isolated murine primary neurons, mouse brain microvascular endothelial cells, murine hippocampal neurons (HT22), and rat primary cortical neurons (Gu et al., 2013; Lotze and Riess, 2021; Luo et al., 2013; Meyer and Riess, 2021; Serbest et al., 2006). In addition, Gu et al. showed an increase in propidium iodine (PI), which is a membrane impermeable nucleic acid dye, the presence of which indicates cell membrane disruption, in HT22 cells that were injured by glucose deprivation and hypoxia, and subsequent reduction of PI-positivity with treatment of 1 μM–1 mM of P188 (Gu et al., 2013). Similarly, post-injury P188 treatment at 100 μM reduced internalization of cell impermeable, aldehyde fixable Lucifer yellow dye by injured embryonic chick forebrain neurons (Kilinc et al., 2008). The measured diffusion of 3 kDa Dextran was also used to calculate the membrane permeability coefficient. After inducing blast injury in brain endothelial cells, Inyang et al. found a two-fold increase in the permeability coefficient of 3 kDa Dextran; which was significantly reduced with treatment with 500 μM of P188 (Inyang et al., 2020).

P188, being an amphiphilic copolymer, is likely able to restore membrane integrity by inserting itself into the plasma membrane (Fig. 2A) (Marks et al., 2001). Marks et al. found an increase in whole cell capacitance of bovine adrenal chromaffin cells following 100 μM of P188 perfusion, suggesting an increase in the surface area of cells perfused with P188 (Marks et al., 2001). The action of P188 on the cell membrane was further elucidated through examination of the neuron megachannel, Pannexin-1. P188 at 100 μM was found to block the megachannel opening associated with cortical spreading depression, or peri-infarct depolarization, in the pinprick model in mice. This suggested that P188's membrane sealing effect may be related to its ability to modify megachannel openings (Yıldırım et al., 2015). The above studies of P188 show promising results for its ability to preserve cell membrane integrity after injury-induced increases in membrane permeability.

3. 1. 2. Cell death

Mechanical injury may lead to cell death through a combination of apoptosis and necrosis (Kaur and Sharma, 2018). Grossly, this can be measured by the volume of total brain tissue loss in animal models (Hånell et al., 2012). In both mice and rat models of TBI, the volume of brain tissue loss in animals treated with 0.4 and 0.8 g/kg P188 or 300 mg/kg of P188 after injury was noted to be significantly less than that of animals who were treated with saline alone (Gu et al., 2013; Zhang et al., 2018).

Microscopically, cell death is often assessed by cell viability assays, usually with a cell membrane permeable fluorescent nucleic acid stain. Multiple studies noted that cell numbers and viability decreased with hypoxic and/or mechanical injuries (Kanagaraj et al., 2018; Lotze and Riess, 2021; Serbest et al., 2005). P188 was found to restore cell viability in a dose-dependent manner after injury with shear stress - the survival rate of injured rat pheochromocytoma cells (PC2 cells) treated with 100 μM of P188 was not different than that of uninjured cells (Serbest et al., 2005). Incubation with 500 μM of P188 after exposure to microcavitation injury resulted in a noticeable improvement in astrocyte cell viability as well (Kanagaraj et al., 2018). Though Meyers et al. found that treatment with 100 μM and 300 μM of P188 during the reperfusion period in an ischemia/reperfusion model did not significantly restore neuron viability, Lotze et al. were able to demonstrate that a high concentration of P188, at 1 mM, did exhibit a protective effect on cells that underwent hypoxic injury. Interestingly, both studies showed that at low doses, P188 decreased cell viability when it was applied to control cells that did not undergo injury (Lotze and Riess, 2021; Meyer and Riess, 2021).

Several studies examined whether P188 can rescue cells from undergoing apoptosis, necrosis, or both (Bao et al., 2016; Marks et al., 2001; Serbest et al., 2005). Marks et al. showed that, in NMDA-induced neuronal death, a model for cell necrosis, incubation with P188 at 100 μM reduced rat hippocampal neuron mortality to 0% (Marks et al., 2001). They further examined P188's effect in cells exposed to kainite, menadione and t-BuOOH, which are all toxins that induce necrosis. P188 was found to significantly increase cell survival after exposure to all these toxins. Given that they found P188 did not influence the NMDA-induced calcium increase or the receptor-mediated inward current, the hypothesis is that P188 may exert its effects further downstream, such as NMDA-induced increase in radical oxygen species (ROS) and subsequent activation of lipid peroxidation (Fig. 2B). In the context of continuous oxidative stimulation with Fe_2 and H_2O_2 via the Fenton reaction, P188 at 30 μM effectively decreased the rate of lipid peroxidation in rat hippocampal and cerebellar neurons (Marks et al., 2001). More studies showed that P188 was able to attenuate the increase in ROS due to chemical and mechanical trauma (Inyang et al., 2020; Kanagaraj et al., 2018; Lotze and Riess, 2021).

On the other hand, Serbest et al. showed that P188 treatment reduced the rate of apoptosis and investigated the mechanisms through which it achieved this (Fig. 2C). They found that P38 phosphorylation, which is associated with apoptosis, is inhibited by the treatment with 100 μM P188 (Serbest et al., 2006). Furthermore, P188 at 100 μM was found to reduce the levels of proteins involved in both the extrinsic and intrinsic pathways of caspase-dependent apoptosis, such as caspase-8, cytochrome-c and caspase-9, respectively (Bao et al., 2012).

Autophagy, the auto-digestion of aggregated proteins and damaged organelles, is a self-catabolic process that cells employ in maintaining

Table 2
Mechanisms and Findings of P188 in TBI.

Injury Category	Mechanism	Findings	Model	References
Membrane permeability	Restores cell membrane integrity	Restores increase in cell membrane permeability	Oxygen–glucose deprivation and reoxygenation (OGD/R) treatment Shock wave generated micro-cavitation Fluid-shear stress injury Hypoxia/reoxygenation (H/R) with and without compression Electroporation Pinprick cortical spreading depression	(Gu et al., 2013; Inyang et al., 2020; Kilinc et al., 2008; Lotze and Riess, 2021; Luo et al., 2013; Marks et al., 2001; Meyer and Riess, 2021; Yıldırım et al., 2015)
		Increases whole cell capacitance	Continuous measurements of whole cell capacitance	
		Blocks mega-channel opening	Pinprick cortical spreading depression	
Cell death	Increases cell viability	Reduces volume of brain tissue loss	Controlled cortical impact Transient middle cerebral artery occlusion	(Gu et al., 2013; Zhang et al., 2018)
		Improves cell viability	Shock wave generated micro-cavitation Hypoxia/reoxygenation (H/R) with and without compression Shear stress by controlled cell shearing device (CCSD)	(Kanagaraj et al., 2018; Lotze and Riess, 2021; Serbest et al., 2005)
	Rescues cells from necrosis	Reduces ROS[a] increase associated with NMDA[b] excitotoxicity	NMDA induced excitotoxity and oxidative stress via the Fenton reaction	(Inyang et al., 2020; Kanagaraj et al., 2018; Lotze and Riess, 2021; Marks et al., 2001)
		Reduces lipid peroxidation	Shock wave generated micro-cavitation TNFα[c] induced chemical trauma Hypoxia/reoxygenation (H/R) with and without compression	
	Rescues cells from apoptosis	Reduces the levels of cytochrome-c, caspase-9, and caspase-8 Inhibits p38 phosphorylation	Controlled cortical impact Shear stress by controlled cell shearing device (CCSD)	(Bao et al., 2012; Serbest et al., 2005)
	Activates autophagy	Increases LC3II/LC3I[d] ratio, downregulates p62	Controlled cortical impact Scratch injury	Bao et al. (2016)
Axonal injury	Maintains normal axon morphology	Prevents microtubule cytoskeleton disruption	Shear stress by in vitro cell shearing device (VCSD) Fluid-shear stress injury	(Kilinc et al., 2008; Luo et al., 2013)
		Blocks axonal bead formation	Fluid-shear stress injury	
		Improves calcium homeostasis	Shear stress by in vitro cell shearing device (VCSD)	
Mitochondrial Dysfunction	Improves mitochondria viability	Increases cellular metabolism	Hypoxia/reoxygenation (H/R) with and without compression	(Kanagaraj et al., 2018; Lotze and Riess, 2021; Luo et al., 2013; Meyer and Riess, 2021)
		Inhibits release of cytochrome *c* from mitochondria to cytosol	Shear stress by in vitro cell shearing device (VCSD)	
		Reduces ROS	Shock wave generated micro-cavitation Isolated mitochondria injury with hydrogen peroxide Asphyxial cardiac arrest induced injury	
Blood Brain Barrier Function	Maintains tight junctions between endothelial cells	Restores levels of transmembrane proteins that form tight junctions – claudin-5, occludin, and zonula occludins-1	Collagenase-induced intracerebral hemorrhage	(Gu et al., 2013; Inyang et al., 2020; Wang et al., 2015)
		Maintains linear structure of tight junction proteins	Collagenase-induced intracerebral hemorrhage	
		Reduces MMP-9[e] & MMP-2 expression.	TNFα induced chemical trauma Transient middle cerebral artery occlusion Collagenase-induced intracerebral hemorrhage	
		Reduces expression of NFkB[f]	Collagenase-induced intracerebral hemorrhage	
	Regulates transport of water	Reduces expression of aquaporin 4	Controlled cortical impact	(Bao et al., 2012; Wang et al., 2015)
		Reduces brain water content	Controlled cortical impact Collagenase-induced intracerebral hemorrhage	
Neuroinflammation		Reduces CD68[g] positive microglial/ macrophages	Controlled cortical impact	Zhang et al. (2018)

[a] ROS – reactive oxygen species.
[b] NMDA – N-methyl-D-aspartate.
[c] TNFα – tumor necrosis factor alpha.
[d] LC3 – light chain 3.
[e] MMP – matrix metalloproteinase.
[f] NFkB – nuclear factor kappa B.
[g] CD – cluster of differentiation.

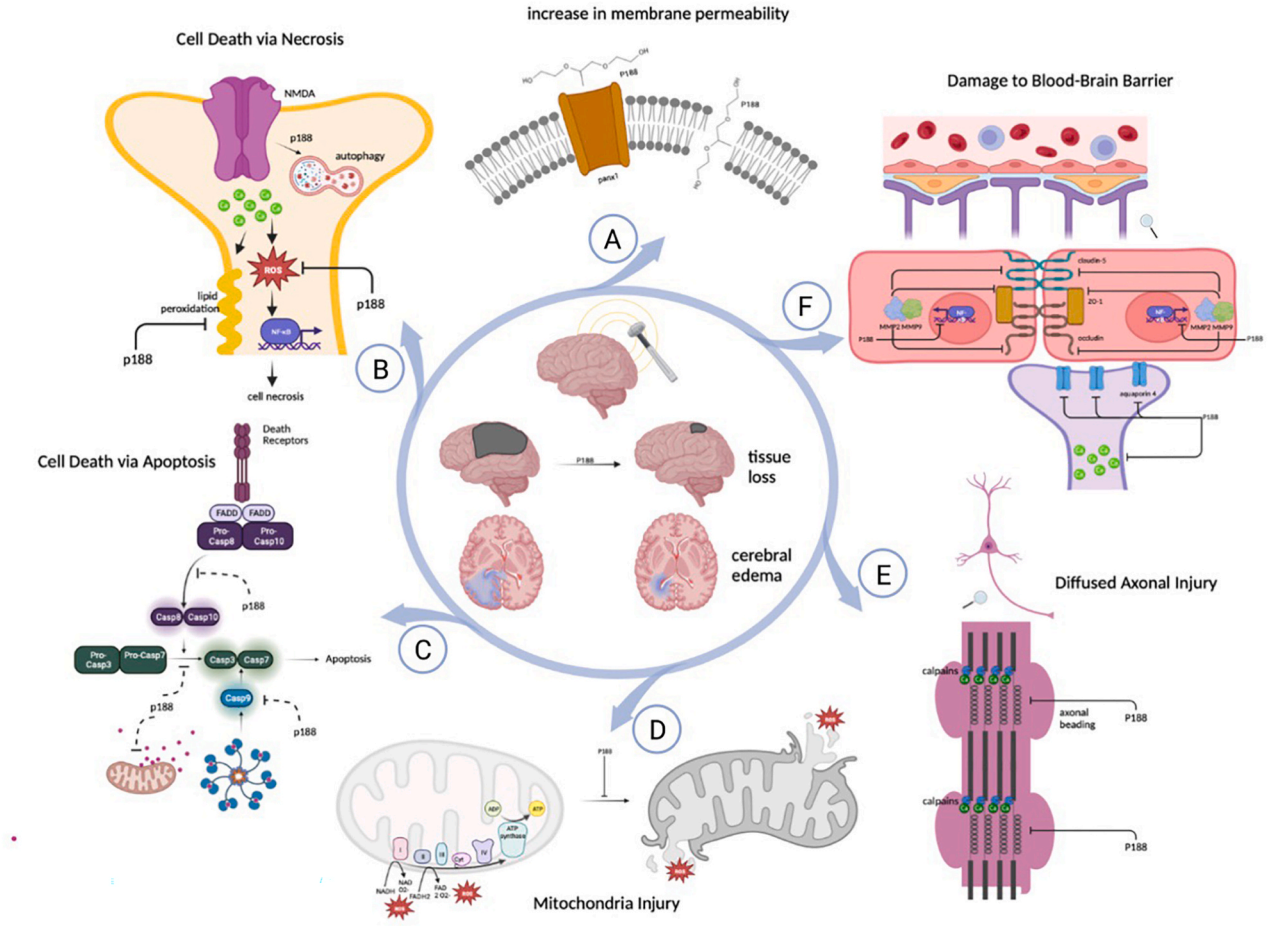

Fig. 2. Schematic depicting the mechanisms of action of P188 in TBI. A) P188 attenuates the increase in cell membrane permeability associated with mechanical, chemical, and hypoxic injuries, as well as modifies opening of mega-channel panx1. B) P188 downregulates cell necrosis and ROS production mediated by NMDA induced excitotoxicity, and subsequent lipid peroxidation. C) P188 reduces protein expression in both intrinsic and extrinsic pathways of caspase-dependent cell apoptosis. D) P188 improves mitochondrial viability by reducing ROS levels. E) P188 blocks the formation of axonal beading and disruptions in microtubule formation. F) P188 upholds integrity of the blood brain barrier by restoring levels of transmembrane protein and decrease expression of aquaporin 4.

their homeostasis. It has been found to both promote cell survival and contribute to cell death. Proteins associated with the regulation of autophagy, such as Beclin-1 and LC3 II have been found to be upregulated after TBI (Bao et al., 2016). While some studies have shown that autophagy plays a protective role in TBI, others have demonstrated support for a rather detrimental role. Inhibition of autophagy after controlled cortical impact model of TBI in mice is associated with increased rate of primary neuronal apoptosis, suggesting the protective role of autophagy in TBI (Sarkar et al., 2014). Using the same model, another group was able to demonstrate that inhibition of autophagy reduced TBI induced neuronal cell apoptosis, and improved the motor and learning deficits otherwise associated with TBI (Bao et al., 2016). When examining the effect of P188 on autophagy, Bao et al. demonstrated that treatment with 100 μM of P188 activated autophagy through upregulating LC3 II and downregulating p62 (Bao et al., 2016) (Fig. 2B). Overall, P188 restores cell viability after mechanical, chemical and hypoxic injuries, and reduces cell death through both apoptosis and necrosis pathways by reducing ROS, inhibiting lipid peroxidation, downregulating proteins associated in caspase-dependent apoptosis, and upregulating proteins associated with autophagy.

3. 1. 3. Axonal injury

To examine the effect of P188 on axonal injury on a cellular level, Kilinc et al. employed the fluid shear stress injury model on primary chick forebrain neurons to reproduce cell morphologies seen in diffuse axonal injuries associated with TBI. In this model, injured neurons were observed to have increased axonal beading, i.e., focal axonal swelling, that is correlated with localized disruption of the microtubule cytoskeleton. Treatment with 100 μM P188 blocked bead formation in response to shear injury. Staining for microtubules and actin filaments revealed that neurons treated with P188 also maintained their microtubule structures without evidence of focal disruption (Kilinc et al., 2008). The attenuation in axonal beading formation with treatment of P188 at 100 μM was also seen in rat primary cortical neurons after

mechanical injury by Luo et al. (Fig. 2E). The axonal beading secondary to the degradation of cytoskeletal protein is associated with the activation of calpain, a neutral protease that is activated by an influx of intracellular calcium (Luo et al., 2013). It is possible that P188's ability to restore cell membrane integrity may lead to improved calcium homeostasis, and in turn, restoration of a myriad of downstream pathways, including microtubule formation, that may have been affected by mechanical injuries.

3. 1. 4. Mitochondrial dysfunction

Mitochondrial alterations after TBI have been well described via ROS production, calcium transmission, autophagy, and apoptosis signaling (Cheng et al., 2012). A decline in mitochondrial viability, as measured with a colorimetric assay, was noted after a combination of hypoxic and compression injury to primary murine neurons. The presence of P188 increased mitochondrial viability at a high concentration of 1000 μM (Meyer and Riess, 2021). Whereas mitochondria play a central role in cellular metabolism, it is not surprising that P188 is able to elevate cellular metabolism in cells that have undergone hypoxia, compression, or both injury types (Lotze and Riess, 2021). In mechanical injury models, treatment with P188 at 100 μM was able to moderate the shearing induced dissipation of mitochondrial membrane potential, and significantly inhibit the release of cytochrome *c* from mitochondria to the cytosol when assessed with Western blot (Luo et al., 2013) (Fig. 2D).

Uncoupling of the mitochondrial electron transport chain in response to mechanical injury is suspected to be a leading source of ROS in astrocytes. Kanagaraj et al. used a highly specific mitochondrial superoxide dye to visualize the formation of superoxide. They found an increase in superoxide levels associated with injury and a subsequent reduction with P188 treatment at 500 μM (Kanagaraj et al., 2018). To evaluate the effect of superoxide on mitochondria, isolated mitochondria were incubated with H_2O_2, with or without P188. P188 showed no effect on the impaired ATP synthesis seen in injured mitochondria for complex I substrates. ATP synthesis for complex II substrates showed no changes in the presence of injury or after treatment with P188. P188 at 250 μM had no effect on the O_2 consumption nor calcium retention capacity for both complex I and II substrates in the presence or absence of injury (Pille and Riess, 2021). This suggests that P188 may improve mitochondrial viability through the reduction of ROS levels and lipid peroxidation rather than its direct interaction with mitochondria (Fig. 2B).

3. 1. 5. Blood-brain barrier function

Disruption in the BBB is thought to contribute to vasogenic edema observed as a sequela of TBI. The brain endothelial cell tight junction complex is an integral part of the blood brain barrier. Much of our understanding of P188's effects on the BBB derives from in vivo models of stroke, including collagenase induced intracranial hemorrhage and transient middle cerebral artery occlusion (Gu et al., 2013; Wang et al., 2015). The tight junction complex consists of transmembrane proteins on one end that interact with other transmembrane proteins on adjacent cells, and on the other, with the cytoskeleton that participate in intracellular signaling pathways. Three of these transmembrane proteins, claudin-5, occludin, and zona occluden-1 (ZO-1), have been studied in intracranial hemorrhage (ICH) models; their levels were all noted to be reduced at 72 h after ICH. Treatment with 12 mg/400 μL of saline P188 at per animal not only partly restored the levels of these three transmembrane proteins, but also rearranged their structure in a continuous and linear fashion, whereas without the treatment of P188, they were noted to be in discontinuity between adjacent endothelial cells (Wang et al., 2015). P188 at 500 μM also restored the decline in ZO-1 levels in endothelial cells that underwent mechanical trauma induced by shockwave generated microcavitations (Inyang et al., 2020).

The expression of tight junction complex transmembrane proteins of endothelial cells is inhibited by the activation of matrix metalloproteinases (MMP). Inyang et al. tested the expression of MMP2 and 9 in brain microvascular endothelial cells, which showed an increase in expression when treated with TNFα. Treatment with P188 at 500 μM was able to reduce the expression of MMP2 and MMP9 in this model (Gu et al., 2013; Inyang et al., 2020). The same effect of P188 was observed in ICH models by Wang et al. Since MMP is a target gene of the NFkB pathway, further investigations have found that P188 reduced the expression of NFkB in the ICH model (Wang et al., 2015).

In addition to endothelial cells, astrocytes form an important part of the BBB. The expression of aquaporin 4, a water channel protein, plays an important role in the formation of cerebral edema after insult, as it aids in the transport of water in an out of the brain parenchyma. Pretreatment with 400 μg of P188 in mice who underwent TBI led to increased aquaporin mRNA expression at 1 and 6 h, but decreased expression at 24 and 48 h after injury (Bao et al., 2012). Chen et al. examined calcium regulation in astrocytes in response to shockwave-generated microcavitations. They found a large increase in intracellular calcium that was associated with exposure to microcavitations, followed by the loss of the ability to regulate calcium dynamics, as seen by lack of calcium spiking. Astrocytes treated with P188 at 500 μM exhibited partially restored calcium homeostasis (Chen et al., 2019) (Fig. 2F).

Grossly, the effect of P188 on the BBB can be evaluated with the Evans blue dye test and measurement of the brain water content. Large doses of P188, at 12mg/mouse, significantly reduced the perihematoma brain water content in the ipsilateral basal ganglia, as well as dye extravasation at 24 and 72 h after injury in the collagenase induced intracranial hemorrhage model in mice (Wang et al., 2015). P188 has the ability to upregulate expression of transmembrane proteins essential in the formation of tight junctions between endothelial cells. This effect on tight junctions, along with downregulation of the water channel, protein aquaporin 4, contribute to its effect in maintaining the BBB and reducing vasogenic edema.

3. 1. 6. Bleeding, vasospasm, and focal microvascular occlusion

In a controlled cortical impact rat model of TBI, P188, at a dose of 300 mg/kg, was able to decrease astrocytes staining positive for GFAP and microglial/macrophages staining positive for CD68, suggesting its role in modulating the secondary neuroinflammation that occurs after TBI. Moreover, it showed the ability to normalize bleeding time 22 h after injury, which was a significant decrease compared to that in mice who received saline treatment (Zhang et al., 2018). Microthrombosis and the area of hemorrhage were both reduced with the treatment of P188 compared to saline. Nitric oxide (NO) is a known smooth muscle relaxer that is a key player in vasodilation. Exposure to hypoxia significantly increased the level of total NO production in brain microvascular endothelial cells, whereas mechanical compression did not significantly alter the total NO production. Interestingly, P188, from 10 μM to 1000 μM, was able further increase NO production in cells that underwent compression injury, but not in cells that underwent hypoxia (Lotze and Riess, 2021).

3.2. Behavioral outcomes

In the controlled cortical impact model of TBI, the modified neurological severity score (mNSS) test was used to assess behavior outcomes of male Wister rats. The mNSS score includes motor, sensory, and reflex tests, and ranges from 0 to 18, with a higher mNSS score indicating a more severe injury (Chen et al., 2001). Sensorimotor function is assessed with foot fault, consisting of a fall or a slip between the wires with each weight-bearing step (Baskin et al., 2003). Using these metrics, Zhang et al. found that P188 treatment, at a dose of 300 mg/kg, significantly improved sensory motor function by reducing the number of foot fault steps, as well as the mNSS score (Zhang et al., 2018).

While traditionally considered ischemic and hemorrhagic stroke models, transient middle cerebral artery (MCA) occlusion model and collagenase induced intracranial hemorrhage (ICH) models provide us

with insights to behavioral outcomes related to ischemic and bleeding injuries that occur in TBI as well. Wire hanging and the pole test were two motor behavioral tasks studied in transient MCA occlusion model in mice (Gertz et al., 2006). Wire hanging assesses the balance and grip strength of mice, while the pole test assesses the amount of time it takes for an animal to turn downward from a head up position, and the total time it takes to reach the floor when placed near the top of a vertically placed pole (Matsuura et al., 1997). In wire hanging tests, mice treated with P188 for three weeks showed increased time on the wire compared that in mice treated with saline. The pole test revealed that mice treated with P188, at a dose of 400 mg/kg and 800 mg/kg completed a turn and reached the floor more quickly than those treated with saline (Gu et al., 2013). The sensorimotor Garcia test and the corner turn test were studied in the collagenase ICH model. The Garcia test consists of a composite assessment evaluating spontaneous activity, axial sensation, vibrissae proprioception, symmetry of limb movement, lateral turning, forelimb outstretching, and climbing (Garcia et al., 1995). A total score is calculated based on the sum of the scores from each category. The corner turn test consisted of observing which direction the animal chooses to turn to exit a 30-degree angled corner, with the score as the number of right turns divided by the total number of turns (Hua et al., 2002). Wang et al. found that a large dose of P188 at 12 mg/mouse significantly reduced neurofunctional deficits as measured by the Garcia test and the corner turn test (Wang et al., 2015).

The Morris water maze (MWM) test is one of the methods used to measure spatial learning impairments (Morris, 1981). It consists of allowing the animal to swim for 90s in a dark blue swimming pool until it finds a platform, which is transparent and invisible to the animal. Clues external to the maze are visible from the pool, and presumably used by the animals for spatial orientation. Spatial learning function is measured by the percentage of time the animal spends in the correct quadrant where a platform is located, and memory function is assessed by the amount of time the animal requires to find the platform. The MWM test is known for its sensitivity and reliability as an index of hippocampal injury (Othman et al., 2022). Zhang et al. found that treatment with P188, at a dose of 300 mg/kg after controlled cortical impact is associated with increased percentage of time spent in the correct quadrant compared to that in rats treated with saline, indicating enhanced spatial learning (Zhang et al., 2018). In vivo models of TBI consisting of the cortical impact and the weight drop models (Xiong et al., 2013), as well as models of intracranial hemorrhage and vaso-occlusive infarcts, two phenomena known to occur in TBI (Bae et al., 2014; Perel et al., 2009), all demonstrated sensory, motor, and learning impairments associated with injury. P188 was able to improve functional outcomes in all the studies examined above.

4. Discussion

The current body of literature provides valuable insight into not only the phenotypic impact of P188 in animal models of TBI, but also its complex mechanism of action in rescuing various types of cellular injuries that occur in TBI. However, we are still far from constructing a comprehensive list of the effects of P188 on cellular functions or using it as a therapeutic agent in humans. Many questions remain unanswered and serve as opportunities for future studies. Here, we examine some of the limitations present amongst the studies reviewed.

While most studies used P188 as a therapeutic treatment by administering it after injury, two studies used it as a prophylactic agent by administering it prior to the injury. Bao et al. determined that administration of P188 30 min prior to TBI in mice resulted in the least amount of brain edema compared to treatment with P188 at 1, 2, or 4 h after injury (Bao et al., 2016). Similarly, Gu et al. added P188 to the culture medium 1 h prior to exposing HT22 cells to oxygen-glucose deprivation (Gu et al., 2013). Though the administration of P188 both prior to, and after the injury resulted in a consistent trend of neuronal protection, there is a paucity of investigation into when P188 exerts its maximum benefit. Clinically, most cases of TBI occur in an unplanned fashion. Hence one could argue that there may not be utility in a prophylactic treatment for TBI, as its occurrence cannot be predicted. However, there are two populations in particular, soldiers and contact-sport-athletes, in whom the incidence of TBI is higher, and its occurrence may be anticipated (McKee and Robinson, 2014; Selassie et al., 2013). Having a prophylactic agent that could minimize the extent of neuronal injuries in these populations could significantly improve outcomes if TBI is more likely to occur. Therefore, the timing of the administration of P188 in TBI warrants further investigation.

Although the incidence of TBI is higher in males than females, any investigation of potential therapies must still consider both sexes and the differences that exist between them. The studies reviewed disproportionally used male mice and rats. This inherently ignores the sexual dimorphism that exists within TBI and subsequent neuroinflammation (Villapol et al., 2017). Despite our limited understanding on why and how such a difference exists, the data confirms this difference. For example, a study examining human CSF markers after TBI found that excitotoxic and ischemic events resulted in greater relative oxidative damage in males than in females (Caplan et al., 2017), while another found that male mice have more robust inflammatory responses to TBI within the first week after injury, which is no longer observed beyond the first week post-injury (Villapol et al., 2017). In order to gain a more complete understanding of the effects of P188 in TBI, we must address its effects in females as well. As a potential therapeutic agent, P188 must be investigated in a fashion that produces generalizable data for the entire population.

The primary injury that occurs in TBI not only triggers neuroinflammation, but also a systemic inflammatory response that is not just limited to the brain. While immune activation is imperative for cell recovery, a dysregulated immune response can lead to further secondary injuries. Systemic inflammatory response syndrome (SIRS) scores have been found to be a predictor for poor clinical outcomes in patients suffering from TBI (Jacome and Tatum, 2018). Apart from the study by Zhang et al. which found that P188 was able to reduce the number of microglial cells in rat models of TBI, there has not been any other investigation into the impact of P188 on neurologic or systemic inflammation (Zhang et al., 2018). Furthermore, studies have found TBI to be associated with chronic neuroinflammation, which can last for years after the injury, and some speculate that this may be involved in the development of neurodegeneration after repeated TBI (Simon et al., 2017). Hence the long-term effects of P188 serve as a point of investigation as well.

P188 is available in a commercial grade form and a purified form, Vepoloxamer. In this literature search, Zhang et al. is the only study that examined the effect of Vepoloxamer, rather than commercial grade P188. Their choice was based on the renal toxicity noted with commercial-grade P188. In 2014, Emanuele et al. found that the use of commercial grade P188 was associated with decreased creatinine clearance and renal dysfunction (Zhang et al., 2018). Given that P188 is excreted in the urine, interference with renal function will have an impact on its pharmacokinetics, prolonging its half-life. As we progress further into testing the efficacy of P188 in animal models and clinically, using Vepoloxamer may allow us to gain a more accurate understanding into dosing and the side effects of P188.

5. Conclusion and future directions

P188 has shown promising results as a possible treatment for TBI and other neurological disorders. A comprehensive review of the existing literature demonstrated that P188 can attenuate neuronal death, improve behavioral outcomes, and reduce brain edema in animal models of TBI. However, in order to consider the use of P188 as a therapeutic agent in the clinical setting, further research is required to gain a more complete understanding of its mechanism of action, pharmacokinetics, and its long-term effects on the delayed and prolonged

injuries that occur in TBI.

Given the ease at which P188 can cross the BBB, there is also a potential for utilizing it as a vehicle for drug delivery systems, particularly with nanotechnologies that are beginning to show therapeutic potential. In particular, theranostic strategies that exploit the advantages of nanotechnologies could offer an alternative to repair the leaky BBB. Application of this type of nanotechnology to TBI is an exciting future direction that could take advantage of the nanoscale cell rescue and repair mechanisms that appear to be facilitated by P188.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors.

CRediT authorship contribution statement

Renqing Wu: Writing – review & editing, Writing – original draft, Conceptualization. **Roopa Koduri:** Writing – review & editing, Writing – original draft, Conceptualization. **Michael Cho:** Writing – review & editing. **Nagham Alatrash:** Writing – review & editing, Writing – original draft, Supervision, Conceptualization. **Vanessa Nomellini:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Conceptualization.

Declaration of competing interest

I do not have any declarations of interest.

Data availability

No data was used for the research described in the article.

References

Bae, D.H., Choi, K.S., Yi, H.J., Chun, H.J., Ko, Y., Bak, K.H., 2014. Cerebral infarction after traumatic brain injury: incidence and risk factors. Korean J Neurotrauma 10, 35–40.

Bao, H.-J., Wang, T., Zhang, M.-Y., Liu, R., Dai, D.-K., Wang, Y.-Q., Wang, L., Zhang, L., Gao, Y.-Z., Qin, Z.-H., 2012. Poloxamer-188 attenuates TBI-induced blood–brain barrier damage leading to decreased brain edema and reduced cellular death. Neurochem. Res. 37, 2856–2867.

Bao, H., Yang, X., Zhuang, Y., Huang, Y., Wang, T., Zhang, M., Dai, D., Wang, S., Xiao, H., Huang, G., 2016. The effects of poloxamer 188 on the autophagy induced by traumatic brain injury. Neurosci. Lett. 634, 7–12.

Bartos, J.A., Matsuura, T.R., Tsangaris, A., Olson, M., McKnite, S.H., Rees, J.N., Haman, K., Shekar, K.C., Riess, M.L., Bates, F.S., 2016. Intracoronary poloxamer 188 prevents reperfusion injury in a porcine model of ST-segment elevation myocardial infarction. JACC (J. Am. Coll. Cardiol.): Basic to Translational Science 1, 224–234.

Baskin, Y.K., Dietrich, W.D., Green, E.J., 2003. Two effective behavioral tasks for evaluating sensorimotor dysfunction following traumatic brain injury in mice. J. Neurosci. Methods 129, 87–93.

Bodratti, A.M., Alexandridis, P., 2018. Formulation of poloxamers for drug delivery. J. Funct. Biomater. 9, 11.

Caplan, H.W., Cox, C.S., Bedi, S.S., 2017. Do microglia play a role in sex differences in TBI? J. Neurosci. Res. 95, 509–517.

Casella, J.F., Barton, B.A., Kanter, J., Black, L.V., Majumdar, S., Inati, A., Wali, Y., Drachtman, R.A., Abboud, M.R., Kilinc, Y., Fuh, B.R., Al-Khabori, M.K., Takemoto, C. M., Salman, E., Sarnaik, S.A., Shah, N., Morris, C.R., Keates-Baleeiro, J., Raj, A., Alvarez, O.A., Hsu, L.L., Thompson, A.A., Sisler, I.Y., Pace, B.S., Noronha, S.A., Lasky III, J.L., de Julian, E.C., Godder, K., Thornburg, C.D., Kamberos, N.L., Nuss, R., Marsh, A.M., Owen, W.C., Schaefer, A., Tebbi, C.K., Chantrain, C.F., Cohen, D.E., Karakas, Z., Piccone, C.M., George, A., Fixler, J.M., Singleton, T.C., Moulton, T., Quinn, C.T., de Castro Lobo, C.L., Almomen, A.M., Goyal-Khemka, M., Maes, P., Emanuele, M., Gorney, R.T., Padgett, C.S., Parsley, E., Kronsberg, S.S., Kato, G.J., Gladwin, M.T., 2021. Effect of poloxamer 188 vs placebo on painful vaso-occlusive episodes in children and adults with sickle cell disease: a randomized clinical trial. JAMA 325, 1513–1523.

Centers for Disease Control and Prevention, N.C.f.I.P.a.C., 2023. Get the Facts about TBI. Centers for Disease Control and Prevention, National Center for Injury Prevention and Control.

Chen, J., Sanberg, P.R., Li, Y., Wang, L., Lu, M., Willing, A.E., Sanchez-Ramos, J., Chopp, M., 2001. Intravenous administration of human umbilical cord blood reduces behavioral deficits after stroke in rats. Stroke 32, 2682–2688.

Chen, B., Tjahja, J., Malla, S., Liebman, C., Cho, M., 2019. Astrocyte Viability and functionality in spatially confined microcavitation zone. ACS applied materials & interfaces 11, 4889–4899.

Chen, W.N., Shaikh, M.F., Bhuvanendran, S., Date, A., Ansari, M.T., Radhakrishnan, A.K., Othman, I., 2022. Poloxamer 188 (P188), A potential polymeric protective agent for central nervous system disorders: a systematic review. Curr. Neuropharmacol. 20, 799.

Cheng, G., Kong, R.H., Zhang, L.M., Zhang, J.N., 2012. Mitochondria in traumatic brain injury and mitochondrial-targeted multipotential therapeutic strategies. Br. J. Pharmacol. 167, 699–719.

Clifton, G.L., Valadka, A., Zygun, D., Coffey, C.S., Drever, P., Fourwinds, S., Janis, L.S., Wilde, E., Taylor, P., Harshman, K., 2011. Very early hypothermia induction in patients with severe brain injury (the National Acute Brain Injury Study: hypothermia II): a randomised trial. Lancet Neurol. 10, 131–139.

Cornelius, C., Crupi, R., Calabrese, V., Graziano, A., Milone, P., Pennisi, G., Radak, Z., Calabrese, E.J., Cuzzocrea, S., 2013. Traumatic brain injury: oxidative stress and neuroprotection. Antioxidants Redox Signal. 19, 836–853.

Corso, P., Finkelstein, E., Miller, T., Fiebelkorn, I., Zaloshnja, E., 2006. Incidence and lifetime costs of injuries in the United States. Inj. Prev. 12, 212–218.

Crupi, R., Cordaro, M., Cuzzocrea, S., Impellizzeri, D., 2020. Management of traumatic brain injury: from present to future. Antioxidants 9, 297.

Curry, D.J., Wright, D.A., Lee, R.C., Kang, U.J., Frim, D.M., 2004. Poloxamer 188 volumetrically decreases neuronal loss in the rat in a time-dependent manner. Neurosurgery 55, 943–949.

Daugherty, J., Waltzman, D., Sarmiento, K., Xu, L., 2019. Traumatic brain injury–related deaths by race/ethnicity, sex, intent, and mechanism of injury—United States, 2000–2017. MMWR (Morb. Mortal. Wkly. Rep.) 68, 1050.

Dewan, M.C., Rattani, A., Gupta, S., Baticulon, R.E., Hung, Y.-C., Punchak, M., Agrawal, A., Adeleye, A.O., Shrime, M.G., Rubiano, A.M., 2018. Estimating the global incidence of traumatic brain injury. J. Neurosurg. 130, 1080–1097.

Garcia, J.H., Wagner, S., Liu, K.-F., Hu, X.-j., 1995. Neurological deficit and extent of neuronal necrosis attributable to middle cerebral artery occlusion in rats. Stroke 26, 627–635.

Gertz, K., Priller, J., Kronenberg, G., Fink, K.B., Winter, B., Schröck, H., Ji, S., Milosevic, M., Harms, C., Böhm, M., Dirnagl, U., Laufs, U., Endres, M., 2006. Physical activity improves long-term stroke outcome via endothelial nitric oxide synthase–dependent augmentation of neovascularization and cerebral blood flow. Circ. Res. 99, 1132–1140.

Gottlieb, R.A., 2011. Cell death pathways in acute ischemia/reperfusion injury. J. Cardiovasc. Pharmacol. Therapeut. 16, 233–238.

Gu, J.-H., Ge, J.-B., Li, M., Xu, H.-D., Wu, F., Qin, Z.-H., 2013. Poloxamer 188 protects neurons against ischemia/reperfusion injury through preserving integrity of cell membranes and blood brain barrier. PLoS One 8, e61641.

Hånell, A., Hedin, J., Clausen, F., Marklund, N., 2012. Facilitated assessment of tissue loss following traumatic brain injury. Front. Neurol. 3.

Hua, Y., Schallert, T., Keep, R.F., Wu, J., Hoff, J.T., Xi, G., 2002. Behavioral tests after intracerebral hemorrhage in the rat. Stroke 33, 2478–2484.

Inyang, E., Abhyankar, V., Chen, B., Cho, M., 2020. Modulation of in vitro brain endothelium by mechanical trauma: structural and functional restoration by Poloxamer 188. Sci. Rep. 10, 3054.

Jacome, T., Tatum, D., 2018. Systemic inflammatory response syndrome (SIRS) score independently predicts poor outcome in isolated traumatic brain injury. Neurocrit Care 28, 110–116.

Jha, R.M., Kochanek, P.M., Simard, J.M., 2019. Pathophysiology and treatment of cerebral edema in traumatic brain injury. Neuropharmacology 145, 230–246.

Kalogeris, T., Baines, C.P., Krenz, M., Korthuis, R.J., 2012. Cell biology of ischemia/reperfusion injury. International review of cell and molecular biology 298, 229–317.

Kanagaraj, J., Chen, B., Xiao, S., Cho, M., 2018. Reparative effects of poloxamer P188 in astrocytes exposed to controlled microcavitation. Ann. Biomed. Eng. 46, 354–364.

Kaur, P., Sharma, S., 2018. Recent advances in pathophysiology of traumatic brain injury. Curr. Neuropharmacol. 16, 1224–1238.

Kilinc, D., Gallo, G., Barbee, K.A., 2008. Mechanically-induced membrane poration causes axonal beading and localized cytoskeletal damage. Exp. Neurol. 212, 422–430.

Kochanek, P.M., Bramlett, H.M., Dixon, C.E., Shear, D.A., Dietrich, W.D., Schmid, K.E., Mondello, S., Wang, K.K.W., Hayes, R.L., Povlishock, J.T., Tortella, F.C., 2015. Approach to modeling, therapy evaluation, drug selection, and biomarker assessments for a multicenter pre-clinical drug screening consortium for acute therapies in severe traumatic brain injury: operation brain trauma therapy. J. Neurotrauma 33, 513–522.

Langham, J., Goldfrad, C., Teasdale, G., Shaw, D., Rowan, K., Group, C.I., 1996. Calcium channel blockers for acute traumatic brain injury. Cochrane Database Syst. Rev. 2010.

Langlois, J.A., Rutland-Brown, W., Wald, M.M., 2006. The epidemiology and impact of traumatic brain injury: a brief overview. J. Head Trauma Rehabil. 21, 375–378.

Lotze, F.P., Riess, M.L., 2021. Poloxamer 188 Exerts direct protective effects on mouse brain microvascular endothelial cells in an in vitro traumatic brain injury model. Biomedicines 9, 1043.

Luo, C.-L., Chen, X.-P., Li, L.-L., Li, Q.-Q., Li, B.-X., Xue, A.-M., Xu, H.-F., Dai, D.-K., Shen, Y.-W., Tao, L.-Y., 2013. Poloxamer 188 attenuates in vitro traumatic brain injury-induced mitochondrial and lysosomal membrane permeabilization damage in cultured primary neurons. J. Neurotrauma 30, 597–607.

Marks, J.D., Pan, C.-y., Bushell, T., Cromie, W., Lee, R.C., 2001. Amphiphilic, tri-block copolymers provide potent, membrane-targeted neuroprotection. Faseb. J. 15, 1107–1109.

Maskarinec, S.A., Hannig, J., Lee, R.C., Lee, K.Y.C., 2002. Direct observation of poloxamer 188 insertion into lipid monolayers. Biophys. J. 82, 1453–1459.
Matsuura, K., Kabuto, H., Makino, H., Ogawa, N., 1997. Pole test is a useful method for evaluating the mouse movement disorder caused by striatal dopamine depletion. J. Neurosci. Methods 73, 45–48.
Mckee, A.C., Daneshvar, D.H., 2015. The neuropathology of traumatic brain injury. Handb. Clin. Neurol. 127, 45–66.
McKee, A.C., Robinson, M.E., 2014. Military-related traumatic brain injury and neurodegeneration. Alzheimers Dement 10, S242–S253.
Meyer, L.J., Riess, M.L., 2021. Evaluation of in vitro neuronal protection by postconditioning with poloxamer 188 following simulated traumatic brain injury. Life 11, 316.
Miller, G.F., Kegler, S.R., Stone, D.M., 2020. Traumatic brain injury–related deaths from firearm suicide: United States, 2008–2017. American journal of public health 110, 897–899.
Mina, E.W., Lasagna-Reeves, C., Glabe, C.G., Kayed, R., 2009. Poloxamer 188 copolymer membrane sealant rescues toxicity of amyloid oligomers in vitro. J. Mol. Biol. 391, 577–585.
Moloughney, J.G., Weisleder, N., 2012. Poloxamer 188 (p188) as a membrane resealing reagent in biomedical applications. Recent Pat. Biotechnol. 6, 200–211.
Morris, R.G.M., 1981. Spatial localization does not require the presence of local cues. Learn. Motiv. 12, 239–260.
Ng, S.Y., Lee, A.Y.W., 2019. Traumatic brain injuries: pathophysiology and potential therapeutic targets. Front. Cell. Neurosci. 13.
Othman, M.Z., Hassan, Z., Che Has, A.T., 2022. Morris water maze: a versatile and pertinent tool for assessing spatial learning and memory. Exp. Anim. 71, 264–280.
Patricia, A.-G., Amos, K., Mabel, K., Martin, S., Robert, V., Daniel, W., Rebekah, C., Suzanne, E., James, B., Martin, E., 1997. RheothRx (poloxamer 188) injection for the acute painful episode of sickle cell disease: a pilot study. Blood 90, 2041–2046.
Perel, P., Roberts, I., Bouamra, O., Woodford, M., Mooney, J., Lecky, F., 2009. Intracranial bleeding in patients with traumatic brain injury: a prognostic study. BMC Emerg. Med. 9, 15.
Pille, J.A., Riess, M.L., 2021. Potential effects of poloxamer 188 on rat isolated brain mitochondria after oxidative stress in vivo and in vitro. Brain Sci. 11, 122.
Quinn, M., Mukhida, K., Sadi, D., Hong, M., Mendez, I., 2008. Adjunctive use of the non-ionic surfactant Poloxamer 188 improves fetal dopaminergic cell survival and reinnervation in a neural transplantation strategy for Parkinson's disease. Eur. J. Neurosci. 27, 43–52.
Sandor, B., Marin, M., Lapoumeroulie, C., Rabaï, M., Lefevre, S.D., Lemonne, N., El Nemer, W., Mozar, A., Français, O., Le Pioufle, B., 2016. Effects of Poloxamer 188 on red blood cell membrane properties in sickle cell anaemia. Br. J. Haematol. 173, 145–149.
Sarkar, C., Zhao, Z., Aungst, S., Sabirzhanov, B., Faden, A.I., Lipinski, M.M., 2014. Impaired autophagy flux is associated with neuronal cell death after traumatic brain injury. Autophagy 10, 2208–2222.
Selassie, A.W., Wilson, D.A., Pickelsimer, E.E., Voronca, D.C., Williams, N.R., Edwards, J. C., 2013. Incidence of sport-related traumatic brain injury and risk factors of severity: a population-based epidemiologic study. Ann. Epidemiol. 23, 750–756.
Serbest, G., Horwitz, J., Barbee, K., 2005. The effect of poloxamer-188 on neuronal cell recovery from mechanical injury. J. Neurotrauma 22, 119–132.
Serbest, G., Horwitz, J., Jost, M., Barbee, K.A., 2006. Mechanisms of cell death and neuroprotection by poloxamer 188 after mechanical trauma. Faseb. J. 20, 308–310.
Simon, D.W., McGeachy, M.J., Bayır, H., Clark, R.S.B., Loane, D.J., Kochanek, P.M., 2017. The far-reaching scope of neuroinflammation after traumatic brain injury. Nat. Rev. Neurol. 13, 171–191.
Teasdale, G., Jennett, B., 1974. Assessment of coma and impaired consciousness. A practical scale. Lancet 2, 81–84.
Thurman, D.J., Alverson, C., Dunn, K.A., Guerrero, J., Sniezek, J.E., 1999. Traumatic brain injury in the United States: a public health perspective. J. Head Trauma Rehabil. 14.
Villapol, S., Loane, D.J., Burns, M.P., 2017. Sexual dimorphism in the inflammatory response to traumatic brain injury. Glia 65, 1423–1438.
Wang, T., Chen, X., Wang, Z., Zhang, M., Meng, H., Gao, Y., Luo, B., Tao, L., Chen, Y., 2015. Poloxamer-188 can attenuate blood–brain barrier damage to exert neuroprotective effect in mice intracerebral hemorrhage model. J. Mol. Neurosci. 55, 240–250.
Winn, H.R., Temkin, N.R., Anderson, G.D., Dikmen, S.S., 2007. Magnesium for neuroprotection after traumatic brain injury. Lancet Neurol. 6, 478–479.
Wright, D.W., Yeatts, S.D., Silbergleit, R., Palesch, Y.Y., Hertzberg, V.S., Frankel, M., Goldstein, F.C., Caveney, A.F., Howlett-Smith, H., Bengelink, E.M., 2014. Very early administration of progesterone for acute traumatic brain injury. N. Engl. J. Med. 371, 2457–2466.
Xiong, Y., Mahmood, A., Chopp, M., 2013. Animal models of traumatic brain injury. Nat. Rev. Neurosci. 14, 128–142.
Yasuda, S., Townsend, D., Michele, D.E., Favre, E.G., Day, S.M., Metzger, J.M., 2005. Dystrophic heart failure blocked by membrane sealant poloxamer. Nature 436, 1025–1029.
Yıldırım, T., Eylen, A., Lule, S., Erdener, S.E., Vural, A., Karatas, H., Ozveren, M.F., 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.
Zaloshnja, E., Miller, T., Langlois, J.A., Selassie, A.W., 2008. Prevalence of long-term disability from traumatic brain injury in the civilian population of the United States, 2005. J. Head Trauma Rehabil. 23, 394–400.
Zargari, M., Meyer, L.J., Riess, M.L., Li, Z., Barajas, M.B., 2023. P188 therapy in in vitro models of traumatic brain injury. Int. J. Mol. Sci. 24, 3334.
Zhang, Y., Chopp, M., Emanuele, M., Zhang, L., Zhang, Z.G., Lu, M., Zhang, T., Mahmood, A., Xiong, Y., 2018. Treatment of traumatic brain injury with vepoloxamer (purified poloxamer 188). J. Neurotrauma 35, 661–670.