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Immune-Based Therapies for Spinal Cord Injury

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ABSTRACT

Traumatic Spinal Cord Injury (SCI) results in both focal and diffuse spinal cord pathologies that are exacerbated by an inflammatory response after the initial injury. Resident and infiltrating immune cells contribute significantly to the growth-refractory environment near the lesion and can intensify damage to spared tissue, resulting in impaired spontaneous functional recovery. Numerous studies have demonstrated that several immunomodulatory therapies administered after experimental SCI may be beneficial in promoting functional recovery. In this review, we focus on the therapeutic potential of the most abundant immune-based therapies e.g., rolipram, liposomal clodronate and TNF-α based therapy including etanercept, thalidomide and adenosine A1 receptor therapy their contribution to eliminating secondary damage and promoting recovery after SCI.

Keywords: Spinal Cord Injury, Immunomodulatory Therapy, Neuroprotection, Rolipram, Liposomal Clodronate, TNF-α Based Therapy

1. INTRODUCTION

Traumatic SCI causes severe and often permanent neurological deficits due to the loss of descending motor and ascending sensory axonal pathways and subsequent demyelination (Bunge *et al*., 1993). The initial primary mechanical insult to the spinal cord includes compression, contusion, shearing and laceration and is followed by a series of destructive cellular processes, known as secondary injury, that accentuate tissue damage at and beyond the original site of trauma (Tator and Fehlings, 1991; Young, 1993; Schwab and Bartholdi, 1996; Sekhon and Fehlings, 2001; Jacobs and Fehlings, 2003; Stys, 2004) (**Fig. 1**).

The cascade of secondary injury events is primarily mediated by a robust cellular inflammatory response (Dusart and Schwab, 1994; Popovich *et al*., 1997; Keane *et al*., 2006) that involves macrophage and microglial activation (Blight, 1992; Popovich *et al*., 1999) and chemokine and cytokine production. Neutrophils are the first circulating leukocytes to infiltrate sites of SCI (~2 h-3 days post-SCI) (Carlson *et al*., 1998; Fleming *et al*., 2006; Kigerl *et al*., 2006; Nguyen *et al*., 2008; Stirling and Yong, 2008). Monocyte-derived macrophages infiltrate~2 days after neutrophils and help clear apoptotic neutrophils from the lesion (Savill *et al*., 1989; Stirling and Yong, 2008). This custodial function of macrophages may be necessary for inducing a subset of functions that include release of resolvins and protectins to suppress further neutrophil recruitment (Nathan, 2006). Unlike neutrophils, macrophages persist in human and mouse SCI lesions as long as any study has examined, months in mice and years in humans (Popovich *et al*., 2003; Fleming *et al*., 2006; Kigerl *et al*., 2006; Chang, 2007). The nonspecific microbicidal activity of neutrophils and monocytes/macrophages can be destructive to host tissue after SCI; both cell types release proteases (e.g., matrix metalloproteases) and oxidative metabolites that can damage cells and compromise the blood-spinal cord barrier (Noble *et al*., 2002; Scholz *et al*., 2007).

Indeed, SCI pathology is reduced and spontaneous recovery of neurological function (motor, sensory and autonomic) is improved when the activation of blood-derived leukocytes is restricted (Giulian and Robertson, 1990; Blight, 1994; Taoka *et al*., 1997; Popovich *et al*., 1999; Gris *et al*., 2004).

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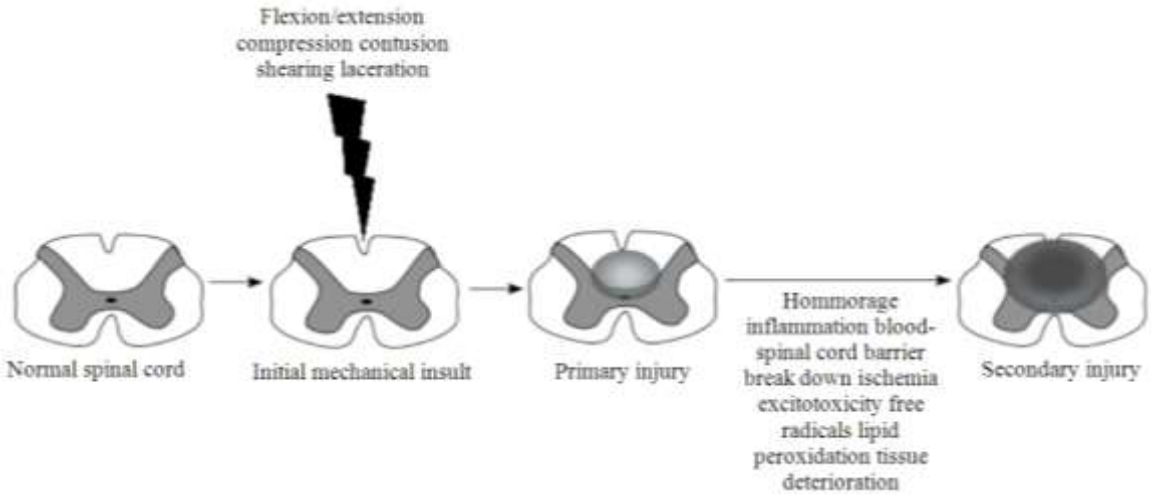

Fig. 1. Acute Spinal Cord Injury (SCI) involves both primary and secondary injuries. Schematic representation of SCI pathophysiology consisting of primary and secondary mechanisms of damage. After the initial mechanical insult, a collection of vascular, biochemical and cellular events occur that not only initiate a secondary set of injury cascades but also induce additional tissue loss and dysfunction that extends beyond the original trauma site

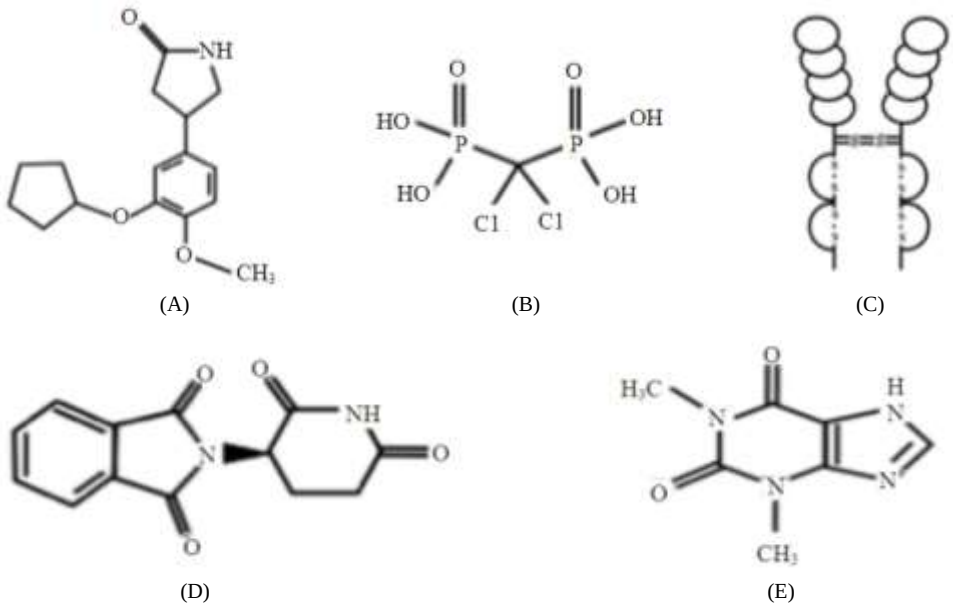

Fig. 2. Chemical structures of immune-based therapeutics discussed including: (A) Rolipram, (B) Liposomal Clodronate, (C) Etanercept, (D) Thalidomide and (E) Theophylline

T and B lymphocytes also infiltrate the injured mammalian spinal cord, although in fewer numbers and at later times post-injury than monocytes (Popovich *et al*., 1997; Sroga *et al*., 2003; Ankeny *et al*., 2006). The functional significance of T and B cells in the injured spinal cord remains a point of controversy (Popovich and Longbrake, 2008; Ankeny and Popovich, 2009).

The spatial extent of secondary injury events spreads both radially and longitudinally along the spinal cord in a rostral-to-caudal manner. The end result is cavitation of

central gray matter along with partial or complete loss of adjacent white matter tracts (Tator, 1998; Norenberg *et al*., 2004). Cell death following SCI occurs by necrosis and apoptosis. While necrosis predominates immediately following the primary traumatic episode, delayed stages of subacute spinal cord pathology induce apoptosis predominantly of oligodendrocytes and to lesser extent neurons (Casha *et al*., 2001). Each event within the secondary injury represents an important therapeutic target for SCI. By focusing on immune-based therapies, injuries including severed and/or demyelinated axons, inflammatory cells and proinflammatory cytokines and glial scar components. Also, considering the multi-faceted nature of secondary pathologic events attributed to SCI, drug cocktails with diverse modes of neuroprotection will likely be useful in preventing or limiting secondary injury progression.

Numerous studies examining the effects of several immunomodulatory drugs, including rolipram, thalidomide, liposomal clodronate, IL-10, etanercept, interferon-β, immunoglobulin G, minocycline and Lipitor support the notion that immunomodulatory therapies after experimental SCI may be beneficial in promoting functional recovery (Iannotti *et al*., 2011; Beaumont *et al*., 2009; Koopmans *et al*., 2009; Whitaker *et al*., 2008; Pannu *et al*., 2007; Gok *et al*., 2007; Genovese *et al*., 2006; Stirling *et al*., 2004).

The purpose of this review is to focus on the therapeutic potential of the most abundant immune-based therapies in SCI e.g. rolipram, liposomal clodronate and TNF-α based therapy including etanercept, thalidomide and adenosine A1 receptor therapy (**Fig. 2**).

1.1. Phosphodiesterase Inhibitors (Rolipram)

The intensity and duration of the inflammatory response directly relates to the intracellular concentration of cAMP (Bruno *et al*., 2004), a second messenger that controls many cellular processes (Beavo and Brunton, 2002). Increased intracellular cAMP contributes to inhibition of proinflammatory cellular functions such as chemotaxis, degranulation, superoxide anion generation, release of IL-8 and phagocytosis in neutrophils (Ottonello *et al*., 1995; Rossi *et al*., 1998; Pryzwansky and Madden, 2003; Pearse *et al*., 2004). Furthermore, monocyte adhesion and migration are inhibited by high cAMP levels, as are phagocytosis and nitric oxide production in macrophages (Rossi *et al*., 1998; Zhu *et al*., 2001; Aronoff *et al*., 2005). Increased cAMP reduces adhesion molecule (CD11b/CD18/L-selectin) expression on leukocytes, leukocyte adhesion to other cells and disrupts chemokine induced chemotaxis (Harvath *et al*., 1991; Derian *et al*., 1995).

Rolipram is a well studied Phosphodiesterase type 4 (PDE4) inhibitor and has been shown to inhibit leukocyte functions, including leukotriene production by monocytes and to have anti-inflammatory effects in vivo that include inhibition of neutrophil migration (Griswold *et al*., 1993). These findings indirectly demonstrate a role for PDE4 in several functions of monocytes and neutrophils. Furthermore, the PDE4B subtype has been identified in activated microglia of the injured spinal cord (Whitaker *et al*., 2008). Targeted inhibition of the PDE4, is a potentially powerful tool (Houslay and Adams, 2003) as PDE4 inhibitors suppress the production of TNF-α, the generation of reactive oxides and the migration of neutrophils (Torphy, 1998; Giembycz, 2000). Rolipram has been shown to decrease the production of TNF-α in homogenates of the injured spinal cord and in activated human mononuclear cells (Semmler *et al*., 1993; Pearse *et al*., 2004).

Rolipram delivery in the first 72 h after SCI in rats has neuroprotective effects, sparing oligodendrocytes from death at 27 h post-injury, an effect that may have involved abrogation of local inflammation (Whitaker *et al*., 2008). Rolipram has been used also in combination with cellular transplant yielding cellular sparing and improved motor outcomes (Pearse *et al*., 2004; Koopmans *et al*., 2009; Beaumont *et al*., 2009; Bretzner *et al*., 2010). Moreover, Iannotti *et al*. (2011) mentioned the ability of rolipram in combination with liposomal-encapsulated clodronate to enhance myelinated tissue sparing and improve hindlimb functional recovery at 4 weeks post-injury. Additionally, histological assessment revealed substantial axonal sparing and/or sprouting from several brainstem motor nuclei and hindlimb motor cortex, a significant reduction in lesion volume (51%) and lesion area at the injury epicenter (45%) and a significant increase in the extent of myelinated tissue sparing (Iannotti *et al*., 2011). Together, these studies suggest a neuroprotective effect of the early administration of this PDE4 inhibitor.

Others have demonstrated the effectiveness of rolipram in promoting regeneration after spinal cord injury due to its effectiveness in blocking growth cone collapse (Nikulina *et al*., 2004; Pearse *et al*., 2004). Kajana and Goshgarian (2008) shown that rolipram can increase phrenic nerve output ipsilateral to an experimental C2 hemisection lesion. Additionally, intravenous rolipram restored respiratory-related activity to the phrenic nerve ipsilateral to the injury and significantly enhanced phrenic nerve inspiratory burst activity in both normal and C2 hemisected animals. These results provided evidence that PDE inhibitors can

strengthen spared ineffective synaptic connections at the level of the phrenic nucleus and may enhance phrenic nerve output and restore respiratory related phrenic nerve function after high cervical SCI (Kajana and Goshgarian, 2008).

These findings emphasize the usefulness of PDE4 inhibitors in promoting axonal regeneration following experimental SCI, particularly in combination with cellular implants (Qiu *et al*., 2002; Nikulina *et al*., 2004; Pearse *et al*., 2004; Kajana and Goshgarian, 2008; Bretzner *et al*., 2010) and in exerting neuroprotective effects like cellular sparing, myelination, improving neurotransmission through the ventrolateral funiculus and functional recovery (Pearse *et al*., 2004; Whitaker *et al*., 2008; Beaumont *et al*., 2009; Iannotti *et al*., 2011).

Rolipram is a "readily-available" drug and its clinical efficacy has been assessed in the treatment of several disorders including depression, systemic lupus erythematosus, rheumatoid arthritis, asthma, chronic obstructive pulmonary disease and multiple sclerosis (Dyke and Montana, 2002). While recent clinical evaluation of rolipram has been hampered by side effects of nausea and vomiting, a new generation of PDE4 inhibitors with a less adverse side effect profile is under evaluation (Dastidar *et al*., 2009; Davis *et al*., 2009).

1.2. Macrophage Depletion (Liposome-Encapsulated Clodronate)

The effect of macrophage infiltration in SCI injury is highly controversial. Several studies have reported both beneficial and harmful effects of macrophages and/or microglia after injury (Prewitt *et al*., 1997; Rapalino *et al*., 1998; Fitch *et al*., 1999; Popovich *et al*., 1999; Schwartz *et al*., 1999; Bomstein *et al*., 2003; Yin *et al*., 2003; McPhail *et al*., 2004; Horn *et al*., 2008; Iannotti *et al*., 2011). Activated microglia and extra-vasated blood monocytes constitute the majority of inflammatory cells present at the site of a SCI (Blight, 1985; Dusart and Schwab, 1994; Popovich *et al*., 1996; Carlson *et al*., 1998). Whether these cells act in a coordinated fashion to resolve the injury site or whether they adopt distinct functional repertoires is unclear. However, the contribution and functional status of one macrophage subset relative to the other is likely to be influenced by several factors including: (i) the nature of the primary insult (e.g., transection, infection, blunt trauma, ischemia); (ii) interactions with resident and infiltrating cells; (iii) distance from the site of the injurious stimulus, related to blood–brain barrier damage and chemotactic gradients; and (iv) exposure to humoral factors or substrates of non-CNS origin (e.g., complement protein, peripheral nervous system components, or drugs). These variables will affect microglial/macrophage activation and whether secondary damage or repair occurs (Popovich *et al*., 1999).

After SCI, the onset of repair precedes hematogenous macrophage infiltration but not the activation of resident microglia. Therefore, interventions that alter the kinetics or nature of the inflammatory response to trauma might affect later stages of repair, including axon regeneration. Since the matrix that forms after injury is not sufficient to maintain axonal growth, it is possible that infiltrating macrophages antagonize the efforts of resident cells to repair the injury site. Fitch and Silver (1997) support the concept that the inefficient progression of endogenous repair and the formation of an axon restrictive growth environment are mediated by blood-brain barrier damage, acute infiltration of blood monocytes and the accumulation of inhibitory extracellular matrix molecules. Acute macrophage depletion limits chondroitin sulfate proteoglycan deposition and reduces the phagocytosis-coupled release of antibacterial agents (e.g., superoxide, hydrogen peroxide, hypochlorous acid), quinolinic acid, or proteolytic enzymes. These latter compounds, although innocuous in the regenerative tissues of the periphery, could cause inefficient repair, progressive necrosis/apoptosis and destruction of healthy tissues and neural/glial progenitors within the CNS (Popovich *et al*., 1999).

Clodronate is a first-generation bisphosphonate drug which, when encapsulated by liposomes, induces the selective apoptotic cell death of monocytes and phagocytic macrophages (Van Rooijen and Sanders, 1994; Selander *et al*., 1996). Horn *et al*. (2008) show that activated macrophages and microglia can induce long-distance retraction of dystrophic axons both in vitro and in vivo after dorsal spinal cord hemisection and that macrophage depletion with liposomal clodronate is capable of attenuating axonal "die-back" following dorsal column lesioning. Moreover, Iannotti *et al*. (2011) reported that an intra-peritoneal injection of liposomal clodronate immediately after injury and on days 1, 3 and 6 post-injury improved myelinated tissue sparing, reduced ED-1+ macrophage infiltration and enhanced locomotor recovery. Additional studies support the notion that depletion or neutralization of neutrophils and macrophages resulted in reduced lesion volume, increased neuronal survival and improved functional recovery (Giulian and Robertson, 1990; Blight, 1994; Rabchevsky and Streit, 1997; Popovich *et al*., 1999; Van Rooijen and Kesteren-Hendrikx, 2002; Gok *et al*., 2007; Iannotti *et al*., 2011).

All of these recent studies strongly suggest that the activated macrophages infiltrating to the injury site act as a regenerative barrier and contradict earlier studies that have described the beneficial effects of macrophage transplants into the injured spinal cord (Rapalino *et al*., 1998; Bomstein *et al*., 2003). The dual nature of the effects of macrophages after SCI is complicated (Popovich and Longbrake, 2008) and suggests that the best approach for treatment following spinal cord injury may be to modify the state of macrophage activation rather than deplete the macrophages altogether (Blight, 1992; Jones *et al*., 2005; Hohlfeld *et al*., 2007).

1.3. TNF-α Therapy

The pathophysiology of spinal cord injury includes an initial mechanical injury that is followed by a cascade of secondary degenerative cellular and molecular processes. The secondary damage is initiated by considerable disruptions in blood supply, breakdown of the blood-spinal cord barrier, a significant release of inflammatory mediators, chemokines, cytokines and neurotoxins within the area; leading to further tissue deterioration (Tyor *et al*., 2002; Chi *et al*., 2008; Sharma, 2011). Amongst the proinflammatory cytokines expressed, expression of tumor necrosis factor-alpha (TNF-α) has been identified as being rapidly upregulated at the lesion site after SCI (Wang *et al*., 1996; Streit *et al*., 1998; Bethea *et al*., 1999; Hayashi *et al*., 2000; Yan *et al*., 2001; Wang *et al*., 2002; Yune *et al*., 2003).

TNF-α influences immunity, inflammation, cell proliferation, differentiation and apoptosis and is found as either a transmembrane protein or soluble cytokine (Bayrakli *et al*., 2012). Within the CNS, TNF-α is generated by astrocytes, microglia and various neuronal populations (McCoy and Tansey, 2008; Caminero *et al*., 2011). Binding to either of the two distinct TNF receptors, TNFR1 and TNFR2, activates separate pathways that either elicits pro-inflammatory or apoptotic signaling or pro-inflammatory and survival signaling, respectively (Bayrakli *et al*., 2012). Both stress and injury induce the release of TNF-α and additional cytokines (Ferguson *et al*., 2008).

Data has shown that TNF-α protein levels are significantly increased within 1 h post-SCI and decline between 8 and 20 h after lesion production (Wang *et al*., 1996). Notably, TNF-α is released more rapidly when compared to other pro-inflammatory cytokines and essentially functions to initiate Wallerian degeneration, activate Schwann cells and assist with the recruitment of macrophage to the injury site (Stoll *et al*., 2002; Esposito and Cuzzocrea, 2011). Furthermore, it is thought that this initial acute phase of TNF-α activity at the lesion site plays a deleterious role in secondary injury (Wang *et al*., 1996; Brewer and Nolan, 2007; Chi *et al*., 2008).

Early studies specifically revealed that TNF-α enhances the permeability of endothelial cells, damages the blood brain barrier, causes apoptosis, activates astrocytes, contributes to glial scar formation, initiates glutamate mediated cellular death and induces the expression of iNOS and NF-α B (Selmaj *et al*., 1990; Schobitz *et al*., 1994; D'Souza *et al*., 1995; Sipe *et al*., 1996; Akassoglou *et al*., 1998; Bethea *et al*., 1999; Lee *et al*., 2000; Beattie *et al*., 2002; Wang *et al*., 2002; Hausmann, 2003; Brewer and Nolan, 2007). When examining tissue damage in chronically injured spinal cords, it was noted that TNF-α transgenic rats had enhanced tissue healing and a persistent baseline level of TNF-α in comparison to wild-type littermates (Chi *et al*., 2008). This chronic data, as well as other studies, suggest that TNF-α may be destructive in the acute phase of injury and beneficial during chronic stages of SCI (Bethea *et al*., 1999; Hausmann, 2003; Brewer and Nolan, 2007; Chi *et al*., 2008).

Because TNF-α acts as signal to trigger apoptosis in the spinal cord following injury, the neutralization of this neurodestructive factor at the most optimal time could be an essential tool to promote neuroprotection in the cord following trauma (Yune *et al*., 2003; Sharma, 2010). Ideally, a therapeutic approach would not only harness the benefits of early TNF-α inhibition but would also take advantage of the potential for contributions to functional recovery in chronic SCI conditions by maintaining optimal physiological levels.

1.4. Etanercept

Etanercept, a TNF-α antagonist, is a genetically engineered fusion protein that is comprised of the extracellular ligand-binding portion of TNFR2 fused to a portion of human IgG1 and functions as a decoy receptor that competitively binds TNF-α and TNF-α (Genovese *et al*., 2006; Kato *et al*., 2010; Caminero *et al*., 2011; Bayrakli *et al*., 2012). Overall, the primary therapeutic objective of TNF antagonist administration is to reduce excessive TNF from circulation and inflammation sites (Genovese *et al*., 2006). Because etanercept functions in vivo as a selective antagonist of TNF, it has been utilized as a treatment option for several inflammatory conditions, traumatic brain injury and neurodegenerative diseases.

The functional significance of etanercept therapy post-SCI has been specifically addressed in several

studies and has revealed remarkable results. An early immunohistochemical and motor study reported that experimental animals treated with etanercept had reduced TNF-α expression, cell apoptosis, neutrophil infiltration and spinal cord damage and improved motor function (Genovese *et al*., 2006). Another investigation examining the outcomes of immediate etanercept therapy after a peripheral nerve injury revealed that experimental groups had a significant enhancement in axonal regeneration when compared to vehicle treated animals (Kato *et al*., 2010). A recent study examined the potential suppression of neuronal and oligodendroglial apoptosis by giving etanercept 1 h after thoracic SCI in rats. Data indicate that treated animals had reduced tissue damage associated with SCI, improved hindlimb locomotor function and myelin regeneration (Chen *et al*., 2011). The neuroelectrophysical effectiveness of entanercept was investigated under partial SCI conditions and results illustrated that the treated groups of animals had significant clinical and electrophysiological recovery that was not seen in controls (Bayrakli *et al*., 2012). Lastly, it was postulated that the significant motor recovery demonstrated in a patient suffering from an initial T7 complete paraplegia, who by chance received etanercept prior to the accident, was possible because of the considerable reduction in post-traumatic spinal cord inflammation (Dinomais *et al*., 2009; Tobinick, 2010).

Together, data from these studies strongly imply that etanercept treatment significantly lessens the degree of inflammation and tissue damage associated with SCI by directly reducing the expression of TNF-α and TNFR during the acute phase of injury. Future studies examining the outcomes of etanercept administration on motor recovery, regeneration and plasticity are essential to not only determine the optimal treatment window but to also take advantage of a promising therapy.

1.5. Thalidomide

An alternative pharmacological strategy utilized in the reduction of secondary tissue damage after SCI is to administer the glutamic acid derivative thalidomide. Thalidomide is a psychoactive drug that easily crosses the blood brain barrier and elicits an inhibitory effect on TNF-α in vitro and in vivo studies (Corral *et al*., 1999). Because it induces anti-inflammatory and immunomodulatory effects, thalidomide has recently been considered as a potential therapeutic in SCI and other diseases (Tseng *et al*., 1996; Esposito and Cuzzocrea, 2011).

In a mouse model of SCI, experimental animals treated with thalidomide had a significant decrease in the development of inflammation and secondary tissue damage (Genovese *et al*., 2008; Esposito and Cuzzocrea, 2011). Another study evaluated the effects of thalidomide on spinal cord ischemia and found that in experimental animals, treatment applied before ischemic insult reduced TNF-α levels and early phase ischemia/reperfusion injury of the spinal cord in rabbits (Lee *et al*., 2007). Lastly, by combining thalidomide with rolipram researches were able to demonstrate a significant attenuation of TNF-α, enhanced sparing of white matter and improved motor function (Koopmans *et al*., 2009).

The overall decrease in secondary tissue degeneration suggests that utilizing a combinatorial therapy to reduce inflammation via different mechanisms compliment one another to elicit optimal results and may further improve functional output. Despite the modifications to TNF-α expression levels and secondary damage induced after SCI, additional studies examining the effectiveness and safety of thalidomide needs to be conducted.

1.6. Adenosine A1 Therapy

Following CNS trauma, a variety of inhibitory substances like adenosine and GABA are released; determining their exact contribution or prevention to secondary damage is of principal importance (Hagberg *et al*., 1987; Fern *et al*., 1994). In order to initiate downstream physiological events, adenosine must binds to one of its specific receptors, which are classified as A1, A2, A3 and A4 (Nantwi and Goshgarian, 2002). Adenosine is recognized as eliciting a tonic inhibitory effect on neuronal excitability (Gundlfinger *et al*., 2007) and by acting through its G-protein coupled receptors can either inhibit (A1 and A3) or promote (A2a and A2b) cAMP synthesis (Dunwiddie and Masino, 2001; Kajana and Goshgarian, 2008). Specifically, activation of the A1 receptor elicits typical Gi/o mediated signaling events as well as proinflammatory effects on adhesion, migration and phagocytosis of neutrophils and monocytes/macrophages (Sawynok and Liu, 2003).

Early investigations sought to determine if adenosine release post-SCI offered a level of neuroprotection. By examining the concentrations of both adenosine and neurotoxic amino acids, data indicated that the release of endogenous adenosine was advantageous to minimizing tissue degeneration (McAdoo *et al*., 2000). While this study confirmed the idea that an increase in adenosine offered no neuroprotection,

future investigations focused on the physiological outcome of adenosine antagonism after SCI.

The specific adenosine A1 and A2 receptor antagonist, theophylline, has been shown to pharmacologically induce plasticity by restoring respiratory muscle function following a spinal cord C2 hemisection (Nantwi *et al*., 1996; Nantwi and Goshgarian, 1998). In a multitude of species, adenosine receptors are localized to carotid bodies and modulate (depress) respiratory activity (McQueen and Ribeiro, 1983; Watt *et al*., 1987; Nikodijevic *et al*., 1991; James and Nantwi, 2006). That being said, application of an A1 receptor agonist has been shown to depress respiration (Eldridge *et al*., 1985; Wesberg *et al*., 1985), whereas inhibition of adenosine receptors is the foundation of the clinical aspect of theophylline treatment for respiratory deficiency (Richmond, 1949; Thithapandha *et al*., 1972; Nantwi and Goshgarian, 2002). Continuing with the C2 hemisection injury model, chronic theophylline treatment was found to elicit results that were similar to acute administration of the therapeutic and suggest that continuous use may not be optimal (Nantwi *et al*., 2003).

Taken together, these results suggest that obtaining a more thorough understanding of the molecular basis of the theophylline mediated recovery of respiratory function post-SCI would most likely guide the development of a comprehensive treatment strategy. While protective and destructive immunity response theories following SCI might appear contradictory, it is possible that both are partially correct. Specifically, some aspects of autoimmunity are beneficial and some are injurious. The ability to disable certain aspects of the immune function while keeping others intact is necessary to solve the complex functions of the immune system following neurotrauma. As new pharmacological tools are developed, the ability to examine these functions will improve. Moreover, the effects of combinational therapy will be pivotal to increase the ability to effectively modulate the immune system and maximize the regenerative potential of the CNS. For example, recent data has shown that immunomediators were capable of controlling the migration, proliferation, quiescence, cell-fate choices and survival of neural stem cells and their progeny and may significantly contribute to the success of other therapeutic treatment strategies post-SCI (Gonzalez-Perez *et al*., 2012).

2. CONCLUSION

Spinal cord injury is first induced by a mechanical insult supporting secondary biochemical and physiological damage that ultimately promotes permanent loss of sensory and motor function (Pajoohesh-Ganji and Byrnes, 2011). The secondary damage initiates a series of degenerative events that results in further tissue destruction, massive cellular death, disrupted vasculature, increased permeability of the blood-spinal cord barrier, axonal demyelination, glial scar formation and neuroinflammation (Fehlings and Nguyen, 2010; Pajoohesh-Ganji and Byrnes, 2011; Jaerve and Muller, 2012). Because the secondary damage is so widespread, the prevention or a reduction in one or several of these secondary events post-SCI could potentially initiate spinal cord tissue repair and promote the overall improvement in functional outcomes.

Table 1. Comprehensive description of each pharmacological agent that includes the molecular formula, class of drug, mode of activity and downstream targets

Agent	Molecular formula	Drug class	Target	Mode of action
Rolipram	$C_{16}H_{21}NO_3$	PDE-4 inhinitor	PDE-4 cAMP phosphodiesterase	cAMP Accumulation
Clodronate	$CH_4Cl_2O_6P_2$	Bisphosphonate	Macrophage and microglia	Apoptoses induction; elimination of macrophage
Etanercept	Recombinant dimmer of human TNF-R proteins bound to human IgG1	Tumor necrosis factor blocker	TNF-α	binds to TNF-α and TNF β
Thalidomide	$C_{13}H_{10}N_2O_4$	Sedative	TNF-α	Anti-inflammatory; Immunomodulatory; anti-angiogenic
Theophylline	$C_7H_8N_4O_2$	Methylxanthine	Adenosine A1 and A2 Receports	PDE inhibitor

Indeed, focusing on any one of the cellular inflammatory responses as a therapy for SCI is of high importance. Extracellular molecules like cyto- and chemokines can be readily targeted by biological agents that demonstrate a high degree of specificity (Esposito and Cuzzocrea, 2011). For example, rolipram has been shown to prevent the reduction in cAMP levels after acute CNS injury as well as promote tissue protection, repair and functional recovery (Pearse *et al*., 2004; Atkins *et al*., 2006; Schaal *et al*., 2012). Additionally, a recent study described the benefits of utilizing a drug-eluting microfibrous patch as means to deliver rolipram into the injured spinal cord and data indicated significant improvements in both functional and anatomical recovery (Downing *et al*., 2012). Clodronate has been shown to induce macrophage apoptosis (Monkkonen *et al*., 1994) and microglia elimination (Kumamaru *et al*., 2012) in vitro but the overall necessity of completely eradicating each is controversial at best. Focusing on the utilization of a TNF-α therapy that neutralizes injury-induced apoptosis and inflammation may be one of the most crucial tools to promote neuroprotection in the cord following trauma (Yune *et al*., 2003; Sharma, 2010). Lastly, functional support has been addressed by treating SCI patients suffering from bradychardia with theophylline (Schulz-Stubner, 2005).

While the above pharmacological agents offer various levels of neuroprotection (**Table 1**), there are disadvantages associated with each therapy. Therefore, it is becoming increasingly clear that a prudent process for therapy design is not only required but that establishing the line between elimination and reduction of secondary molecules is critical. Ideally, this will involve thoroughly investigating and harnessing the benefits of combinatorial therapies, optimization of the dosage and delivery mode and establishment of the best therapeutic window so that clinical feasibility can be examined (Rabchevsky *et al*., 2011; Schaal *et al*., 2012).

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