9-Review

Glial scar-modulation as therapeutic tool in spinal cord injury in animal models[1]

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Abstract

Purpose: Spinal Cord injury represents, in veterinary medicine, most of the neurological attendances and may result in permanent disability, death or euthanasia. Due to inflammation resulting from trauma, it originates the glial scar, which is a cell interaction complex system. Its function is to preserve the healthy circuits, however, it creates a physical and molecular barrier that prevents cell migration and restricts the neuroregeneration ability.
Methods: This review aims to present innovations in the scene of treatment of spinal cord injury, approaching cell therapy, administration of enzyme, anti-inflammatory, and other active principles capable of modulating the inflammatory response, resulting in glial scar reduction and subsequent functional improvement of animals.
Results: Some innovative therapies as cell therapy, administration of enzymes, immunosuppressant or other drugs cause the modulation of inflammatory response proved to be a promising tool for the reduction of gliosis
Conclusion: Those tools promise to reduce gliosis and promote locomotor recovery in animals with spinal cord injury.
Key words: Stem Cells. Inflammation. Neuroprotection. Models, Animal.

■ Spinal cord injury in veterinary medicine

Spinal Cord Injury (SCI) may have an endogenous or exogenous origin. Regardless of the cause, SCI are related to injury, compression, transaction, laceration, traction of the neural tissue, hemorrhage and hematoma, hypoxia, spinal cord laceration or the associated roots and others injuries resulting in varying degrees of neurological disorders[1,2]. Furthermore, physical interruption of nerve impulses and loss of blood flow and auto regulation, other biochemical, vascular and inflammatory events are involved in the neuronal destruction and necrosis[3,4].

Endogenous capacity of self-repair and regenerate of the spinal cord is limited after injury[5,6], due to the minor capacity of the replacement of damaged nerve cells[7], as well as the production of growth inhibitory myelin associated axon and the formation of glial scar[8].

The consequences of SCI in veterinary medicine, depending on the injured segment can lead to permanent disability or euthanasia.

■ Glial scar

Glial scar consists predominantly of reactive astrocytes, macrophages, microglia and Chondroitin Sulfate Proteoglycans (CSPGs)[9], that leads to a dense deposit of extracellular collagen matrix, acting as protective barrier scar, however, inhibits cell and axonal migration[10].

Damage of the blood-brain barrier, leukocytes extravasations and accumulation of inflammatory cells in the center of the lesion are crucial events in the formation of the gliosis. Several molecules derived from blood or produced via inflammatory has been identified as a trigger for their induction, including interleukin-1, Transformation Growth Factor beta (TGFβ) and fibrinogen[11-15].

After the injury, fibroblasts migrate into the epicenter of the lesion, forming a fibrotic scar filled with extracellular fibronectin, collagen and laminin[16]. The proliferation of A-type pericytes contributes to the formation of the fibrosis, even in contused injuries, when meninges are intact and responsible for most of the components of the fibrotic scar. The glial scar appears in its mature form within two weeks after injury[17,18].

Actived macrophages and microglia increase significantly the expression of matrix metalloproteinases (MMPs), which contributes to vascular permeability and accumulation of more inflammatory cells in the lesion, which reaches its peak around thirty days after injury[19-21]. Therefore, these activated cells, although important for the debridement of injured tissue, may also lead to secondary damage by inflammatory process[22]. Studies have shown that activated macrophages are responsible for the gradual and progressive death of axons after injury, trough the activity of MMPs and direct physical interaction with injured cells[19,20].

The glial response is mainly characterized by hypertrophy of astrocytes migrate out of the inflammatory epicenter, where they increase in size and present high gene expression of Glial Fibrillary Acidic Protein (GFAP), vimetin and nestin[23,24]. Hyperatrophic astrocytes are restructured into a network of tangled filamentous process, which acts protecting viable neural cells, however, resulting in a major physical barrier for axonal regeneration. Furthermore, studies suggest that glial scar prevents the inflammatory process to spread the healthy tissue[25,26]

■ Inflammatory response modulation as a therapeutic approach

Several authors associated gliosis modulation with the clinical response of spinal

cord injury in animais. In one study, Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF) was administered intraperitoneally, from 3 to 4 weeks after spinal cord injury in rats. There was a decrease in the expression of CSPGs and neurocan, intense expression of GFAP, preservation of axonal arrangement and structure in inflammatory myelin and improved gray matter and gliosis reduction[27].

Yazdani *et al.*[28] compared the transplantation of cells from the olfactory epithelium and bone marrow-derived mesenchymal stem cells, neurally induced in rats with spinal cord injury. They concluded that the induced cells caused significantly motor improvement, reduction of the size of injury and axonal regeneration, making this strategy promising candidate for future therapies.

Another study has shown that Hepatocyte Growth Factor (HGF) has curative capacity by regulating TGFβ, completely blocking the secretion of these factors on reactive astrocytes in vitro. The transplantation of cells capable of secrete HGF reduced neurocan expression and glycosaminoglycan deposition in the lesion and promoted axonal growth around the gliosis and functional improvement of the hindlimbs in rats[29].

Ahmed *et al.*[30] used decorin – a proteoglycan associated with collagen fibers – to block the glial scar and cystic cavitation and induce fibrotic dissolution of gliosis in rats with chronic spinal cord injury. These mechanisms have been attributed to the induction capacity of MMPs and plasminogen activity, modulation of inflammation, removal of growth inhibitors and axonal regeneration promotion in the lesion.

Another study demonstrated the efficacy of transplantation dedifferentiated adipocytes in promoting locomotor improvement, remyelination, glial scar reduction and increased expression of neurotrophic factors in mice with spinal cord injury[31].

Studies using curcimun – an active component of turmeric, which acts as an anti-inflammatory – demonstrated the ability of the substance to reduces local inflammation, suppressing the formation of glial scar by inhiniting the process of reactive astrocytes cytokines and pro-inflammatory such as TNF-TNF-α, IL-1β e NK- κb, in addition to promote protection of neurons and axons after spinal cord injury in rodents[32,33].

Rapamycin – an immunosuppressant used for the prophylaxis of organ transplant rejection – reduces infiltration of neutrophils and macrophages in the lesion, microglial activation, secretion of TNFβ, the number of cells expressing GFAP, inhibited the proliferation of astrocytes and promoted neuronal survival and axiogenesis around the injury, being a good tool in the treatment of spinal cord injury in mice[34].

Naïve Schwann cells and Schawann cells transduced to express GDNF which were seeded into guidance channels and implanted at the spinal cord injury by Do-Thi *et al.*[35], inhibit the formation of glial scar by promoting functional improvement in rats, when expressed Lv-shGFAP (lentiviral-mediated RNA-interference against GFAP). It was also observed growing axons and increased serotonergic innervation, suggesting that this type of therapy aids in the treatment of spinal cord injury.

Several studies using the enzyme chondroitinase ABC[36-39] in ratis demonstrated their potential in digesting CSPGs – inhibitory molecules predominant in glial scar – modifying the intra and extracellular architecture, reducing the formation of gliosis, regenerating axons injured by improving neural connections and promoting neuroprotection.

By intrathecal bone marrow cells transplantation, Zhu *et al.*[40] demonstrated that gliosos is more associated with macrophages than microglia in mice. Depletion of these

macrophages resulted in a reduction of fibroblasts and the formation of basal lamina, leading to a scar less fibrotic and more conducive to axonal growth.

The transplantation of neural progenitor cells in the spinal Cord injury showed the ability of these cells to inhibit astrocyte activation, reduce gliosis and promote improved locomotor in treated rats[41-43].

In the following Table 1, a summary of the therapies addressed in this review can be observed:

Table 1 - Therapies to promote glial scar-modulation.

Author	Therapy	Animal	Route of administration
Zhu *et al.*[40]	Bone Marrow cells	Mice	Intrathecal
Yamada *et al.*[31]	Mature adipocyte-derived dedifferentiated fat cells	Mice	Intramedullary
Yazdani *et al.*[28]	Olfactory epithelium and bone marrow-derived mesenchymal stem cells (neurally induced)	Rats	
Jeong *et al.*[29]	HGF overexpressing mesenchymal stem cells derived from human bone marrow (HGF-MSCs)	Rats	
Ahmed *et al.*[30]	Decorin	Rats	
Do-Thi *et al.*[35]	Schwann cells	Rats	
Yick[36], Xia *et al.*[37], Huang *et al.*[38], Ni *et al.*[39]	Chondroitinase ABC	Rats	
Bonner *et al.*[41], Jin *et al.*[42], Mitsui *et al.*[43]	Neural progenitor cells	Rats	
Huang *et al.*[27]	Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF)	Rats	Intraperitoneally
Yuan *et al.*[33]	Curcimun	Rats	
Wang *et al.*[32]	Curcimun	Mice	
Goldshmit *et al.*[34]	Rapamycin	Mice	

■ Conclusion

Spinal Cord injuries represent the majority of neurological manifestations in veterinary medicine. Gliosis is characterizes by replacement of functional tissue by fibrous after injury, in order to promote protection of healthy cells. This process, however, implies a physical and molecular barrier that inhibits cell and axon migration and thus prevents the functional improvement.

As a way of reversing or deflecting this event, studies have shown that modulation of the inflammatory response at the wound site results in a reduction in lesions size, neuronal survival, protection growth, remyelination and increased innervations, leading to a reduction, inhibition or reversal of glial scar and promotes improved locomotor to treated animals.

We conclude that either by cell therapy, administration of enzymes, immunosuppressant or other drugs, the

modulation of inflammatory response proved to be a promising tool for the reduction of gliosis, aiding locomotor recovery in animals with spinal cord injury.

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