JOURNAL OF NEUROTRAUMA 28:1545–1588 (August 2011)

DOI: 10.1089/neu.2009.1149

A Systematic Review of Non-Invasive Pharmacologic Neuroprotective Treatments for Acute Spinal Cord Injury

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Abstract

An increasing number of therapies for spinal cord injury (SCI) are emerging from the laboratory and seeking translation into human clinical trials. Many of these are administered as soon as possible after injury with the hope of attenuating secondary damage and maximizing the extent of spared neurologic tissue. In this article, we systematically review the available pre-clinical research on such neuroprotective therapies that are administered in a non-invasive manner for acute SCI. Specifically, we review treatments that have a relatively high potential for translation due to the fact that they are already used in human clinical applications, or are available in a form that could be administered to humans. These include: erythropoietin, NSAIDs, anti-CD11d antibodies, minocycline, progesterone, estrogen, magnesium, riluzole, polyethylene glycol, atorvastatin, inosine, and pioglitazone. The literature was systematically reviewed to examine studies in which an *in-vivo* animal model was utilized to assess the efficacy of the therapy in a traumatic SCI paradigm. Using these criteria, 122 studies were identified and reviewed in detail. Wide variations exist in the animal species, injury models, and experimental designs reported in the pre-clinical literature on the therapies reviewed. The review highlights the extent of investigation that has occurred in these specific therapies, and points out gaps in our knowledge that would be potentially valuable prior to human translation.

Key words: animal model; neuroprotection; spinal cord injury; systematic review; translational research

Introduction

The spinal cord injury (SCI) field is replete with therapeutic candidates that potentially enhance neurologic recovery by attenuating the pathophysiological processes triggered after acute injury, thereby minimizing secondary damage. In the pre-clinical testing of such therapies, the treatment is typically administered immediately or very soon after the injury is induced in an animal model (mainly rat or mouse). Neurologic efficacy in such models suggests that the therapy in question may have a "neuroprotective" role in acute human SCI. While many treatments are administered in this fashion, those that can be delivered systemically via intravenous infusion or even orally have the potential to reach the cord injury site most rapidly (as compared to a surgically delivered treatment). Since the methylprednisolone clinical trials, the SCI community has sought additional interventions that would follow the similar strategy of administration as early as possible after injury to minimize secondary damage. Such "non-invasive" therapies are the focus of this review.

Given the explosion of interest in acute SCI and the seemingly endless number of therapies that are reportedly found to be beneficial in animal models of SCI, the purpose of this initiative was to review specific non-invasive treatments in a systematic fashion so as to depict the current body of pre-clinical literature that might support the translation of such treatments into clinical trials. The field is large, and in this regard, it is difficult – if not impossible – to cover it comprehensively. Our strategy, therefore, was to select therapies that have reasonable "translational potential" by virtue of the fact that they are already in human clinical use for other indications, or because of the extent and depth of their pre-clinical investigation (or both). While it was not the intention to attempt to cover the entire SCI field, our goal was to apply the tenets of systematic review to the specific therapies that met

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these conditions. By performing a systematic review of each of these therapies, we hoped to provide the field with an overview of the body of pre-clinical evidence that supports (or fails to support) the translation of the therapy into human trials.

Methodology

First, we selected acute SCI treatments that fit the following criteria: (a) a treatment that is already in human clinical use for some other related or unrelated condition, or if not currently in human use, is currently available in a form that could be given to humans; (b) a treatment that could be administered systemically (i.e., non-invasively) to an SCI patient (either orally or intravenously). A PubMed search was then conducted on "the therapy" and "spinal cord injury" (e.g., "minocycline and spinal cord injury"). From the list of studies generated through this fairly indiscriminant search, we applied the following criteria to review systematically the pre-clinical literature on these therapies:

- Studies in which the testing of the therapy was performed in an *in-vivo* animal model of SCI. Studies that were exclusively *in-vitro* experiments were excluded.
- Studies in which the spinal cord is traumatically injured. Non-traumatic local or global ischemia models and photochemical reaction models were excluded, as were traumatic root avulsion or dorsal root entry zone models.
- Studies in which the application of the therapy was via the systemic circulation. This included agents administered orally, or via subcutaneous, intraperitoneal, or intravenous injection. Studies in which the therapy was applied directly to the cord or via intrathecal injection/infusion were excluded.
- At least two peer-reviewed publications available on the therapy.

The data from those studies that fitted the criteria were then extracted into a table format to depict the animal model, injury model, the treatment's dose and timing, the experimental groups tested in the study and the "*n* per group," and the reported behavioral and non-behavioral outcomes (e.g., histological, biochemical, or physiological outcomes). A summary statement about the body of literature was then generated.

Results

Using this selection process, we identified the following therapies: erythropoietin, systemic hypothermia, non-steroidal anti-inflammatory agents (NSAIDs), anti-CD11 antibodies, minocycline, progesterone, estrogen, magnesium sulfate, riluzole, polyethylene glycol, atorvastatin, inosine, and piaglitazone (Table 1).

The PubMed searches on these therapies were conducted in the spring/summer of 2008 by SCI researchers across Canada and updated in June 2009. By applying the previously described criteria (essentially, *in-vivo* animal studies utilizing a traumatic model of SCI to test a pharmacologic or non-invasive therapy), the following studies were generated, and the tables for each of these respective therapies are listed below.

Erythropoietin (Table 2)

Erythropoietin (EPO) has been studied quite extensively in acute SCI. The systematic review produced 19 studies, all of which utilized Sprague Dawley (10), Wistar (eight), or Long Evans (one) rats, with one study utilizing a knockout mouse (Brines et al., 2004). Some form of contusion SCI was employed in 12 studies, aneurysmal clip or rod compression was employed in six studies, and one study utilized a unilateral hemisection (King et al., 2007). All injuries occurred in the thoracic spine; to date there is no evaluation of EPO in a cervical SCI model. EPO was administered at a variety of doses but most commonly at 1000 IU/kg or 5000 IU/kg, either intraperitoneally, or intravenously. A dose effect was demonstrated in experiments by Gorio and colleagues (2005), Kaptanoglu and colleagues (2004), and Kontogeorgakos and colleagues (2009). Interestingly, in the studies by both Gorio and colleagues (2005) and Kontogeorgakos and colleagues (2009) in which different doses of EPO were tested intravenously or subcutaneously respectively, the optimal results were observed with the lower doses. With respect to time window of effective intervention, EPO was administered at the time of injury in half of the studies and within 30–60 min in the remainder. Improvements in behavioral outcomes were reported by Boran and colleagues (2005) and Gorio and colleagues (2002) with a 60-min delay in intervention, although this was not found by others (Mann et al., 2008; Pinzon et al., 2008).

Non-behavioral outcomes included improved white and grey matter sparing, reduced apoptosis and lipid peroxidation, reduced ERK phosphorylation, and decreased inflammatory cytokine release and neutrophil invasion. Many authors reported improved locomotion with Basso, Beattie, and Breshnan (BBB) scores, or improved function on the swimming test. Of note, the recent reports of Guìzar-Sahagún and colleagues (2009), Mann and colleagues (2008), and Pinzon and colleagues (2008) revealed no histological or behavioral improvements with EPO administered either 12 h pre-injury, immediately at the time of injury, or 1 h post injury respectively. The study by Pinzon and colleagues (2008) is notable as an NIH-sponsored attempt to reproduce the positive effects of EPO described previously, particularly in the studies by Gorio and colleagues. The study by Mann and colleagues (2008) examined both erythropoietin and darbepoetin, a more bioavailable version of EPO, but found neither to have any neuroprotective efficacy. Like minocycline, it is unclear how to interpret these contradictory reports on the efficacy of EPO in acute SCI.

Systemic hypothermia (Table 3)

While technically not a "drug," systemic hypothermia was included in this review as it is a relatively "non-invasive" treatment that is applied to the cord in a systemic, non-operative manner (as compared to local hypothermia, which requires intrathecal cooling devices or open surgery). Systemic hypothermia has had a long history of study, stemming from the early 1990s. A recent resurgence in the interest in systemic hypothermia has arisen due to its well-publicized application in Kevin Everett, an NFL football player who suffered a cervical SCI in the fall of 2007.

We identified 17 papers that fulfilled the criteria of studying systemic hypothermia after traumatic SCI (although some describe the same set of experimental animals but report different outcomes in different papers). These mostly utilized Sprague Dawley rats, although the injury models were quite variable, and included one generic weight-drop device, two

Table 1. Non-invasive Therapies Systematically Reviewed

Therapy	*Current human use*	*Mode of human administration*	*Published studies meeting criteria*
Erythropoietin	For increasing red blood cell production	Subcutaneous	19
Systemic Hypothermia	Neuroprotection in cardiac arrest, neonatal hypoxic encephalophathy	Endovascular or body surface cooling	17
NSAIDs	Anti-inflammatory/analgesic agent	Oral	17
Anti-CD11d mAB	Humanized antibody in development (currently not used clinically)	IV	11
Minocycline	Antibiotic and treatment of acne	Oral (but IV formulation exists)	10
Progesterone	Hormone replacement	Oral	10
Estrogen	Hormone replacement	Oral	8
Magnesium	Pre-eclampsia, cardiac arrest	IV	9
Riluzole	Amyotrophic lateral sclerosis	Oral administration	8
Polyethylene Glycol	Excipient in many drugs, also used in many laxatives, toothpaste, skin creams	Oral or IV	5
Atorvastatin	Cholesterol lowering agent	Oral	3
Inosine	Dietary supplement	Oral	3
Pioglitazone	Type II diabetes	Oral	2

with the NYU weight-drop impactor, one with the OSU impactor, 10 with cord compression induced by a balloon or clip, and one cord transection. Three studies (Jou et al., 2000; Lo et al., 2009; Strain and Waldrop, 2005) utilized a cervical injury model, while the remainder studied midthoracic to upper lumbar injuries. Systemic hypothermia was typically induced to a level of 30–34°C, which was maintained for as little as 20 min to as long as 4 h, or, in some cases, until the experiment was completed. In four studies, hypothermia was induced prior to the injury; in an additional 11 studies, hypothermia was induced at the time of injury or just after injury without any significant delay in intervention; in the remaining two studies, hypothermia was started at 15 min (Westergren et al., 2001) or 30 min (C.G. Yu et al., 2000) post injury. The study by Yu and colleagues (2000) in which hypothermia was begun 30 min post injury described improved behavioral recovery in the hypothermia-treated animals.

With respect to outcomes, 11 out of 17 studies only reported on histological/biochemical/physiological outcomes and did not report any behavioral outcomes. In general, these studies reported reduced apoptosis, myeloperoxidase activity, vasogenic edema, and tissue damage. For the six papers that assessed behavioral recovery, four reported on improvements in hindlimb function (Jou et al., 2000; Lo et al., 2009; Morino et al., 2008; C.G. Yu et al., 2000), while two reported no improvements (Martinez-Arizala and Green, 1992; Westergren et al., 1999).

NSAIDs (Table 4)

NSAIDs have had a long history of investigation stemming back to the 1980s, indicative of the long-standing appreciation for the role of inflammation in the secondary injury cascade post SCI. The most extensively studied NSAID is indomethacin. The systematic review revealed 10 papers published from 1991 to 2006 that reported on the effects of indomethacin in rat models (Wistar, Lewis, SD) and also a rabbit model. Standard contusion injury devices were not available at the time that many of these studies were performed, and hence, a wide range of injury models were described (weight drop, clip or forcep compression, longitudinal incision). A very wide range of intraperitoneal or intravenous indomethacin doses have been tested, from 0.1 mg/kg to 20 mg/kg, with most in the 2–10 mg/kg range. Strangely, in many cases, indomethacin treatment was started 30–60 min *prior* to injury. In the remaining studies, the drug was given at the time of injury, and hence, no time window of efficacy has been established. Many of the indomethacin studies examined non-behavioral outcomes only, and showed decreased edema, BBB permeability, and less overall tissue damage. Simpson and colleagues in 1991 reported improved performance on an inclined plane test with indomethacin, but more recently, Harada and colleagues (2006) reported decreased performance on the inclined plane test with 5, 10, and 20 mg/kg doses given 1 h prior to injury.

Other NSAIDs that have been reported in either one or two studies include BW755C, celebocid, SC58125, NS-398, naproxen, aspirin, and ibuprofen. Again, in many of these studies, the drug was given either prior to or within minutes of injury. Ibuprofen, however, has more recently been found to promote both histologic and behavioral improvements in thoracic contusion SCI models when administered 3 days post injury (Wang et al., 2009) or even 7 days post injury (Fu et al., 2007). These two studies also revealed that ibuprofen was superior to naproxen in both behavioral and non-behavioral outcomes. Interestingly, the role of ibuprofen in reducing RhoA activation raises the potential that this drug may have therapeutic benefit through the promotion of axonal sprouting (i.e., not exclusively attenuation of inflammation). The doses utilized in the preclinical studies (60–70 mg/kg/day) merit discussion. At these doses, a 70 kg individual would receive 4,200 to 4,900 mg of ibuprofen per day, which does slightly exceed the 3,200 mg/day maximum does recommendation for rheumatologic and arthritic conditions. However, FDA guidelines for the conversion of a therapeutic dose in rats to a "human equivalent dose" suggest a human equivalent dose of closer to the 700–800 mg/day range (FDA, 2005). Hence the therapeutic dose in human SCI patients may be well within a range known to be clinically safe.

Anti-CD11d antibodies (Table 5)

The anti-CD11d antibody approach has been pioneered by Dr. Lynne Weaver and colleagues, and all 11 papers found

Table 2. Erythropoietin

Paper	*Animal model and injury model*	*Intervention and timing*	*Experimental groups*	*Reported outcomes:* • *Histologic/biochemical/physiological* • *Behavioral*
Guízar-Sahagún *Spinal cord* 2009	*Model:* Adult Female Long–Evans Rats *Injury:* T9 Contusion NYU Impactor 10 g×12.5 mm	**EPO** IP • 2000 U/kg @ 0h PI; 12 h pre-injury; 24 h PI **Methylprednisolone (MP)** • 30 mg/kg @ 0h PI; 2 h pre-injury; 2 h PI **Cyclosporine-A** IP • 5 mg/kg@ 0h PI; 12 h pre-injury; 24 h PI	SCI + • **EPO** • MP • Cyclosporine-A • Saline n = 7 per group/time point	*Histologic/Biochemical/Physiological:* Neither drug improved the amount of spared tissue at the injury site at 6w PI. *Behavioral:* No differences were observed in the number of rearing events in the open field at at 5w and 6w between the rHuEPO, MP and saline groups. No differences in BBB from 1 d to 6w. The number of rears was significantly less in the group treated with Cyclosporine-A.
Huang *J Int Med Res* 2009	*Model:* Male SD Rats, 210 g *Injury:* T10 Weight Drop 10 g×50 mm for 20 sec	**EPO** IP • 1000 IU/kg @ 0 h	SCI + • **EPO** (n = 20) • Saline (n = 20) Sham (n = 20)	*Histologic/Biochemical/Physiological:* EPO increased spared tissue, decreased the cavity volume at injury site. • Some neuronal regeneration was observed in the EPO-treated group. • In the saline group, ERK phosphorylation was increased at 7d PI while mitogen-activated protein kinase phosphatase (MKP-1) was inactivated. The EPO-treated group showed significantly lower p-ERK and significantly higher MKP-1 at 7d PI. *Behavioral:* EPO significantly improved BBB score in comparison to the saline group (~11 vs. ~9, $p < 0.01$) at 7d PI.
Kontogeorgakos *Arch Orthop Trauma Surg* 2009	*Model:* Female Wistar Rats, 270 – 300 g *Injury:* T10 Aneurysm Clip 0.7 N×1 min	**EPO** SC • Low total dose (EPO-L): 1,000 IU @ 0h and 24 h PI • High total dose (EPO-H) 1,000 IU @ 0 h, then every 2nd day×13 doses	SCI + • Low **EPO** (EPO-L) • High **EPO** (EPO-H) • Saline n = 10/group	*Histologic/Biochemical/Physiological:* Not reported. *Behavioral:* The mean score 17.3 +/- 1.2 in the EPO-L group and 14.7 ± 1.8 in the the EPO-H vs. 8.2 ± 0.8 in the control at 5w and 6w. The difference between all three groups was significant.
Yazihan *Injury* 2008	*Model:* Adult Wistar Rats, 200 - 220 g *Injury:* T9-11 Epidural Clip Compression 40g×30 sec	**EPO** IP • 150 IU/kg @ 1 hr PI **Ketamine IP** • 100 mg/kg @ 30 min PI (NMDA receptor antagonist)	SCI + • **EPO** (n = 7) • **EPO** + Ketamine (n = 7) • Ketamine (n = 7) • Untreated (n = 7) • Sham-operated (n = 7)	*Histologic/Biochemical/Physiological:* SCI decreased the levels of antioxidant enzyme catalase and glutathione (GSH), and increased the levels of TNFα and the lipid peroxidation product malonyldialdehyde (MDA). 12h PI, EPO significantly increased the catalase and GSH levels and decreased the TNF-α and MDA levels. • Application of ketamine before EPO treatment decreased effects of EPO. *Behavioral:* Not reported.
Fumagalli *Eur J Pharmacol* 2008	*Model:* Adult SD Rats, 240 - 260 g *Injury: T9* Univ of Trieste Impactor 1N×1sec	**EPO** IP • 1000 U/kg @ 30 min PI **Methylprednisolone (MP)** • 30 mg/kg IP	SCI + • **EPO** • MP • Untreated Laminectomized n = at least 8 per group	*Histologic/Biochemical/Physiological:* EPO, but not MP, significantly up-regulated nerve growth factor (NGF) expression both caudally and rostrally to the lesion at 3d PI. At 1w PI, both rEPO and MP increased the NGF expression in the epicenter. • No effect of either drug on BDNF or basic fibroblast growth factor-2 expression. Glial-derived neurotrophic factor expression was enhanced by both drugs at 7d. *Behavioral:* EPO caused significant motor function recovery at 1w to 4w (BBB scores of 13.9 ± 0.5, 9.0 ± 0.2, and 9.5 ± 0.5 in EPO, MP, and Control groups respectively).

Mann, *Exp Neurol* 2008	*Model:* Male SD Rats, 320 - 340 g *Injury:* T9/T10 Contustion OSU Impactor 200-260 kdyn	**EPO** IV • 5000 IU/kg @ 1 h PI **Darbepoetin** IV • 10 mg/kg @ 1 h PI	SCI + • **EPO** (n = 12) • **Darbepoetin** (n = 11) • Vehicle (n = 11)	*Histologic/Biochemical/Physiological:* Neither EPO nor darbepoetin improved white or grey matter sparing (EC staining) over controls. *Behavioral:* Neither EPO nor darbepoetin yielded any behavioral improvements on BBB scoring, footfall analysis, footprint analysis, or hindpaw sensation.
Pinzon, *Exp Neurol* 2008	*Model:* • Adult female Wistar Rats, 220 - 280 g • Adult Female SD Rats, 240 - 260 g *Injury:* • T3 Aneurysm Clip, 50 g×60 sec or 20 g×10 s • T9 Contusion NYU Impactor 10 g×12.5 mm	**EPO** IP • 1000 IU/kg @ 0, 24, or 48 h PI for aneurysm clip SCI • 5000 IU/kg @ 0 h PI, then q24 h×7d for contusion SCI	SCI (Aneurysm Clip) + • **EPO** • Saline control SCI (Contusion) • **EPO** • Saline IP once • Saline IP, q24 h×7 days n = 10-15/compression group n = 11/ contusion group	*Histologic/Biochemical/Physiological:* In the aneurysm clip model, EPO did not result in improved preservation of tissue at the injury epicenter. In the contusion injury model, EPO resulted in better preservation of tissue at the epicenter and reduced cavitation, but the differences were not statistically significant compared to control animals. *Behavioral:* In the aneurysm clip model, EPO did not improve BBB scores up to 4w post-injury. In the contusion model, EPO did not improve BBB scores up to 7 weeks post-injury.
Vitellaro-Zuccarello, *Neuroscience* 2008	*Model:* Male SD Rats, 240 - 270 g *Injury:* T9 Contusion with a modified Univ of Trieste Impactor	**EPO** IP • 5000 IU/kg @ 30 min PI	SCI + • **EPO** • Saline n = 14/group	*Histologic/Biochemical/Physiological:* EPO treatment increased AQP4 immunoreactivity and decreased GFAP immunoreactivity. EPO induces an increase in the total volume occupied by microvessels in the injured cord. *Behavioral*: 4w PI, the BBB locomotor scores were ~15 and ~8 for the EPO and control groups respectively (statistically significant).
King, *Eur J Neurosci* 2007	*Model:* Male Wistar Rats, 200 - 220 g *Injury:* T10/T11 Unilateral Hemisection	**EPO** or **cEPO** IP • 40 μg/kg twice @ 30 min and 24 h PI	SCI + • **EPO** • **cEPO** • Vehicle n = 6/group/timepoint	*Histologic/Biochemical/Physiological:* Lesion size was significantly reduced by both cEPO and EPO. In addition, TUNEL (apoptosis) and b-APP (damaged axons) staining was decreased around the lesion site in response to either EPO or cEPO treatment. Schwann cell infiltration was increased with either treatment, however macrophage infiltration remained unchanged. *Behavioral:* Not reported.
Okutan, *J Clin Neurosci* 2007	*Model:* Female WistarRats, 210 - 250 g *Injury:* T8 Weight Drop Contusion 40 g×cm	**EPO** IP • 1000 IU/kg @ 0 h PI **Methylprednisolone (MP)** IP • 30 mg/kg @ 0 h PI	SCI + • **EPO** • **MP** • Vehicle n = 8/group	*Histologic/Biochemical/Physiological:* 24h PI, MPO activity (neutrophil infiltration) was equally decreased by MPSS or EPO treatment. Activated caspase3 activity (apoptosis) was also similarly decreased by either drug. *Behavioral:* BBB scores were reported 24h PI. EPO treatment increased locomotor recovery (~8) when compared to MP (~4) or vehicle control (~3).
Vitellaro-Zuccarello, *Neuroscience* 2007	*Model:* Male SD Rats, 240 - 270 g *Injury:* T9 Contusion with a modified Univ of Trieste Impactor	**EPO** IP • 5000 IU/kg @ 30 min PI	SCI + • **EPO** • Saline n = 7/group	*Histologic/Biochemical/Physiological:* 4w PI, EPO preserved ventral white matter compared to saline, increased the number of NG2 expressing OPCs in the cord, increased 5-HT immunoreactivity, and reduced phosphacan immunoreactivity. *Behavioral:* 4w PI, the BBB locomotor scores were ~15 and ~8 for the EPO and control groups respectively (statistically significant).

(continued)

Table 2. Erythropoietin (Continued)

Paper	Animal model and injury model	Intervention and timing	Experimental groups	Reported outcomes: • Histologic/biochemical/physiological • Behavioral
Arishima, *Spine* 2006	*Model:* Male Wistar Rats, 380 – 500 g *Injury:* T8-T9 Weight Compression 120 g×2 min	■ **EPO** IP • 5000 IU/kg @ 15 min and 24 h PI, sacrificed at 0, 6, 12 h, 1, 3, 5, or 7d PI	SCI + • **EPO** • Saline n = 4/group/sacrifice time	*Histologic/Biochemical/Physiological:* The number of apoptotic cells (mainly believed to be oligodendrocytes and motor neurons) was significantly reduced with EPO treatment (assessed by TUNEL or activated caspase3 positive cells). These anti-apoptotic effects were observed up to 7d PI. Cavitation volume was also reduced at the injury epicenter (H&E stain). *Behavioral:* Not reported.
Cetin, *Eur Spine J* 2006	*Model:* SD Rats, 200 - 300 g *Injury:* T3 Aneurysm Clip 0.6 N×1 min	**EPO** IP • 1000 IU/kg @ 5 min PI • @ 5 min PI, then q24h×3d. • @ 40 min PI • @ 40 min PI, then q24h×3d **Methylprednisolone (MP)** IP • 30 mg/kg @ 5 min PI, then 5.4 mg/kg/h q8h starting @ 50 min PI	SCI + • **EPO** • Saline n = 8/group	*Histologic/Biochemical/Physiological:* Histological scores indicated that a daily dose of 1000 IU/kg for 3d preserved tissue better than a single 1000 IU/kg treatment. However, the combination of three 1000 IU/kg doses with methylprednisolone provided the best results. *Behavioral:* Locomotor recovery was analyzed using the swimming test 24h and 72h after injury. A daily dose of 1000 IU/kg for 3d promoted better behavioral recovery than a single 1000 IU/kg treatment. Contrary to Gorio (2005), the combination of three 1000 IU/kg doses with methylprednisolone provided the best results.
Grasso, *J Neurosurg Spine* 2006	*Model:* SD Rats, 275 - 300 g *Injury:* T3 Clip Compression 58g×1 min	**EPO** or **asialoEPO** IV • 10 μg/kg @ 24h PRIOR to SCI • 10 μg/kg @ 0 h PI • 10 μg/kg 3x/d, then twice per week	SCI + • **EPO** • **asialoEPO** • Saline n = 6/group	*Histologic/Biochemical/Physiological:* EPO increased GFAP expression (Western Blot) and the GFAP positive cells (immunoreactivity). Serum analysis confirmed that EPO **does not need** to be present within systemic circulation at the time of injury. *Behavioral:* BBB analysis at 6w PI revealed that EPO given at the time of injury was superior to EPO pretreatment (BBB of ~16 vs ~13), but both were better than saline control (BBB of ~9). No benefit was observed from administering multiple doses of EPO as opposed to just once at the time of injury.
Boran *Restor Neurol and Neurosci* 2005	*Model:* Male Wistar Rats, 180-220 g *Injury:* T6-T7 Weight Drop Contusion 50 g×cm	**EPO** IP • 5000 IU/kg @ 1 h PI **Methylprednisolone (MP)** IP • 30 mg/kg	SCI + • **EPO** • **MP** • Vehicle n = 20/group	*Histologic/Biochemical/Physiological:* Not reported *Behavioral:* At 14d PI (experimental endpoint), only EPO increased motor recovery as measured by a swimming test. Significant improvements began at day 4 and were observed until day 14.

Gorio, *PNAS* 2005	*Model:* SD rats (gender unspecified), 240 - 260 g *Injury:* T9 modified Univ of Trieste Impactor	**EPO** IV • 100, 500, or 5000 IU/kg within 30 min PI **EPO** IP • 100, 500, or 5000 IU/kg within 30 min PI • 5000 IU/kg @ 24 h PI • 5000 IU/kg @ 48 h PI **Methylprednisolone (MP)** IP • 30 mg/kg	SCI + • **EPO** • MP • Saline IP n ≥ 18/group	*Histologic/Biochemical/Physiological:* EPO at 5000 IU/kg IP significantly increased spared tissue at and around the injury epicenter, an effect that was negated by co-treatment with MP. Both EPO and MP given alone both reduced pro-inflammatory cytokines MIP-2, TNF-α, IL-1β, and IL-6. *Behavioral:* Optimal effects on BBB locomotor scores were achieved with EPO 5000 IU/kg IP or 500 IU/kg IV given by 30min post injury. BBB scores at 28d PI were: 13 for EPO and 9 for saline. Delay in treatment by 24h or 48 h PI yielded a small benefit that lasted only for the first 3 weeks. Co- treatment with MP negated the positive behavioral effects of EPO.
Brines, *PNAS* 2004	*Model:* • C57/BL6 WT and bcR KO Mice; 8-16 weeks of age *Injury:* T3 Steel Rod Compression 4 min	**EPO** IP • 10 μl/kg @ 0 h PI **cEPO** IP • 10 μl/kg @ 0 h PI	SCI + • **EPO** • **cEPO** • PBS n = 10/strain/group	*Histologic/Biochemical/Physiological:* The KO vs WT experiment confirmed that the bc-Receptor (bcR) mediates EPOs protective effect. *Behavioral:* The BMS locomotor scale was used to evaluate locomotion. EPO or cEPO mediated complete locomotor recovery in WT mice; however this was not seen in the bcR KO mice or PBS controls.
Kaptanoglu, *Neurosurg Rev* 2004	*Model:* Male Wistar Rats, 215 - 260 g *Injury:* T7-T8 Weight Drop Contusion 50 g×cm	**EPO** IP • 100, 1000, or 5000 IU/kg @ 0 h PI **Methylprednisolone (MP)** IP • 30 mg/kg	SCI + • **EPO** • **MP** • Vehicle n = 8/group	*Histologic/Biochemical/Physiological:* 2h post injury – thiobarbituric acid-reactive substances were used to estimate lipid peroxidation. Out of all the treatments, EPO (5000 IU/kg) reduced peroxidation and protected spinal cord ultrastructure the greatest. *Behavioral:* Not reported.
Gorio, *PNAS* 2002	*Model:* Female SD Rats, 180-300 g *Injury:* • T3, Aneurysm Clip 0.6 N×1 min • T9 modified Univ of Trieste Impactor 1 N×1 s	**EPO** IP *1st Model (aneurysm clip)* • 1,000 IU/kg @ 0 h PI • 1,000 IU/kg/d @ 0 h PI, then q24h for 3d *2nd Model (contusion)* • 500 IU/ kg/d IP @ 1 h PI, then q24 h for 7d • 5000 IU/kg/d IP @ 1 h PI, then q24 h for 7d	SCI (Clip) + • **EPO** • Saline @ 0 h PI SCI (Contusion) + • **EPO** • Saline @1 h PI n = 14/treatment group n = 6/saline group	*Histologic/Biochemical/Physiological:* 7d after contusion injury, EPO resulted in a significantly smaller cavitation volume (~25%) at injury site and significantly fewer apoptotic (TUNEL+) cells rostral to the injury site. *Behavioral:* After clip compression SCI, EPO as single or 3d dose significantly improved motor function at 7d PI. The final motor scores were near normal at 28d in treatment groups, but stay quite poor (10) in the saline group. After contusion SCI, a single 5000 IU/kg dose was just as effective as the 7d dosing, and both were more effective than a daily 500 IU/kg injection. Day 28 BBB scores were roughly 18 for the EPO treated (5000 IU/kg) and 10 for saline.

SCI: spinal cord injury; d: day, days; h: hour, hours; w: week, weeks; PI: post-injury; q24h: interval 24 hours;
T8: thoracic vertebra 8; C5: cervical vertebra 5; IP: intraperitoneal; IV: intravenous; SC: subcutaneous.
BBB: Basso, Beattie and Bresnahan locomotor test; BDNF: brain-derived neurotrophic factor; EPO: Erythropoietin; GFAP: glial fibrillary acidic protein; KO: knockout; IL: interleukin; MDA: malondialdehyde; MIP-2: macrophage inflammatory protein-2; MP(SS): Methylprednisolone (Sodium Succinate); MPO: Myeloperoxidase; SD rats: Sprague-Dawley rats; TNF: tumor necrosis factor; WT: wild type.

Table 3. Systemic Hypothermia

Paper	*Animal model and injury model*	*Intervention and timing*	*Experimental groups*	*Reported outcomes:* • *behavioral* • *Histologic/biochemical/physiological*
Lo *J Comp Neurol* 2009	*Model:* Adult Female Fischer Rats, 180–200 g *Injury:* C5 OSU Impactor displacement of 0.95 mm	**Hypothermia** • 33°C @ 5 min PI for 4 h with gradual rewarming (1°C per hour)	SCI + • **Hypothermia** (n = 13) • Normothermia (n = 14) Uninjured control (n = 15)	*Histologic/Biochemical/Physiological:* Hypothermia spared gray and white matter • Decreased the loss of ventral motor neurons ($NeuN^+$) at 0.9-1.5 mm from the epicenter, but not in the epicenter. • The loss of retrogradely traced neurons is significantly retarded (28.1% vs 61.2%) • Hypothermia caused no significant changes in physiological variables. *Behavioral:* Improved forelimb strength (weight-supported hanging and grip strength). No difference in the incline plane. Significant improvement in BBB scores only during weeks 1–3, but not at 8w PI.
Duz *Neurochem Res* 2009	*Model:* female Wister Rats, 250–350 g *Injury:* T9 clip compression 50 g×60 sec	**Hypothermia** • 27–29°C @ 5 min PI for 1 h	SCI + • **Hypothermia** (n = 7 + 7) • Normothermia (n = 7 + 7) Sham (n = 7) Tissue harvested 1 h or 24 h PI	*Histologic/Biochemical/Physiological:* 1 h hypothermia reduced lipid peroxidation (thiobarbituric acid reaction) and GSH-peroxidase levels. This beneficial effect reversed at 24h following systemic hypothermic treatment. *Behavioral:* Not reported.
Morino *Spinal Cord* 2008	*Model:* Adult Male SD Rats, 200–300 g *Injury:* T11 clip compression 20 g×20 min	**Hypothermia** • 33°C @ 0 h from beginning of compression for 1 h	SCI + • **Hypothermia** (n = 5 for behaviour, n = 15 for histology) • Normothermia (n = 5 for behaviour, n = 14 for histology) Sham (n = 8)	*Histologic/Biochemical/Physiological:* 1 h hypothermia significantly decreased microglia (isolectin-positive cells) at 48 and 72h PI. Marked but not significant dcrease in the TNF-α in the compressed portion of the spinal cord was observed during this period of time. *Behavioral*: Motor function measured as standing frequency returned to the normal levels at 72h PI in the hypothermic rats, while no improvement was observed in the normothermic rats.
Strain *Dig Dis Sci* 2005	*Model:* Adult Male SD Rats, 300–500 g *Injury:* C6-7 transection	**Hypothermia** • 23.8 ± 0.9°C • 19.4 ± 0.7°C cooling started @ 0 h PI and target temperature reached @ 6 h PI	SCI + • **Hypothermia** 23.8°C (n = 12) • **Hypothermia** 19.4°C (n = 12) • Normothermia (n = 12) Sham (n = 12)	*Histologic/Biochemical/Physiological*: Hypothermia significantly increased gastric ulcerogenesis compared to euthermia, probably primarily due to decreased circulation, while maintained euthermia produced ulcer indices not different from sham surgery. The mean ulcer index was 1.62 ± 0.96 for Group 1; 2.00 ± 0.71 for Group 2; 0.25 ± 0.45 for Normothermia, 0.09 ± 0.30 for the sham-operated animals. *Behavioral*: Not reported.
Shibuya *Spinal Cord* 2004	*Model:* Male SD Rats, 280–320 g *Injury:* T11 Compression 25 g×10 min	Hypothermia • 32°C, @ 0 h PI for 4 h	SCI + • **Hypothermia** • Normothermia Sham n = 15/group SCI n = 9/group Sham	*Histologic/Biochemical/Physiological*: At 3 and 7 days post-injury, significantly fewer TUNEL positive cells around injury site with hypothermia. *Behavioral:* Not reported.

Westergren *Spinal Cord* 2001	*Model:* Male SD Rats, 350-420 g *Injury:* T8 Compression 35 g×5 min	Hypothermia • 30°C, @ 15 min PI and maintained during measurements	SCI + • **Hypothermia** • Normothermia Sham • **Hypothermia** • Normothermia n = 6/group	*Histologic/Biochemical/Physiological:* Hypothermia reduces spinal cord blood flow (SCBF) compared to normothermic uninjured controls, but in the trauma groups, hypothermia did not significantly reduce SCBF compared to normothermia (11.3% difference, $p = 0.09$). *Behavioral:* Not reported.
Chatzipanteli *J Neurotrauma* 2000	*Model:* Female SD Rats, 250-300 g *Injury:* T10 NYU Impactor 10 g×12.5 mm	Hypothermia • 32°C, @ 0 h PI for 3 h	SCI + • **Hypothermia** • Normothermia Sham • **Hypothermia** • Normothermia n = 4/group	*Histologic/Biochemical/Physiological:* Hypothermia significantly reduces myeloperoxidase activity (a marker of neutrophil accumulation) (143.1 ± 23.4 vs 511.3 ± 39.0) *Behavioral:* Not reported.
Jou *Spine* 2000	*Model:* SD rats, 290-340 g *Injury:* C6/7 Clip Compression 10 or 60 s	Hypothermia • 34°C, PRIOR Injury for 1 h • 30°C, PRIOR Injury for 1 h	SCI + • **Hypothermia** • Normothermic n = 12/group	*Histologic/Biochemical/Physiological:* Moderate hypothermia resulted in less gross tissue destruction at injury epicenter. *Behavioral:* Moderate hypothermia improved neurophysiological recordings during cord compression, and improved hindlimb motor function 3 days post-injury with moderate hypothermia.
Westergren *Acta Neurochir* 2000	*Model:* Male SD Rats, 350-500 g *Injury:* T8 Compression 35 g×5 min	Hypothermia • 30°C, @ 0 h PI for 2 h	SCI + • **Hypothermia** (n = 14) • Normothermia (n = 12) Sham • **Hypothermia** (n = 4)	*Histologic/Biochemical/Physiological:* Not reported. *Behavioral:* No improvement in hindlimb motor scores or inclined plane performance in hypothermic animals at 2 weeks post-injury.
W.R. Yu *Acta Neuropathol* 2000 a	*Model:* Male SD Rats, 387 ± 37 g *Injury:* T8 Compression 35 g×5 min	Hypothermia • 30°C, @ 0 h PI for 20 min	SCI + • **Hypothermia** • Normothermia Sham • **Hypothermia** n = 5/group	*Histologic/Biochemical/Physiological:* 24 h PI, significantly larger cord cross sectional area in normothermic SCI animals compared to hypothermia SCI and sham animals (suggestive of reduced edema in hypothermia). Microtubule-associated protein 2 (MAP2) immunostaining was significantly less in normothermia animals at injury site. *Behavioral:* Not reported.
C.G. Yu *J Neurosurg Spine* 2000 b	*Model:* Female SD Rats, 225-275 g *Injury:* T10 NYU Impactor 10 g×12.5 mm	Hypothermia • 32-33°C, @ 30 min PI for 4 h	SCI + • **Hypothermia** (n = 19) • Normothermia (n = 17)	*Histologic/Biochemical/Physiological:* 16 longitudinal sections through injured cord 6 weeks postinjury revealed decreased lesion size (15.8% difference, $p < 0.01$). *Behavioral:* BBB scores in hypothermia animals were significantly better by 9 days post-injury and then thereafter, including the final 6 week evaluation (13.3 ± 0.5 vs 10.8 ± 0.4, $p = 0.0024$).
Westergren *Spinal Cord* 1999	*Model:* Male SD Rats, 387 ± 37 g *Injury:* T8 Compression 50 g×5 min	**Hypothermia** • 30°C, @ 0 h PI for 20 min	SCI + • **Hypothermia** • Normothermia Sham • **Hypothermia** n = 5/group	*Histologic/Biochemical/Physiological:* Semi-quantitative analysis around the injury site revealed decreased b-APP, ubiquitin, and PGP9.5 in hypothermia animals. *Behavioral:* Not reported.

(continued)

Table 3. Systemic Hypothermia (Continued)

Paper	Animal model and injury model	Intervention and timing	Experimental groups	Reported outcomes: • Behavioral • Histologic/biochemical/physiological
Yu *Acta Neuropathol* 1999 a	*Model:* Male SD Rats, 387 ± 37g *Injury:* T8 Compression 50 g×5 min	Hypothermia • 30°C, @ 0h PI for 20min	SCI + • **Hypothermia** • Normothermia Sham • **Hypothermia** n = 5/group	*Histologic/Biochemical/Physiological:* Semi-quantitative analysis of albumin, fibrinogen, and fibronectin shows decreases in all three in hypothermia-treated animals, suggesting that there is less vasogenic edema; cord cross sectional area was also reduced (suggestive again of decreased edema). *Behavioral:* Not reported.
Yu *Neuropatho* 1999 b	*Model:* Male SD Rats, 387 ± 37 g *Injury:* T8 Compression 50 g×5 min	Hypothermia • 30°C, @ 0h PI for 20min	SCI + • **Hypothermia** • Normothermia Sham • **Hypothermia** n = 5/group	*Histologic/Biochemical/Physiological:* Semi-quantitative analysis around the injury site revealed decreased vimentin in capillaries and decreased GFAP. *Behavioral:* Not reported.
Yamamoto *Neuroreport* 1998	*Model:* Male SD Rats, 350-375 g *Injury:* L1 Epidural Balloon Compression 60 s	Hypothermia • 33°C, PRIOR Injury for 4h	SCI + • **Hypothermia + Nicaraven** (n = 5) • **Hypothermia** (n = 5) • **Nicaraven** (n = 12) • Normothermia (n = 5)	*Histologic/Biochemical/Physiological:* Microdialysis revealed significantly lower CSF glutamate levels in hypothermia-treated animals compared to normothermic controls, with no additional benefit with Nicaraven (free radical scavenger). *Behavioral:* Not reported.
Farooque J *Neurotrauma* 1997	*Model:* Male SD Rats *Injury:* T8 Severe Cord Compression	Hypothermia • 30-31°C, PRIOR injury and then during entire observation period	SCI + • **Hypothermia** • Normothermia n = 6/group	*Histologic/Biochemical/Physiological:* Microdialysis revealed no significant difference in lactate levels in hypothermia animals, and they had (surprisingly) increased extracellular levels of glutamate and aspartate compared to normothermic animals. *Behavioral:* Not reported.
Martinez-Arizala J *Neurotrauma* 1992	*Model:* Female SD Rats, 250-300 g *Injury:* T8 Weight Drop 50 g×cm	Hypothermia • 31-32°C, PRIOR injury for 4 h	SCI + • **Hypothermia** • Normothermia n = 3/group	*Histologic/Biochemical/Physiological:* Hypothermia-treated animals had less hemorrhage and parenchymal damage than controls, but no description is provided about how this was analyzed or quantified. *Behavioral:* All animals remained completely paralyzed during the 4d observation period (no functional benefit reported with hypothermia).

SCI: spinal cord injury; d: day, days; h: hour, hours; w: week, weeks; PI: post-injury
T8: thoracic vertebra 8; C5: cervical vertebra 5
BBB: Basso, Beattie and Bresnahan locomotor test; CSF: cerebrospinal fluid; GFAP: glial fibrillary acidic protein; SD rats: Sprague-Dawley rats; TNF: tumor necrosis factor.

TABLE 4. NSAIDS

Paper	*Animal model and injury model*	*Intervention and timing*	*Experimental groups*	*Reported outcomes:* • *Histologic/biochemical/physiological* • *Behavioral*
Wang *J Neurotrauma* 2009	*Model:* Female SD Rats, 250-270 g *Injury:* T7 MASCIS Impactor 10 g×25 mm	**Ibuprofen** SC • 70mg/kg/d (osmotic minipump) @ 3d PI, continuously for 28 days **Naproxen** • 25mg/kg/d (osmotic minipump) @ 3d PI, then daily×28d	SCI + • **Ibuprofen** (n = 25) • **Naproxen** (n = 6) • Saline (n = 22)	*Histologic/Biochemical/Physiological:* Ibuprofen suppressed twofold RhoA activation induced by injury to the level below than that in the uninjured animals. *Behavioral:* After 3w of treatment and up to 7w of observation, ibuprofen (but not naproxen) showed a significant improvement in BBB scores (9.4 ± 0.5 vs. 7.7 ± 0.7 for saline vs. 6.8 ± 0.5 for naproxen at 7w). At 7w, 13 of 25 animals in the ibuprofen group had hindlimb weight support vs. 7 of 28 animals in the two other groups.
Fu *J Neurosci* 2007	*Model:* Female SD Rats, 180-250 g *Injury:* • T6-T7 Dorsal Over-Hemisection, 1.9 mm deep (CST lesion) • T8/T9 NYU Moderate Contusion	**Ibuprofen** SC • 60 mg/kg/d (osmotic pump) @ 0 h PI for CST lesion or @ 7d PI, then daily×28d for contusion **Naproxen** SC • 50 mg/kg/d (osmotic pump) @ 0 h PI for CST or 7d PI, then daily×28d for contusion	SCI (CST lesion) + • **Ibuprofen** (n = 12) • **Naproxen** (n = 7) • Vehicle (n = 7) SCI (Contusion) + • **Ibuprofen** (n = 6) • Vehicle (n = 6)	*Histologic/Biochemical/Physiological:* Ibuprofen (but not naproxen) enhanced corticospinal and serotonergic sprouting caudal to the injury in both injury models. Ibuprofen reduces RhoA activation at the injury site, 5-7 days after SCI. (Supported with *in vitro* neurite outgrowth and neuronal RhoA expression assays) *Behavioral:* 42d PI, Ibuprofen (but not naproxen) significantly improved locomotor recovery (BBB, Grid walk, and footprint) in both injury models, even in the contusion model where the drug delivery was delayed for 7 days. BBB scores were ~15.5 vs. ~13.5 for Ibuprofen and control respectively.
Harada *J Neurotrauma* 2006	*Model:* Male Wistar Rats, 300-350 g *Injury:* T12 Compression 20 g×20 min	**Indomethacin** SC • 5, 10, and 20 mg/kg @ 1sh PRIOR to SCI **NS-398** oral gavage • 30 mg/kg @ 1 h PRIOR to SCI **Iloprost** IP infusion • 100 ng/kg/min @ 30 min PRIOR to SCI, continued for 3 h PI.	SCI + • **Indomethacin** • **NS-398** • **Iloprost** • Vehicle Locomotor: n = 10/group Biochemistry: n = 6/group	*Histologic/Biochemical/Physiological:* Indomethacin, not NS-398, attenuated increases in prostacyclin thromboxane metabolites. Furthermore, COX inhibition after SCI with indomethacin increased MPO activity and TNF expression in the spinal cord. These outcomes were reversed with iloprost, a prostacyclin analogue (prostacyclin is a product of COX). *Behavioral:* 56d PI, indomethacin treatment at 5, 10, and 20 mg/kg resulted in decreased performance on inclined plane test and footprint analysis. No effect was seen with NS-398. Lastly, iloprost, a prostacyclin analogue, increased motor recovery.
Pantovic *Spinal Cord* 2005	*Model:* Adult Rabbits, 2.5-3.5 kg *Injury:* L2 Weight Drop Contusion 12.5 g×120 mm/ 150 g-cm	**Indomethacin** IV • 0.1, 0.3, 1.0, or 3.0 mg/kg @ 0 h PI	SCI + • **Indomethacin** • Vehicle Sham n = 6/group	*Histologic/Biochemical/Physiological:* The measurement of free fatty acids in the cord was used as a measure of overall tissue damage and/or protection. The elevation of free fatty acids after SCI was significantly reduced with indomethacin treatment in a dose dependent manner. *Behavioral:* Indomethacin had a positive effect on locomotor recovery measured on the Tarlov scale. Significant improvements were observed in a dose dependent manner, with 3.0mg/kg having the greatest effect (2.7 vs. 1.3).

(continued)

TABLE 4. NSAIDS (CONTINUED)

Paper	*Animal model and injury model*	*Intervention and timing*	*Experimental groups*	*Reported outcomes:* • *Histologic/biochemical/physiological* • *Behavioral*
Schwab *Glia* 2004	*Model:* Male Lewis rats, 250 - 300 g *Injury:* T8 Dorsal spinal cord Symmetrical four-fifth Overhemisection	**Indomethacin** IP • 2 mg/kg/day×3d (no description of start time)	SCI + • **Indomethacin** (n = 5) • Vehicle (n = 5) • Sham operated (n = 3)	*Histologic/Biochemical/Physiological:* Indomethacin significantly decreases the number of RhoA+ cells, and these mainly include granulocytes, ED1 + macrophages/microglia, and GFAP + astrocytes. Lastly, GAP-43 + neurites at the injury site are also increased with indomethacin treatment. *Behavioral:* Not reported.
Sharma *Muscle and Nerve* 2002	*Model:* Male SD Rats, 350-420 g *Injury:* T10-T11 Longitudinal incision into the Right Dorsal Horn	**Indomethacin** IP • 10 mg/kg @ 30 min PRIOR to SCI **Ibuprofen** IP: • 10 mg/kg @ 30 min PRIOR to SCI	SCI + • **Indomethacin** (n = 6) • **Ibuprofen** (n = 7) • Vehicle (n = 6)	*Histologic/Biochemical/Physiological:* Both indomethacin and ibuprofen significantly reduced edema, BSCB permeability, and changes in spinal cord blood flow 5 h after injury. Both attenuated reductions in somatosensory evoked potentials 5h after SCI *Behavioral:* Not reported.
Hains *J Neurotrauma* 2001	*Model:* Male SD Rats, 200-225 g *Injury:* T13 NYU Weight Drop Contusion 10 g×12.5 mm	**NS-398** IP (selective COX2 inhibitor) • 5 mg/kg @ 15 min PRIOR to injury	SCI + • **NS-398** • Vehicle n = 20/group	*Histologic/Biochemical/Physiological:* From day 14 – 28 post injury, NS-398 promoted significantly greater tissue sparing as compared to the control treatment, and also decreased PGE2 in the caudal spinal cord. *Behavioral:* From day 14 – 28 post injury, NS-398 promoted significantly higher BBB locomotor score (~17.4) than control animals (~14.7). NS-398 treatment also attenuated the development of mechanical allodynia.
Resnick Spine J 2001	*Model:* Long Evans Rats, 2.5 months old *Injury:* T9, NYU Impactor 25 g×cm	**Celebocid** oral gavage (selective COX2 inhibitor) • 3 mg/kg @ 20 min PI, sacrificed at 2, 4, 6, 12, 24, 48, and 72 h (no perfusion) or 4, 24 h (with perfusion)	SCI + • **Celebocid** Sham operated n = 4/group/timepoint	*Histologic/Biochemical/Physiological:* 5-mm segment centered at the region of injury was harvested. In saline perfused animals, celebocid resulted in statistically significant reduction in spinal cord PGE_2 and TxB_2 levels 4 and 24 h post injury *Behavioral:* Not reported.
Guven *Pediatr Neurosurg* 1999	*Model:* Female Wistar Rats 160-200 g *Injury:* T7-T8 Aneurysm Clip 150 g×1 min	**Indomethacin** IP • 3 mg/kg @ 0 h PI	SCI + • **Indomethacin** • Vehicle n = 8/group	*Histologic/Biochemical/Physiological:* The absorption of thiobarbituric acid reactive substances was measured as an indicator of lipid peroxidation at 1, 15, 30, 60, and 90 min after SCI. Indomethacin significantly increased lipid peroxidation after SCI, suggesting a harmful role for this drug. *Behavioral:* Not reported.

Resnick *J Neurotrauma* 1998	*Model:* Male Long Evens Rats, 275-340 g *Injury:* T8-T9 NYU Impactor 12.5 or 25 g×cm	**SC58125** oral gavage (selective COX2 inhibitor) • 3 mg/kg @ 15 min PI	SCI + • SC58125 • Vehicle n = 8/group	*Histologic/Biochemical/Physiological:* There was an increased expression of COX2 up to at least 48 hours after SCI (Western Blot). Furthermore, this COX2 expression occurs in neurons and blood vessels. *Behavioral:* BBB locomotor scores were significantly improved by SC58125 treatment only in the 12.5 g-cm injury model.
Guth *PNAS* 1994 & Guth *Exp Neurol* 1994	*Model:* Female SD rats, 170-200 g *Injury:* T8 Compression with jewelers forceps 2 sec	**Indomethacin** (IM) IP • 0.2 mg @ 0 h PI, then q24 h×21d. **Pregnenolone** (PREG) **Lipopolysaccharide** (LPS)	SCI + • IM (n = 6) • IM + LPS (n = 4) • IM + PREG + LPS (n = 6) • PREG (n = 6) • LPS (n = 4) • Vehicle (n = 6)	*Histologic/Biochemical/Physiological:* Indomethacin alone did not reduce lesion area 21 days post injury. However, the cavity area was reduced when Indomethacin was combined with pregnenolone and lipopolysaccharide *Behavioral:* A modified Tarlov's motor scale was used to assess animals (day 21 PI). Indomethacin treatment alone did not improve locomotor recovery. However, it did improve locomotor recovery markedly when combined with pregneolone and lipopolysaccharide.
Sharma *Acta Neuropath* 1993	*Model:* Male SD Rats, 200 – 300 g *Injury:* T10-T11 2mm Longitudinal incision into the Right Dorsal Horn	**Indomethacin** IP • 1,5, and 10 mg/kg @ 30 min PRIOR to SCI	SCI + • **Indomethacin** • Vehicle n = 5/group	*Histologic/Biochemical/Physiological:* Only pretreatment with 10 mg/kg significantly reduced spinal cord edema. Indomethacin reduced the sponginess of tissue and the number of neurons exhibiting cytological changes, and immuno-staining for MBP was improved. *Behavioral:* Not reported.
Sharma *Neuroscience* 1993	*Model:* Adult Wistar Rats *Injury:* T10-T11 2mm Longitudinal incision into the Right Dorsal Horn	**Indomethacin** IP • 5 and 10 mg/kg @ 30 min PRIOR to SCI	SCI + • **Indomethacin** • Vehicle	*Histologic/Biochemical/Physiological:* Pretreatment with 10 mg/kg indomethacin but not 5mg/kg significantly reduced edema (water content), microvascular permeability (Evens blue extravasation), and attenuates increases in serotonin levels. *Behavioral:* Not reported.
Winkler *Neuroscience* 1993	*Model:* Male SD Rats, 350-400 g *Injury:* T10-T11 2mm Longitudinal incision into the Right Dorsal Horn.	**Indomethacin** IP • 5 and 10 mg/kg @ 30 min PRIOR to SCI	SCI + • **Indomethacin** (n = 11) • Vehicle (n = 6)	*Histologic/Biochemical/Physiological:* Edema formation was significantly decreased by indomethacin treatment. Electrophysiologically, 5h after injury, indomethacin pretreated animals showed significant decreases in trauma-induced changes of spinal cord evoked potentials. *Behavioral:* Not reported.
Simpson *J Spinal Disord* 1991	*Model:* SD Rats, 250-350 g *Injury:* Midthoracic Weight Drop Contusion 50 g×cm	**Indomethacin** IP • 2 mg/kg @ 0h PI, then q24 h, subcutaneously, ×7d	SCI + • **Indomethacin** (n = 10) • Vehicle (n = 12)	*Histologic/Biochemical/Physiological:* Indomethacin treatment resulted in less histopathological changes in the spinal cord 6 weeks after injury (tissue loss). No improvements in somatosensory evoked and corticomotor evoked potentials were observed with the treatment compared to vehicle control. *Behavioral:* Indomethacin significantly improved locomotor recovery 6 weeks after SCI (inclined plane test). No improvements in sensory recovery.

(continued)

Table 4. NSAIDS (Continued)

Paper	*Animal model and injury model*	*Intervention and timing*	*Experimental groups*	*Reported outcomes:* • *Histologic/biochemical/physiological* • *Behavioral*
Fadenl *Brain Res* 1988	*Model:* SD Rats, 275-325g *Injury:* T9 Weight Drop 50 g×cm	**BW755C** IV (dual lipoxygenase/COX inhibitor) • 10 mg/kg @ 15 min PI	SCI + • **BW755C** • Vehicle n = 15/group for behavior n = 8/group for biochemistry	*Histologic/Biochemical/Physiological:* BW755C did not lower water (edema) or cation content in the spinal cord after injury. However, this treatment did significantly lower tissue levels of thromboxane B2 after SCI. *Behavioral:* Groups were analyzed with the incline plane test and for locomotor recovery. BW755C treated animals had significantly better neurological recovery than saline controls on both tests.
Fujita *Paraplegia* 1985	*Model:* Rats (no details) *Injury:* T2-T3 Aneurysm Clip	**Aspirin** IV or IP • 5, 15, or 50 mg/kg @ 30 min PRIOR to injury	SCI + • **Aspirin** • Saline	*Histologic/Biochemical/Physiological:* 30 minutes PI, Na +/K +-ATPase activity and the levels of 6-keto-PGF1 and thromboxane B2 in the cord were determined. Aspirin, starting at 5mg/kg, significantly prevented decreases in the activity of Na+/K+-ATPase, and decreased total 6-keto-PGF1 and thromboxane B2 levels in the spinal cord in a dose dependent manner. *Behavioral:* Motor performance was assessed using the incline plane test. 7 days after injury, aspirin delivered at 50mg/kg enhanced motor recovery.

SCI: spinal cord injury; d: day, days; h: hour, hours; w: week, weeks; PI: post-injury; q24h: interval 24 hours;
T8: thoracic vertebra 8; C5: cervical vertebra 5; IP: intraperitoneal; IV: intravenous; SC: subcutaneous;
BBB: Basso, Beattie and Bresnahan locomotor test; COX: - cyclooxygenase; GAP: growth-associated protein; GFAP: glial fibrillary acidic protein; MPO: Myeloperoxidase; PG: prostaglandin; SD rats: Sprague-Dawley rats; TNF: tumor necrosis factor; Tx - thromboxane.

in this systematic review were published from this single laboratory. The CD11/CD18 integrin family has several members, and this antibody is directed against the CD11d subunit of the CD11d/CD18 integrin that is expressed only by certain leukocytes such as neutrophils and monocytes. The selectivity and specificity of targeting this subunit with this antibody is key to its effectiveness. Other CD11 integrins containing the subunits a–c have more widespread cellular distributions and antibodies directed against them have not been particularly useful as neuroprotective treatments. Relative homogeneity in animal species (Wistar) and injury model (T4 clip compression) exists amongst these studies. The antibody treatment is administered intravenously in all cases. A dose response has been demonstrated insofar as 1 mg/kg appeared to be better than 5 mg/kg in an early study, and has been the dose used in all subsequent studies. The dosing regimen most extensively studied is 1 mg/kg started 2 h post injury and repeated at 24 and 48 h post injury. Given that multiple studies report a beneficial effect with this approach started 2 h post injury, a 2-h time window of intervention has been established. Recently, however, therapeutic efficacy has been shown when starting the treatment at 6 or 12 h post injury, which are important findings with respect to clinical applicability. Early studies focused on non-behavioral outcomes (and did not report behavioral outcomes at all). These largely confirmed the mechanism of action in terms of reducing neutrophil and macrophage invasion, decreasing inflammation and lipid peroxidation, and improving tissue sparing. Behavioral outcomes revealed increased BBB locomotor scores, decreased autonomic dysreflexia, and reduced mechanical allodynia.

Minocycline (Table 6)

Minocycline has been evaluated in a relatively extensive manner in acute SCI models. The systematic review resulted in 10 papers that met the inclusion criteria. Most of these (6 of 10) utilized Sprague Dawley rats, although three used adult Wistar rats, and one mouse SCI model was described. Most of the injury models employed a thoracic contusion at T9 with the NYU impactor, although balloon compression, clip compression, and partial transection models were also utilized by individual investigators. Minocycline was administered in a range of doses, and the effect of different doses was demonstrated by some authors (Festoff et al., 2006; Teng et al., 2004). The most common dose utilized was 90 mg/kg given intraperitoneally, although doses of 50 mg/kg were also utilized by Stirling and colleagues (2004) and Wells and colleagues (2003) in their studies. Most of the studies administered minocycline immediately after injury, but a window of efficacy was observed at 1 h post injury (Teng et al., 2004; Wells et al., 2003) and at 2 h post injury (Yune et al., 2007).

Non-behavioral outcome measures revealed tissue sparing, decreased cytochrome c release, decreased caspase 3 positive oligodendrocytes, and macrophages/microglia. Behavioral outcome measures revealed improved BBB, inclined plane, hindlimb coordination. The recent study by Marchand and colleagues (2008) reported an attenuation of neuropathic pain with intraperitoneally administered minocycline, which is an extension of important work by Hains and colleagues who previously demonstrated that intrathecally delivered minocycline inhibited the activation of microglia reduced pain behavior on mechanical and thermal stimulation of the paw (Hains and Waxman, 2006; Zhao et al., 2007a, 2007b). These papers were not included in the table because of the intrathecal (and chronic) mode of minocycline administration, but are noted here for their important insights on the role of inflammatory mediators in the generation of pain behavior.

Of note, two recent papers (Pinzon et al., 2008; Saganová et al., 2008) revealed limited neuroprotection and no significant functional benefit to minocycline. The study by Pinzon and colleagues was an NIH-sponsored attempt to reproduce the positive effects of minocycline that had been demonstrated by others; it failed to reveal any benefit to either intraperitoneally or intravenously administered minocycline at the 90 mg/kg dose. Reconciling the contradictory results from this attempted reproduction is difficult. Presently, intravenously administered minocycline is under evaluation as a neuroprotective compound for the management of SCI in a phase I/II pilot study in Calgary, Alberta.

Progesterone (Table 7)

The systematic review identified 10 studies that evaluated progesterone in traumatic SCI models. All of these studies utilized Sprague Dawley rats. Eight of 10 studies utilized a complete thoracic transection SCI model, and were from the same laboratory. One utilized the Infinite Horizon impactor (Fee et al., 2007) and one utilized the NYU impactor (Thomas et al., 1999) to induce clinically relevant thoracic contusion injuries. Progesterone was administered at 4 mg/kg in almost all studies, but also at 8 mg/kg by Fee and colleagues (2007) and 16 mg/kg/day by Labombarda and colleagues (2009). In all cases, the progesterone was started either 30 to 60 min post injury and was typically repeated daily two, three, or more times. The "*n* per group" ranged from 4 to 19, but was most often approximately five. All eight studies that utilized a transection injury reported only on non-behavioral outcomes, and typically within a few days post injury. These reported that progesterone treatment can alter gene and protein expression, cell morphology, and receptor and neurotransmitter expression in the injured spinal cord. Of the two studies that utilized contusion injuries, one reported no histologic improvement in progesterone-treated animals (Fee et al., 2007), while the other reported more white matter sparing (Thomas et al., 1999). The former reported no behavioral improvement on BBB testing, while the latter reported significant improvement.

Estrogen (Table 8)

The systematic review of estrogen turned up eight papers, all published within the last 5 years. Sprague Dawley rats were utilized in five studies, Wistars in one, and mice in two studies. Injury models were quite variable, and included midthoracic contusion with the NYU weight drop (three) or Infinite Horizon impactor (one), or a clip compression injury (two), forceps crush injury (one), and a thoracic transection injury (one). Estrogen was administered in an extremely wide range of doses, from 0.1 mg/kg to 600 mg/kg, and in some cases subsequent dosing was also done. A dose effect was demonstrated in some studies (Cuzzocrea et al., 2008; Ritz and Hausmann, 2008; Yune et al., 2004). In seven of eight studies, the estrogen was started prior to, at the time of, or within 15 min of the injury. The most recent study by

Table 5. Anti-CD11d Antibodies

Paper	*Animal model and injury model*	*Intervention and timing*	*Experimental groups*	*Reported outcomes:* • *Histologic/biochemical/physiological* • *Behavioral*
Gris *Neuroimmunol* 2009	*Model:* Female Wistar Rat, 250-300 g *Injury:* T4 Clip Compression 50 g×1 min	**Anti-CD11d mAb217L** IV • 1 mg/kg @ 2, 24, and 48 h PI IB7 control mAb IV • 1 mg/kg @ 2, 24, and 48 h PI	SCI + • **Anti-CD11d mAb** • IB7 No SCI (n = 10) n = 3 for RNA analysis and n = 5 for histology per group per time points at 12h, 3d, 7d or 21d PI	*Histologic/Biochemical/Physiological:* CD11d mAb reduced hematogenous macrophage infiltration (CD8α expression) in the lesion by ~35% at 3d PI, 7d and 21d. • Up to 1500 genes were differentially expressed in CD11d mAb-treated vs. IB7-treated animals. CD11d mAb reduced expression of pro-inflammatory cytokines like IL-6, Il-1β and others and increased expression of mediators that promote adaptive immune cell responses and wound healing. • CD11d mAb decreased apoptosis (TUNEL) and increased expression of pro-survival, anti-apoptotic genes like Survivin, Selenium Glutathione Peroxidase and IGF-1. *Behavioral:* Not reported
Ditor *J Neurosurg Spine* 2006	*Model:* Female Wistar Rat, 250-300 g *Injury:* T4 Clip Compression 50 g	**Anti-CD11d mAb 217L** IV • 1 mg/kg @ 2, 24, and 48 h PI or @ 6, 24, and 48 h PI or @ 12, 24, and 48 h PI or @ 24 and 48 h PI IB7 control mAb IV • 1 mg/kg @ 2, 24, and 48 h PI or @ 6, 24, and 48 h PI or @ 12, 24, and 48 h PI or @ 24 and 48 h PI	SCI + • **Anti-CD11d mAb** (n = 43) • IB7 (n = 44) No SCI (n = 10)	*Histologic/Biochemical/Physiological:* MPO activity and ED-1 expression showed decreases in intraspinal neutrophils and macrophages after treatments at 2, 6 & 12 h PI. Oxidative enzyme expression decreased with treatment onset delayed up to 12h. Lipid peroxidation and DNA oxidation were reduced with treatment onset up to 6 h. Protein nitration and oxidation were decreased with onsets of 2 h and 12 h, respectively. *Behavioral:* BBB scores and autonomic dysreflexia were examined after treatments commencing at 6h PI and additional doses at 24 & 48 h. BBB scores were increased by anti-CD11d mAb treatment at 3-5 wk after injury. At 5w after SCI, mAb decreased autonomic dysreflexia.
Bao *J Neurochem* 2005	*Model:* Female Wistar Rat, 220 g *Injury:* T4 Clip Compression 50 g	**Anti-CD11d mAb** 217L IV • 1 mg/kg @ 2h or 2, 24, and 48 h PI IB7 control mAb IV • 1 mg/kg @ 2h or 2, 24, and 48 h PI	SCI + • **Anti-CD11d mAb** (n = 35) • IB7 (n = 37) No SCI (n = 14)	*Histologic/Biochemical/Physiological:* Used immunohistochemistry, fluorescent methods of detecting reactive oxygen species (ROS), & western blotting. Tissue sections or homogenates of the lesion were examined at 6 & 24 h after SCI (1 treatment at 2 h) or 72 h & 1w after SCI (3 treatments at 2, 24, 48 h). Treatments reduced ROS production at 6 h-1w after SCI. The single treatment decreased expression of the oxidative enzyme (NDAPH) subunit gp91phox, production of hydroxynonenal -bound proteins (indicating lipid peroxidation), activation of caspase-3 (apoptosis) and numbers of dead cells at 6-72 h after SCI. ROS and gp91phox were predominant in neutrophils. *Behavioral:* Not reported.

Gris *J Exp Neurol* 2005	*Model:* Female Wistar Rat, 250 g *Injury:* T4 Clip Compression 50 g	**Anti-CD11d mAb** 217L IV • 1 mg/kg @ 2, 24, and 48 h PI **Methylprednisolone** IV • 30 mg/kg @ 2h PI, then 15 mg/kg @ 24 and 48 h PI IB7 control mAb IV • 1 mg/kg @ 2, 24, and 48 h PI	SCI + • **Anti-CD11d mAb** (n = 17) • **Methylprednisolone** (n = 13) • **Anti-CD11d mAb + MP** (n = 7) • IB7 or saline control (n = 21)	*Histologic/Biochemical/Physiological*: 2w PI, the anti-CD11d mAb and MP treatments caused an increase in CGRP-immunoreactive fibres in laminae III-V of T9 but not at L4/5. At T9, the lesion area tended to be smaller after MP treatment and was significantly smaller after anti-C11d mAb. The treatments also increased resting mean arterial pressure at 2w. *Behavioral:* MP and combined MP/anti-CD11d mAb treatments decreased autonomic dysreflexia and increased resting mean arterial pressure at 2 but not 6w PI. Lack of additive advantage of combined treatment was notable.
Oatway *J Neurosci* 2005	*Model:* Male Wistar Rats, 250–350 g *Injury:* T12 Clip Compression 50 g	**Anti-CD11d mAb** 217L IV • 1 mg/kg @ 2, 24, and 48 h PI Saline • @ 2, 24, and 48 h PI	SCI + • **Anti-CD11d mAb** (n = 19) • Saline (n = 22) No SCI (n = 6)	*Histologic/Biochemical/Physiological:* Buildup of rostral serotonergic fibres in the dorsal horn was reduced by treatment and more fibres were found caudually in the IML and dorsal and ventral horns. Lateral serotonergic projections imply treatment-induced sparing of fibres with collateral sprouting. Compact myelin was also increased. Retrograde labeling of raphe-spinal axons showed more axons traversing the lesion site in some treated rats. *Behavioral:* BBB scores and mechanical allodynia correlated with changes in rostral and caudal serotonergic projections. Anti-CD11d mAb led to higher BBB scores from 1-4 wk PI. Mechanical allodynia was reduced from 2-4 wk after injury.
Weaver *J Neurotrauma* 2005	*Model:* Male or Female, Wistar Rat, 250–300 g *Injury:* • T4 Severe Clip Compression 50 g • T12 Moderately Severe Clip Compression 35 g	**Anti-CD11d mAb 217L** IV • 1 mg/kg @ 2, 24, and 48 h PI **Methylprednisolone** IV • 30 mg/kg @ 2 h PI, then 15 mg/kg @ 24 and 48 h PI IB7 control mAb IV • 1 mg/kg @ 2, 24, and 48 h PI	SCI + • Anti-CD11d mAb (n = 29, from Gris, et al., 2004) • Methyl prednisolone (n = 26) • Anti-CD11d mAb + MP (n = 7) • IB7 or saline control (n = 33) Males used for motor and pain Females used for motor	*Histologic/Biochemical/Physiological:* Adjacent to the lesion centre, both treatments increased neurofilament at 6wk after T4 and at 12w after T12 injury. MP and mAb increased myelin staining, at 2w after T4 SCI, but the combination had no effect. At 6w after T4 and 12w after T12, MP treatment increased myelin adjacent to but not within the lesion centre, while mAb treatment increased myelin at sites closer to the lesion centre. *Behavioral:* MP and combinatory treatment had no effect on BBB scores 6w after T4 SCI or 12w after T12 SCI. MP had no consistent effect on at-level or below-level mechanical allodynia when monitored for 12 w after T12 injury.
Bao *J Neurochem* 2004 (a)	*Model:* Female Wistar Rat, 220 g *Injury:* T4 Clip Compression 50 g	**Anti-CD11d mAb** 217L IV • 1 mg/kg @ 2 h or 2, 24, and 48 h PI IB7 isotype control mAb IV • 1 mg/kg @ 2 h or 2 h + 24 h + 48 h PI	SCI + • **Anti-CD11d mAb** (n = 43) • IB7 (n = 41) No SCI (n = 15)	*Histologic/Biochemical/Physiological:* Used immunohistochemistry on spinal cord sections & Western Blotting of homogenates of the lesion area at 6, 24 & 72 h after SCI (1 treatment at 2 h) or at 1 w after 3-dose regimen. ED-1 expression (macrophage marker) and MPO activity (neutrophils) decreased at 6-72 h after SCI. Anti-CD11d mAb treatment decreased lipid peroxidation, expression of iNOS & protein nitration at 6-72 h post injury. By one week MPO activity and lipid peroxidation in control and anti-CD11d mAb-treated rats were similar to those of uninjured rats. *Behavioral:* Not reported.

(continued)

Table 5. Anti-CD11d Antibodies (Continued)

Paper	*Animal model and injury model*	*Intervention and timing*	*Experimental groups*	*Reported outcomes:* • *Histologic/biochemical/physiological* • *Behavioral*
Bao *J Neurochem* 2004 (b)	*Model:* Female Wistar Rat, 220 g *Injury:* T4 Clip Compression 50 g	**Anti-CD11d mAb** 217L IV • 1 mg/kg @ 2 h PI IB7 control mAb IV • 1 mg/kg @ 2 h	SCI + • **Anti-CD11d mAb** • IB7 No SCI n = 17/group	*Histologic/Biochemical/Physiological:* Used immunohistochemistry on spinal cord sections & western blotting or chemical staining of homogenates of the lesion area at 6, 24 & 72h after SCI (1 treatment at 2 h). Anti-CD11d mAb treatment decreased COX-2 expression, RNA & DNA oxidation, and protein carbonylation. Expression of APE/Ref-1, a DNA repair enzyme increased. All effects likely result from treatment-induced reduction of free radical formation after SCI. *Behavioral:* Not reported
Gris *J Neurosci* 2004	*Model:* Model: Male and Female Wistar Rat, 250-300 g *Injury:* • T4 Severe Clip Compression 50g • T12 Moderate Clip Compression 35 g	**Anti-CD11d mAb** 217L IV • 1 mg/kg @ 2, 24, and 48 h PI IB7 control mAb IV • 1 mg/kg @ 2, 24, and 48 h PI	SCI + • **Anti-CD11d mAb** (n = 29) • IB7 (n = 28) n = 16 male (motor and pain) n = 41 female (motor and autonomic)	*Histologic/Biochemical/Physiological:* Anti-CD11d mAb treatment led to tissue sparing PI. In the T4 severe injury: 2w levels of compact myelin and 6w levels of myelin and neurofilaments were increased. In the T12 moderate injury: 12w levels of necrosis were decreased, while myelin and neurofilaments were increased. *Behavioral:* BBB scores improved 5 & 6w after severe T4 SCI (females) and autonomic dysreflexia was attenuated at 2 & 6w PI. BBB scores were higher 4-12 w after moderately severe T12 SCI (9.7 vs 7.8), as were inclined plane scores at 5-12w and grid walk scores at 8-12w. Neuropathic pain was reduced following T12 injury in mAb-treated rats from 4-12w PI.
Saville *J Neuro-Immunology* 2004	*Model:* Female Wistar Rat, 250g *Injury:* T4 Clip Compression • Severe (50 g) • Moderate (35 g)	**Anti-CD11d mAb** IV 217L • 1.0 mg/kg @ 2, 24, 48, 72, and 96h PI **Anti-CD11d mAb** IV 236L & 226H • 1.0 mg/kg @ 24 h or 2 and 24 h or 2, 24, and 48 h PI **MP** IV • 30 mg/kg @ 2 h PI, then 15 mg/kg @ 24, and 48 hPI Control mAb IV • 1.0 mg/kg @ 24 h or 2 and 24 h or 2, 24, and 48 h PI	SCI + • **Anti-CD11d mAbs** • **MP** • Control mAb n = 5-10/group (175 total)	*Histologic/Biochemical/Physiological:* Flow cytometry and immunohistochemistry demonstrated CD11d expression by splenic macrophages & blood leukocytes using 217L & 236L. 217L reduced macrophages and neutrophils in lesion site from 18-72h post injury after severe or moderate injury. Dosing of 217 mAb was most effective at 2, 24, 48 h regimen. 236L & 226H had effects similar to 217L. At 7d after SCI, neutrophil and macrophage numbers in lesion were like controls. MP also reduced inflammatory cells in lesion from 18-72h. At 7d after MP treatment neutrophils were increased and macrophages decreased in lesion. *Behavioral:* Not reported.
Mabon *Exp Neurol* 2000	*Model:* Male Wistar Rat, 250-300g *Injury:* T4 Transection	**Anti-CD11d mAb** IV • 1 mg/kg or 5 mg/kg @ 24 h PRIOR SCI, 2h, and 24 h PI **Methylprednisolone** IV • 15 mg/kg or 30 mg/kg @ 30 min, 2h, and 24 h PI IB7 control mAbs • 1 mg/kg @ 24 h PRIOR SCI, 2 h, and 24 h PI	SCI + • **Anti-CD11d mAbs** (226H, n = 9; 236L, n = 8; 226B, n = 8) • **MP** (n = 5) • IB7 (n = 9) • No Treatment Control (n = 4)	*Histologic/Biochemical/Physiological:* At 2d after SCI, macrophages: decreased at injury site with 226H &236L anti-CD11d mAbs when compared to isotype control (1 mg/kg had a greater effect than 5 mg/kg). No effect of 226B. Neutrophils: decreased at injury site only with 236L (both doses equally effective). MP: decreased macrophages < the mAb treatments, did not decrease neutrophils. Staining of cord revealed macrophages and neutrophils in/near the lesion expressing CD11d. *Behavioral:* Not reported.

SCI: spinal cord injury; d: day, days; h: hour, hours; w: week, weeks; PI: post-injury;
T8: thoracic vertebra 8; C5: cervical vertebra 5; IP: intraperitoneal; IV: intravenous; SC: subcutaneous;
Ab: antibody; BBB: Basso, Beattie and Bresnahan locomotor test; COX: cyclooxygenase; MP: Methylprednisolone; MPO: Myeloperoxidase; ROS: reactive oxygen species.

Table 6. Minocycline

Paper	Animal model and injury model	Intervention and timing	Experimental groups	Reported outcomes: • Histologic/biochemical/physiological • Behavioral
Marchand *Eur J Pain* 2008	*Model:* Adult Male Wistar Rats, 220–250 g *Injury:* T13 Left Hemisection	**Minocycline** IP • 40 mg/kg @ 30 min PI, then 3 times q12 h.	• SCI + **Minocycline** (n = 8) **Saline** IP (n = 8) Sham operated + **Minocycline** IP (n = 8) **Saline** IP (n = 8)	*Histologic/Biochemical/Physiological:* Minocycline significantly attenuated microglial activation by 30-50% in the lumbar dorsal horns (OX-42 expression, n = 4) and evoked neuronal activity (c-Fos expression after noxious stimulation, n = 4) at 7d and 14d PI. *Behavioral:* Minocycline treatment prevented the development of mechanical allodynia and thermal hyperalgesia in both ispilateral and contralateral paws during first 2 w PI.
Ha *Eur Spine J* 2008	*Model:* Male SD Rats, 300–350 g *Injury:* T9/10 NYU Impactor 10 g×25 mm	**Minocycline** IP • 30 mg/kg @ 30min PI, then 12 h, 24 h, 36 h and 48 h PI **Methylprednisolone** IP • 30 mg/kg IP, @ 30 min, then at 12h and 24 h PI	• SCI + **Minocycline** (n = 8) **MP (n = 8)** **Saline** IV (n = 8)	*Histologic/Biochemical/Physiological:* Minocycline decreased lesion volume, attenuated microglial activation (anti-OX-42+ cells) and apoptosis (TUNEL). *Behavioral:* Minocycline significantly improved locomotor function at 7d PI (2.6 ± 0.5 vs. 1.0 for saline and 2.4 ± 0.5 for MP group in 6-point locomotion scale) and also improved incline plane test score at 7d PI.
Pinzon *Brain Res* 2008	*Model:* Male SD Rats, 220–280 g *Injury:* T9/10 NYU Impactor 10 g×12.5 mm	**Minocycline** IP or IV • 90 mg/kg @ 0h PI, then 45 mg/kg IP at 12h and 24h PI	• SCI + **Minocycline** IP (n = 15) **Minocycline** IV (n = 15) Vehicle IP (n = 8) Vehicle IV (n = 7)	*Histologic/Biochemical/Physiological:* Minocycline did not improve spared tissue areas or total cavity areas as calculated from horizontal sections. *Behavioral:* Minocycline did not lead to behavioral improvement at any point in time (BBB: minocycline IP 12.1 ± 0.18 vs saline control 11.8 ± 0.2; differences in BBB subscores were also not observed).
Saganová *Neurosci Lett* 2008	*Model:* Adult Wistar Rats 300–330 g *Injury:* T9 Balloon Compression (12.5μl×5 min)	**Minocycline** IP • 90 mg/kg @ 1h PI, then 45 mg/kg IP at 12h and 24h • 90 mg/kg @ 1h PI, then 45 mg/kg IP q12h×5 days	SCI + **Minocycline**×1 day **Minocycline**×5 days Saline IP (n = 12/group)	*Histologic/Biochemical/Physiological:* Minocycline at both the 1 and 5 day administration regimen increased gray and white matter sparing in sections 2-4 mm rostral to injury epicenter, but did not influence tissue sparing at the epicenter or at any sections caudal to epicenter. *Behavioral:* Minocycline at both the 1 and 5 day administration regimen did not result in any BBB improvement over 28 days of observation.
Yune *J Neurosci* 2007	*Model:* Male SD Rats, 250–300 g *Injury:* T9/10 NYU Impactor 10 g×25 mm	**Minocycline** IP • 90 mg/kg @ 0h or 2h PI, then 45 mg/kg q12h for 3d. **Methylprednisolone** IP: • 30 mg/kg @ 0h or 2h PI, then 30 mg/kg q12h for 3d.	• SCI + **Minocycline** (n = 44) MP (n = 20) Vehicle (n = 44) Sham (n = 3)	*Histologic/Biochemical/Physiological:* Minocycline significantly reduced levels of pro-NGF by 5 d post-SCI, protein levels of p-p38MAPK and p-MAPKAPK-2 by 3d and 5d post-SCI, significantly inhibited p75NTR mRNA and protein, and GTP-bound RhoA at 3d and 5d, significantly decreased the number of caspase-3-positive (CC1-positive) oligodendrocytes, all while sparing myelin and reducing axonal loss. *Behavioral:* Minocycline treatment (immediate or 2h delay) but not MP resulted in significantly improved BBB, inclined plane and grid walk stepping. Delayed (2 h) minocycline also improved foot coordination (foot print analysis), whereas MP did not.

(continued)

Table 6. Minocycline (Continued)

Paper	Animal model and injury model	Intervention and timing	Experimental groups	Reported outcomes: • Histologic/biochemical/physiological • Behavioral
Festoff *J Neurochem* 2006	*Model:* Female SD Rats, 300–325 g *Injury:* T9 NYU Impactor 10 g×25 mm	• **Minocycline** IP Single dose 90 mg/kg @ 30min, 1h or 24 h PI. 3 doses of 30 mg/kg @ 30min, 1h and 24 h PI. **Tetracycline** IP 30 mg/kg @ 1 h PI.	SCI + **Minocycline** (90 mg/kg @ 0.5,1 or 24 h post-SCI) (n = 12) **Minocycline** (30 mg/kg×3) (n = 4) Tetracycline (n = 3) Sham (n not indicated)	*Histologic /Biochemical/Physiological:* Minocycline significantly spares tissue, reduces tissue damage, cavity size, gliosis, necrosis, apoptosis, caspase-3 activation and caspase-3 cleavage products. Minocycline also reduced microglial activation and TNF-α levels. *Behavioral:* Minocycline 30 mg/kg×3 doses yielded ∼5 point BBB improvement over tetracycline controls. Similarly, minocycline (single dose 90 mg/kg) given at 0.5, 1, or 24 h post-SCI improved BBB scores over tetracycline-treated injured controls (Day 28: 14.6 ± 0.62 vs 8.33 ± 0.66). Timing of minocycline post-injury did not significantly affect functional recovery.
Stirling *J Neurosci* 2004	*Model*: Adult Wistar Rats *Injury:* Spinal Cord Dorsal Column C7/C8 Transection	**Minocycline** IP • 50 mg/kg @ 30 min PI and 8 h PI, then q12×2d	SCI + **Minocycline** Saline n = 6-8/group for 7d survival n = 4-5/group for 14d survival	*Histologic/Biochemical/Physiological:* Minocycline greatly reduces active caspase-3-positive oligodendrocytes and microglia/macrophages profiles in proximal and distal ascending sensory tracts (AST), inhibited transection-induced glial cell death within the distal and proximal AST, reduced ED1-positive (microglial/macrophage) density 7d PI, and reduced corticospinal tract dieback and lesion size both 7d and 14d PI. *Behavioral:* Footprint analysis revealed improved interlimb coordination and reduced hindlimb angle of rotation with minocycline treatment.
Teng *PNAS* 2004	*Model:* Female SD Rats, 280–330g *Injury:* T9 NYU Impactor 10g×25 mm	**Minocycline** IP • 8, 90 or 180 mg/kg @ 1h PI, collected @ 4h PI (dose response study) • 90 mg/kg @ 1h PI, then 45 mg/kg q12h×5d	• SCI + **Minocycline** Vehicle n = 3/group/dose and time point)	*Histologic/Biochemical/Physiological*: A dose-dependent effect of minocycline on cytochrome c release at SCI site was seen at 4h PI, reducing cytochrome c release to the negligible pre-SCI level. At 28d PI, minocycline perserved residual white matter, protected ventral horn neurons and oligodendrocytes, and reduced reactive astroglia within the ventral funiculi. *Behavioral:* Minocycline-treated rats demonstrated significantly increased coordinated hindlimb motor function; 3w and 4w BBB scores were significantly greater for minocycline treatments vs. vehicle.
Lee *J Neurotrauma* 2003	*Model:* SD Rats, 230-250 g *Injury:* T10 NYU Impactor 10g×12.5 mm	**Minocycline** IP • 90 mg/kg @ 0h PI, then 45 mg/kg q12 h×2 doses	SCI + **Minocycline** (n = 41) Vehicle (n = 41) Sham (n = 13)	*Histologic/Biochemical/Physiological:* Minocycline reduced cavitation between 28 and 38 days, caspase-3 activity, TUNEL-postive cells, and DNA laddering. *Behavioral:* Minocycline significantly increased BBB scores at 24 - 38 days PI (18.6 ± 0.7 vs. 15.6 ± 0.5).
Wells *Brain* 2003	*Model:* Male CD-1 Mice ∼3 months of age *Injury:* T3/T4 Clip Compression 8 g	**Minocycline** IP • 50 mg/kg @ 1 h PI, then 25 mg/kg q24 h×6 d.	• SCI + **Minocycline** (n = 43) Vehicle (n = 41)	*Histologic/Biochemical/Physiological:* Minocycline treatment yielded increased rubrospinal tracts, and reduced lesion area. *Behavioral:* Minocycline improved murine survival, BBB and inclined plane.

SCI: spinal cord injury; d: day, days; h: hour, hours; w: week, weeks; PI: post-injury; q24h: interval 24 hours;
T8: thoracic vertebra 8; C5: cervical vertebra 5; IP: intraperitoneal; IV: intravenous; SC: subcutaneous;
BBB: Basso, Beattie and Bresnahan locomotor test; MP: Methylprednisolone; SD rats: Sprague-Dawley rats; TNF: tumor necrosis factor.

Table 7. Progesterone

Paper	Animal model and injury model	Intervention and timing	Experimental groups	Reported outcomes: • Histologic/biochemical/physiological • Behavioral
Gonzalez, *Cell Mol Neurobiol* 2009	Model: Male SD Rats, 250–300 g Injury: T10 Complete Transection	**Progesterone** (PROG) SC • 4 mg/kg in vegetable oil @ 1, 24, 48 and 72 h PI Vehicle (oil) SC • @ 1, 24, 48 and 72 h PI * 1h doses given IP	SCI + • **Progesterone** • Vehicle Sham + • **Progesterone** • Vehicle n = 5/group	Histologic/Biochemical/Physiological: Spinal cords at L1 were examined by immunoelectron microscopy at 75 h PI. After SCI nuclear changes include dispersion of the nucleoplasm and eccentric location within the cell. Cytoplasm became chromatolytic. These changes were not present or reduced by PROG treatment. SCI also caused reduced MAP2 staining in dendrites, reflecting cytoskeletal disruption. PROG treatment reduced these changes. Behavioral: Not reported.
Labombarda *Glia* 2009	Model: Male SD Rats, 250–300 g Injury: T10 Complete Transection	**Progesterone** (PROG) SC • 16 mg/kg/day in vegetable oil started @ 3 h PI, for 3 or 21d Vehicle (oil) SC • @ 3 h PI, for 3 or 21d	SCI + • **Progesterone** (n = 6) • Vehicle (n = 6) Sham + • **Progesterone** (n = 5) • Vehicle (n = 5) Animals sacrificed at 3d or 21d PI, 4 sections per animal.	Histologic/Biochemical/Physiological: Short-term PROG treatment significantly increased the number of oligodendrocyte precursor cells (NG2+/Ox42-cells) in the injured spinal cord and induced their differentiation into mature oligodendrocytes by increasing the expression of Olig2 and Nkx2.2. • 21d of threatment increased the number of mature oligodendrocytes and favored remyelination by increasing proteolipid protein expression and Olig1m transcription factor involved in myelin repair. Behavioral: Not reported.
Fee, *Brain Res* 2007	Model: Male or Female SD Rats, 192-300 g Injury: T10 IH Impactor 150 kdynes	**Progesterone** (PROG) IP • 4 mg/kg in DMSO @ 0.5, 6, 24, 48, 72, 96, and120h PI • 8mg/kg @ 0.5, 24, 48, 72, 96, and 120h PI • 8 mg/kg or 16mg/kg @ 0.5, 6 h, then q24h for 14d Vehicle DMSO IP • @ 0.5, 6, 24, 48, 72, 96, and 120 h PI or @ 0.5h, 6h, then q24 h for 14d	SCI + • **Progesterone** • DMSO n = 18male, 19female/group for 14d n = 19male/female/group for 5d	Histologic/Biochemical/Physiological: No effects of PROG treatment or gender were found in injury length or sparing of white or grey matter. Behavioral: BBB scores did not differ between groups with respect to PROG treatment or gender.
Labombarda, *J Endocrinol* 2006	Model: Male SD Rats, 250–300g Injury: T10 Complete Transection	**Progesterone** (PROG) SC • 4 mg/kg in vegetable oil @ 1, 24, 48 and 72h PI * 1 h doses given IP	SCI + • **Progesterone** (n = 6) • No treatment (n = 11) Sham (n = 5)	Histologic/Biochemical/Physiological: Spinal cords at L1 were examined at 75h PI. Steroid levels were measured by GC/Mass Spec in the spinal cord and plasma. SCI caused increased PROG, 5αhydroprogesterone and 3α5α-tetrahydroprogesterone in the spinal cord without increases in plasma. PROG treatment mirrored these outcomes in plasma and spinal cord. 3β5α-tetrahydroprogesterone levels increased in the spinal cord but were barely detected in plasma. The ratio of reduced metabolites to PROG was 65-times higher in spinal cord than plasma, suggesting that the metabolites originated from local spinal cord biosynthesis. Behavioral: Not reported.

(continued)

Table 7. Progesterone (Continued)

Paper	*Animal model and injury model*	*Intervention and timing*	*Experimental groups*	*Reported outcomes:* • *Histologic/biochemical/physiological* • *Behavioral*
Labombarda, J *Neurotrauma* 2006	*Model:* Male SD Rats, 250–300 g *Injury:* T10 Complete Transection	**Progesterone** (PROG) SC • 4 mg/kg in vegetable oil @ 1, 24, 48 and 72 h PI Vehicle (oil) SC • @ 1, 24, 48 and 72 h PI * 1h doses given IP	SCI + • **Progesterone** • No treatment Sham + • **Progesterone** • No treatment n = 4-6/group	*Histologic/Biochemical/Physiological:* L4 spinal cords examined at 75h PI. SCI decreased immunostaining for myelin basic protein (MBP) in the corticospinal tract and dorsal ascending tract but not in the ventral funiculus. PROG treatment after SCI reversed this response. MBP mRNA in these tracts increased with SCI + PROG but not in other groups. SCI increased the number of NG2 immunoreactive cells (oligodendrocyte precursors) in grey and white matter and PROG + SCI further increased this number. Immunostaining for mature oligos (RIP) revealed no changes after SCI or SCI + PROG. *Behavioral:* Not reported.
Gonzalez, *Neuroscience* 2004	*Model:* Male SD Rats, 250–300 g *Injury:* T10 Complete Transection	**Progesterone** (PROG) SC or IP (1st dose) • 4 mg/kg SC @ 1, 24, 48 and 72 h PI	SCI + • **Progesterone** • No treatment Sham + • **Progesterone** • No treatment n = 4-5/group	*Histologic/Biochemical/Physiological:* L1 spinal cords examined at 75h PI. SCI produced severe chromatolysis of L1 motoneurons (Nissl staining). PROG reversed this response. SCI decreased BDNF mRNA (in situ hybridization) and protein (immunocytochemistry) expression. PROG reversed this response. In sham operated spinal cords, PROG increased BDNF protein expression without changing mRNA. *Behavioral*: Not reported.
Labombarda, *J Neurochem* 2003	*Model:* Male SD Rats, 250–300 g *Injury:* T10 Complete Transection	**Progesterone** (PROG) SC • 4 mg/kg in vegetable oil @ 1, 24, 48 and 72 h PI * 1h doses given IP	SCI + • **Progesterone** (n = 4) • No treatment (n = 10) Sham (n = 6)	*Histologic/Biochemical/Physiological:* Spinal cords at L1 were examined at 72h PI. SCI reduced the mRNA expression of the classical intracellular progesterone receptor (PR) but did not change that of 25-Dx, a membrane binding site (RT-PCR). PROG treatment after SCI had no effect on the low levels of PR but increased 25-Dx. Immunostaining of PR showed it inside neurons and glia whereas 25-Dx was on cell membranes of dorsal horn and central canal neurons. Numbers of PR-immunoreactive neurons was unchanged by SCI or SCI + PROG. In contrast, numbers of 25-Dx-immunoreactive neurons decreased after SCI and this number was restored after PROG treatment. *Behavioral:* Not reported.

Labombarda, *J Neurotrauma* 2002	*Model:* Male SD Rats, 250–300 g *Injury:* T10 Complete Transection	**Progesterone** (PROG) SC • 4 mg/kg in vegetable oil @ 1, 24, 48 and 72h PI **Vehicle (oil)** SC • @ 1, 24, 48 and 72h PI * 1h doses given IP	SCI + • **Progesterone** • No treatment Sham + • **Progesterone** • No treatment n = 6/group	*Histologic/Biochemical/Physiological:* Spinal cords at L1 were examined at 75h PI. SCI reduced the expression of choline acetyl transferase (ChAT) in L1 segment motorneurons and PROG restore this immunoreactivity. SCI also reduced the expression of mRNA (in situ hybridization) for the $\alpha 3$ and $\beta 1$ regulatory subunits of neuronal Na-K-Atpase and PROG restored both subunit mRNA to normal levels. Upregulation of GAP-43 in motorneurons of SCI rats was further enhanced by PROG. None of these effects were observed in sham-operated rats. *Behavioral:* Not reported.
Labombarda, *J Steroid Biochem Mol Biol*2000	*Model:* Male SD Rats, 250-300 g *Injury:* T10 Complete Transection	**Progesterone** (PROG) SC • 4 mg/kg in vegetable oil @ 1, 24, 48 and 72h PI * 1h dose given IP	SCI + • **Progesterone** • No treatment Sham + • **Progesterone** • No treatment n = 4-6/group	*Histologic/Biochemical/Physiological:* Spinal cords immediately above, immediately below and caudal to the SCI were examined at 75h PI. In SCI rats PROG increased the number of NADPH-diaphorase active (NOS) astrocytes in grey and white matter above and below the injury but not far caudal to it. In contrast, the number of GFAP-expressing astrocytes (activated cells) was increased by PROG in sham-injured but not SCI rats. GFAP was increased significantly in all areas measured by SCI alone. *Behavioral:* Not reported
Thomas, *Spine* 1999	*Model:* Male SD Rats, 295-305 g *Injury:* T8 NYU Impactor 10g×25 mm	**Progesterone** (PROG) IP • 4mg/kg in DMSO @ 0.5, 6, 24, 48, 72, 96, and120h PI Vehicle DMSO IP • @ 0.5, 6, 24, 48, 72, 96, and 120h PI	SCI + • **Progesterone** • Vehicle • No treatment Sham n = 10/group	*Histologic/Biochemical/Physiological:* At 6w PI, SCI + PROG had significantly more intact white matter (H&E + Luxol, 53%) than SCI only (24%) or SCI + vehicle (31%). *Behavioral:* BBB: SCI + PROG (15.5 pt) different from SCI only (12) and SCI + vehicle (10.3) only at 6w PI.

SCI: spinal cord injury; d: day, days; h: hour, hours; w: week, weeks; PI: post-injury
T8: thoracic vertebra 8; C5: cervical vertebra 5; IP: intraperitoneal; SC: subcutaneous
BBB: Basso, Beattie and Bresnahan locomotor test; BDNF: brain-derived neurotrophic factor; DMSO: dimethyl sulfoxide; GAP: growth-associated protein; GFAP: glial fibrillary acidic protein; MAP-2: Microtubule-associated protein 2; SD rats: Sprague-Dawley rats.

Table 8. Estrogen

Paper	Animal model and injury model	Intervention and timing	Experimental groups	Reported outcomes: • Histologic/biochemical/physiological • Behavioral
Cuzzocrea *Shock* 2008	*Model:* Male CD1 Mice, 25-30g *Injury:* T6 Clip Compression 24g	**17 Estradiol (E2)** SC • 300 μg/kg, @ 1h PRIOR Injury, then 3 and 6h PI **17 Estradiol (E2)** SC • 300 μg/kg @ 3 and 6h PI • 600 μg/kg @ 3 and 6h PI **E2 receptor antagonist ICI 182,780** • @ 1h before E2	SCI + • **E2** • **E2 + E2R antagonist** • Vehicle Sham • **E2** • **E2 + E2R antagonist** • Vehicle n = 10/group	*Histologic/Biochemical/Physiological:* All animals assessed at 24h. All significant effects were blocked by an E2R antagonist. E2 treatment decreased loss of tissue and myelin at lesion area as well as MPO activity and expression. Western blotting and immunocytochemistry showed that E2 treatment decreased expression of iNOS, nitrotyrosine, COX2, BAX, TUNEL, mRNA for TNFα, IL6, IL1β and MCP-1 (by Q-real time PCR) but increased expression of Bcl-2. *Behavioral:* Murine modified BBB scores were evaluated for 10 days. Authors report signficant improvement in hindlimb motor function from 5 to 10d post-injury in "E2-pretreated and posttreated mice", and improved function in animals receiving 600 μg/kg of E2 post-treatment. No further description is provided in the text, and the Figure to illustrate motor recovery (Figure 11), does not support this claim (although there may be a typographical error in the legend).
Ritz *Brain Res* 2008	*Model:* Male Wistar Rats, 250-300g *Injury:* T8-9 Clip Compression 20g×15s	**17 Estradiol (E2)** IP • 0.1 mg/kg @ 0h PI • 4.0 mg/kg @ 0h PI	SCI + • **E2** • Saline n = 3-6/group, 27 total	*Histologic/Biochemical/Physiological:* No treatment effects of 0.1 mg/kg E2 were found. 4.0 mg/kg reduced IL-1α in lesion at 6h, but not on IL-6. Changes were not found in cytokines or in IL-1receptor antagonist or IL-1β/IL-1ra ratio at 3 or 7d. Lesion size, GFAP, vimentin and macrophages in lesion were assessed at 3d, 2, and 4w. Lesion size decreased at 1w. Area of GFAP in lesion increased at 1wk and Vimentin appeared earlier. Area of CD68 decreased at 1 and 2w. *Behavioral:* BBB score of E2-treated rats increased more rapidly than controls at 3d and 7d post. Only E2 at 4 mg/kg had a higher score at 28d and improved narrow beam crossing was seen at 7d only.
Swartz *J Neurotrauma* 2007	*Model:* Male and Female SD Rats, 192-330g *Injury:* T10 IH Impactor 150 kdyne	**17 Estradiol (E2)** SC • 25 pg/ml or 75 pg/ml @ 7d PRIOR Injury, capsules replaced at 7d PI for 21d Sesame oil vehicle	SCI + • OVX female + **E2** • OVX female plus vehicle • Male + **E2** • Male + vehicle n = 6-8/group	*Histologic/Biochemical/Physiological:* Lesion assessed at 21d post injury. No effect of E2 on lesion length, total tissue sparing or epicenter sparing. Females had significantly shorter lesions and more total sparing due to grey matter preservation. No gender effects were found at the lesion centre. *Behavioral:* BBB score of E2-treated rats (both doses) was higher than controls at 7 d post injury only (11 vs. 8). No gender differences in motor function were found.

Chaovipoch *J Neurotrauma* 2006	*Model:* Female SD Rats, Adult 200–250g and Middle-Age, 350-400g *Injury:* • Midthoracic Forceps Crush 3s • Lateral Compression 0.5 mm	**17 Estradiol (E2)** SC • 180 g/ml in capsule, 1w PRIOR Injury Sesame oil vehicle capsule	SCI + (pre and post-menopausal) • **E2** + Ovariectomy • Vehicle + ovariectomy • Intact ovary Sham (pre and post-menopausal) • **E2** + Ovariectomy • Vehicle + ovariectomy • Intact ovary (n not indicated)	*Histologic/Biochemical/Physiological.* White matter sparing at 21d was greater in E2 treated rats, pre or post menopausal. Number of ventral horn neurons (Nissl) was greater, but apoptotic neurons (TUNEL) was less at 1 and 7d in E2 OVX rats and premenopausal rats, and in post menopausal E2 OVX rats. No effect of E2 was found on degeneration detected by fluorojade or on urine voiding. E2 treatment decreased body weight gains after SCI. *Behavioral:* E2 treatment improved BBB scores at 14 and 211 in treated postmenopausal and premenopausal OVX rats. Cycling rats (premenopausal) with intact ovaries had higher scores than vehicle treated OVX rats.
Sribnick *J Neurosci Res* 2006	*Model:* Male SD Rats, 250–300g *Injury:* T12 NYU Weight Drop 5g×8 cm	**17 Estradiol (E2) in DMSO** IV • 4 mg/kg, 15min and 24h PI DMSO • 15min and 24h PI	SCI + • **E2** • DMSO Sham n ≥ 4/group, 45 total	*Histologic/Biochemical/Physiological:* All assessed at 48h post-injury, with expression or activity data obtained by Western blotting. Neurofilament in lesion not changed by E2 treatment and less decreased in caudal penumbra compared to vehicle. Calpain expression and activity not changed in lesion, and less increased in penumbra than vehicle. Percent cytosolic cytochrome c not changed by E2 treatment in lesion, and increased less in penumbra than vehicle. Estrogen receptor β less decreased in lesion and penumbra compared to vehicle. *Behavioral:* Not reported.
Webb *Behav Brain* Res 2006	*Model:* Male Mice, 3-4 mo. B6; 129-UCP2^{tm1low1}(UCP-2KO) *Injury:* T2 Transection	**17 Estradiol (E2)** SC • 0.5 mg @ 1w PRIOR Injury Placebo pellet	SCI + • **E2** UCP-2 KO (n = 7) • **E2** WT (n = 7) • Placebo UCP-2 KO (n = 7) • Placebo WT (n = 8) n = 4/group for 1wk n = 6/group for 2wk	*Histologic/Biochemical/Physiological.* Lesion assessed at 21d post injury. No effect of E2 on the size of the CGRP-immunoreactive primary afferent arbour, nor on the fibrous scar in the injured cord or on CD11b immunoreactivity for microglia and macrophages or between KO and WT. Serum concentrations were greatly increased by the E2 pellets in all groups but with as much as a 3-fold difference between groups. *Behavioral:* E2 reduced autonomic dysreflexia caused by colon distension in UCP-KO and WT mice. Placebo mice had blood pressure increases of 25-30 mmHg whereas E2-treated mice had increases of 9-12 mmHg. Dysreflexia by tail pinch was not affected by E2.
Sribnick *J Neurosci* Res 2005	*Model:* Male SD Rats, 250–300g *Injury:* T12 NYU Weight Drop 5g×8 cm	**17 Estradiol (E2) in DMSO** IV • 4 mg/kg, 15 min and 24h PI DMSO • 15 min and 24h PI	SCI + • **E2** • DMSO Sham n ≥ 3/group, 54 total	*Histologic/Biochemical/Physiological:* All done at 48h post injury. Edema in lesion (water content) was decreased by E2 treatment. Microglia and macrophage accumulation (OX42 + and ED2 + in tissue sections) in lesion and caudal penumbra decreased by E2 treatment. NFκB expression (assessed with Western blotting) was not changed in lesion but in the penumbra it was increased in cytosol and decreased in nuclei compared to vehicle (similar to sham). E2 treatment increased myelin in lesion and penumbra by histologic analysis. *Behavioral*: Not reported.

(continued)

Table 8. Estrogen (Continued)

Paper	Animal model and injury model	Intervention and timing	Experimental groups	Reported outcomes: • Histologic/biochemical/physiological • Behavioral
Yune *J Neurotrauma* 2004	*Model:* Male SD Rats, 250–300g *Injury:* T9/10 NYU Weight Drop 10g×12.5 mm	**17 Estradiol (E2)** IV • 3 μg/kg @ 2h PRIOR Injury • 100 μg/kg @ 2h PRIOR Injury • 300 μg/kg @ 2h PRIOR Injury **E2 IV** • 100 μg/kg @ 0h PI	SCI + • **E2** • Vehicle Sham n = 8/group for behavior n = 3/group for sham and other analyses	*Histologic/Biochemical/Physiological:* All done on animals receiving estrogen pre-injury. Lesion area decreased at 18 & 28d from 2.5-2.0 mm^2 with 100 μg/kg. Apoptosis (TUNEL) and DNA laddering decreased @ 24 h post injury with 100 & 300 μg/kg doses. Caspase-3 activity decreased @ 4 h post injury with 100 μg/kg. Bcl-2 increased with all doses at 24 h post injury and increased at 6 & 12h post injury with 100 μg/kg. At 24h post injury, Bcl-2 expression in tissue sections was in putative neurons. *Behavioral:* E2 (100 μg/kg, given 2h pre-injury) significantly improved BBB @ 15-30d (scores E2 vs saline: 18 vs. 15) E2 (100 μg/kg immed post I) improved BBB @ 19-30 d (scores E2 vs saline: 18 vs. 14).

SCI: spinal cord injury; d: day, days; h: hour, hours; w: week, weeks; PI: post-injury;
T8: thoracic vertebra 8; C5: cervical vertebra 5; IP: intraperitoneal; Iv: intravenous; SC: subcutaneous;
BBB: Basso, Beattie and Bresnahan locomotor test; COX: cyclooxygenase; CGRP: calcitonin gene related protein; DMSO: dimethyl sulfoxide; GFAP: glial fibrillary acidic protein; IL: interleukin; iNOS: inducible nitric oxide synthase; KO: Knockout; MCP-1: monocyte chemoattractant protein-1; MPO: Myeloperoxidase; NFκB: nuclear factor κB; SD rats: Sprague-Dawley rats; TNF: tumor necrosis factor; WT: wild type.

Cuzzocrea and colleagues (2008) was the only one to include a more substantive delay prior to beginning estrogen (3 h). The "*n* per group" was variable as well, and ranged from 3 to 10. Non-behavioral outcomes reported included decreased overall secondary tissue damage, reduced MPO activity, microglial/macrophage accumulation, and reduced apoptosis. Behavioral outcomes reported improved BBB scores (although these were measured out to a few weeks post injury in a number of cases). Webb and colleagues (2006) reported reduced autonomic dysreflexia as well. Important to note that almost all of these beneficial behavioral and non-behavioral outcomes were observed in paradigms of estrogen pretreatment (or treatment at the time of injury). Only the Cuzzocrea paper utilized a time window of 3 h post injury. These authors report improved BBB scores at 5–10 days post injury in animals treated after injury, but this claim is difficult to verify in the figure provided in the article.

Magnesium (Table 9)

For its long-understood role as a physiologic antagonist of NMDA receptors, magnesium has had a long history of investigation as a potential neuroprotectant in stroke and brain injury. The systematic review resulted in eight studies in which magnesium was used for acute SCI. These have utilized Wistar and SD rats, and in one study a rabbit model was employed (Özdemir et al., 2005). Cord injury has been induced largely by some form of weight-drop contusion, although a clip compression injury model was utilized by Ditor and colleagues (2007). Systemic administration of magnesium has typically been with magnesium sulfate at doses of 100, 300, or 600 mg/kg given immediately after injury. In terms of a dose response, the 600 mg/kg dose was found by Süzer and colleagues (1999) to be more effective than the 300 mg/kg dose (in non-behavioral outcomes only). Kaptanoglu and colleagues (2003b) also found the 600 mg/kg dose to be more effective than the 100 mg/kg dose (in both histologic and behavioral outcomes). A time window of intervention for magnesium sulfate alone had not been extended beyond 30 min until the recent study by Wiseman and colleagues (2009), in which magnesium administration up to 8 h post injury (but not 12 or 24 h post injury) resulted in significantly improved BBB scores over controls. Non-behavioral outcomes include improved tissue sparing, reduced apoptosis and lipid peroxidation, and restoration of BBB integrity. Behavioral outcomes revealed improvements on incline plane testing, but these were evaluated at extremely early time points (e.g., 24 h post injury) and longer-term outcomes are lacking.

It should be noted that the 600 mg/kg dose of magnesium sulfate reported to be beneficial in rodent SCI far exceeds the tolerable human dose. The studies by Ditor and colleagues (2007) and Kwon and colleagues (2009) evaluated lower doses of magnesium in combination with polyethylene glycol (PEG). In both studies, magnesium in a PEG formulation was reported to increase tissue sparing at the site of injury and resulted in modest BBB improvements at 6 weeks post injury. The study by Kwon and colleagues (2009) evaluated a dose of magnesium chloride similar to that given to human patients for preeclampsia and cardiac arrest. This study included a dose-response experiment to optimize the dose and infusion regimen, and additionally reported a 4-h window of efficacy for a magnesium chloride in PEG formulation administered as five infusions 8 h apart.

Riluzole (Table 10)

The systematic review of riluzole revealed eight studies, which utilized Wistar, Long Evans, or Sprague Dawley rats and a variety of thoracic and cervicothoracic injury models, including weight drop (five), clip compression (one), and balloon compression (one). A complete sacral spinal-cord transection model was employed in the study of Kitzman and colleagues (2009) to examine the effect of riluzole on tail spasticity. Riluzole was most commonly administered intraperitoneally in doses of 5–8 mg/kg, although a dose of 2 mg/kg administered intravenously was also described (Stutzmann et al., 1996). A dose response has not been reported in the thoracic contusion SCI models. In the study by Kitzman and colleagues (2009), both 8 and 10 mg/kg doses reduced signs of tail spasticity, but the higher dose was associated with systemic side effects (lethargy, locoomotor ataxia). Therapeutic neuroprotective efficacy has been reported with a delay in intervention of 15 min (Springer et al., 1997) and 30 min (Stutzmann et al., 1996). Mu and colleagues (2000b) reported that with a delay of 2 h in post-thoracic contusion SCI, riluzole alone did not have a beneficial effect either histologically or behaviorally, but did when given in combination with methylprednisolone. Riluzole was administered 4 weeks post injury in the study by Kitzman and colleagues (2009), but this was to examine the effect on established tail spasticity not on local tissue protection or locomotor behavior. Non-behavioral outcomes included improved tissue sparing, reduced MAP-2 loss, decreased lipid peroxidation, and improved electrophysiological recordings. Behavioral outcomes included improved BBB locomotor scores and improved performance on inclined plane testing. Additionally, after 3 days of riluzole treatment, metrics of tail spasticity (e.g., response to stretch, pinch, or light touch) were significantly reduced, but this effect was limited to 3 h after the final riluzole dose and gone by 6 h.

Of note, a clinical trial for acute human SCI is being initiated through the North American Clinical Trials Network based on this pre-clinical body of evidence and facilitated by the fact that oral riluzole is FDA approved for the treatment of ALS. A similar oral dose of 50 mg twice a day has been proposed, but may be doubled to 100 mg twice a day to provide a typical 70-kg patient with a 2.9 mg/kg dose – one that more closely approximates the 5–8 mg/kg dose given intraperitoneally in the animal SCI studies.

Polyethylene glycol (Table 11)

Polyethylene glycol (PEG) has well-recognized fusogenic properties, and has been evaluated as a neuroprotective agent for its potential ability to repair damaged axons. The systematic review resulted in five studies in which PEG was administered systemically, although this excludes six additional studies by Dr. Richard Borgens in which PEG was directly applied to the damaged spinal cord (Borgens and Shi, 2000; Borgens et al., 2002; Duerstock and Borgens, 2002; Luo and Shi, 2004; Luo and Shi, 2007; Luo et al., 2004). The extensive body of literature from Dr. Borgens' lab on the use of PEG in a guinea-pig model of SCI has arguably served as the basis for interest in PEG as a neurooprotective agent.

Of the five studies identified in this systematic review, three utilized Wistar or Sprague Dawley rats with either clip compression or contusion injuries, one utilized a guinea-pig

Table 9. Magnesium

Paper	*Animal model and injury model*	*Intervention and timing*	*Experimental groups*	*Outcomes:* • *behavioral* • *Histologic/biochemical/physiological*
Kwon *J Neurotrauma* 2009	*Model:* Female SD rats, 200–225g *Injury:* T10 Infinite Horizon Impactor, 150 kdynes	**$MgSO_4$** IV • 60 mg/kg@15min & 6h PI **PEG** IV • 1 g/kg@15min & 6h PI **$MgCl_2$ in PEG** IV • 127 or 254 μmol/kg @ 2h PI, then 1, 3, or 5 infusions q6h or q8h **$MgCl_2$ in PEG** IV • 190 μmol/kg @ 15min, 2, 4 or 8h, then 4 infusions with the interval 8h. Methylprednisolone 30 mg/kg	SCI + • **$MgSO_4$** (n = 10) • **$MgSO_4$ in PEG** (n = 10) • PEG (n = 10) • Saline (n = 10) Sham (n = 10) SCI + • **$MgCl_2$ in PEG:** 127 or 254 μmol/kg $MgCl_2$, 2, 4 or 6 infusions started @ 2h PI (n = 5-10 /group) SCI + • **$MgCl_2$ in PEG:** 190 μmol/kg $MgCl^2$ at 15min, 2h, 4h or 8h PI (n = 5-10 /group) • Methylprednisolone	*Histologic/Biochemical/Physiological:* $MgSO_4$ in PEG formulation significantly decreased the lesion volume and improved BBB scores as compared to saline controls. PEG alone was ineffective at descreasing lesion volume. • 254 μmol/kg $MgCl_2$ in PEG was more neuroprotective than 127 μmol/kg dose. in PEG significantly decreased the lesion volume by 67% with 5 infusions at 190 μmol/kg. • 5 infusions of 190 μmol/kg $MgCl_2$ in PEG decreased lesion volume by 67%. Neuroprotection conferred with delay of up to 8 hours PI. *Behavioral:* $MgCl_2$ in PEG significantly improved scores (10.0 ± 0.2 for 5 infusions of 190 μmol/kg vs 7.8 ± 0.4 in saline group; Fergusson-BBB 12-grade scale). Behavioral efficacy time window observed with a delay of 4 h PI. • Methylprednisolone had inferior behavioral and histologic effect compared to $MgCl_2$/PEG, but did not interfere with it. • Improved locomotion was highly correlated with the lesion volume.
Wiseman *J Neurosurg Spine* 2009	*Model:* Female SD rats, 250–300g *Injury:* T9/10 NYU Impactor 10gx25 mm or 10gx12.5 m	**$MgSO_4$** IP • 600 mg/kg @ 10 min PI or @ 8, 12, 24 hr PI **Methylprednisolone** IP • 30 mg/kg @ 10 min PI or @ 8, 12, 24 hr PI	SCI (10g×25 mm) + • **$MgSO_4$** (n = 20) • **$MgSO_4$ + MP** (n = 20) • MP (n = 17) • Saline (n = 13) (Treatments started 10 min PI) SCI (10g×12.5 mm) + • **$MgSO_4$** @ 8, 12, or 24 PI • Saline (n = 13 per group)	*Histologic/Biochemical/Physiological:* Histology only performed on animals in more severe injury (10g×25 mm); here, all treatments significantly increased volume of white matter sparing at injury site (Mg, 32.3%; Mg + MP, 42.3%; MP, 30.3%, Saline, 20.2%). *Behavioral:* For more severe injury (10g×25 mm), BBB scores in Mg treatment were significantly better than saline at 4 weeks PI (6.9 vs 3.9). Significant morbidity and mortality in MP groups precluded assessment of MP or MP + Mg. Severe autophagia was seen in 25% of Mg treated animals. • For less severe injury (10g×12.5 m), BBB scores were significantly improved over saline controls with treatment by 8 hours (13.8 vs 8.6), but not at 12 or 24 hours PI.
Ditor *J Neurosci Res* 2007	Model: *Male Wistar Rats, 200–250g* Injury: *T4 Extradural Clip Compression* 50g×1 min	**$MgSO_4$** IV • 300 mg/kg @ 15 min & 6h PI **PEG IV** • 1 g/kg @ 15 min & 6h PI	SCI + • PEG (n = 11) • **$MgSO_4$** (n = 5) • **$MgSO_4$ in PEG** (n = 6) • Vehicle (n = 10)	*Histologic/Biochemical/Physiological:* PEG alone did not significantly reduce lesion volume. Only the combination of $MgSO_4$ in PEG was able to significantly reduce lesion volume. Dorsal compact myelin sparring, assessed by staining with solochrome cyanin, was improved by PEG/$MgSO_4$ and $MgSO_4$, but not PEG alone. *Behavioral:* At 42d post-injury BBB scores for PEG, $MgSO_4$, PEG/$MgSO_4$ and vehicle were 7.3 ± 0.2, 7.7 ± 0.4, 7.6 ± 0.2 and 6.4 ± 0.6, respectively. Mechanical allodynia was significantly reduced by all interventions in comparison to vehicle controls. None of the interventions attenuated the increase of mean arterial pressure triggered by colonic distention (a measure of autonomic dysreflexia).

Gok *Chin J Physiol* 2007	*Model:* Female Wistar Rats, 210–250g *Injury:* T7-T9 Weight Drop Contusion 40g×cm	**$MgSO_4$** IP • 600 mg/kg @ 0h PI **Methylprednisolone** IP • 30 mg/kg @ 0h PI	SCI + • **$MgSO_4$** • MP • Vehicle, 1 ml of saline • No treatment Sham n = 7/group	*Histologic/Biochemical/Physiological:* Magnesium sulphate or MPSS treatment decreased neutrophil infiltration (MPO activity) 24h after acute contusion injury. *Behavioral:* Magnesium sulphate (not MPSS) treatment significantly improved early functional scores (inclined plane technique of Rivlin and Tator) at 24h after trauma.
Solaroglu *Surg Neurol* 2005	*Model:* Adult Female Wistar Rats, 210–250g *Injury:* T8 Allen Weight Drop 40g×cm	**$MgSO_4$** IP • 600 mg/kg @ 0h PI **Methylprednisolone** IP • 30 mg/kg @ 0h PI	SCI + • **$MgSO_4$** • MP • Saline • No treatment Sham N = 8/group	*Histologic/Biochemical/Physiological:* The levels of apoptosis measured as the caspase-3 activity were significantly increased in spinal cord after contusion injury. Magnesium sulfate or MPSS treatment significantly reduced the caspase-3 activity, the latter having more pronounced effect. *Behavioral:* Not reported.
Ozdemir *Magnes Res* 2005	*Model:* Adult New Zeland Rabbits *Injury:* T8-T10 Weight Drop	**$MgSO_4$** IV • 100 mg/kg @ 5min PI	SCI + • **$MgSO_4$** • No treatment Sham n = 10/group	*Histologic/Biochemical/Physiological:* Contusion significantly increased the lactate and malondialdehyde (MDA) levels in contused spinal cord tissue at 60 min post-trauma ($p < 0.05$). Treatment with 100 mg/kg magnesium normalized the lactate levels and significantly decreased the MDA levels, although the latter still stayed significantly higher than in the control group. Magnesium significantly decreased heart rate which was increased by SCI. *Behavioral:* Not reported.
Kaptanoglu *Neurosurg Rev* 2003 (a)	*Model:* Female SD Rats, 220–270g *Injury:* T8, Allen Weight Drop 50g×cm	**$MgSO_4$** IP • 600 mg/kg @ 0h PI	SCI + • **$MgSO_4$** • No treatment Sham Intact control n = 8/group	*Histologic/Biochemical/Physiological:* The trauma increased tissue Evans blue content. 24h samples showed more remarkable tissue Evans blue content, than 2h samples. 600 mg/kg magnesium normalized the blood-spinal cord barrier after SCI. Magnesium markedly reduced Evans blue content in the spinal cord at both time points. Laminectomy did not affect the spinal cord Evans blue content in 2h and 24h groups. *Behavioral:* 600 mg/kg magnesium significantly improved locomotor function according to inclined plane, Tator and BBB 24h post-trauma.
Kaptanoglu *J Clin Neurosci* 2003 (b)	*Model:* Female SD Rats, 180–230g *Injury:* T7-T8, Allen Weight Drop 50g×cm	**$MgSO_4$** IP • 100 mg/kg @ 0h PI • 600 mg/kg @ 0h PI	SCI + • **$MgSO_4$** • Saline No SCI: • Sham • Intact control n = 10/group	*Histologic/Biochemical/Physiological:* Grading system for electron microscopic evaluation has been developed, including edema, nucleus damage, axonal and vascular changes. Application of high dose MgSO4 revealed better ultrastructural findings than low dose. Axonal myelination in the both $MgSO_4$ groups was significantly undistinguishable from controls. Both doses significantly improved axonal scores. *Behavioral:* Magnesium treatment significantly improved neurological outcome with inclined plane, Tarlov motor scale and BBB scale tests at 24h after SCI with essentially better results for 600 mg/kg than for 100 mg/kg.

(continued)

Table 9. Magnesium (Continued)

Paper	Animal model and injury model	Intervention and timing	Experimental groups	Outcomes: • behavioral • Histologic/biochemical/physiological
Süzer 1999 *Spinal Cord*	*Model:* Adult Male albino Rats, 210-310g *Injury:* T9 Extradural Aneurysm Clip Compression 50g×30s	**$MgSO_4$** SQ • 300 mg/kg @ 30min PI • 600 mg/kg @ 30min PI	SCI + • **$MgSO_4$** • Saline n = 10/group	*Histologic/Biochemical/Physiological:* The amplitudes of somatosensory evoked potentials (SSEPs) were signifcantly decreased at 30min post injury, and were signifcantly improved 3h after administration of 600 mg/kg $MgSO_4$ (one half of the drop), but not of saline or 300 mg/kg of MgSO4. The lipid peroxide measured as MDA levels significantly decreased at 24h after injury in 600 mg/kg $MgSO_4$ group as compared to saline or 300 mg/kg $MgSO_4$ groups. *Behavioral:* Not reported.

SCI: spinal cord injury; d: day, days; h: hour, hours; w: week, weeks; PI: post-injury; q8h: interval 8 hours;
T8: thoracic vertebra 8; C5: cervical vertebra 5; IP: intraperitoneal; IV: intravenous; SC: subcutaneous;
BBB: Basso, Beattie and Bresnahan locomotor test; MDA: malondialdehyde; MP(SS): Methylprednisolone (Sodium Succinate); PEG – polyethylene Glycol; SD rats: Sprague-Dawley rats.

Table 10. Riluzole

Paper	Animal model and injury model	Intervention and timing	Experimental groups	Reported outcomes: • Histologic/biochemical/physiological • Behavioral
Kitzman *Neurosci Lett* 2009	*Model:* Female Adult SD Rats, 200–250g *Injury:* S2 Cord Transection (through L1/2 laminectomy)	**Riluzole** IP • 8 mg/kg @ 4w PI, then q24h×3 days • 10 mg/kg @ 4w PI, then q24h×3 days	SCI + • **Riluzole** 8 mg/kg • Saline controls for 8 mg/kg • **Riluzole** 10 mg/kg • Saline controls for 10 mg/kg n ~ 4-5 per group	*Histologic/Biochemical/Physiological:* Not reported. *Behavioral:* At 1h and 3h post-administration, but not later, 8 mg/kg riluzole significantly decreased the responsiveness of the tail musculature to a pinch and light stimuli (representative of decreased spasticity). The drug at 8 mg/kg did not produce any overt indications of locomotor ataxia or lethargy in SCI animals. Riluzole at 10 mg/kg significantly decreased the responsiveness of the tail to a pinch, stretch and light stimuli. However, this dose produced indications of lethargy and locomotor ataxia in approximately 67% of the SCI animals tested.
Ates *J Clin Neurosci* 2007	*Model:* Adult Male Wistar albino Rats, 200–250g *Injury:* T7/T10 Weight Drop Contusion 5g×10 cm	**Riluzole** IP • 8 mg/kg @ 0h PI **Mexiletine** IP • 80 mg/kg @ 0h PI **Phenytoin** IP • 200 mg/kg @ 0h PI	SCI + • **Riluzole** • **Mexiletine** • **Phenytoin** • Saline 1 ml Sham n = 18/group	*Histologic/Biochemical/Physiological:* All 3 drug treatment groups showed greater myelin and neuronal gray matter sparing and smaller lesion areas, MDA levels, and spinal cord water content than controls. However, MDA and water levels in the phenytoin-treated group were higher than the other 2 drug groups. *Behavioral:* Sodium channel blockers resulted in improved motor function scores and inclined plane angles. However, phenytoin-treated rats revealed significantly lower improvement rates than mexiletine and riluzole-treated rats for the inclined plane.
McAdoo *Brain Res* 2005	*Model:* Male SD Rats, 240–320g *Injury:* • T9/T10 MASCIS Impactor 25 mm×10 g • Infinite Horizons 150 kdyn	Sodium channel blockers **Riluzole** via Fibre • 2 mM @ 0h PI **Mexiletine** IP • 80 mg/kg @ 0h PI **QX-314** • 1.5 μl of a 2.0 mM solution @ 0h PI NMDA receptor blockers **Memantine** **MK-801** AMPA/ kainate receptor blockers **Cyclothiazide** **NBQX** **GYK 52466**	SCI + • Riluzole • Mexilentine • QX-314 • MK-801 • Memantine • Cyclothiazide • NBQX • GYK 52466 • ACSF control n = 6/group	*Histologic/Biochemical/Physiological:* The effect of the sodium channel blockade on trauma-induced glutamate release was determined using microdialysis. Sodium channel blockade did not decrease glutamate release within the injured spinal cord. Infact, none of the agents tested had an appreciable effect on glutamate release following SCI. *Behavioral:* Not reported.
Schwartz *J Neurosurg* 2001	*Model:* Female Adult Wistar Rats; 225-280g *Injury:* C7/T1 Extradural Clip Compression 53g	Sodium channel blockers **Riluzole** IP • 5 mg/kg @ 0h PI **CNS5546A** IP • 15 mg/kg @ 0h PI **Phenytoin** IP@ 0h PI • 30 mg/kg 2Hpβ CD Vehicle • 5 mg/kg @ 0h PI	SCI + • **Riluzole** • **CNS5546A** • **Phenytoin** • Vehicle n = 15/group	*Histologic/Biochemical/Physiological:* Riluzole treated rats consistently yielded more FG-positive retrograde labeled neurons in the brain stem than controls, with significantly more neurons counted within the red nucleus. The other sodium channel blockers did not improve the integrity of descending axons. LFB and H&E stained transverse spinal cord sections demonstrated that all drugs were able to significantly reduce cavitation, with riluzole treatment offering the greatest protection. *Behavioral:* Only riluzole was able to improve BBB scores above controls. All sodium channel blockers resulted in greater inclined plane angles; however significance was only reached with riluzole.

(continued)

Table 10. Riluzole (Continued)

Paper	Animal model and injury model	Intervention and timing	Experimental groups	Reported outcomes: • Histologic/biochemical/physiological • Behavioral
Mu *Brain Res* 2000 a	*Model:* Female Long-Evans Rats, 225-250g *Injury:* T10 NYU Impactor 12.5 mm Drop (mean value for cord compression was 1.66 ± 0.11)	**Riluzole** IP • 8 mg/kg @ 15min and 2h PI **Methylprednisolone** (MP) IV • 30 mg/kg @15min and 2h PI	SCI + • **Riluzole** • **Riluzole + MP** • **MP** • Vehicle n = 9/group	*Histologic/Biochemical/Physiological:* At 4h PI, spinal cords were removed and synaptosomes prepared and examined using five measures of oxidative stress: 1) mitochondrial function; 2) reactive oxygen species levels; 3) thiobarbituric acid reactive product levels; 4) glutamate and 5) glucose levels. Riluzole and MP treatments improved mitochondrial function and enhanced glutamate and glucose uptake. MP treatment also reduced lipid peroxidation. MP, alone or in combination with riluzole, reduced malondialdehyde levels. The combination treatment was effective in improving all five measures of oxidative stress. *Behavioral:* Not reported.
Mu *J Neurotrauma* 2000 b	*Model:* Female Long-Evans Rats, 225-250g *Injury:* T10 SCI Impactor rod 12.5 mm drop	**Riluzole** IP • 8 mg/kg @ 2h and 4h PI and then daily×1 week **Methylprednisolone** (MP) IV via the femoral vein • 30 mg/kg @ 2h and 4h PI	SCI + • **Riluzole** • **Riluzole + MP** • **MP** • Vehicle n = 9/group	*Histologic/Biochemical/Physiological:* The overall appearance of spinal cord cavitation for transverse sections was similar for vehicle controls, Rriluzole and MP. However, the combination of Rriluzole + MP yielded significantly increased spared tissue. *Behavioral:* Riluzole or MP alone was not able to improve BBB scores above vehicle controls. At 4, 5 and 6w post-SCI the combination of riluzole + MP yielded significantly improved BBB scores (vehicle = 9.7 & 10.5; riluzole + MP = 12.5 & 13.7 at 5 and 6w, respectively).
Springer *J Neurochem* 1997	*Model:* Rats; (No further details of animals used) *Injury:* T10 NYU Impactor 25 mm Drop	**Riluzole** IP • 8 mg/kg @ 15min PRIOR to and 2h PI	SCI + • **Riluzole** • Saline n = 42/group	*Histologic/Biochemical/Physiological:* Immunocytochemical and Western Blot approaches were used to determine the ability of riluzole to attenuate microtubule-associated protein (MAP2) loss post-SCI. Riluzole treatment was associated with significant ability to reduce the loss of MAP2 at 24 h post-SCI. *Behavioral:* Not reported.
Stutzmann *NeuroReport* 1996	*Model:* Male Wistar Rats (280 ± 20g) *Injury:* T10/T12 Compresison via Fogarty balloon catheter 2.5 bars×8 min	**Riluzole** IV • 2 mg/kg @ 30min PI, then twice daily×10d Vehicle (1.5% pluronic F68 in 0.9% NaCl @ 30min PI, then twice daily×10d	SCI + • **Riluzole** • Vehicle n = 10/group	*Histologic/Biochemical/Physiological:* Vehicle-treated controls showed hemorrhage within the sub-dural space and necrosis of the grey and white matter. This damage was attenuated with riluzole treatment, especially in the white matter. Control somatosensory evoked potentials (SSEPs) recorded in all rats prior to SCI were abolished following injury. Vehicle treated rats did not recover SSEPs, however riluzole treatment led to significant recovery of SSEP amplitude, duration and latency. *Behavioral:* Riluzole treatment improved behavioral recovery. Qualitatively the riluzole-treated rats were able to use their paws to sit upright. Vehicle-treated controls could not sit upright.

T8: thoracic vertebra 8; C5: cervical vertebra 5; IP: intraperitoneal; IV: intravenous; SC: subcutaneous;
AMPA: α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate; BBB: Basso, Beattie and Bresnahan locomotor test; FG: fluorogold; LFB: Luxol Fast Blue; MDA: malondialdehyde; MP: Methylprednisolone; NMDA: N-methyl D-aspartate; SD rats: Sprague-Dawley rats.

Table 11. Polyethylene glycol

Paper	Animal model and injury model	Intervention and timing	Experimental groups	Reported outcomes: • Histologic/biochemical/physiological • Behavioral
Baptiste *J Neuropathol Exp Neurol 2009*	Model: Female Wistar rats 200–250g Injury: C8 *Extradural Clip Compression*35g×1 min	**PEG** IV • 1 g/kg @ 1h PI (for animals surviving < 24hr) • 1 g/kg @ 1h & 3 h PI (for animals surviving > 24hr) (PEG: 2,000 Da, 30%w/w in sterile lactate Ringer's (SLR))	SCI + • **PEG** (n = 14) • SLR (n = 14)Sham (n = 14)(for TUNEL/immunos 7d PI and Western blots at 2,4,8 24h PI) SCI + • **PEG** (n = 12) • SLR (n = 11)Sham (n = 9) (for long term survival 42d PI)	Histologic/Biochemical/Physiological: PEG decreased NF200 degradation (Western blot protein analysis 24 h PI) and reduced neuronal apoptosis (TUNEL staining) at injury site. Counts of retrograde labeled brainstem neurons was greater in PEG treated animals versus SLR controls (55.9 ± 8.76 vs. 33.8 ± 6.42, respectively; $p < 0.05$), particularly within the reticular formation. Spared tissue at the lesion epicenter and 700 mm rostral and caudal to it was greater in the PEG treated animals. Behavioral: PEG improved locomotor recovery on open field testing, with BBB scores of 7.2 ± 0.56 versus 4.5 ± 0.86 for SLR controls ($p = 0.006$). No significant difference was observed with inclined plane testing.
Kwon*J Neurotrauma*2009	Model: Female SD rats, 200–225g Injury: T10 Infinite Horizon Impactor,150 kdynes	**PEG** IV • 1 g/kg@15min & 6h PI **$MgSO_4$** IV • 60 mg/kg@15min & 6h PI	SCI + • **PEG** (n = 10) • $MgSO_4$ (n = 10) • $MgSO_4$ in PEG (n = 10) • Saline (n = 10) Sham (n = 10)	Histologic/Biochemical/Physiological: PEG alone did not significantly reduce lesion volume at the site of injury when compared to saline controls, 6 weeks post-injury. The combination of $MgSO_4$ in PEG resulted in a significant decrease in lesion volume. Behavioral: PEG alone did not significantly improve open field locomotion as compared to saline controls, 6 weeks post-injury. The combination of $MgSO_4$ in PEG resulted in a significant improvement in BBB scores.
Ditor *J Neurosci Res* 2007	Model: *Male Wistar Rats, 200-250g* Injury: *T4 Extradural Clip Compression*50g×1 min	PEG **IV** • 1 g/kg @ 15 min & 6h PI **$MgSO_4$** IV • 300 mg/kg @ 15 min & 6h PI	SCI + • PEG (n = 11) • **$MgSO_4$** (n = 5) • **$MgSO_4$ in PEG** (n = 6) • Vehicle (n = 10)	Histologic/Biochemical/Physiological: PEG alone did not significantly reduce lesion volume. Only the combination of $MgSO_4$ in PEG was able to significantly reduce lesion volume. Dorsal compact myelin sparring, assessed by staining with solochrome cyanin, was improved by PEG/$MgSO_4$ and $MgSO_4$, but not PEG alone. Behavioral: At 42d post-injury BBB scores for PEG, $MgSO_4$, PEG/$MgSO_4$ and vehicle were 7.3 ± 0.2, 7.7 ± 0.4, 7.6 ± 0.2 and 6.4 ± 0.6, respectively. Mechanical allodynia was significantly reduced by all interventions in comparison to vehicle controls. None of the interventions attenuated the increase of mean arterial pressure triggered by colonic distention (a measure of autonomic dysreflexia).
Laverty *J Neurotruama* 2004	Model: Clinical Dogs, ≤40 lbs; 2–8 years old Injury: T3–L3 paraplegia resulting from acute intervertebral disk herniation	**PEG IV** • 2 ml/kg of 3,500 Da, 30% w/w in sterile water **Poloxamer 188 (P188)** IV • 2 ml/kg of 150 mg/mL (given at the time of Xray exam, then 4-6h later) **Methylprednisolone** IV • 30 mg/kg Dogs included if within 72 hours of paraplegia	SCI + • **PEG + MP** (n = 19) • **P188 + MP** (n = 16)	Histologic/Biochemical/Physiological: SSEPs for PEG or P188-treated dogs demonstrated 63% improvement in recovered nerve conduction versus 0% in 36 historical control dogs who suffered paraplegia from acute intervertebral disk herniations. Behavioral: The occurance for a return of deep pain in hindlimbs and digits (indication of neurological recovery in complete paraplegia canine cases) was significantly greater for PEG and P188 treated dogs than historical controls.

(continued)

Table 11. Polyethylene Glycol (Continued)

Paper	*Animal model and injury model*	*Intervention and timing*	*Experimental groups*	*Reported outcomes:* • *Histologic/biochemical/physiological* • *Behavioral*
Borgens *J Neurosci Res* 2001	*Model:* Guinea Pigs < 300g *Injury:* Midthoracic spinal cord Crush (dura removed) with blunted forceps possessing a détente	**FITC decorated** PEG (FL-PEG); in sterile lactated ringers (SLR) applied to the exposed cord • 1 cc of 50% w/w @ 0h PI **FL-PEG** in SLR, SQ, IP or jugular vein IV • 1 cc of 30% w/w @ 0h PI **FL-PEG** in SLR SQ • 1 cc of 30% w/w @ 6h PI(for functional study)	SCI + • **FL-PEG** local (n = 2) • **PEG in SLR** SQ (n = 3) • **PEG in SLR** IP (n = 1) • **PEG in SLR** IV (n = 2) • **PEG in SLR**, 6h PI (n = 10) • Vehicle (n = 10)	*Histologic/Biochemical/Physiological:* FL-PEG is distributed to the injured spinal cord following topical, subcutaneous, intraperitoneal and intravenous delivery. *Behavioral:* Subcuteaneous PEG treatment restores SSEP and CTM reflex post-injury even with a single 6 hr PI injection.

SCI: spinal cord injury; d: day, days; h: hour, hours; w: week, weeks; PI: post-injury; q8h: interval 8 hours;
T8: thoracic vertebra 8; C5: cervical vertebra 5; IV: intravenous;
BBB: Basso, Beattie and Bresnahan locomotor test; CTM: cutaneus trunci muscle; FITC: fluorescein isothiocyanate; MDA: malondialdehyde; MP(SS): Methylprednisolone (Sodium Succinate); PEG – polyethylene Glycol; SD rats: Sprague-Dawley rat; SSEP: somatosensory evoked potentials.

model with a forcep crush injury, and one utilized canines who suffered paraplegia from intervertebral disc herniations. A dose of 1 g/kg was most commonly administered. Subcutaneously injected PEG was reported to be effective with a delay in intervention of 6 h post injury (Borgens and Bohnert, 2001), and dogs treated with PEG within 72 h of injury were reported to recover better than historical controls. In a rat SCI model, a 1-h time window of efficacy was reported by Baptiste and colleagues (2009).

With respect to non-behavioral outcomes, PEG was reported to decrease NF200 degradation, reduce apopotosis, and improve tissue sparing at the lesion site (Baptiste et al., 2009). In contrast, Kwon and colleagues (2009) and Ditor and colleagues (2007) found no significant tissue neuroprotection with PEG alone. With respect to behavioral outcomes, Borgens and Bohnert (2001) reported improved electrophysiologic recordings and the return of the cutaneous truncal muscle reflex after PEG treatment, and reported improved hindlimb recovery in PEG-treated dogs as compared to historical controls. Baptiste and colleagues (2009) reported modest improvements in BBB scores in PEG-treated animals, but no improvement on inclined plane testing. Ditor and colleagues (2007) also reported very modest improvement in BBB scores and a reduction in allodynia with PEG treatment.

Atorvastatin (Table 12)

Atorvastatin for acute SCI has been reported in three studies from two laboratories. All studies utilized female SD rats, employed thoracic weight-drop contusion injuries at T12 (Pannu et al., 2005, 2007) and T10 (Dery et al., 2009), and evaluated a 5 mg/kg dose of atorvastatin. The drug was administered via oral gavage in the two studies by Pannu and colleagues, and by intraperitoneal injection in the study by Dery and colleagues. In terms of a time window for therapeutic efficacy, the first of the two Pannu studies (2005) administered the drug prior to injury, while the subsequent study (2007), included delays of 2, 4, or 6 h post injury. Daily oral-gavage treatments were continued for 42 days. A 2-h delay in intervention was studied by Dery and colleagues, who continued the atorvastatin injections daily for 2 weeks.

Atorvastatin was found by Pannu and colleagues to reduce the expression of inflammatory cytokines, increase tissue sparing, and reduce Rho activity and GFAP expression and apoptosis. Dery and colleagues also reported a reduction in caspase 3 activation and decreased TUNEL staining, although statistically signficant tissue sparing at the injury site was not achieved in the atorvastatin-treated animals. In all three studies, atorvastatin was found to induce significant behavioral recovery, which was noted to be quite extraordinary in the articles by Pannu and colleagues. While the neuroprotective benefits reported by Dery and colleagues (2009) may not have been as dramatic as those described in the Pannu studies, the fact that this was done independently but with some similarities in experimental design (same species, comparable weight-drop force, same daily dose) reflects, to some degree, a robustness of the treatment.

Inosine (Table 13)

Inosine is a nucleoside that is commonly taken as a dietary supplement to improve athletic performance. Its potential for SCI was first introduced by Benowitz and colleagues (1999) in a study that revealed that local infusion of inosine to the motor cortex promoted sprouting of corticospinal axons in the medulla. Because of its invasive mode of administration, this study is excluded from this systematic review. However, three subsequent studies have been published that utilize a systemic administration starting at either an acute time point (15 min to 2 h post injury) or at a chronic time point (3 months post injury).

As an acute therapy for SCI, inosine was reported to decrease apoptosis, and, after 6 weeks of administration, reduce the invasion of ED-1 positive cells into the injury site. No reports of improved tissue or axonal sparing are made in either study. No behavioral assessments were made in either study. In a chronic (3 months post injury) setting, inosine reportedly increases sprouting of ascending and descending tracts, and was associated with recovery of the cutaneous truncal muscle reflex. No locomotor behavior has been reported. In summary, there is little pre-clinical evidence available to support the use of inosine as an acute therapy for SCI. While inosine has been studied by independent labs, behavioral outcomes were not assessed in either of the acute studies, and there is a paucity of histologic outcomes. A time window of 2 h was indicated by Liu and colleagues (2006), but the histologic outcomes were performed at 3 days post injury.

Pioglitazone (Table 14)

Pioglitazone is a PPAR (peroxisome proliferator-activated receptor) gamma agonist. PPARs are part of the nuclear hormone receptor superfamily that, upon ligand activation, regulate gene expression. The PPARγ agonists such as pioglitazone and rosiglitazone are in common clinical use for type II diabetes (marketed under the names Actos® and Avandia® respectively). Beyond their metabolic effects, interest in PPARγ agonists has grown in the last decade due to their anti-inflammatory, neuroprotective, and even anti-neoplastic properties (Kapadia et al., 2008; Ondrey, 2009).

Pioglitazone has not yet had extensive testing in models of SCI. Two papers from independent laboratories were identified in the literature search. Both utilized thoracic contusion injuries induced by either the OSU or NYU impactors in Sprague Dawley rats. Pioglitazone was administered intraperitoneally in a range of doses, from 0.5 to 10 mg/kg. Dose-response effects were observed in both studies, with McTigue and colleagues (2007) reporting better neuroprotection with 10 mg/kg versus 1.0 mg/kg, and Park and colleagues (2007) reporting better neuroprotection with 3.0 and 1.5 mg/kg versus 0.5 mg/kg. Park and colleagues (2007) additionally demonstrated that for a 1.5 mg/kg dose, four administrations over 48 h were better than one administration. While the optimal dose and treatment regimen remains to be established, it is noteworthy and commendable that these studies both looked at dose, with the Park study examining both dose and regimen. Importantly, the Park study established a 2-h time window of efficacy for the 1.5 mg/kg dose of pioglitazone; neuroprotective efficacy was lost when the drug was started 4 h post injury. Interestingly, while Park and colleagues (2007) observed significant recovery on the BBB with pioglitazone at 1.5 mg/kg and 3.0 mg/kg, McTigue and colleagues (2007) did not see recovery on the BBB in either the 10 mg/kg or 1 mg/kg, although they did report other behavioral outcome measures that showed some benefit of pioglitazone. Group sizes in both

Table 12. Atorvastatin

Paper	Animal model and injury model	Intervention and timing	Experimental groups	Reported outcomes: • Histologic/biochemical/physiological • Behavioral
Déry *Neurosci Lett* 2009	*Model:* Female SD Rats, 225–250g *Injury:* T10 Weight Drop 5g×6 cm	**Atorvastatin (AT)** IP • 5 mg/kg @ 2h PI, single injection for 4h PI sacrifice • 5 mg/kg @ 2h PI, then 5 mg/kg daily x15d for 4w sacrifice	SCI + • **Atorvastatin** ($n=8$) • Saline (n = 11) (sacrificed at 4h PI) • **Atorvastatin** ($n=6$) • Saline ($n=6$) (sacrificed at 4w PI) Sham operated controls (n = 11)	*Histologic/Biochemical/Physiological:* • Atorvastatin prevented the elevation of the caspase-3 activity in the injured spinal cord and significantly decreased the number of TUNEL-positive cells at the injury site as compared to vehicle-treated rats. • Atorvastatin did not result in significantly greater spared spinal cord tissue at injury site compared to vehicle-treated rats ($42 \pm 6\%$ vs $34 \pm 8\%$, $p = 0.12$). *Behavioral:* Significant improvement in locomotion at week 4 post-SCI in the statin-treated group compared to the saline-treated group (average BBB score 14 vs. 10 in control).
Pannu *J Neurochem* 2007	*Model:* Female SD Rats, 225–250g *Injury:* T12 Weight Drop 40g×cm	**Atorvastatin (AT)** oral. • 5 mg/kg/day, @ 2h, 4h, or 6h PI, then daily x42d	SCI + • **Atorvastatin** • Saline n = 6/group for behavior n = 3/group for histology/biochemistry	*Histologic/Biochemical/Physiological:* • 4h PI - AT reduced the expression (mRNA) of iNOS, TNFα, and IL-1β. • 6h PI - AT reduced Rho activity • 24h PI - AT treatment promoted tissue sparing (H&E stain), reduced neutrophil and macrophage infiltration, MMP 9 activity and expression, and Evans Blue extravasation. • 5d PI - AT reduced GFAP expression (mRNA). *Behavioral:* Significant locomotor recovery 6w PI. BBB scores were ~19 for each of the three AT treated groups and ~8 in controls; animals treated after 2h had the fastest recovery.
Pannu J Neurosci Res 2005	*Model:* Female SD Rats, 225–250g *Injury:* T12 Weight Drop 30g×cm	**Atorvastatin (AT)** oral • 5mg/kg/day started 7d PRIOR to SCI, then daily×15d	SCI + • **Atorvastatin** (n = 9) • Saline (n = 9) n = 9/group for behavior n = 3/group for histology/biochemistry	*Histologic/Biochemical/Physiological:* • AT decreased secondary tissue damage; • Reduced iNOS, TNFα, and IL-1β mRNA expression (acute); • Reduced macrophage invasion, GFAP reactive astrocytes, TUNEL positive apoptotic cells (1 week); Note: there was no quantification in these outcomes, only representative images were shown. *Behavioral:* Significant locomotor recovery. At 15 days post-SCI, BBB scores were ~19 in AT treated and ~9 in saline treated animals.

SCI: spinal cord injury; d: day, days; h: hour, hours; w: week, weeks; PI: post-injury;
T10: thoracic vertebra 10; IP: intraperitoneal;
BBB: Basso, Beattie and Bresnahan locomotor test; IL: interleukin; iNOS: inducible nitric oxide synthase; SD rats: Sprague-Dawley rats; TNF: tumor necrosis factor.

Table 13. Inosine

Paper	Animal model and injury model	Intervention and timing	Experimental groups	Reported outcomes: Histologic/biochemical/physiological Behavioral
Conta *Spinal Cord* 2008	*Model:* "Adult" Female Long-Evans Rats *Injury:* T9/T10 NYU Impactor 10g×25 mm	**Inosine** IP • 100 mg/kg starting @ 15min or 3w PI, then twice daily for 1 week **Inosine** SQ • 100 mg/kg starting @ 15min PI, then twice daily for 6 weeks	SCI + • **Inosine** IP (n = 10) • **Inosine** SQ (n = 8) • Vehicle (n = 28)	*Histologic/Biochemical/Physiological:* Reduced the volume fraction of ED1 positive profiles around the lesion site only in the group with continuous 6 week inosine delivery. *Behavioral:* Not reported.
Bohnert *J Neurotrauma* 2007	*Model:* Female Guinea Pigs, ~400g *Injury:* T10 Lateral Hemisection	**Inosine** SQ through an osmotic minipump • 10 mM at 0.25 μL/h for 1 month started @ 3 months PI	SCI + • **Inosine** • Vehicle n = 15/group	*Histologic/Biochemical/Physiological:* Retrograde and anterograde tracing of spinal cord tracts indicated that inosine induced regenerative sprouting of ascending and descending tracts up to the lesion site. This regeneration was enhanced when inosine therapy was combined with oscillating field stimulation (OFS). *Behavioral:* 5 months after injury or 2 months after treatment, Inosine mediated a significant recovery of CTM receptive fields.
Liu *Spinal Cord* 2006	*Model:* Male SD Rats, 200–220g *Injury:* T8-T9 Clip Compression 95g×1 min	**Inosine** IP • 75 mg/kg @ 2h, 12h or 24h	SCI + • **Inosine** • Saline n = 6/group	*Histologic/Biochemical/Physiological:* At 3 days post injury, Inosine given as late as 12h, significantly reduced apoptosis (TUNEL labeling) and degenerative areas in the spinal cord (H&E stain). *Behavioral:* Not reported.

SCI: spinal cord injury; h: hour, hours; w: week, weeks; PI: post-injury;
T8: thoracic vertebra 8; IP: intraperitoneal; SC: subcutaneous;
CTM: cutaneus trunci muscle; SD rats: Sprague-Dawley rats.

Table 14. Pioglitazone

Paper	Animal model and injury model	Intervention and timing	Experimental groups	Reported outcomes: • Histologic/biochemical/physiological • Behavioral
McTigue *Exp Neurol* 2007	*Model:* Female SD Rats, 214–245g *Injury:* T8 OSU Impactor Contusion 0.7 or 0.8 mm displacement	**Pioglitazone** IP • 10 mg/kg @ 15min PI, then q12h×7d • 1 mg/kg @ 15min PI, then q12h×7d	SCI (0.7 mm) + • **Pioglitazone** • PBS n = 6/group SCI (0.8 mm) + • **Pioglitazone** • PBS n = 6/group, 5 wk survival, or n = 7/group, 1 wk survival	*Histologic/Biochemical/Physiological*: High dose pioglitazone reduced lesion area rostral to epicenter, increased spared white matter, and increased motor neurons (much moreso than low dose). It did not alter apoptosis (caspase-3+ cells count) or macrophage density at 7d post-injury. *Behavioral:* BBB scores at 5w PI were not significantly improved with high or low dose pioglitazone. BBB subscores were greater in high-dose treated animals at 7, 14 and 28d PI in 0.7 mm injury. Pioglitazone rats had better coordination, toe clearance, earlier stepping and more parallel paw position.
Park *J Pharmacol Exp Ther* 2007	*Model:* Adult SD Rats, 300–325g *Injury:* T9 NYU Impactor Contusion 10g×12.5 mm	**Pioglitazone** IP (PIO) • 0.5, 1.5, or 3.0 mg/kg @ 5 min, 2, or 4h PI, then no more, or another dose @ 12, 24, and/or 48h PI **Rosiglitazone** IP (ROSI) • 0.5, 1.5, or 3.0 mg/kg @ 5 min, 2, or 4h PI, then no more, or another dose @ 12, 24, and 48h PI **PPARγ antagonist** (GW9662) • 2 mg/kg @ 1h prior to each pioglitazone injection	SCI + • **PIO** • **ROSI** • **GW9663** • PBS n = 5-9/group	*Histologic/Biochemical/Physiological*: Pioglitazone and rosiglitazone decreased the lesion size, motor neuron loss (by 3 to 10 fold), myelin loss, astrogliosis and microglial activation. Pioglitazone attenuated the induction of inflammatory genes and enhanced the induction of neuroprotective heat-shock proteins and anti-oxidant enzymes. Pretreatment with the PPAR antagonist GW9662 prevented this neuroprotection. Pioglitazone neuroprotection maintained when given at 5min and 2h PI, but lost when started at 4h PI. Dose response shown, with neuroprotection improved with 1.5 and 3.0 mg/kg (versus 0.5 mg/kg), and with more total doses. *Behavioral:* The drugs improved BBB scores with a time window of 2h PI. Better BBB recovery with 4 total doses, and with 1.5 and 3.0 mg/kg versus 0.5 mg/kg. (BBB of ~13 vs ~10) At 28d after SCI, chronic thermal hyperalgesia was decreased significantly in the pioglitazone group.

SCI: spinal cord injury; d: day, days; h: hour, hours; w: week, weeks; PI: post-injury;
T8: thoracic vertebra 8; IP: intraperitoneal;
BBB: Basso, Beattie and Bresnahan locomotor test; CTM: cutaneus trunci muscle; SD rats: Sprague-Dawley rats.

experiments were relatively small in both studies. For example, many of the experiments done by Park and colleagues (for example, the dose and time-window studies) were with an n of only five per group. As a side note, a study of an endogenous PPARγ agonist, 15-deoxy-delta-12,14-prostaglandin J2, found beneficial effects with a 200 μg/kg dose, but deleterious effects with a high doses of 1 mg/kg (Kerr et al., 2008), illustrating the complexity of this dose issue in such pharmacotherapies for SCI.

Conclusion

With the urgency to establish novel treatments for acute SCI, it is recognized that the process of bringing forth a new human therapy takes a great deal of time. Novel therapeutics require substantive safety evaluations, which add expense and time to the translation of new treatments. Along this line, the idea of exploring the neuroprotective potential of treatments that are already in human clinical application remains an inviting strategy. This review summarizes the available pre-clinical literature on the use of such agents in *in-vivo* models of acute SCI. It is not a comprehensive list of every therapy that has ever been tried in pre-clinical models of SCI, but rather, a systematic review of specific therapies that are currently being considered for human translation. In some cases (such as systemic hypothermia and minocycline), the human barrier has been broken and clinical evaluation has actually begun.

The obvious question to ask when viewing this large body of pre-clinical data is "which are the most promising?" and thus best candidates to take forward into clinical trials. This is a difficult question to answer, for a variety of reasons. The systematic review of these therapies and the description of the individual studies in this tabular form make it evident that significant variability exists across the scientific community in the conduct of these studies. This variability includes animal sex, weight, strain, and species, SCI level, severity, and mechanism, therapeutic dose, dose regime, timing, and survival, and a myriad of different histologic and behavioral outcomes. These differences make it very difficult, if not impossible, to compare across studies to determine which agent had the greatest "effect." While this variability is bemoaned for the difficulty that arises in comparing results from one laboratory to the next, it is at least partly consistent with the variability inherent to human injuries. If something is to "work" in human SCI, it should conceivably "work" in a variety of pre-clinical injury models that utilize different animal strains, weights, and ages, and different injury severities and levels; that is, the beneficial effect of the treatment must be "robust" enough that it can be demonstrated in a variety of experimental settings.

This issue of robustness is central to the discussion of translation, and is reflected in part by the number of independent laboratories that have reported on beneficial effects of a therapy, often with different rodent species, injury models, and doses. In this regard, most of the agents documented in this review achieved this level of "robustness" (e.g., minocycline, erythropoietin, riluzole, systemic hypothermia, progesterone, estrogen, atorvastatin, and magnesium sulfate). However, this has important distinctions from a formal attempt at replicating the positive results of a particular study from another laboratory, in which great efforts are made to simulate as many of the exact experimental conditions as possible. Such replication attempts sponsored by the NIH have been unsuccessful in the case of erythropoietin (Pinzon et al., 2008) and minocycline (Pinzon et al., 2008). In fact, to our knowledge, no formal replication study has yet to reproduce the efficacy reported in the original research article.

The second obvious translational issue for neuroprotective agents administered in the acute SCI setting is that of the time window of efficacy. The length of the time window of efficacy that needs to be established in pre-clinical animal studies in order to suggest that a drug would be effective in a realistic timeframe in human SCI is not currently understood, and it likely differs depending on the precise mechanism of action of the therapeutic agent (which also may not be that well understood). As the therapies reviewed in this paper are largely neuroprotective treatments to be utilized in acute SCI, it is quite revealing to note the paucity of time window of intervention studies that are actually performed. Most studies employ extremely short time windows, in which the intervention is applied either at the time of injury or very soon after. The therapy with the most number of animal studies that met the inclusion criteria of our systematic review was erythropoietin (19 studies), and yet, a time window of behavioral efficacy beyond 1 h has not yet been established.

Finally, the issue of what actually constitutes "clinically meaningful efficacy" in pre-clinical research is something that is not so well defined. Given that human SCI is ultimately a functional problem, it would seem obvious that in pre-clinical studies, a therapy should, above all else, demonstrate some improvement in behavioral outcomes. As indicated in the tables, most studies (but certainly not all) actually measured behavioral outcomes, the most common being the open field locomotor scores (BBB scale). The tables make it evident that the scientific community places much emphasis on the recovery of locomotor function, which of course is well understood not to be the top priority of patients with spinal cord injuries (Anderson, 2004). From a translational standpoint, at least the use of the BBB allows for a common metric amongst experiments of inevitably varied design, and provides some context to the extent of behavioral recovery that has actually occurred. Exactly what extent of BBB recovery is "clinically meaningful," however, remains controversial, given the recognized differences in locomotor control between rodents and humans.

In summary, this manuscript portrays the state of the pre-clinical literature on a number of potential acute therapies for SCI that have been studied to various degrees in animal models. Some have already gone into clinical trials (e.g., minocycline in Calgary, Alberta, and systemic hypothermia in Miami, Florida), and others are poised to be soon translated into human SCI (e.g., riluzole, and a magnesium/PEG formulation). While the body of literature on many therapies is not insignificant, a casual review of this systematically collected literature reveals that there are some obvious gaps to fill with respect to demonstrating robustness and confirming relevant time windows of efficacy. Developing guidelines analogous to those that exist within the stroke field to provide direction to the pre-clinical development of these therapies prior to human translation would be valuable.

Acknowledgment

This work was supported by the Rick Hansen Institute (formerly, The Canadian SCI Solutions Network). BKK holds

a CIHR New Investigator Award, and WT is the Rick Hansen Man in Motion Chair in Spinal Cord Injury Research.

Author Disclosure Statement

No competing financial interests exist.

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