N-acetyl proline-glycine-proline: implications for neurological disorders

N-acetyl proline-glycine-proline (ac-PGP) is a matrix-derived chemoattractant, or matrilysin, produced by the degradation of collagen. Ac-PGP was first identified in studies of alkali eye injury in rabbits (Pfister et al., 1995). The molecule was found to be a potent neutrophil chemoattractant and application of ac-PGP to the eye results in intense neutrophilia equivalent to that observed in acute alkali injury (Pfister et al., 1998). Recent studies identified matrix metalloproteinase-8 (MMP-8) and -9 and prolyl endopeptidase as requisite proteases in the formation of ac-PGP from the proteolytic destruction of collagen and demonstrated that the molecule is a chemokine (C-X-C motif) receptor 1 (CXCR1)/2 chemokine receptor ligand with structural similarity to proinflammatory chemokine IL-8 (CXCL8) (Weathington et al., 2006; Gaggar et al., 2008). A growing number of studies have identified the molecule in inflamed tissues and suggest a role for ac-PGP in injury associated with acute and chronic inflammation.

The studies that identified ac-PGP provided the first evidence for its role in inflammation and tissue injury. In the alkali eye injury model, neutrophilia after alkali exposure is associated with corneal ulceration and perforation. The abundance of ac-PGP in the injured eye and its ability to drive neutrophilia strongly support a role for the peptide in alkali-induced inflammation and tissue injury processes. Further, specific antagonists of ac-PGP significantly reduce corneal ulceration in alkali injury (Haddox et al., 2001). Studies of lung inflammatory disorders cystic fibrosis and chronic obstructive pulmonary disease have demonstrated significant levels of ac-PGP in affected tissues in human clinical samples, but not in healthy controls (Weathington et al., 2006; Gaggar et al., 2008). Chronic administration of ac-PGP to normal lung in animals induces neutrophilia and inflammatory tissue remodeling, further suggesting a role for ac-PGP in the pathology of these diseases (Weathington et al., 2006). Both diseases are associated with neutrophilia and elevated levels of MMP-9 in affected tissues. In addition to stimulating neutrophil chemotaxis, ac-PGP stimulates neutrophil release of MMP-9 and CXCL8, suggesting that the production of ac-PGP at sites of injury may result in a forward-feeding inflammatory cycle in which the production of ac-PGP acts to sustain inflammation and promote progressive tissue injury in inflammatory disorders (Overbeek et al., 2011; Xu et al., 2011).

We recently reported significantly increased levels of ac-PGP in infarcted brain after ischemic stroke in rats and further demonstrated that ac-PGP induces neuronal apoptosis through its binding at neuronal CXCR2 receptors (Hill and Nemoto, 2015). These findings suggest that ac-PGP may be a novel mediator of inflammation and neuronal injury in stroke. Inhibitors of MMPs and CXCR2 antagonists reduce infarction and improve outcome in animal stroke models. Our findings suggest that neuroprotection mediated by these therapies may involve decreased production of ac-PGP and inhibition of ac-PGP binding to leukocyte and neuronal CXCR2 receptors, respectively.

In addition to stroke, MMPs are implicated in the pathology of numerous neurological disorders, including Alzheimer’s disease, Parkinson’s disease, amyotrophic lateral sclerosis (ALS), and multiple sclerosis (MS). Upregulation of MMP expression and activities in brain and spinal cord, cerebrospinal fluid (CSF), and blood has been reported in these disorders and MMPs are believed to participate in injury processes in these disorders through several mechanisms, including opening of the blood-brain barrier, direct injury to neurons through anoxia, and downregulation of neuronal DNA repair enzymes (Hill and Nemoto, 2015; Brkic et al., 2015). As MMP-9 has been shown to be upregulated in several neurological disorders (stroke, Alzheimer’s disease, Parkinson’s disease, ALS, MS, and traumatic brain injury) and collagens are expressed by the neuroepithelium, endothelial cells, reactive astrocytes, and neurons, the potential for increased peripheral and central nervous system (CNS) ac-PGP levels in these disorders is significant.

Chemokines and their receptors, particularly the CXCR2 receptor, are implicated in numerous neuropathologies. Chemokines are small secreted proteins (~8–15 kDa) that play roles in normal immunosurveillance and mediate leukocyte activation and trafficking into the CNS in disease processes. Upregulation of CXCR2 receptors and CXCR2 ligands CXCL1, CXCL2, and CXCL8 has been observed in plasma, CSF, and brain in stroke and neurodegenerative disorders (Semple et al., 2010; Hill and Nemoto, 2015). Since ac-PGP has been shown to be neurotoxic in addition to its chemoattractant function, ac-PGP may mediate both inflammation and neurodegeneration in inflammatory disorders of the CNS. The potential involvement of ac-PGP in

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**Figure 1: Potential involvement of ac-PGP in forward-feeding cycles of neuroinflammation and neuronal injury in stroke and neurological disorders.**

Solid lines indicate established roles of ac-PGP and CXCR2 receptor binding chemokines in neuronal death and neuroinflammation. Dotted lines indicate putative roles of ac-PGP in the CNS based on established consequences of CXCR2 receptor activation and peripheral functions of ac-PGP. Circular arrows indicate potential forward-feeding pathways involving ac-PGP. In addition to chemokine IL-8 (CXCL8), neutrophils, astrocytes, microglia, and neurons express the CXCR2 receptor and prolyl endopeptidase (PREP). Rodents express CXCL8 functional homologs CINC-1/KC (CXCL1) and macrophage inflammatory protein 2 (MIP-2) (CXCL2). Lymphocytes and oligodendrocytes also express the CXCR2 receptor and are anticipated to be responsive to ac-PGP signaling. The function and expression of other CXCR2 binding chemokines (CXCL1-3 and 5-7) in the CNS may be modulated by ac-PGP. BBB: Blood-brain barrier; IL-8: interleukin-8.
forward-feeding cycles of inflammation and neuronal injury is illustrated in Figure 1. The CXCR2 receptor is expressed on numerous cell types in the CNS and the contribution of ac-PGP to neurodegeneration and leukocyte trafficking and signaling in the CNS may be significant. Non-acetylated PGP, which is several-fold less chemoattractant for neutrophils than ac-PGP, has also been detected in clinical samples from cystic fibrosis patients (Gagger et al., 2008) and may be present in the CNS. Interestingly, leukotriene A4 hydrolase (LTA4H), an enzyme responsible for generating inflammatory mediator leukotriene B4 (LTB4), was shown to limit pulmonary neutrophilic inflammation by degrading PGP and yet possesses no activity towards ac-PGP (Snelgrove et al., 2010). While LTA4H expressed by CNS-resident leukocytes and neurons may regulate PGP, it is unclear how ac-PGP may be regulated in the CNS.

Our results in primary neurons and studies in CXCR2 knockout mice, respectively, suggest that ac-PGP mediates its effects on neurons and neutrophils primarily through the CXCR2 receptor (Weathington et al., 2006; Hill and Nemoto, 2015). Activation of CXCR2 on CNS-resident or peripheral leukocytes facilitates leukocyte activation and chemotaxis, resulting in increased CNS and peripheral cytokine levels which may potentiate neuronal injury through sustained activation of neuronal CXCR2 receptors. Additionally, increased production of ac-PGP mediated by elevated MMP-9 associated with leukocyte activation may further propagate inflammation and neurodegeneration. As ac-PGP was recently shown to induce vascular permeability in endothelial cells (Hahn et al., 2015), activation of CXCR2 on cerebral vascular endothelial cells by ac-PGP may promote opening of the blood-brain barrier and leukocyte extravasation and infiltration into the CNS during neuroinflammation. Thus, ac-PGP may be involved in CNS inflammatory responses and neuronal death pathways at numerous junctures.

In addition to potential roles in neuronal death and neuroinflammation, activation of CXCR2 receptors by ac-PGP may have roles in neurogenesis and neuroprotection in developmental and disease processes. CXCR2 signaling has pivotal roles in processes as diverse as brain development, neurotransmission, neuroprotection, and neurogenesis (Semple et al., 2010). For example, stromal cell-derived factor 1 (SDF-1) (CXCL12) expressed by astrocytes is neuroprotective and promotes tissue repair in cerebral ischemia and neurodegenerative diseases by mediating the migration of neural progenitor cells to sites of tissue damage. CXCL12 expression in astrocytes is mediated through the CXCR2 receptor by CXCL1 and CXCL5 (Shin et al., 2014). In a model of Alzheimer’s disease, CXCR2 ligand CXCL2 protects neurons against amyloid-β-induced neuronal death (Watson et al., 2005). In other models, activation of neuronal CXCR2 receptors by CXCL2, CXCL8, or ac-PGP induces neuronal death (Hill and Nemoto, 2015). It is clear that responses to CXCR2 ligands are contextually dependent and vary according to cell type, ligand, concentration, and duration of exposure. Accordingly, ac-PGP in the CNS, by its propensity to bind CXCR2 receptors, may couple destruction of the extracellular matrix to modulation of developmental, degenerative, and regenerative processes.

In summary, following the recent discovery of ac-PGP in the CNS, studies are needed to examine the roles of ac-PGP in chemokine signaling pathways that influence outcomes in stroke and neurological disorders. Such studies may provide novel mechanistic insights and suggest novel therapeutic strategies to decrease neuroinflammation and provide neuroprotection in inflammatory disorders of the CNS.

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