Serine Proteases and Chemokines in Neurotrauma: New Targets for Immune Modulating Therapeutics in Spinal Cord Injury

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Abstract: Progressive neurological damage after brain or spinal cord trauma causes loss of motor function and treatment is very limited. Clotting and hemorrhage occur early after spinal cord (SCI) and traumatic brain injury (TBI), inducing aggressive immune cell activation and progressive neuronal damage. Thrombotic and thrombolytic proteases have direct effects on neurons and glia, both healing and also damaging bidirectional immune cell interactions. Serine proteases in the thrombolytic cascade, tissue- and urokinase-type plasminogen activators (tPA and uPA), as well as the clotting factor thrombin, have varied effects, increasing neuron and glial cell growth and migration (tPA), or conversely causing apoptosis (thrombin) and activating inflammatory cell responses. tPA and uPA activate plasmin and matrix metalloproteinases (MMPs) that break down connective tissue allowing immune cell invasion, promoting neurite outgrowth. Serine proteases also activate chemokines. Chemokines are small proteins that direct immune cell invasion but also mediate neuron and glial cell communication. We are investigating a new class of therapeutics, virus-derived immune modulators; One that targets coagulation pathway serine proteases and a second that inhibits chemokines. We have demonstrated that local infusion of these biologics after SCI reduces inflammation providing early improved motor function. Serp-1 is a Myxomavirus-derived serine protease inhibitor, a serpin, that inhibits both thrombotic and thrombolytic proteases. M-T7 is a virus-derived chemokine modulator.

Here we review the roles of thrombotic and thrombolytic serine proteases and chemoattractant proteins, chemokines, as potential therapeutic targets for SCI. We discuss virus-derived immune modulators as treatments to reduce progressive inflammation and ongoing nerve damage after SCI.

Keywords: Neurotrauma, spinal cord injury, immune, inflammation, serine protease, serpin, thrombosis, thrombolysis, chemokine.

1. INTRODUCTION

1.1. Trauma in the Central Nervous System; Spinal Cord Injury (SCI) and Traumatic Brain Injury (TBI)

Damage after traumatic brain injury (TBI) or spinal cord injury (SCI) can be extraordinarily severe, causing prolonged morbidity and mortality and often leaving victims with sustained neurological deficits [1-6]. It is estimated that approximately 288,000 people in the United States are currently living with severe penetrating and / or compressive neuronal damage after SCI [3]. The World Health Organization (WHO) estimates that approximately 250,000-500,000 people worldwide suffer a spinal cord injury each year [7]. Injury to the central nervous system is associated with initial bleeding and / or clotting, followed by an aggressive inflammatory response and a final organization of the damaged area into cystic spaces and scarring, which can be both protective and healing or cause further damage [8-14]. Neuronal damage is extensive and progressive with SCI and TBI, due to ongoing hemorrhage as well as micro-thrombotic changes in small vessels with secondary persistent immune damage limiting recovery. High-dose methylprednisolone (steroid) is given in some centers for SCI; however, a significant amount of published data has indicated that the risk of adverse effects and morbidity consistently outweighs benefit. Further, many clinical trials have indicated that steroids for SCI lead...
to variable results that are dependent upon individual patients [15-25]. Thus, current cervical SCI guidelines strongly advise against the use of steroids and many experts advocate against their use [26–28]. The American Association of Neurosurgeons issued a Level 1 recommendation against the use of methylprednisolone to treat acute SCI, cautioning about the use of high-dose steroids due to their negative side effect profile [29]. The use of steroids is thus now at the discretion of treating physicians, remaining a topic for debate [15-30]. There is a clear and current need for effective treatment of SCI and for a standard of medical care for these patients. Many new treatments are under active investigation and some hold great promise, but none have proven clinical benefit at this time. Patients with SCI are often left with permanent disabilities, such as paralysis, painful radiculopathy, and paraplegia. In some cases, patients have ongoing progressive damage requiring mechanical or motorized support for mobility. Newer experimental approaches to treatment include stem cells, either implanted alone or implanted in hydrogels. These hydrogels are designed to provide a surface for neuronal outgrowth across cystic spaces [31-57]. Other treatments have been developed that range from modification of local electrolyte composition [54-56] to altering protease activation. Some approaches specifically target serine proteases in the thrombotic and thrombotic cascades, in addition to modulation of proteases that induce inflammatory damage or cause fibrous scar formation and further oxidative damage. Conversely, other potentially beneficial treatments include the release of growth factors that encourage neuronal growth and extension [58-61]. These newer treatments remain investigational.

An improved understanding of the basic cellular and molecular mechanisms that induce progressive neurological damage after SCI is central to developing effective treatments that will improve short- and long-term outcomes after CNS injury. In this review, we begin with an overview of two pathways that have been implicated in early injury after spinal cord trauma and that are also implicated in the ongoing damage leading to worsening function and pain. We will discuss the roles of activation and suppression of the clot-dissolving (thrombolytic, also termed fibrinolytic) and also the clot-forming (thrombotic) serine proteases after SCI [62, 63], as well as the roles of the small proteins that act to direct immune cell migration and invasion to sites of trauma, the chemokines, in SCI [62-68]. We will discuss studies relevant to these specific pathways in SCI and TBI as well as relevant findings after ischemic damage in strokes (cerebrovascular accidents, CVA). We will then review the potential for treating early SCI with drugs that target serine protease and chemokine pathways, as well as a new class of virus-derived immune-modulating proteins that selectively target coagulation (thrombosis and thrombolysis) and also chemokine pathways [62-67]. Two such virus-derived immune modulators have been tested for efficacy in rat spinal cord compression models for SCI and have demonstrated some early benefit. Other mammalian proteins that target these same pathways are also under investigation and have shown promise in preclinical studies. These findings underscore the central roles that these molecular pathways have in SCI and the potential to design new approaches to the treatment of SCI that modulate these pathways and improve long term outcomes.

1.2. Coagulation Pathway Activation; Thrombosis and Thrombolysis after Neurotrauma

When there is trauma in the mammalian body, the initial response is hemorrhage due to vessel damage and leak. There is also a risk for vascular thrombotic occlusion in larger or smaller vessels (macro- and micro-thrombotic occlusion) due to compression and / or stasis of blood flow. Each of these initial clot-forming breakdown responses is designed to prevent excess bleeding by activating clot-forming proteases, and to prevent excess clotting by activating thrombolysis, representing a natural balance of thrombosis and thrombolysis throughout the body and in the CNS. The activation of these clot forming and clot-dissolving cascades, in turn, can induce innate (inflammatory) and (antibody mediated) immune cell responses. Thus, initial trauma is followed by repair responses through activation of both the coagulation pathways and through innate and adaptive immune pathways, the restorative inflammatory and immune responses. In contrast, the persistence of inappropriate or excess thrombosis, thrombolysis or immune responses can also lead to damage. Persistent hemorrhage or clotting can cause further tissue destruction and activation of immune cells that invade nervous system tissues, causing escalating damage to the CNS. Thrombosis, hemorrhage and excess immune cell activation can initiate further progressive damage after brain or spinal cord trauma.

Sequela thrombosis pathway and immune responses to injury are seen throughout the mammalian body and cause ongoing severe damage in clotting or inflammatory disorders, as is seen in inflammatory atherosclerosis and vasculitis, or infectious disorders, such as acute respiratory distress syndromes in SARS-CoV-2 infections or bacterial sepsis with disseminated intravascular coagulation. This response to injury has been described for hundreds of years by two well-established medical axioms: 1) the inflammatory response to trauma referred to as ‘tumor, rubor, calor and dolor’ in Latin, representing swelling, erythema or redness, warmth, and pain respectively and 2) Virchow’s triad, specifically venous stasis, activation of coagulation, and venous damage. The inflammatory response after injury was first described by Galen and recorded by Celsus the Roman scholar in the first century AD and more recently ‘functio laesa’, or loss of function, has been added as the fifth sign of inflammation and seems very appropriate when discussing damage to the CNS [69]. Virchow’s triad was described by Rudolf Virchow in the 1800’s in Berlin, Germany. This triad is described in greater depth as transient hypercoagulability with platelet activation, venous stasis from paralyzed venous muscle pumps, and vascular endothelial damage from accompanying injury, venous dilation, and pressure on the veins, which all contribute to venous thromboembolism (VTE). Patients who suffer from acute cervical SCI have a very high risk of systemic venous thromboembolism, and spinal trauma guidelines recommend both mechanical and chemical blood clot prophylaxis [70, 71], with the potential to increase bleeding at the site of SCI. In SCI, these elements of hemorrhage and thrombosis, and the subsequent aggressive
also has a wide array of functions outside of clot formation, growth, glial cell activity, the endothelium in the vasculature, and neuroplasticity after CNS damage [71-75] as well as indirect effects on immune cell responses to injury. Thus, both thrombolytic and thrombotic serine proteases have direct effects on neuron growth, extension, and neuroplasticity after CNS damage [71-75] as well as indirect effects on immune cell responses to injury.

Current understanding of the roles of serine proteases and serine protease inhibitors, termed serpins, in neurotrauma after SCI and, more generally in TBI, is reviewed in the following sections.

1.3. General Overview of Immune Cell Responses to CNS Injury

The first stage in the immune response to spinal cord injury is infiltrating neutrophils and monocytes that enter the spine from circulating blood and are recruited to sites of SCI through glial chemokine and cytokine release. Subsequent upregulation of chemotactic adhesion molecules such as ICAMs and VCAMs which are Ig-superfamily cell adhesion molecules as well as upregulation of selectins such as P-selectins and L-selectins on endothelial cells attracts cells in the circulating blood (the arterial lumen) to migrate into sites of neurological damage. This is a complex response in that both peripheral immune cells from the circulating blood, along with endogenous microglia, drive the inflammatory response to SCI [76]. If the immune or inflammatory response is prolonged or excessive, this can cause progressive damage. Damage is produced by cellular invasion, breakdown of cells, connective tissue and vascular barriers and also activation of coagulation pathway responses (Figs. 1-3). However, the innate immune responses are also the first-line response to injury and healing and can enable cellular and neurological tissue recovery, leading to nervous system tissue repair through this mixed reparative and pathological responses [77]. The immune response related to circulating blood mononuclear cells invading at sites of trauma may provide an early response that is lost once the area of damage is sealed off, leading to a reestablished predominant role of the intrinsic glial cell responses.

The general role of glial cells, which includes multiple cell subsets in the CNS, is to support signaling and neuron activity, as well as acting as a resident immune response network within the CNS (Fig. 1). Microglia are CNS immune cells that police the CNS environment, phagocytosing pathogens and secreting cytokines and growth factors [76-80]. Oligodendrocytes produce myelin sheaths that insulate axons, creating quick and efficient action potential conduction. Satellite oligodendrocytes are situated in the grey matter of the CNS and do not myelinate axons; however, during demyelinating injuries, satellite oligodendrocytes are recruited to aid in remyelinating damaged axons. This oligodendrocyte recruitment is advantageous in times of injury because oligodendrocytes support the metabolic needs of neurons and protect against neuronal apoptosis [78]. Astrocytes act as links between the vasculature and neurons, providing interfaces between the vasculature and neurons in the brain and spinal column. Astrocytes provide neurotransmitters, ions, and nutrients for neuronal signaling. Pericytes sheath endothelial cells in capillaries in the vascular networks and modulate capillary diameter and alter vascular coupling and function.

2. THROMBOLYTIC SERINE PROTEASES

2.1. Tissue-type Plasminogen Activator, tPA

Tissue-type plasminogen activator, tPA, has displayed both beneficial and also harming actions in the CNS after neurotrauma. tPA is a highly effective thrombolytic, well-known for clinical applications as a highly effective treatment, improving lives and cardiac function as well as recovery after strokes by dissolving clots and opening arterial occlusions after acute thrombotic occlusion. tPA, as well as urokinase and streptokinase, act rapidly to dissolve sudden coronary occlusions in ST elevation myocardial infarctions (STEMI), disrupting occlusive coronary arterial clot and more recently tPA has been used for cerebrovascular thrombotic occlusions, in strokes, termed cerebrovascular accidents (CVA or stroke) [80]. The fibrinolytic (thrombolytic) plasminogen activator, termed fibrokinase, was first reported by Astrup and Permin in 1947 [75]. Later Pennica, et al.
cloned and expressed tPA in Escherichia coli in 1983, leading to clinical applications for treatment in ST Elevation Myocardial Infarction (STEMI heart attack) [81]. Thrombolysis for heart attacks is now widely displaced by direct mechanical revascularization, termed primary percutaneous coronary intervention (PCI) with stent implants for heart attacks. However, tPA is used and is highly effective, when PCI is not accessible. tPA is also used in acute thrombotic CVAs, if detected early within a narrow therapeutic window. In treatment for early-detection of CVA, tPA is highly beneficial, but does have the attendant risk of bleeding. Not only can tPA treatment lead to reperfusion of an ischemic cerebral vascular occlusion, but tPA also has a risk of secondary hemorrhage, due in part to increased collateral supply to the ischemic area or disruption of the BBB at sites of infarction with hemorrhagic transformation. Thus, the use of tPA is highly beneficial in reducing ischemic thrombotic infarction in the brain (CVA) and restoring blood flow to the brain, but tPA can also cause harm due to excess bleeding produced by an imbalance in the thrombotic and thrombolytic pathways. Thus, the use of tPA for CVA has remained a subject of debate, and this risk of hemorrhage after tPA treatment for CVA further illustrates the risks of excess damage when there is ongoing bleeding in the CNS.

tPA is a 69kDa protease that interacts with low-density lipoprotein receptor-related protein (LRPR) and NMDA (N-Methyl-D-Aspartate) receptors that modify cell activation [77]. tPA not only functions to break down blood clots [82-85], increasing oxygen and nutrient supply for neuronal health, but tPA is also associated with directly aiding in neuronal growth (Fig. 2) and has shown early benefit in neurotrauma with initial effects of improved motor function in induced pluripotent stem cell treatments in rats [86] tPA has pleiotropic, wide-ranging effects in the CNS including bene-
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Fig. (2). Postulated protective effects of serine proteases after SCI. Thrombin leads to decreased bleeding through thrombus formation. tPA and uPA breakdown excessive clotting. tPA enhances neurite outgrowth. Serp-1 balances thrombotic or thrombolytic serine protease activity by targeting activated proteases in both pathways and can reduce inflammatory cell activation and invasion. (A higher resolution / colour version of this figure is available in the electronic copy of the article).

With neurotrauma and specifically SCI, tPA is reported to produce early beneficial effects after SCI with improved motor function (Fig. 2). tPA is also reported to lead to a loss of integrity of the BBB, the cerebrovascular barrier that provides functional protection against excess toxicity, systemic [94-98] immune responses, as well as local aggressive immune damage and toxicity [97]. It has been demonstrated that tPA deficient (tPA−/−) mice have smaller stroke volumes suggesting that tPA also has damaging functions after CVA. Supportive studies have demonstrated that tPA treatment leads to adverse effects in tPA−/− mice by increasing stroke volumes after middle cerebral artery occlusion (Fig. 3) [96-100]. There are reports that tPA breaks down endothelial cell-to-cell connections, the basis for the protective BBB. This is supported by studies demonstrating that tPA−/− mice have higher preserved integrity of the BBB [82, 83]. tPA regulation of the BBB is controlled through activation of...
platelet-derived growth factor receptor alpha (PDGFRα) on perivascular astrocytes mediating increased vascular permeability [83]. The endothelial cell layer with smooth muscle cells and perivascular astrocytes maintains the integrity of the BBB, maintaining tight control over concentrations of molecules in the CNS.

Thus, tPA is highly multifunctional, with multiple and extensive activities separated from plasmin activation and clot breakdown and separate from the dissolution of fibrin degradation in clots. There is a high expression of tPA in vascular endothelium, as well as throughout the brain in the microglia, astrocytes, oligodendrocytes and neurons [84, 87]. tPA is considered an acute phase reactant, upregulated with increased secretion in the endothelium after CNS injury, but also upregulated in neurons, microglia, astrocytes and oligodendrocytes after CNS injury [83]. In neurons, tPA is expressed by excitatory neurons as well as perivascular inter-neurons [88]. tPA is reported to activate growth factors such as nerve growth factors (pro-NGF and pro-BDNF), activated protein C, platelet-derived growth factor (PDGF) and matrix metalloproteinases (MMPs) (Fig. 2) [87]. tPA binds and/or activates multiple receptors such as low-density lipoprotein, receptor-related protein (LRP), NMDA-receptor, annexin-II, and epidermal growth factor receptors (EGFRs) [82, 89]. tPA is cleared from the blood by the low-density lipoprotein receptor-related protein (LRP1), with this interaction activating microglial cells in the ischemic brain [87]. tPA also activates matrix metalloproteinases (MMPs) that break down

Fig. (3). Postulated potential adverse effects produced by serine proteases after SCI. Thrombin can increase neuronal apoptosis, cell death, and increase local inflammation. tPA and uPA can induce excess bleeding and breakdown of the BBB as well as enhancing immune cell activation and invasion. (A higher resolution / colour version of this figure is available in the electronic copy of the article).
local connective tissue, allowing both innate inflammatory cell and neuron migration or invasion [92].

tPA is reported to release connective tissue stores of growth factors leading to enhanced, directed neuronal migration, growth, and rejuvenation. It is hypothesized that tPA leads to neuronal cell growth and migration through the breakdown of connective tissue (CT) [93]. tPA is detected as a protein released in the neuron growth cone, altering neurite outgrowth and remodeling (Fig. 2) [94]. tPA treatment and tPA deficient mouse models have thus demonstrated a role for tPA in responses to neuron excitation by altering cerebrovascular integrity and neurovascular blood flow, altering BBB permeability and providing energy supplies necessary for active neurons. tPA is thus believed to play a role in maintaining neuronal activity, potentially through control of the BBB and local energy supply to neurons, termed neumometabolic coupling [95-97]. Plasminogen activators and plasmin are closely linked to progressive hemorrhagic conversion (PHC) after TBI or SCI, again illustrating the dual roles of tPA (and their receptors) in outcomes after CNS injury [100]. tPA and uPA also activate plasmin which in turn breaks down fibrin clots. tPA and uPA also act individually via plasmin to increase matrix metalloproteinase (MMPs) activation. MMPs break down the connective tissue surrounding endothelial cells and in surrounding neurons to allow for immune cell invasion. As noted, tPA also separates endothelial cell connections leading to increased breakdown of the BBB and vascular leak [83]. These effects of tPA on connective tissue breakdown and immune cell invasion are also reported with the urokinase-type plasminogen activator, uPA.

2.2. Urokinase-type Plasminogen Activator, uPA

Similar to tPA, uPA also has many diverse functions, acting as a thrombolytic to break down clots, but with a reported greater role in cell activation and enabling immune cell migration. uPA is now considered to act predominantly in cellular activation and migration, as opposed to acting as a primary thrombolytic, particularly in tumor cells [101]. uPA interacts with a differing set of cell surface receptors. uPA binds to the uPA receptor (uPAR), a non-transmembrane, glycosylphosphatidylinositol (GPI) linked protein receptor that sits in a large lipid raft of proteins on the surface of inflammatory cells. uPAR interacts with circulating uPA and tissue vitronectin, but also has cis interactions with proteins in the uPAR lipid raft, specifically integrins, lipoprotein LDL-receptor-related protein (LRP), and chemokine receptors [102]. uPAR alters cell activity via interactions with other members of the lipid raft as well as via GPI linking. uPAR interacts with the actin motility machinery in cells and alters gene expression, signaling and cell activation via JAK-STAT and other intracellular pathways [103]. uPAR increases chemotaxis and beta2-integrin-dependent adhesion. Monocyte recruitment and neutrophil migration are significantly impaired in uPAR-deficient (uPAR−/−) mice [102]. uPAR alters cell migration and adhesion via interactions with the extracellular matrix, specifically collagens I, III and IV, fibronectin, fibrin and vitronectin. uPA and uPAR act in concert to alter uPA, PAI-1 (Plasminogen Activator Inhibitor-1) and uPAR expression [104, 105]. uPA also has the capacity to activate pro-neurotrophic factors, including the hepatocyte growth factor (HGF), a motor neuron survival factor [106, 107], or to activate pro-BDNF (brain-derived neurotrophic factor) and pro-NGF (nerve growth factor) via plasmin [108]. Furthermore, both uPA and tPA have been implicated in synaptic remodeling associated with cerebellar motor learning, visual cortex ocular dominance columns, and both hippocampal and corticostriatal long-term potentiation (LTP) [109-111].

uPA, when bound to the uPAR on the cell surface, activates plasmin, similar to tPA activation of plasmin. uPA, together with plasmin, activates MMPs as reported for tPA leading to the breakdown of collagen and elastin in the connective tissue (CT) and the endothelial glyocalyx, allowing cell invasion [112, 113] from the vasculature. With connective tissue breakdown, immune response cells can more readily invade connective tissue surrounding cells leading to either cell and tissue repair, disruption of the BBB, or further immune-mediated damage. This CT breakdown is also posited to enhance neuron outgrowth after damage to the spine [113]. CT breakdown also releases stores of growth factors such as PDGF and TGF, further potentiating neurite outgrowth [114]. Of interest, proteases activate chemokines and release them from cell surfaces initiating neuronal migration as further discussed in the section on chemokines.

3. THROMBOTIC SERINE PROTEASES, THROMBIN

Thrombin, factor two in the coagulation cascade, is similarly acutely activated with endothelial damage. Clots form on damaged endothelium in the inner arterial layer as well as on the surface of activated platelets and adherent macrophages. α-Thrombin is a 36 kDA serine protease composed of two chains linked by a disulfide bond [115, 116]. Thrombin is a central mediator of intrinsic and extrinsic clotting cascades, initiating fibrinogen conversion to fibrin with deposition at sites of endothelial damage and platelet activation and adhesion along damaged endovascular and platelet cell surfaces [116]. Thrombin acts through four protease-activated receptors (PARs 1,2,3, and 4), with prothrombin and PAR1 being upregulated at sites of SCI and TBI [117].

In addition to clot formation detected after spinal cord injury (SCI) and traumatic brain injury (TBI), thrombin is also increased in Experimental Autoimmune Encephalomyelitis (EAE) models in mice models, another inflammatory disease of the CNS. Thrombin was dramatically elevated during the peak of EAE, before the impaired motor function is detected [118]. Thrombin has reported effects on neurons after neurotrauma, post-stroke ischemia, and degenerative nerve disease. Thrombin acts on the endothelial cells of the BBB, astrocytes, and microglia as a pro-inflammatory mediator, promoting vascular dysfunction and neurodegeneration [119]. Thrombin is associated with membrane lipid peroxidation (MLP), reactive oxygen species (ROS), and platelet activation.

Thrombin has direct adverse effects on neurons. At select concentrations, thrombin induces apoptosis in neurons via PAR-1 and MLP via caspase 3 (Figs. 2 and 3). When bound to PAR-1, thrombin induces apoptosis in motor neurons and can induce neurite retraction as well as astrogliosis [120]. PAR-1 also induces the expression of adhesion molecules
such as P-selectin, leading to immune/inflammatory cell recruitment and secondary vascular tissue damage [120].

Thrombin-induced secondary injury is mediated through its receptor, protease-activated receptor-1 (PAR-1), by "biased agonism" [121]. Activated protein C (APC) acts through the same PAR-1 receptor but functions as an anticoagulant and anti-inflammatory protein, which counteracts many of the effects of thrombin and is neuroprotective [122]. Effects of thrombin on recovery after neurotrauma have been linked to interaction with PAR-1 receptors, and detrimental functions of thrombin/PAR-1 binding are reported to be opposed by APC, also working via the PAR-1 receptor. Thrombin concentrations above 100U/mL have been reported to directly induce neuronal apoptosis [122, 123].

4. SERINE PROTEASE INHIBITORS, SERPINS THAT REGULATE THROMBOLYTIC AND THROMBOTIC PROTEASES

Serine proteases in the thrombotic and thrombolytic cascades, in addition to balancing clot formation and dissolution, are, regulated by serine proteinase inhibitors, termed serpins. [63, 124-129], as reviewed in the following section. These regulators of the thrombotic and thrombolytic serine proteases in the coagulation pathways have also reported differing effects on recovery after neurotrauma as for their targeted serine proteases. Serpins are suicide inhibitors that bind to activated serine proteases, forming inactivated suicide complexes where the activity of both the protease and the serpin are lost. The serine protease binds and cleaves the P1-P1’sequence in the reactive center loop (RCL) of the serpin and forming a covalent bond between the cleaved RCL and the protease. The protease is then dragged to the opposite pole of the serpin molecule, and the cleaved RCL becomes part of the A beta sheet in the serpin. These serpins regulate clotting throughout the circulating blood and represent up to 2-10% of the proteins in the circulating blood.

Additionally, some genetic disorders are caused by serpin deficiencies; for example 1) alpha-1-antitrypsin (α1AT, SERPINA1) deficiency, which causes severe pulmonary disease, chronic hepatitis, cirrhosis, and hepatocellular carcinoma, 2) C1 Esterase Inhibitor (C1INH, SERPING1) deficiency which causes complement pathway activation and excess immune cell activation, and 3) antithrombin (AT, SERPINC1) deficiency which leads to excess thrombosis. Serpins are necessary for control of these pathways and serpin replacement therapy has been developed for some of these serpin deficiency syndromes [124-130]. Serpins represent vital controls for the regulation of normal human homeostasis and function.

Not only can serpins be used for replacement therapy, but they have also shown additional potential as therapeutics, α1AT has been assessed for the treatment of sepsis caused by Pseudomonas aeruginosa sepsis. C1INH has been developed for inflammation-related complications as a replacement for complement-activated inflammatory state and associated metabolic acidosis. C1INH has been reported to decrease tumor necrosis factor α and to attenuate renal, intestinal, and lung injury in a dose-dependent manner. Pretreatment of Wistar rats with human plasma-derived C1INH exhibited protective effects in ischemia/ reperfusion injury of lower extremities and associated lung damage, significantly reducing edema in re-perfused muscle and lungs, improved muscle viability, and decreased plasma levels of pro-inflammatory cytokines.

4.1. Plasminogen Activator Inhibitor Type 1 (PAI-1), PAI-2, and PEDF

Plasminogen activator inhibitor-1 (PAI-1 or SERPIN E1) is only weakly expressed in a healthy brain, whether from man or mouse. However, PAI-1 expression is markedly increased after TBI or SCI or other pathologic neurologic conditions, such as cerebral ischemia [124, 125]. PAI-1, as well as PAI-2 and neuroserpin (NSP), regulate tPA and uPA activity in the CNS, and PAI-1 and NSP are now considered important in the regulation of tPA in the CNS [83, 126-132]. As noted above, tPA−/− mice have smaller stroke volumes. However, as a principle regulator with wide-ranging pleiotropic effects, tPA in the CNS has a neuroprotective function, promoting axonal generation in mice. tPA/PAI-1 complexes increase BBB permeability after TBI in mouse models [83]. Of interest, when the serpin PAI-1 binds to uPA / uPAR complexes on the cell surface, detachment of cell surface integrins from extracellular matrix (ECM) ligands occurs, leading to internalization in an LRPI-uPA / uPAR-dependent manner. This alters intracellular signaling such as the Jak/STAT pathway, with subsequent signaling in cell activation, motility and response and ultimately culminating in either enhanced or suppressed cell migration. When PAI-1 binds in a non-uPA/uPAR-dependent manner to LRPI, this also triggers Jak/STAT cellular signaling events that culminate in enhanced cell migration [124, 125]. This increased cell migration is hypothesized to increase BBB permeability following SCI and TBI. Treatment with glucagon and a PAI-1 peptide is also reported to improve outcomes after SCI through inhibition of ERK and JNK/MAPK intracellular pathways [123, 131]. Another serpin, Pigment Epithelial Derived Factor (PEDF or SERPIN F1) has angiogenic, anti-apoptotic, and neurotrophic functions, with many effects seen in the retina. PEDF also has been reported to alter PAI-1 activity and to be regulated by plasminogen [132].

4.2. Neuroserpin (Nsp)

Another mammalian serpin, neuroserpin (SERPIN II), also binds to and inhibits tPA and uPA and is considered one of the main regulators for thrombolytic proteases [59-61]. Neuroserpin (Nsp) has been assessed in models of vascular inflammation after aortic allograft transplant in mice and reduced inflammation, altering Th2 to Th1 ratios, in these vascular models, nsp has known effects after injury to the CNS with reported protective functions [133]. Mice with neuroserpin deficiency have larger stroke volumes after cerebrovascular occlusion, eg. strokes. In serpinopathies caused by genetic anomalies in the NSP RCL sequence, clumps of serpins become adherent to one another in inactive deposits, termed inclusion bodies, believed to be caused by the insertion of the RCL of one serpin into an adjacent A beta-sheet on adjacent serpin molecules [126-129]. NSP deficiency due to aggregation and loss of function leads to epilepsy and neurodegenerative disorders. Studies have also detected improved outcomes with neuroserpin after neurotrauma [59-
61]. NSP is reported to reduce autophagy and decrease neuronal apoptosis in rat models of SCI.

4.3. Antithrombin III (AT-III)

Anti-thrombin III (termed AT or AT-III, SERPIN C1) is a serpin that has also been assessed in SCI models. When treating patients with arterial or venous thrombosis, atrial fibrillation (risk of transient ischemic strokes), or implant (placement) of percutaneous coronary stent implants, heparin is routinely administered as an anticoagulant. Heparin is derived from tissue extracts of heparan sulfate, which is a mixture of differing length glycosaminoglycans or polysaccharides termed heparin, extracted form mammalian tissues. Heparan sulfate is the predominant endothelial glycolayx in arterial wall connective tissue. Heparin, as a therapeutic, functions by increasing the activity of the mammalian serpin, AT. AT binds and inhibits factor IIa, IXa, Xla. XIIa, and Xa, in particular factors II and X, in the clotting cascade [127]. Three studies have reported improved outcomes in rodent models treated with AT-III after SCI [134-137]. AT-III may improve functional outcome after injury comparison to the SC due to decreased tumor necrosis factor-alpha (TNF-α) and increased prostaglandin-I2 (PG12) expression and release in the vascular endothelium, with reduced inflammatory neutrophil responses. AT’s anti-inflammatory effects are not detectable after treatment with Indomethacin, a non-steroidal anti-inflammatory drug (NSAID) that blocks prostaglandin (PG) activity [136, 137]. AT has also been found to reduce ischemia reperfusion injury (IRI) in models of ischemic neuronal damage. In this IRI model, AT reduced the numbers of microthrombi and increased the numbers of motor neurons in animals subjected to SCI [137].

5. CHEMOKINES IN CNS INJURY

Chemokines are small 8-12 kDa proteins classified into 4 chemokine classes: C, CC, CXC, and CXC3C based upon the sequence of amino acids interposed between the first two CC amino acids in the sequence. CXC chemokines are often linked to neutrophil and macrophage activation and migration, while CC chemokines are linked to monocyte and lymphocyte activation/migration [138-140]. Chemokines form chemoattractant gradients by binding to tissue glycosaminoglycans (GAGs) and lining up in the tissue to attract leukocytes. Leukocytes bind to chemokines via cell surface chemokine receptors, 7 transmembrane G protein-coupled chemokine receptors [139] that bind to a differing area on the chemokine. These are the so-called “chemokine gradients” that direct innate and acquired immune cells into areas of tissue damage or infection [140]. It is important to note that the interaction of chemokines with both GAGs and also with leukocyte receptors is not a selective, one-to-one interaction. There is extensive promiscuity among chemokines with chemokine cross-reactivity with multiple GPCR’s, chemokine receptors, as well as individual receptors. The combined interaction of chemokines with GAGs and with receptors may provide a more specific interaction, but this remains to be determined. These chemokine GAG receptor interactions attract leukocytes to areas of tissue damage, and in some cases, chemokine also activates cells outside of their binding to receptors.

It is also now known that chemokines are involved in the CNS response to injury. Chronic neurodegeneration has been shown to prime astrocytes to release large amounts of chemokines when acutely stimulated with cytokines [141]. Chemokines have been identified as markers for neurodegenerative changes in the CNS [142-144]. Altered chemokine expression is also reported in other neurological disorders, including stroke, schizophrenia, and depression, in addition to neurodegenerative disorders [145-150]. Chemokine signaling is now reported to play a key role in astrocyte, glial cell and neuronal responses to injury and well as functioning as neurotransmitters [145-148]. Of great interest, serine proteases can release cell surface chemokines and activate chemokines in the nervous system linking chemokine activity back to the thrombotic serine proteases. Chemokines may also direct neuronal extension and growth following spinal cord injury [147-148], and thus may have some beneficial functions similar to what is seen with the serine protease tPA. In SCI, the CC chemokine subtype CCL2 has been associated with neuroprotection mechanisms of mesenchymal stem cell implants after SCI. CCL2 provides a protective function by driving macrophage recruitment to sites of neurotrauma and increasing conversion of macrophage to an M2 anti-inflammatory, neuroprotective phenotype. In a mouse model, human CCL2 prevented motor neuron degeneration in vitro and delivered in mice after SCI is reported to improve motor performance [147, 148]. In contrast, others have reported that blockade of the chemokine MCP-1 reduced inflammation and neuronal damage after SCI in a rat model. siRNA blockade of MCP-1 expression in this SCI model reduced neuronal apoptosis [149]. CCL20 is also linked to aggressive neuroinflammation after SCI. Neutralizing antibodies reduced edema and inflammation and improved motor function after compressive injury in a rat model [150].

Thus chemokine activation is a double-edged sword, chemokines as noted above, are closely associated with immune cell activation as well as inflammatory cell invasion after SCI, which can lead to damage with excessive inflammation (Fig. 3) while other studies have demonstrated protective functions [134-152]. It should also be noted that the innate and acquired immune responses are a protective mechanism designed to protect and heal after severe trauma or infections. The innate immune, the inflammatory response is activated early and leads to the initial healing or tissue repair responses. Excess immune cell activation can be damaging but the mammalian body relies on these early innate immune responses to allow for the rebuilding of damaged organs.

6. CONNECTIVE TISSUE (CT)

The brain is a soft tissue, lacking much of the usual connective tissue and collagen scaffolding seen in other structures. The one obvious exception being the external layers surrounding the brain, such as the meninges or the vascular structures associated with arteries, veins and the blood brain barrier. Thus the brain soft tissue lacks typical collagen scaffold but does have glycosaminoglycan and proteoglycan in the supportive tissues. Collagen IV is seen in soft nervous system scarring. Mechanical stresses are the subject of ongoing investigations [153-157]. All of these changes occurring
in the brain, however, may differ from the connective tissue changes seen in damaged or inflamed arteries where a more typical connective tissue containing collagen I, II, IV and VI around the damaged artery will differ from that seen in the brain and can alter immune cell responses and invasion after injury. Altering the endothelial layer and damage or leaking in the BBB also alters both connective tissue composition and immune responses at sites of trauma.

Fibrous or glial scars build-up at sites of SCI, walling off the area of damage and ongoing inflammation and lead to a central cystic space in the spine. This scar and the central cystic space is a soft tissue and is reported to prevent neuron growth across cystic areas of damage and necrosis due to a lack of support for axon extension. However, walling off this space together with the gradual removal of the encased fluid in the damaged area can also prevent extension of damage beyond the original injury site protecting adjacent areas of the spine not initially damaged by the original trauma.

Glial scars have long been thought to form physical and chemical barriers to nerve growth. These physical barriers encompass deposits of the extracellular matrix that interfere with axonal growth [150, 151], barriers that include chondroitin sulfate proteoglycans and other inhibitory matrix molecules that bind to receptors and can inhibit axonal growth [146-155]. Fibronectin together with chondroitin sulfate (CS GAG) inhibits axonal outgrowth in vitro. CS proteoglycan-rich regions also block axonal regeneration in vivo [151-157]. Furthermore, intrathecal injection of Chondroitinase ABC, an enzyme that degrades CS GAG at sites of nervous system injury, upregulates a regeneration-associated protein in injured neurons and promotes regeneration of both ascending sensory projections and descending corticospinal tract.

In contrast, transgenic manipulation of SOX9 and N-acetylgalactosaminyl transferase are reported to reduce GAGs after SCI, neuroprotection and axon regeneration. Laminin supports neuron outgrowth both in vitro and supports outgrowth in human embryonic stem cells [156-159]. Chemokines are guided along glycosaminoglycans (GAGs) to form gradients that direct leukocyte trafficking into sites of damage. As noted above, these chemokine and immune cell responses can provide both protective actions to enhance nerve growth, but also can lead to progressive nerve damage in the case of excess immune cell activation and aggression. As with thrombotic and thrombolytic serine proteases and chemokines, the connective tissues at sites of spinal cord injury can have both beneficial reparative functions as well as functions that limit regrowth and healing after SCI.

7. VIRUS-DERIVED IMMUNE-MODULATING PROTEINS

Viruses have studied mammalian immune response systems, developing highly effective immune-modulating proteins through trial and error as they have evolved to highly effective pathogens [63, 160-162]. These millions of years of evolution have led to the development of potent immune modulators that target central pathways. The viral immune modulators regulate immune responses, allowing viruses to invade and replicate by blocking or evading the host responses designed to inhibit the viral infection. Thus, evolution has allowed viruses to design reagents that very effectively inhibit host immune responses often targeting key steps in immune reactions and working in picomolar or lower concentrations, allowing the virus to fight back against a host immune response that attacks the virus. Viruses have been used as therapeutics as vaccines and as agents for gene expression. We have been developing these naturally evolved viral immune-modulating proteins as a new class of therapeutics. Two of the most effective pathways around which virus-derived immune modulators have been developed are the serpins [160-174] and the chemokine modulators [175-181].

Poxviruses are large DNA viruses and highly effective pathogens. Myxoma virus is a poxvirus that selectively infects and kills European rabbits but does not infect other mammals [63, 161-181]. Several genes identified in Myxomavirus enhance virus pathogenicity and have proven highly effective in targeting key immune response pathways. The immune response pathways targeted by viruses are often central regulatory pathways, allowing the virus to effectively escape the host immune response [63, 160-174].

Serp-1 is a secreted Myxomavirus serpin that inhibits tPA, uPA, plasmin, as well as factor X and thrombin, proteases in both thrombotic and thrombotic cascades, respectively. Poxviruses as well as herpesviruses, both large DNA viruses, have also developed chemokine modulating proteins (CMPs), immune modulators which inhibit chemokines.

We have examined both virus-derived serpins and chemokine modulators as new therapeutic approaches to improve outcomes after SCI, testing these in rat models of SCI.

7.1. Virus-derived Serpins

Serpins expressed by poxviruses target thrombotic and thrombolytic proteases as noted above, as well as apoptotic pathways, often displaying marked efficacy and potency. Myxoma virus encodes two such serpins, Serp-1 and Serp-2 that have been studied in animal models of inflammatory disease [160-174]. Serp-1 is a 55 kDa glycosylated secreted serpin that binds tPA, uPA, plasmin, factor X, and thrombin inhibiting immune responses driven by both clots forming and clot-dissolving cascades (Fig. 4). Serp-2 is a cross-class serpin that blocks both serine and cysteine proteases, inhibiting both apoptosis and the inflammamsome through binding granzyme B as well as caspases 1 and 8. Cowpox virus encodes a similar cross-class serpin known as Crm A that also binds and inhibits caspases 1 and 8 and granzyme B. Over millions of years of evolution, virus-derived serpins have developed the advantage of targeting coagulation, inflammamsome, and apoptotic pathways, blocking these host immune responses essential to effective viral infections, allowing the virus to survive and infect other cells (Fig. 4) [63, 163-174]. We have examined the efficacy of blocking both the thrombotic and thrombotic pathways as well as associated aggressive inflammation in multiple animal models of disease [63, 163-174]. Serp-1 improved outcomes in preclinical animal models of vascular disease, including atherosclerosis, restenosis after angioplasty and stent implant, allograft vasculopathy after organ transplant, and rare in-
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Flammary vasculitic syndromes (IVS) such as Takayasu’s and Giant cell arteritis. Serp-2 also improved outcomes in models of atherosclerosis in Apolipoprotein E (ApoE) knockout mice with carotid cuff injury, as well as aortic allograft transplants and liver ischemia models [169]. In more recent work, we have demonstrated accelerated wound healing when applying Serp-1 with saline through a collagen-chitosan hydrogel [175].

Of interest, Serp-1 has also been assessed as a treatment to reduce arterial inflammation in patients with acute unstable coronary syndromes with coronary stent implants. A randomized, double-blinded Phase 2a clinical trial performed at 7 sites in Canada and the US demonstrated an early and significant reduction in markers of myocardial damage. In addition, no significant adverse events were demonstrated with zero Major Adverse Cardiovascular Effects (MACE) and no detected neutralizing antibodies [172].

Both the preclinical and the Phase 1 safety trial with Serp-1 in healthy volunteers also demonstrated remarkable safety. The fact that these findings have been proven in a wide range of animal vascular and inflammatory models as well as up to phase 2 clinical trial ascertainment that these virus-derived immune modulators can be used safely as therapeutics in human diseases [172].

Prolonged inflammation leads to continued damage after SCI, providing an avenue for Serp-1 as a potential therapeutic [173-175]. Serp-1 has been shown to be neuroprotective with subdural infusion and when introduced after SCI in a chitosan collagen hydrogel. Serp-1 treatment reduced tissue damage with associated early benefit, improving neurological function [173-175]. While Serp-1 has also been tested in rat models of SCI, other virus-derived serpins such as Serp-2 or CrmA have yet to be examined in SCI models.

7.2. Virus-derived Chemokine Modulating Proteins (CMPs)

Myxovirus has also developed two highly potent chemokine modulating proteins, M-T1 and M-T7 [63, 176-183]. The murine gamma Herpesvirus (MHV68) expresses a chemokine modulating protein (CMP), M3, required to establish a normal latent viral load [176-183]. M-T1 binds at the receptor binding domain of chemokines in the CC chemokine class. M-T7 is a 37kDa glycosylated protein that shares sequence homology with the rabbit interferon gamma (IFN-γ) receptor gamma (IFN-γ) and selectively binds rabbit IFNJ. M-T7 inhibits chemokines in rats, mice, and humans through binding and inhibition of the glycosaminogycan (GAG) binding domain of C, CC, and CXC chemokines [176-183]. M-T7 also inhibits rabbit IFN-γ, but this is species-specific to rabbits and does not binds IFN-γ from other species. M3 from MHV68 blocks both chemokine receptor binding as well as chemokine GAG binding [184-187]. M-T7 and M3, through blocking chemokine binding to GAGs in the glyocalyx around endothelial cells in arteries, can effectively inhibit immune cell activation.

M-T1, M-T7 and M3 reduce vascular inflammation in simple rodent balloon injury models [63, 176-183]. M-T7 has further demonstrated improved outcomes in aortic and renal transplants in mouse models and after stent implant in rabbit models of restenosis. M-T7 has more recently been demonstrated to accelerate wound healing and to reduce SCI after balloon crush injury in a rat SCI model [173].
7.3 Treatment with Virus-derived Immune Modulators after SCI

Both Serp-1 and M-T7 have been examined as potential therapeutics in rat models of balloon crush spinal cord injury. Each protein was tested individually as a local subdural infusion started at the time of SC crush injury [173]. Serp-1 has been more extensively assessed in the rat SCI model. When Serp-1 was administered as a short-term local infusion at sites of distal T12 SCI crush injury, there was reduced inflammation and improved motor function as assessed by a modified motor scale devised by Kwiecien et al. [1745, 175].

Fig. (5). Upper panel - Illustration of potential therapeutic targets for Serp-1 and M-T7 in areas of neurotrauma with neuronal damage and excess progressive immune-mediated damage. Bottom panel - Micrographs demonstrate reduced tissue damage and reduced inflammation after SCI in the rat model of lower thoracic forceps crush injury; 7 days follow up after implant of Serp-1 in a chitosan-collagen hydrogel. Implant was at the site of injury immediately after crush injury [174-176]. Left - Control CCH - chitosan collagen hydrogel illustrates large area damage and inflammation. Right - CCH Serp-1 - hydrogel implant illustrates reduced area of damage and inflammation. 10X Magnification. H&E Staining. (*A higher resolution / colour version of this figure is available in the electronic copy of the article.*
Longer-term infusions also displayed benefit, as did a chitosan-collagen hydrogel implant after forceps crush injury [174]. However, systemic or intraperitoneal injections did not improve outcomes nor reduce inflammation at the site of balloon crush injury. More prolonged local SC infusions of Serp-1 again displayed early benefit, however, this benefit was lost after 14-21 days postinjury. Application of a hydrogel that provided local Serp-1 releases again demonstrated improved early motor function after balloon crush SCI.

As noted, the administration of Serp-1 via a chitosan-collagen hydrogel in rats demonstrated a significant reduction of the extent of spinal cord damage and reduced inflammation. Analysis by immunohistochemical staining for Neurofilament Medium polypeptide (NF-M) at day 7 post-injury indicated that high-dose Serp-1 (delivered through the hydrogel) significantly reduced the area of neuronal loss in comparison to the control (hydrogel alone). This treatment also limited neuronal damage at day 28 post-injury when compared to the hydrogel alone. These areas of NF-M staining displayed reduced apoptosis as indicated by fewer caspase-3 positive cells for rats treated with Serp-1. This suggests that the reduced area of injury may have been mediated through suppression of apoptosis of neuronal cells. Additionally, immunohistochemical staining for CD3+ T-cells indicated that treatment with Serp-1 also significantly reduced the number of immune cells in the area of injury at 28 days postinjury (Fig. 5) [173-175].

In our Serp-1 model, the effects on the extent of astrogliosis were determined by staining for glial fibrillary acidic protein (GFAP). On day 7 post-injury, the number of GFAP-positive cells proximal to the injury was significantly increased for rats treated with Serp-1 via the chitosan-collagen hydrogel. A histological scoring system for GFAP-positive compartmentalization also demonstrated that the Serp-1 chitosan-collagen hydrogel (CCH) stimulated earlier protection of spinal cord lesions (Fig. 5). This indicates that treatment with Serp-1 may promote earlier astrogliosis and compartmentalization of the injury.

Astrocytes are critical inflammatory regulators in the CNS. Astrogliosis is the process by which trauma, ischemia, infection, stroke, autoimmune, or neurodegenerative change induce an increase in the number of astrocytes. Astrogliosis has a dual role in spinal cord injury with both detrimental effects as well as recent discovery of its protective effects in limiting damage and extent of damage. It is important to note that astrogliosis does not occur in isolation but is a coordinated part to a multicellular reaction to CNS trauma involving other glial cells, neurons, and non-neuronal cells. More recently, scientists recognize that the process of astrogliosis is not a simple generic response, it is rather a fine-tuned unique response that occurs on a spectrum from reversible alterations in gene expression to pronounced cell proliferation with compact scar formation and permanent tissue rearrangement [188]. Astrocyte scarring plays a critical role in walling off inflammatory cells from the area of trauma or autoimmune damage into nearby healthy tissue.

The mechanism involves a wide range of specific molecular signaling mechanisms that regulate specific aspects of astrogliosis. Thus, it is not a simple on-off reaction. Astrocytes not only attract inflammatory cells by opening the blood-brain barrier through the release of chemokines but also directly have strong immunosuppressive effects on inflammatory constituents. In fact, more recent studies are revealing that CNS autoimmune diseases that target astrocytes have a more severe course than autoimmune diseases that attack other cell lines.

This improved function and healing with Serp-1 hydrogel may also be the end result of inhibition of excess inflammation and tPA or uPA mediated immune cell responses and/or inhibition of thrombin-mediated inflammation and apoptosis. Serp-1 is a serpin and thus acts at sites where serine proteases are activated. With the combined activation of thrombotic (thrombin) and thrombolytic (tPA and uPA) Serp-1, my function to balance and or damp down excess protease activation. Much further work will be required to identify the specific pathways targeted by Serp-1 and M-T7 as well as the optimal timing for treatment in the benefits detected with treatments for acute SCI and to determine methods by which longer term improvement can be identified and developed.

One small study has also demonstrated the benefit with M-T7 when infused locally at sites of balloon crush SCI in rats. We are currently investigating the local effects of Serp-1 and M-T7 treatment on dorsal root ganglion neuronal outgrowth in vitro. Effects of Serp-1 or M-T7, when applied in the presence and absence of the thrombolytic and thrombotic proteases, will be assessed in vitro, allowing an analysis of the potential molecular mechanisms of action of each immune modulator when used after SCI.

**CONCLUSION**

What has become clear in reviewing these specific serine proteases, serpin, and chemokine pathways in CNS injury is that these pathways have proven to be highly active, with significant effects on the responses in the CNS to injury and healing. It is also evident that many of the agents functioning in these protease and chemokine responses have both direct effects on neurons and glial cells as well as indirect effects on the systemic and locally mediated immune responses to injury. Each of these pathways has been demonstrated to have some benefits and also some adverse effects dependent upon the specific neurological and immunological responses. With two very powerful immune modulators derived from viruses we have seen some early benefits with reduced damage and size of cystic spaces as well as reduced inflammation and preserved neuronal tissue and function. However, these effects are demonstrated as an early benefit, but not a long term benefit, as has been seen with many treatment approaches. This suggests that these newer biologics targeting serine protease and chemokine pathways may well provide an early benefit that would allow more prolonged neuronal regrowth when combined in the future with other approaches such as stem cell implants and hydrogels to support axon extension and growth. Treatments for SCI may thus require a staged approach. A greater understanding of the molecular responses and their specific effects on repair after neurotrauma is in great need, and focused work on these areas will benefit future approaches to new treatments.
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Serine Proteases and Chemokines in Neurotrauma

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effects of tPA in the CNS?

The effects of tPA in the central nervous system (CNS) are complex and multifaceted. Tissue plasminogen activator (tPA), a member of the serine protease family, is known to promote fibrinolytic activity in the CNS. This protease has been shown to play a critical role in the resolution of blood clots and the maintenance of normal vascular function. However, excessive tPA activity can lead to hemorrhagic events, particularly in the context of ischemic brain injury. The balance between tPA-driven fibrinolysis and its potential prothrombotic effects is a critical area of investigation.

Intriguingly, studies have reported that tPA expression and activity are upregulated in the ischemic brain, where it promotes fibrinolysis and clears the path for neuroprotective processes. However, increased tPA levels may also contribute to hemorrhage, a significant complication in stroke patients. The mechanisms by which tPA regulates clot resolution and hemorrhage remain an active area of research, with potential implications for developing effective therapeutic strategies for stroke and other neurovascular disorders.

In summary, the role of tPA in the CNS is multifaceted, with both beneficial and detrimental effects, depending on the context and dosage. Further investigations into the precise mechanisms underlying tPA’s actions in the ischemic brain are essential for developing targeted therapeutic approaches for stroke and other neurovascular disorders.

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