Investigating the use of PrP^C as an immediate treatment to reduce hemin toxicity in hemorrhagic stroke

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Abstract

Hemorrhagic strokes occur when a blood vessel ruptures or leaks, which leads to the damage or death of brain cells resulting in a range of symptoms. Hemin, a hemoglobin-derived molecule, is metabolized by enzymes to produce biliverdin, iron, and carbon monoxide, all of which can be lethal at high concentrations. The neurological, physical, and cognitive abilities in addition to attention, memory, problem-solving, and decision-making can all be impacted long-term by a hemorrhagic stroke. The cellular prion protein (PrP^C) helps form synapses, protect nerve cells from reactive oxygen species (ROS) damage, and prevent the buildup of excessive copper in the nervous system. In contrast to hemorrhagic stroke, which results from bleeding in the brain, ischemic stroke is brought on by a blood clot or plaque obstructing blood flow to the brain. This review explores the utilization of PrP^C and other neuroprotective proteins as a treatment for hemorrhagic stroke. In brief, the hemin-PrP^C interaction inhibits hemin from wreaking havoc intracellularly by preventing its release of oxidized metals such as iron; furthermore, this interaction increases hemoglobin (Hb) synthesis in hematopoietic cells, indicating a special role for PrP^C that may have an effect on the treatment of Cerebral hemorrhage and sCJD. However, PrP^C is not the only neuropeptide to demonstrate neuroprotective abilities. Secretoneurin (SN), a neuropeptide derived from secretogranin II, stimulates neuronal outgrowth of immature cerebellar granule cells and helps in the growth and repair of neuronal tissue. It was demonstrated that SN-mediated neuroprotection, which also promotes the expression of the antiapoptotic proteins, Bcl-2 and Bcl-xL, blocking apoptosis. These findings show that PrP^C and SN directly aid in the survival of neurons after hemorrhagic or ischemic injury.

Introduction

Hemorrhagic and ischemic strokes are two different types of strokes with distinct causes, symptoms, and treatment options. A hemorrhagic stroke occurs when a blood vessel in the brain ruptures or leaks, causing bleeding into the surrounding brain tissue. (Kumar et al 2022) This can lead to damage or death of brain cells and can cause a wide range of symptoms, depending on the location and severity of the bleed. (DeSai et al 2022) Whereas ischemic stroke is caused by a blood clot or plaque that blocks blood flow to the brain. (Stroke causes and risk factors. *NIH.*) Both types of strokes have similar symptoms such as sudden weakness or numbness on one side of the body, difficulty speaking or understanding speech, and vision problems. (DeSai et al 2022) However, hemorrhagic stroke may also cause severe headache, neck pain, and vomiting, while ischemic stroke may cause confusion or loss of consciousness. (Abdu et al 2021) Hemorrhagic strokes are less common than ischemic strokes, but they are often more severe and are life-threatening if not treated promptly. (Kumar et al 2022).

The current treatment for ischemic stroke typically involves dissolving the blood clot using clot-busting drugs or removal with a device, while treatment for hemorrhagic stroke may involve controlling the bleeding, reducing pressure on the brain, and repairing the damaged blood vessel. (Stroke treatment. *NHS*.) The prognosis for ischemic stroke is generally better than for hemorrhagic stroke as it is more treatable. However, the severity of the stroke, the speed of diagnosis, and the overall health of the patient also play a role in recovery. (Abdu et al 2021) Ischemic stroke is more common than hemorrhagic stroke, accounting for approximately 87% of all strokes.(Donkor et al 2018) While both ischemic and hemorrhagic stroke are widely important to study, this review will solely be focusing on the effects and treatments of hemorrhagic stroke.

Long term consequence of hemorrhagic stroke

The long-term consequences of a hemorrhagic stroke can vary depending on the location and severity of the bleed, as well as the overall health of the individual. Some long term effects of hemorrhagic stroke are physical impairments including weakness or paralysis on one side of the body, difficulty with coordination and balance, and problems with fine motor skills.(Raghavan et al 2015) The long term effects of hemorrhagic stroke are also cognitive, impairing a person's ability to think, communicate, and remember things.(Al-Qazzaz et al 2014) They may also have difficulty with attention, memory, problem-solving, and decision making.(Shinichiro Maeshima et al 2021). Long term effects of hemorrhagic stroke can be severe and potentially permanent. This high severity of long term effects of hemorrhagic stroke is more catastrophic than ischemic strokes due to the blood toxicity that occurs when the pH change between the blood vessel and neuronal extracellular space causes the heme in hemoglobin to oxidize to hemin (Figure 1).

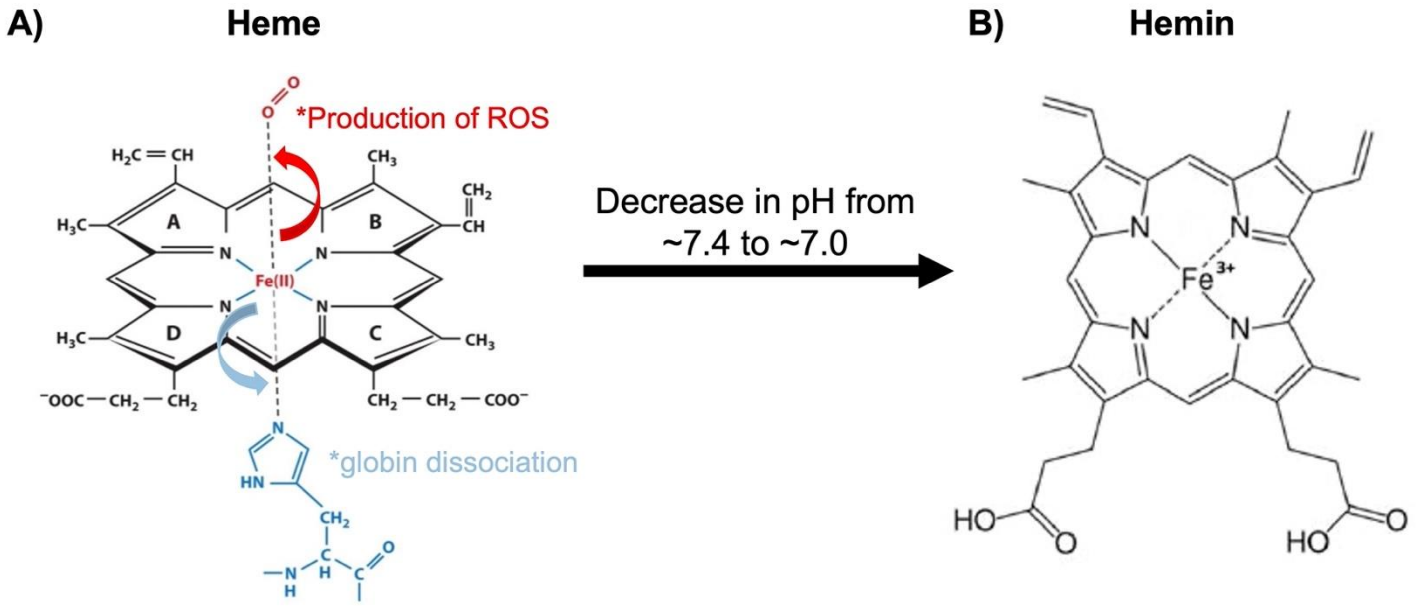


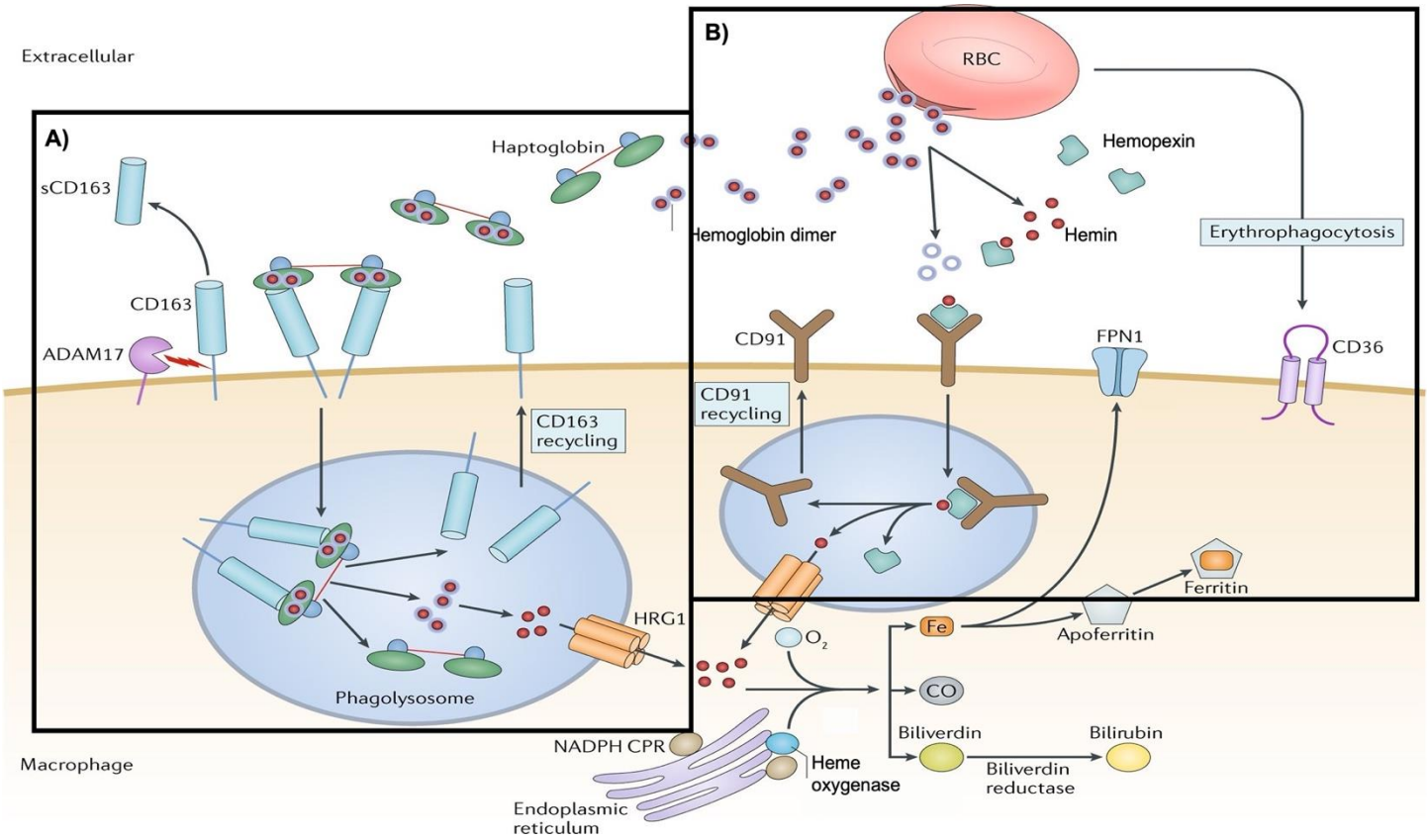
Figure 1. *Write figure caption.*

Hemin toxicity and hemorrhagic stroke

Normally when hemolysis occurs due to a vascular injury, hemoglobin is released from red blood cells (RBCs) into the extracellular space, where haptoglobin uptakes hemoglobin dimers, which triggers endocytosis upon binding to CD163 cell surface receptor. Upon endocytosis, CD163 receptors are recycled and hemoglobin is sorted to the phagolysosome to be degraded, with minor levels of hemin being transported into the cytosol to be further degraded by heme oxygenase, which converts it into iron, carbon monoxide, and biliverdin, which is subsequently metabolized into bilirubin by biliverdin reductase. The iron that is produced can be stored intracellularly or be exported out of the cell. The carbon monoxide that is produced can act as a signaling molecule, but it can also be toxic in high concentrations (Figure 2A).

However, when hemolysis occurs due to a vascular injury in the brain, the change in pH between the blood vessel and the neuronal extracellular space causes the porphyrin molecule heme to dissociate from the globin protein and become oxidized to hemin, creating a reactive oxygen species (ROS) as a byproduct. Hemopexin then binds to hemin and is uptaken intracellularly via endocytosis upon hemopexin binding to the CD91 cell surface receptor. In the sorting endosome, hemopexin is recycled and hemin is transported to the cytosol via HRG1 channels where it is subsequently broken down by heme oxygenase and biliverdin reductase like the normal vascular injury uptake and depletion mechanism (Figure 2B). The issue here is that normal cellular uptake of hemoglobin usually results in little hemin production, and therefore ROS. Whereas neuronal vascular injury results in the excess production of ROS due to hemin oxidation. Therefore, the toxicity of hemin in astrocytes is thought to be related to the generation of reactive oxygen species, which can damage cell membranes, nascent

peptides, DNA, and other cellular components. Furthermore, the accumulation of bilirubin, a byproduct of hemin metabolism, has been linked to oxidative stress and inflammation in astrocytes. (Dang et al 2011).



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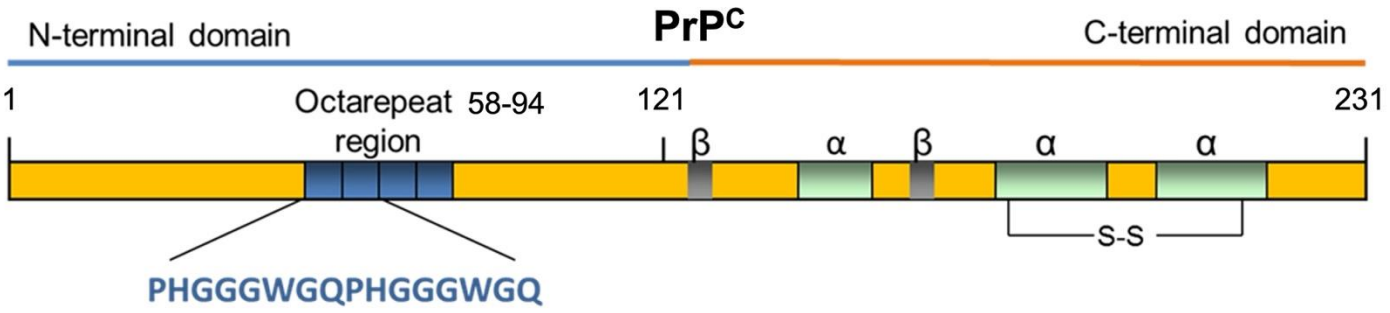
PrP^{C} neuroprotective properties

The cellular prion protein (PrP^{C}) is known to play a role in the growth and development of nerve cells through several protective mechanisms. PrP^{C} has antioxidant properties demonstrated to protect nerve cells from damage caused by ROS, which can damage neuronal cells and contribute to the development of neurodegenerative diseases. (Pham-Huy et al 2008). PrP^{C} also promotes the formation of synapses, which are the connections between nerve cells, making this crucial to the proper functioning of the nervous system. (Garmo et al 2021) Another important property of PrP^{C}, is that it has been demonstrated that it can bind copper ions. Copper is an essential trace element that is involved in many physiological processes, but excess of it can lead to oxidative stress and neurodegeneration. PrP^{C} binding to copper ions can prevent the accumulation of excess copper in the nervous system, which could be toxic. (Garmo et al 2021). Therefore, scientists are looking to the use of PrP^{C} in the immediate treatment of hemin toxicity caused by hemorrhagic stroke.

Neuroprotective mechanisms of intrinsically disordered peptides

Current evidence points to hemin inducing endocytosis of PrP proteins from the neuronal plasma membrane, potentially limiting propagation of the PrP-scrapie (PrP^{Sc}) isoform, which causes Mad Cow's Disease. The hemin-PrP^{C} interaction is also of interest in cerebral-hemorrhage (CH), this condition proposes the same issue of blood, and henceforth hemin toxicity again due to the pH change in the neuronal extracellular space causing the dissociation of heme and globin to result in the accumulation of hemin. Interestingly, PrP^{C} is upregulated in penumbric neurons surrounding CH pooling and is known to confer neuroprotection in a dose-dependent manner. (Tripathi et al 2016) However, the underlying mechanism is not clear. Tripathi et al (2016) reports that PrP^{C} binds hemin consistently across diverse cell lines, resulting in its aggregation or degradation in a cell-type specific manner. PrP^{C}-hemin interaction also upregulates Hb synthesis in hematopoietic cells, a response that is abolished by deleting the intrinsically disordered hemin-binding octa-repeat region of PrP^{C} (Figure 3). (Tripathi et al 2016) A similar response occurs in brain organotypic cultures where exposure to hemin upregulates α-

globin expression in PrP^C wild-type (WT) relative to PrP^C knockout (KO) samples. Furthermore, red blood cells and brain tissue from PrP^C KO mice show significantly less α-globin relative to WT controls, indicating a positive effect of PrP^C on Hb synthesis under physiological conditions as well. (Tripathi et al 2016) Conversely, levels of α-globin are significantly higher in sCJD brain tissue relative to controls, suggesting compensatory upregulation of Hb synthesis by surviving neurons or misregulation in diseased brains. These observations reveal a unique function of PrP^C that is likely to impact the therapeutic management of CH and sCJD, and putatively hemorrhagic stroke due to the similarities with hemin toxicity due to blood pooling. (Tripathi et al 2016)



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~~It has been postulated that the development of amyloid plaques in AD may result from an imbalance between the generation and clearance of the amyloid-beta peptide (α-beta). Although familial AD appears to be caused by α-beta overproduction, whereas the most prevalent form of AD, sporadic AD, may result from impairment in α-beta clearance (Yin et al 2006). Recent evidence suggests that several proteases may contribute to the degradation of Abeta. Furthermore, astrocytes have recently been implicated as a potential cellular mediator of Abeta degradation. In this study, we examined the possibility that matrix metalloproteinases (MMPs), proteases known to be expressed and secreted by astrocytes, could play a role in extracellular Abeta degradation *(Ke-Jie Yin et. al. 2006)*. We found that astrocytes surrounding amyloid plaques showed enhanced expression of MMP-2 and MMP-9 in aged amyloid precursor protein (APP)/presenilin 1 mice. Moreover, astrocyte-conditioned medium (ACM) degraded Abeta, lowering levels and producing several fragments after incubation with synthetic human Abeta(1-40) and Abeta(1-42). This activity was attenuated with specific inhibitors of MMP-2 and -9, as well as in ACM derived from mmp-2 or -9 knock-out (KO) mice *(Ke-Jie Yin et. al. 2006).* In vivo, significant increases in the steady-state levels of Abeta were found in the brains of mmp-2 and -9 KO mice compared with wild-type controls. Furthermore, pharmacological inhibition of the MMPs with N-[(2R)-2-(hydroxamidocarbonylmethyl)-4-methylpentanoyl]-L-tryptophan methylamide (GM 6001) increased brain interstitial fluid Abeta levels and elimination of half-life in APPsw mice. These results suggest that MMP-2 and -9 may contribute to extracellular brain Abeta clearance by promoting Abeta catabolism. *(Ke-Jie Yin et. al. 2006)*~~

While the known effects of the intrinsically disordered octa-repeat region of PrP^C on hemin toxicity and clearance is limited, there are other intrinsically disordered peptides (IDPs) that have been demonstrated to confer neuroprotection that we can draw parallels with to learn more about the possible mechanism of action of PrP^C clearance of hemin. Secretoneurin (SN), a primarily intrinsically disordered neuropeptide derived from secretogranin II, promotes neurite outgrowth of immature cerebellar granule cells. SN also aids in the growth and repair of neuronal tissue, although the precise mechanisms underlying the promotion of brain tissue neuroprotection and plasticity by SN are not understood. A rat model of ischemic stroke and in ischemic human brain tissue cultures, PrP^C was markedly upregulated in both neurons and endothelial cells upon SN treatment. (Shyu et al 2008) SN-mediated neuroprotection rescued primary cortical cell cultures from oxygen/glucose deprivation and induced expression of the antiapoptotic proteins Bcl-2 and Bcl-xL through the Jak2/Stat3 pathway, inhibiting apoptosis by blocking caspase-3 activation. (Shyu et al 2008) Furthermore, SN treatment enhanced stem cell targeting to the injured brain tissue in mice and promoted angiogenesis to increase local cortical blood flow in the ischemic hemisphere. (Shyu et al 2008) Both in vitro and in vivo, SN not only promoted neuroprotection, but also enhanced neurogenesis and angiogenesis. These results demonstrate that SN acts directly on neurons after hypoxia and ischemic insult to further their survival by activating the Jak2/Stat3 pathway.

(Shyu et al 2008) In summation, while the use of neuropeptides to confer neuroprotection against hemin toxicity is a cutting-edge field with very little experimental results, we can infer that in general intrinsically disordered neuropeptides confer neuroprotection in the immediate use after vascular injury in the brain.

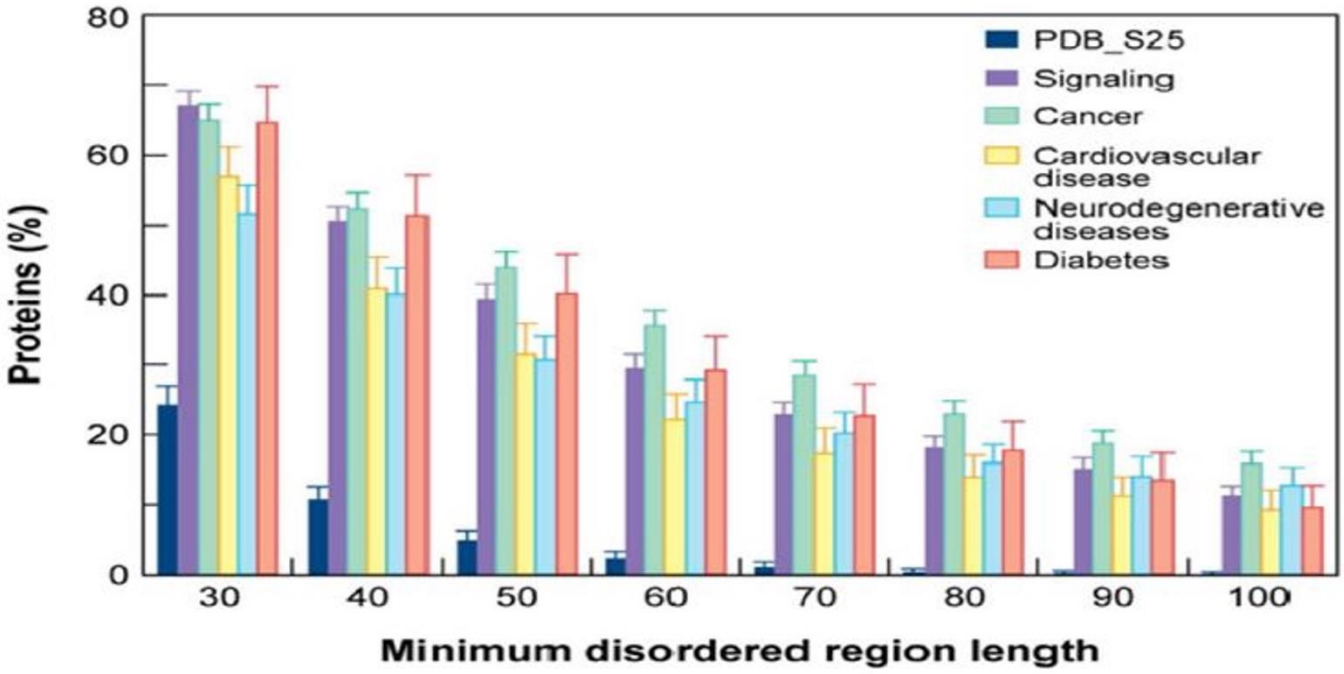
Discussion

Hemorrhagic strokes occur when a blood vessel ruptures or leaks, leading to damage or death of brain cells and a range of symptoms including vision issues, sudden headaches, unconsciousness, weakness or numbness on one side of the body, and trouble speaking or understanding speech. Although hemorrhagic strokes are less frequent than ischemic strokes, they are frequently more serious and, if untreated, can be fatal due to the hemin toxicity associated with the bleed. When hemin is catabolized by heme oxygenase, which converts it into biliverdin, iron, and carbon monoxide, biliverdin which is then further metabolized into bilirubin, results in the accumulation of iron, bilirubin, and carbon monoxide, which can all act as signaling molecules at low concentrations, become toxic in high concentrations. While all these byproducts contribute to hemin toxicity, the primary source of toxicity is thought to be in the generation of ROS upon the dissociation of heme and globin, which can damage cell membranes and other cellular components.

Hemorrhagic stroke is known to have more severe long-term consequences than ischemic stroke due to the hemin toxicity, which can result in coordination, balance, and fine motor skills impairments as well as impairing cognitive and communication skills. Therefore, finding immediate treatments to combat hemin toxicity while surgeons fix the bleed, is the next step to improve hemorrhagic stroke treatment to result in better long-term recovery outcomes.

The intrinsically disordered octa-repeat region of PrP^C has been demonstrated to confer neuroprotection against hemin toxicity in both rat and human brain tissue cultures. While the mechanism of action regarding this neuroprotection is unknown, we speculate that it follows a similar mechanism to the normal hemoglobin recycling mechanism, where PrP^C bound to hemin would be endocytosed and sorted into a phagolysosome for further hemin degradation, versus hemin catabolism that occurs when hemin is uptaken by hemopexin and further broken down intracellularly. Furthermore, the use of PrP^C against hemin toxicity is a viable strategy for the treatment of CH, due to the hemin- PrP^C interaction upregulating Hb synthesis in hematopoietic cells, putatively contributing to neurogenesis and angiogenesis repair upon intracerebral damage.

Outside of the use of PrP^C to confer neuroprotection, IDPs in general can confer protection against many physiological diseases through an unknown mechanism (Figure 4). This concept was further demonstrated by the use of the intrinsically disordered secretoneurin peptide, to improve stroke outcomes in a mouse model. They found that secretoneurin increased the expression of PrP^C and protected against brain damage caused by stroke, through an unknown mechanism, but activation of antiapoptotic pathways likely play a role as well, concluding that PrP^C may play a key role in the neuroprotective effects of secretoneurin.



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It is the authors opinion that the most viable treatment for hemorrhagic stroke depends on the severity and the location of the hemorrhage. Furthermore, it is likely that the future of stroke treatment be a multi-faceted approach through the use of medications to control blood pressure within the brain to prevent it from rupturing further, in addition to a drug treatment to combat hemin toxicity, while also treating the stroke surgically. While the use of intrinsically disoreded peptides to combat hemin toxicity is still in its infancy, there is great potential for the exploitation of these neuroprotective properties to increase long-term recovery outcomes from hemorrhagic stroke.

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