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Axonal Degeneration and Demyelination Following Traumatic Spinal Cord Injury; A Systematic Review and Meta-analysis

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Highlights

- The pathophysiology of SCI are poorly understood.
- Axonal and myelin sheath properties was changed as time elapsed from the injury.
- The pathophysiology of axons and myelin sheath differ in various phases of SCI.
- These changes are affected by multiple factors related to the injury.

Abstract

The pathophysiology of spinal cord injury (SCI) related processes of axonal degeneration and demyelination are poorly understood. The present systematic review and meta-analysis were performed such to establish quantitative results of animal studies regarding the role of injury severity, SCI models and level of injury on the pathophysiology of axon and myelin sheath degeneration. 39 related articles were included in the analysis. The compiled data showed that the total number of axons, number of myelinated axons, myelin sheath thickness, axonal conduction velocity, and internode length steadily decreased as time elapsed from the injury ($P_{\text{for trend}} < 0.0001$). The rate of axonal retrograde degeneration was affected by SCI model and severity of the injury. Axonal degeneration was higher in injuries of the thoracic region. The SCI model and the site of the injury also affected axonal retrograde degeneration. The number of myelinated axons in the caudal region of the injury was significantly higher than the lesion site and the rostral region. The findings of the present meta-analysis show that the pathophysiology of axons and myelin sheath differ in various phases of SCI and are affected by multiple factors related to the injury.

Keywords: Animal studies; Degeneration; Myelin sheath; Spinal cord injuries

1 Introduction:

Events following spinal cord injury (SCI) are classified in three general phases of acute, sub-acute and chronic (1). Pathophysiology of SCI recovery and reorganization are different in these three phases and lead to the manifestation of different clinical symptoms (2). Axonal degeneration is considered one of the major mechanisms of the degeneration process and occurs differently in each phase. Currently, the presence of axonal degeneration after spinal cord injury has been established and different treatments for neurologic injury have been implemented (3). Unfortunately, these treatments have not been effective in most cases.

Some researchers believe that the creation of new rostral and caudal connections through the injury site with the induction of sprouting and axonal regeneration does not necessarily lead to improvement in sensory and motor functions but could lead to exacerbation of irritating syndromes such as neuropathic pain (4, 5). It is possible that this response is due to the process of axonal and myelin changes as a result of the injury. In addition, it has not fully addressed how the severity of the injury, SCI model, and level of injury (cervical, thoracic and thoracolumbar) affect the axonal and myelin pathophysiology. Further, does axonal degeneration status differ in rostral and caudal regions of the injury? Therefore, this systematic review and meta-analysis aim to gather existing quantitative animal findings in the field of axon and myelin sheath pathophysiology following SCI.

2 Methods:

2.1 Search strategy

In this systematic review and meta-analysis, by using words related to SCI in combination with keywords related to pathophysiology, a search was done in Medline and Embase from 1946 until December 2015. These articles were supplemented with a further search utilizing a Google search engine and Google scholar and the references of related articles. Keywords were selected based on Mesh and Emtree databases, using the titles of related articles, and consultation with experts. The search query in Medline and Embase databases has been shown in table 1.

Table 1: Search strategies used in Medline and Embase

Medline via PubMed	EMBASE via Ovid SP
("Spinal Cord Injuries/pathology"[Mesh] OR "Spinal Cord Injuries/physiopathology"[Mesh]) OR (((Trauma*[tiab] OR Injur*[tiab]) AND (Spinal[tiab] AND Cord[tiab])) AND (Pathophysiolog*[tiab] OR Physiopatholog*[tiab] OR Patholog*[tiab] OR Pathobiolog*[tiab] OR Histopatholog*[tiab])) AND ("Time"[Mesh:NoExp] OR "Time Factors"[Mesh] OR "Chronology as Topic"[Mesh] OR "Acute Disease"[Mesh] OR Time[tiab] OR Timing[tiab] OR Chronolog*[tiab] OR Min[tiab] OR Minute*[tiab] OR Hour[tiab] OR Hours[tiab] OR Day[tiab] OR Days[tiab] OR Week*[tiab] OR Month*[tiab] OR Year*[tiab] OR Phase[tiab] OR Phases[tiab] OR Stage[tiab] OR Stages[tiab] OR Early[tiab] OR Late[tiab] OR Primary[tiab] OR Secondary[tiab] OR Acute[tiab] OR Subacute[tiab] OR Subchronic[tiab]) AND ("Animals"[Mesh])	1. spinal cord injury/ 2. (Spinal adj Cord adj (Trauma\$ or Injur\$)).ti,ab. 3. or/1-2 4. exp pathology/ 5. pathophysiology/ 6. histopathology/ 7. (Pathophysiolog\$ or Physiopatholog\$ or Patholog\$ or Pathobiolog\$ or Histopatholog\$).ti,ab. 8. or/4-7 9. time/ 10. chronology/ 11. acute disease/ 12. (Time or Timing or Chronolog\$ or Min or Minute? or Hour? or Day? or Week? or Month? or Year? or Phase? or Stage? or Early or Late or Primary or Secondary or Acute or Subacute).ti,ab. 13. or/9-12 14. 3 and 8 and 13 15. limit 14 to animals

2.2 Inclusion criteria

We included all animal studies in which axonal or myelin sheath pathophysiology was the main subject of the study. Exclusion criteria consisted of human studies, review articles and studies lacking quantitative report of the findings.

2.3 Data extraction and the quality control

A detailed review of the methodology of searching, screening, and summarizing articles is in our previous studies (6-13). In summary, two independent reviewers selected related articles based on the inclusion and exclusion criteria by reading the title and abstract and then the full text. A checklist was used to extract the related data of full papers. This checklist was designed based on the PRISMA Guideline. These variables included the name of the first author of the article, year of publication, the number of samples, species and genus of the animals, SCI model including compression injury, contusion (weight-drop apparatuses, electromagnetic impactors) model, crush injury, hemisection, and transection, as well as severity of injury, level of injury, tracer of axon and myelin, follow up durations and outcomes. Classification of injuries and determining injury severity was done based on the definition given in the article by

Cheriyian et al. (14). In addition, duration of follow up was divided into three groups of immediate-acute, sub-acute, and chronic phases.

In many articles, data were presented graphically. In these cases for extraction of data plot digitizer software version 2.0 was applied; a method that has higher accuracy and speed compared to the manual method (15). Finally, the quality control of the studies was performed according to the guidelines provided by Hassannejad et al. (16).

2.4 Outcomes

The outcomes in the present study were classified in six sections, including 1) total number of axons, 2) number of myelinated axons, 3) rate of axonal retrograde degeneration, 4) myelin sheath thickness based on G-ratio (inner region of axon/total thickness of axon including myelin sheath), 5) internode length (distance between adjacent Ranvier nodes), and 6) axon conduction velocity. The effect of SCI on each evaluated outcome was assessed in three phases of immediate-acute (0 to 4 days after SCI), sub-acute (5 to 13 days), and chronic (14 and later).

2.5 Statistical analyses

Data were analyzed in STATA statistical software 14.0. Data were summarized as mean and standard deviation and effect size with 95% confidence interval (95% CI) was calculated using “metan” command. In case of the presence of heterogeneity ($I^2 \geq 50\%$ or $p < 0.1$) random effect model was used and in other cases fixed-effect model was applied. The presence of publication bias was assessed based on Egger’s suggested method and drawing funnel plot. It should be noted that subgroup analysis was performed based on location of assessment (lesion site, rostral or caudal to injury), injury model, level of injury (cervical, thoracic and thoracolumbar), severity of injury, and animals’ species. Findings of subgroup analyses were reported as standardized mean difference (SMD) and for comparing the subgroups, odds ratio (OR) and 95% CI were reported. Subgroup analysis was performed when the results were reported in at least three separate experiments. In all analyses, $p < 0.05$ was considered as the level of significance.

3 Results:

3.1 Characteristics of included studies

Thirty nine related articles were included involving 789 experimental animals (17-55) (Figure 1). Twenty nine studies were performed on rats, eight on mice, one on cats, and one on monkeys. Models used to develop SCI were contusion in 11 studies, hemisection in nine, transection in seven, compression in six, crush in six, and dislocation and distraction in only one study. Thoracic region injury (25 papers) and cervical region (11 papers) were the most common levels of injury induction. Duration of follow up of the animals varies between one and 450 days (Table 2).

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Table 1: Summary of included studies

Authors, Year	Sample size* control / SCI	Gender; Species	Injury model	Level of injury	Staining	Follow up duration (days)	Outcome
Anthes et al., 1995 (17)	6 / 6	F; Wistar rats	Compression	C8-T1	Toluidine blue	1	Axon number
Arvanian et al., 2009 (18)	4 / 4	F; Sprague–Dawley rats	Hemisection	T10	Toluidine blue	14, 42	Axon number; G-ratio
Bretzner et al., 2008 (19)	NA / 7	M; Sprague– Dawley rats	Crush	C4-C5	BDA	42	Dieback
Busch et al., 2009 (21)	NA / 3	F; Sprague–Dawley rats	Crush	T1	Texas Red-conjugated	2, 4, 7, 14, 28	Dieback
Busch et al., 2011 (20)	NA / 4	F; Sprague–Dawley rats	Crush	T1	Texas Red-conjugated	2, 4, 7	Dieback
Choo et al., 2008 (22)	9 / 10	M; Sprague– Dawley rats	Contusion; dislocation; distraction	C4-C5	Fluorescein-dextran and cascade blue-dextran	1	Axon number
Darlot et al., 2012 (23)	13 / 19	F; Sprague–Dawley rats	Hemisection	C2-C3	Fluorogold-fluororuby	7, 90	Axon number
Ek et al., 2010 (24)	3 / 3	M; Sprague– Dawley rats	Contusion	T10	Methylene blue	1, 7, 28, 70	Axon number
Ek et al., 2012 (25)	4 / 4	NR.; Rats	Contusion	T10	Luxol Fast Blue	1, 7, 28, 70	Axon number
Evans et al., 2014 (26)	NA / 3	M and F; Transgenic mice	Crush	T10	CX3CR1 GFP/+	1, 2, 5, 8	Dieback
Fehlings et al., 1995 (27)	5 / 5	F; Wistar rats	Compression	T1	Horseradish peroxidase	42	Axon number
Gensel et al., 2015 (28)	NA / 3	F; Sprague–Dawley rats	Crush	C8	Texas Red-conjugated	4, 8	Dieback
Gledhill et al., 1977 (29)	3 / 14	NR; Cat	Compression	T9-T10	NR	180	Internode length
Guest et al., 1997 (30)	NA / 3	F; Wistar rats	Transection	T11-T12	BDA	35	Dieback
Hesp et al., 2015 (31)	4 / 5	F; Sprague–Dawley rats	Contusion	T8	GFP-NF	28	Axon number; G-ratio

Horn et al., 2008 (32)	NA / 3	F; Sprague–Dawley rats	Crush	C8	Texas Red-conjugated	2, 4, 7, 14, 28	G-ratio
Houle and Jin, 2001 (33)	NA / 3	F; Sprague–Dawley rats	Hemisection	C3	BDA	7, 28, 56, 98	Dieback
Huang et al., 2014 (34)	3 / 3	NR; Sprague–Dawley rats	Compression	L1	Osmic acid staining	1, 3, 7	Axon number; G-ratio
James et al., 2011 (35)	5 / 5	F; Sprague–Dawley rats	Contusion	T10	Eriochrome cyanine R	1, 7, 14, 28, 84, 180	Axon number; G-ratio; velocity
Kerschensteiner et al., 2005 (36)	NA / 10	NA; Transgenic GFP-S mice	Transection	C2-C6	GFP-labeled axon	1, 2	Dieback
Lasiene et al., 2008 (37)	8 / 8	F; C57BL/6; Mice	Contusion	T9	BDA	56	Axon number; G-ratio; velocity; Internode length
Muradov et al., 2013 (38)	4 / 4	F; Sprague–Dawley rats	Contusion	T9	Choleratoxin B	0, 1, 2, 7	Axon number
Nashmi and Fehlings, 2001 (39)	8 / 7	F; Wistar rats	Compression	T7	Toluidine blue	42	Axon number; G-ratio; velocity
Oudega et al., 1999 (40)	NA / 4	F; Fischer rats	Transection	T8	BDA	7, 14, 28, 56	Dieback
Pallini et al., 1988 (41)	NA / 4	F; Wistar rats	Transection	T9	HRP	5, 14, 28, 56	Dieback
Powers et al., 2012 (42)	3 / 18	F; Gt(ROSA)26Sor mice	Contusion	T9-T10	Tetramethylrhodamine dextran	90	Velocity; Internode length
Powers et al., 2013 (43)	5 / 5	F; Gt(ROSA)26Sor mice	Contusion	T9-T10	mG+ sheaths	30, 90, 180	Axon number; G-ratio; Internode length
Rosenberg and Wrathall, 1997 (44)	4 / 3	F; Sprague–Dawley rats	Contusion	T8	Toluidine blue	1	Axon number
Seif et al., 2007 (45)	N / 5	F; Sprague–Dawley rats	Hemisection	T8	DiI	7, 14, 28, 56, 112	Dieback
Siegenthaler et al., 2007 (46)	24 / 24	F; Sprague–Dawley rats	Contusion; Hemisection	T10	Resin	60	Axon number; G-ratio
Stirling et al., 2004 (48)	NA / 6	NA; Wistar rats	Transection	C7	BDA	7, 14	Dieback

Stirling et al., 2013 (47)	NA / 7	NA; Cx3cr1 mice	Transection	brainstem	Nile Red	1	Dieback
Tang et al., 2015 (49)	NA / 6	M; Transgenic mice	Hemisection	T12	Texas Red dextran	1, 2	Dieback
Totoiu and Keirstead, 2005 (50)	4 / 4	F; Sprague–Dawley rats	Contusion	T10	Toluidine blue	7, 14, 28, 70, 120, 450	Axon number; G-ratio
Wang et al., 2009 (52)	6 / 3	F; Sprague–Dawley rats	Transection	T8	NF200	0, 10, 30	Axon number
Wang et al., 2012 (51)	NA / 6	F; Sprague–Dawley rats	Hemisection	T9	BDA	56	Dieback
Wang et al., 2015 (53)	NA / 8	F; C57BL/6 mice	Hemisection	C5	NF200	5, 56	Dieback
Ward et al., 2014 (54)	5 / 4	F; Sprague–Dawley rats	Compression	T12	NF200 and SMI31 and SMI32	1, 3, 7	Axon number
Wu et al., 2013 (55)	3 / 3	M; Macaca fascicularis; Monkey	Hemisection	T8-T9	SMI31	7, 30	Axon number

*, number of animals per group. BDA: Biotinylated dextran amines; DiI: Diiododecyl-tetramethyl-indocarbocyanine; GFP: Green fluorescent protein; HRP: Horseradish peroxidase; NF200: Neurofilament-200; NR: Not reported.

3.2 The risk of bias

The quality assessment of included studies is presented in figure 2 and table 3. The status of most studies regarding bladder expression (19 studies), blinding of assessor (27 studies), reporting genetic background (22 studies), description of treatment allocation (32 studies) and description of the reasons to exclude animals from the experiment during the study (32 studies) are at high risk of bias. The status of other items is low risk in most of the articles. Some degree of publication bias exists only when mean axonal retrograde degeneration (coefficient=-0.93; $p<0.0001$) and probably internode length (coefficient= 5.14; $p=0.058$) (Figure 2).

Table 3: The quality assessment of included studies

Author, Year	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Anthes et al., 1995 (17)	+	+	+	+	+	+	+	+	+	+	+	?	?	?	?
Arvanian et al., 2009 (18)	+	+	+	+	+	+	+	+	+	+	+	+	?	?	+
Bretzner et al., 2008 (19)	+	+	+	+	+	+	+	+	+	+	?	+	+	?	?
Busch et al., 2009 (21)	+	+	+	+	+	+	+	+	+	+	?	?	+	?	?
Busch et al., 2011 (20)	+	+	+	+	+	+	+	+	+	+	?	?	+	?	?
Choo et al., 2008 (22)	+	+	+	+	+	+	+	+	?	+	?	+	?	?	+
Darlot et al., 2012 (23)	+	+	+	+	+	+	+	+	+	+	?	+	?	?	+
Ek et al., 2010 (24)	+	+	+	+	+	+	+	+	+	+	?	+	?	?	+
Ek et al., 2012 (25)	+	+	+	+	+	+	?	+	+	+	?	?	?	?	?
Evans et al., 2014 (26)	+	+	+	+	+	+	+	+	+	+	?	?	+	?	?
Fehlings et al., 1995 (27)	+	+	+	+	+	+	+	+	+	?	?	+	?	+	?
Gensel et al., 2015 (28)	+	+	+	+	+	+	+	+	+	+	+	?	+	?	?
Gledhill et al., 1977 (29)	+	+	+	+	?	+	?	+	+	?	?	?	?	?	?
Guest et al., 1997 (30)	+	+	+	+	+	+	+	+	+	+	?	?	+	?	+
Hesp et al., 2015 (31)	+	+	+	+	+	+	+	+	+	+	+	?	?	?	?
Horn et al., 2008 (32)	+	+	+	+	+	?	+	?	+	+	?	?	?	?	?
Houle and Jin, 2001 (33)	+	+	+	+	+	+	+	?	?	+	?	?	?	?	?
Huang et al., 2014 (34)	+	+	+	+	+	?	+	+	+	+	?	+	?	+	?
James et al., 2011 (35)	+	+	+	+	+	+	+	?	+	+	+	?	?	?	?
Kerschensteiner et al., 2005 (36)	+	+	+	+	+	+	+	+	+	+	?	?	+	?	?
Lasiene et al., 2008 (37)	+	+	+	+	+	+	+	+	+	+	+	?	+	?	?

Muradov et al., 2013 (38)		+	+	+	+	+	+	+	+	+	+	+	+	?	+	?	?
Nashmi and Fehlings, 2001 (39)		+	+	+	+	+	+	+	+	+	+	+	?	?	?	?	?
Oudega et al., 1999 (40)		+	+	+	+	+	+	+	+	+	+	+	+	?	+	?	?
Pallini et al., 1988 (41)		+	+	+	+	+	+	+	?	?	?	+	?	?	?	?	?
Powers et al., 2012 (42)		+	+	+	+	+	+	?	+	+	+	?	?	?	?	?	?
Powers et al., 2013 (43)		+	+	+	+	+	+	+	+	+	+	+	+	?	+	?	?
Rosenberg and Wrathall, 1997 (44)		+	+	+	+	+	+	+	+	+	?	+	?	?	?	?	?
Seif et al., 2007 (45)		+	+	+	+	+	+	+	+	+	+	+	+	?	?	?	?
Siegenthaler et al., 2007 (46)		+	+	+	+	+	+	+	?	+	+	+	+	?	?	?	?
Stirling et al., 2004 (48)		+	+	+	+	+	+	+	+	+	+	+	+	+	+	?	+
Stirling et al., 2013 (47)		+	+	+	+	+	+	+	+	+	+	+	?	+	+	?	?
Tang et al., 2015 (49)		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Totoiu and Keirstead, 2005 (50)		+	+	+	+	+	+	+	?	+	+	+	+	?	?	+	?
Wang et al., 2009 (52)		+	+	+	+	+	+	+	+	+	+	+	+	?	?	?	?
Wang et al., 2012 (51)		+	+	+	+	+	+	+	+	+	+	+	+	?	+	+	?
Wang et al., 2015 (53)		+	+	+	+	+	+	+	+	+	+	+	?	?	+	+	?
Ward et al., 2014 (54)		+	+	+	+	+	+	+	+	+	+	+	+	+	?	?	?
Wu et al., 2013 (55)		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	?
1. Species; 2. Using appropriate tests; 3. Severity of injury; 4. Level of injury; 5. Age/weight; 6. Number of animals per group; 7. Designation of strain; 8. Definition of control; 9. Description of statistical analysis; 10. Regulation and ethics; 11. Bladder expression; 12. Blindness of assessor; 13. Genetic background; 14. Method of allocation to treatments; 15. Description of the reasons to exclude animals from the experiment during the study (attrition) +: indicates no risk of bias; ?: the presence of risk of bias is unclear due to insufficient descriptions in the article																	

3.3 Meta-analysis

3.3.1 *The number of axons decreases following SCI*

Analyses showed that immediately after SCI (immediate-acute phase) the total number of axons decreased in the lesion site of the injured spinal cord (SMD= -3.86; 95% CI: -5.02 to -2.69). This decreasing trend continued in the sub-acute phase (SMD= -4.95; 95% CI: -6.88 to -3.88) and reached its maximum in the chronic phase (SMD= -5.98; 95% CI: -7.56 to -4.40) ($P_{\text{for trend}} < 0.0001$) (figure 3).

Subgroup analysis revealed that in the immediate-acute phase, the rate of losing axons in the lesion site was higher compared to 1-10 mm rostral ($p < 0.0001$) and 1-10 mm caudal regions ($p = 0.001$). In addition, the decrease of axon number in the injuries induced by the compression-contusion model was more evident than that in other models ($p < 0.0001$). Yet, the rate of axonal degeneration was higher in severe injuries ($p = 0.009$).

In the sub-acute phase, it was revealed that in injuries caused by the compression-contusion model ($p < 0.05$) the rate of axonal degeneration was higher than other models, while the rate of axonal degeneration in thoracic ($p = 0.21$) and thoracolumbar ($p = 0.013$) injuries was higher than cervical injuries. Finally, in the chronic phase, it was found that the number of degenerated axons in the lesion site ($p < 0.05$) and in the thoracic level were higher than other levels (table 4).

3.3.2 *The number of myelinated axons declines following SCI*

The findings of the meta-analysis showed that the number of myelinated axons also was altered after SCI. In the immediate-acute phase of SCI, the number of myelinated axons in the lesion site decreased significantly (SMD= -2.55; 95% CI: -3.12 to -1.98; $p < 0.0001$). The decrease was many times greater in sub-acute (SMD= -4.70; 95% CI: -6.29 to -3.10; $p < 0.0001$) and chronic injuries (SMD= -7.29; 95% CI: -9.67 to -4.91; $p < 0.0001$) phases ($P_{\text{for trend}} < 0.0001$).

The analyses performed in the immediate-acute phase showed that the number of myelinated axons in the lesion site was less than caudal regions of injury ($p = 0.01$). In the sub-acute phase, none of the evaluated factors affected the number of myelinated axons. However, in the chronic phase, the myelinated axons number in the 1 to 10 mm caudal region of the injury was greater than the lesion site ($p = 0.004$) (table 5).

3.3.3 *Axonal retrograde degeneration (dieback) is progressively seen after SCI*

Mean axonal retrograde degeneration in the immediate-acute phase in the rat was 461.65 μm . This amount was 734.07 μm and 1155.86 μm in sub-acute and chronic phases, respectively

(figure 3). Analyses showed that as time passed after injury, the extent of axonal retrograde degeneration progressively increased ($P_{\text{for trend}} < 0.0001$).

Subgroup analysis showed that in the immediate-acute phase, the grade of axonal retrograde degeneration in the transection ($p=0.031$) model was lower than the crush model. In the sub-acute phase, none of the factors had any effect on axonal retrograde degeneration. However, in the chronic injuries, the extent of axonal retrograde degeneration observed in the thoracic region was up to 3.58 times greater than the cervical region ($p=0.002$) (table 6).

3.3.4 Myelin Sheath thickness decreases following SCI

For assessment of the effect of SCI on myelin sheath thickness, G-ratio scale was applied. Analyses showed that the amount of G-ratio in the immediate-acute (SMD=4.35; 95% CI: 1.86 to 6.85; $p < 0.0001$), sub-acute (SMD=3.43; 95% CI: 2.26 to 4.60; $p < 0.0001$), and chronic (SMD=2.01; 95% CI: 1.43 to 2.60; $p < 0.0001$) phases were higher than in healthy animals (figure 4). G-ratio decreased with time passed since the injury and gradually became closer to the measures in normal animals ($P_{\text{for trend}} = 0.035$). The data also revealed that G-ratios in severe injuries ($p=0.04$) were higher than other injury intensities (table 7).

3.3.5 Following SCI, internode length significantly decreases

Internode length is considered as a factor for evaluating myelination status. Following SCI, with the presence of oligodendrocytes at the site of injury, myelination was initiated (29, 35, 37, 42, 43). Yet, the myelinated segments of regenerated axons were shorter than uninjured axons (SMD=-2.15; 95% CI: -2.68 to -1.62; $p < 0.0001$) (figure 4).

3.3.6 Conduction velocity of regenerated axons is less than healthy axons

In evaluating the conduction velocity, it was shown that the conduction of neural messages in regenerated axons was many times slower than unaffected axons (SMD=-5.38; 95% CI: -7.40 to -3.36; $p < 0.0001$). These findings are in line with the other two findings that showed both myelin sheath thickness and internode length decreased significantly following SCI.

Table 4: Subgroup analysis of total number of axon after spinal cord injury compare to intact animals

Variable	Effect size			Significance among subgroups	
	SMD (95% CI)	p value	Heterogeneity (p value)	Odds ratio (95% CI)	p value
Immediate and acute phase					
Overall	-1.21 (-1.56 to -0.86)	<0.0001	78.0% (<0.0001)	NA	NA
Location of assessment					
Lesion site	-3.86 (-5.02 to -2.69)	<0.0001	84.2% (<0.0001)	<i>Ref.</i>	<i>Ref.</i>
1 to 10 mm rostral	-0.48 (-0.94 to -0.03)	0.037	71.0% (<0.0001)	14.41 (3.51 to 59.13)	<0.0001
1 to 10 mm caudal	-0.69 (-1.09 to -0.28)	0.001	64.7% (<0.0001)	12.57 (3.09 to 51.18)	0.001
Injury model					
Compression-contusion	-2.36 (-2.92 to -1.79)	<0.0001	89.5% (<0.0001)	<i>Ref.</i>	<i>Ref.</i>
Transection	0.32 (-0.39 to 1.05)	0.374	46.5% (0.07)	14.13 (2.70 to 74.01)	0.002
Other	-0.17 (-0.47 to 0.14)	0.284	47.9% (0.013)	8.27 (2.58 to 26.45)	0.001
Level of Injury					
Cervical	-0.38 (-0.72 to -0.06)	0.022	69.2% (<0.0001)	<i>Ref.</i>	<i>Ref.</i>
Thoracic	-0.64 (-1.26 to -0.01)	0.045	70.2% (<0.0001)	0.04 (0.003 to 2.00)	0.816
Severity					
Moderate	-0.35 (-0.67 to -0.03)	0.034	66.6% (<0.0001)	<i>Ref.</i>	<i>Ref.</i>
Severe	-2.22 (-2.89 to -1.54)	<0.0001	80.4% (<0.0001)	0.21 (0.07 to 0.68)	0.009
Subacute phase					
Overall	-2.67 (-3.37 to -1.98)	<0.0001	81.9% (<0.0001)	NA	NA
Location of assessment					
Lesion site	-4.95 (-6.88 to -2.02)	<0.0001	78.2% (<0.0001)	<i>Ref.</i>	<i>Ref.</i>
1 to 10 mm rostral	-1.88 (-2.76 to -0.99)	<0.0001	82.8% (<0.0001)	12 .05 (0.90 to 160.70)	0.059
1 to 10 mm caudal	-2.39 (-3.50 to -1.28)	<0.0001	71.2% (<0.0001)	8.28 (0.53 to 130.03)	0.128
Injury model					
Compression-contusion	-4.35 (-5.46 to -3.23)	<0.0001	73.4% (0.081)	<i>Ref.</i>	<i>Ref.</i>
Hemisection	-0.21 (-0.78 to 0.36)	0.470	55.5% (0.028)	32.31 (4.18 to 249.42)	0.001
Transection	-1.61 (-2.85 to -0.37)	0.011	73.9 (<0.0001)	11.41 (1.44 to 90.20)	0.022
Level of Injury					
Cervical	0.015 (-0.21 to 0.5)	0.418	0.0% (0.817)	<i>Ref.</i>	<i>Ref.</i>
Thoracic	-3.15 (-4.17 to -2.14)	<0.0001	78.3% (<0.0001)	0.04 (0.003 to 0.61)	0.021
Thoracolumbar	-3.52 (-4.52 to -2.51)	<0.0001	41.5% (0.081)	0.02 (0.001 to 0.41)	0.013

Species					
Rat	-2.71 (-3.43 to -1.99)	<0.0001	82.6% (<0.0001)	<i>Ref.</i>	<i>Ref.</i>
Other	-2.55 (-5.8 to 0.7)	0.124	73.0% (0.025)	1.43 (0.02 to 103.04)	0.867
Chronic phase					
Overall	-3.78 (-4.56 to -3.01)	<0.0001	83.3% (<0.0001)	NA	NA
Location of assessment					
Lesion site	-5.98 (-7.57 to -4.40)	<0.0001	76.2% (<0.0001)	<i>Ref.</i>	<i>Ref.</i>
1 to 10 mm rostral	-2.48 (-3.44 to -1.51)	<0.0001	80.5% (<0.0001)	17.24 (1.42 to 208.24)	0.026
1 to 10 mm caudal	-2.95 (-4.42 to -1.48)	<0.0001	82.1% (<0.0001)	17.14 (1.20 to 245.41)	0.037
Injury model					
Compression-contusion	-4.76 (-5.87 to -3.65)	<0.0001	81.4% (<0.0001)	<i>Ref.</i>	<i>Ref.</i>
Hemisection	-1.06 (-1.98 to -0.133)	0.025	71.6% (0.001)	17.69 (0.97 to 322.78)	0.052
Transection	-3.43 (-5.45 to -1.41)	0.001	84.2% (<0.0001)	3.31 (0.18 to 62.50)	0.416
Level of Injury					
Cervical	-0.27 (-0.69 to 0.14)	0.21	0.0% (0.726)	<i>Ref.</i>	<i>Ref.</i>
Thoracic	-4.47 (-5.39 to -3.54)	<0.0001	81.3% (<0.0001)	0.01 (0.0005 to 0.38)	0.012
Severity					
Moderate	-4.94 (-6.01 to -3.87)	<0.0001	0.0% (0.443)	<i>Ref.</i>	<i>Ref.</i>
Severe	-3.63 (-4.45 to -2.82)	<0.0001	82.4% (<0.0001)	3.91 (0.11 to 134.85)	0.442
Species					
Rat	-3.69 (-4.48 to -2.91)	<0.0001	83.6% (<0.0001)	<i>Ref.</i>	<i>Ref.</i>
Other	-6.09 (-11.19 to -0.99)	0.019	64.1% (0.062)	6.04 (0.02 to 154.21)	0.516

CI: Confidence interval; NA: Not applicable; SMD: Standardized mean difference.

Table 5: Subgroup analysis of number of myelinated axon after spinal cord injury compare to intact animals

Variable	Effect size		Significance among subgroups		
	SMD (95% CI)	p value	Heterogeneity (p value)	Odds ratio (95% CI)	p value
Immediate and acute phase					
Overall	-1.86 (-2.27 to -1.44)	<0.0001	50.2 (<0.0001)	NA	NA
Location of assessment					
Lesion site	-2.55 (-3.12 to -1.98)	<0.0001	0.6% (0.440)	<i>Ref.</i>	<i>Ref.</i>
1 to 10 mm rostral	-1.76 (-2.48 to -1.03)	<0.0001	54.5% (0.010)	2.56 (0.93 to 7.08)	0.067
1 to 10 mm caudal	-1.33 (-1.98 to -0.67)	<0.0001	51.6% (<0.0001)	3.81 (1.41 to 10.30)	0.010
Subacute phase					
Overall	-3.32 (-4.12 to -2.52)	<0.0001	65.4% (<0.0001)	NA	NA
Location of assessment					
Lesion site	-4.70 (-6.29 to -3.10)	<0.0001	54.8% (0.024)	<i>Ref.</i>	<i>Ref.</i>
1 to 10 mm rostral	-3.52 (-4.42 to -2.64)	<0.0001	9.9% (0.353)	1.78 (0.22 to 14.43)	0.576
1 to 10 mm caudal	-1.87 (-2.99 to -0.75)	0.001	66.8% (0.002)	12.63 (1.76 to 90.72)	0.128
Level of Injury					
Thoracic	-4.63 (-6.32 to -2.94)	<0.0001	77.6% (<0.0001)	<i>Ref.</i>	<i>Ref.</i>
Thoracolumbar	-2.57 (-3.23 to -1.90)	<0.0001	28.7% (0.156)	0.27 (0.04 to 1.88)	0.18
Chronic phase					
Overall	-5.00 (-6.25 to -3.74)	<0.0001	82.5% (<0.0001)	NA	NA
Location of assessment					
Lesion site	-7.98 (-9.50 to -4.91)	<0.0001	81.4% (<0.0001)	<i>Ref.</i>	<i>Ref.</i>
1 to 10 mm rostral	-4.73 (-6.45 to -3.01)	<0.0001	63.3% (0.004)	6.91 (0.26 to 184.98)	0.239
1 to 10 mm caudal	-2.20 (-4.22 to -0.17)	0.034	84.6% (<0.0001)	128.16 (5.28 to 310.48)	0.004
Severity					
Moderate	-7.06 (-8.98 to -5.14)	<0.0001	0.0% (0.705)	<i>Ref.</i>	<i>Ref.</i>
Severe	-4.91 (-6.29 to -3.53)	<0.0001	81.9% (<0.0001)	0.01 (0.0001 to 5.90)	0.115

CI: Confidence interval; NA: Not applicable; SMD: Standardized mean difference.

Table 6: Subgroup analysis of mean axonal retrograde degeneration (dieback) after spinal cord injury in rat model

Variable	Effect size			Significance among subgroups	
	Mean* (95% CI)	p value	Heterogeneity (p value)	Odds ratio (95% CI)	p value
Immediate and acute phase					
Overall	461.65 (348.93 to 574.37)	<0.0001	92.8% (<0.0001)	NA	NA
Injury model					
Crush	501.87 (411.69 to 592.05)	<0.0001	84.9% (<0.0001)	<i>Ref.</i>	<i>Ref.</i>
Transection	181.00 (102.60 to 259.40)	<0.0001	0.0% (>0.99)	0.85 (0.74 to 0.98)	0.031
Level of Injury					
Cervical	504.64 (338.16 to 671.12)	<0.0001	91.1% (<0.0001)	<i>Ref.</i>	<i>Ref.</i>
Thoracic	434.20 (289.79 to 578.60)	<0.0001	91.0% (<0.0001)	0.93 (0.70 to 1.24)	0.570
Subacute phase					
Overall	734.07 (585.01 to 883.13)	<0.0001	96.0% (<0.0001)	NA	NA
Injury model					
Crush	667.96 (623.99 to 711.93)	<0.0001	0.0% (0.929)	<i>Ref.</i>	<i>Ref.</i>
Hemisectomy	896.08 (332.19 to 1459.98)	0.002	93.9% (<0.0001)	1.27 (0.79 to 2.06)	0.300
Transection	709.35 (438.30 to 980.40)	<0.0001	97.7% (<0.0001)	1.06 (0.70 to 1.62)	0.785
Level of Injury					
Cervical	644.63 (581.52 to 707.74)	<0.0001	9.0% (0.359)	<i>Ref.</i>	<i>Ref.</i>
Thoracic	820.72 (611.64 to 1029.81)	<0.0001	97.2% (<0.0001)	1.28 (0.89 to 1.86)	0.172
Chronic phase					
Overall	1155.86 (853.58 to 1715.43)	<0.0001	96.7% (<0.0001)	NA	NA
Injury model					
Crush	960.54 (547.28 to 1373.81)	<0.0001	75.2% (0.018)	<i>Ref.</i>	<i>Ref.</i>
Hemisectomy	1284.51 (853.58 to 1715.43)	<0.0001	94.3% (<0.0001)	1.34 (0.30 to 5.94)	0.685
Transection	1096.90 (613.89 to 1579.91)	<0.0001	98.4% (<0.0001)	1.07 (0.22 to 5.34)	0.929
Level of Injury					
Cervical	573.55 (438.29 to 708.81)	<0.0001	56.9% (0.010)	<i>Ref.</i>	<i>Ref.</i>
Thoracic	1885.10 (1413.53 to 2356.66)	<0.0001	95.7% (<0.0001)	3.58 (1.70 to 7.52)	0.002

*, Data are presented as micrometer (μm); CI: Confidence interval; NA: Not applicable.

Table 7: Subgroup analysis of mean G-ratio after spinal cord injury

Variable	Effect size			Significance among subgroups	
	SMD (95% CI)	p value	Heterogeneity (p value)	Odds ratio (95% CI)	p value
Chronic phase					
Overall	2.01 (1.43 to 2.60)	<0.0001	82.0% (<0.0001)	NA	NA
Severity					
Moderate	1.56 (0.92 to 2.19)	<0.0001	65.6% (<0.0001)	<i>Ref.</i>	<i>Ref.</i>
Severe	3.05 (1.99 to 4.1)	<0.0001	66.7% (<0.0001)	4.37 (1.08 to 17.73)	0.040
Species					
Rat	2.56 (1.94 to 3.18)	<0.0001	62.4% (<0.0001)	<i>Ref.</i>	<i>Ref.</i>
Mice	0.32 (-0.21 to 0.85)	0.231	0.0% (0.957)	0.12 (0.4 to 0.36)	0.001

CI: Confidence interval; NA: Not applicable; SMD: Standardized mean difference.

4 Discussion:

A quantitative analysis was performed on axonal pathophysiology and the changes in myelin sheath following SCI. The results indicate that the number of axons and myelin structure changes after SCI. The number of axons (both total number of axons and myelinated axons) progressively decreases after SCI and remaining axons also gradually show retrograde degeneration, the highest rate of which is seen in the chronic phase of SCI. Regarding myelin, findings varied a little. After SCI, myelin sheath thickness decreased but in the chronic phase of injury, remyelination was induced and myelinated sheath reappeared around the axons gradually. However, thickness and length of this regenerated myelin were smaller than intact axons, leading to functional abnormalities. The evidence for this claim was the decrease in axon conduction velocity after SCI in the chronic phase. Table 8 depicts the most important findings of the present study in various phases of injury.

After SCI, degeneration of axon is observed and gradually exacerbates. The decrease of the axons number as well as significant axonal retrograde degeneration can be seen. However, regeneration of injured axons or sprouting of spared fibers is limited due to the presence of numerous endogenous barriers. For example, after SCI, nociception receptors such as ORL1 and Nogo receptors show an up-regulation, which is a preventive factor in axon growth (56, 57). Additionally, other inhibitory molecules related to myelin such as MAG, OMgp, and CSPGs are intensively expressed, which delay the axon regrowth after SCI (58). Among cellular factors, the presence of astrocytes, fibroblasts, microglia, macrophages, and other immune cells at the site of injury can be pointed out. The role of each of these mechanisms in SCI is under debate. For example, the presence of astrocytes at the site of injury and its adjacent tissues were reported to have a beneficial role for axon growth (59); some other studies showed that it could lead to the intensification of gliosis and inflammatory responses and delay in recovery (60, 61). Similar differences were also reported regarding the presence of microglia (62). Overall, it is likely that the factors restricting growth and axon elongation outweighed the factors inducing axonal regeneration (57).

Table 8: Summary of pathophysiological changes in axon and myelin after spinal cord injury according to injury phase.

Phase	Changes	Injury model	Level of injury	Severity of injury	Species
Immediate and acute					
1- Total number of axons	↓↓	Compression-contusion injury caused highest axon lost	Axon numbers is lower in thoracolumbar injuries	Axon numbers is lower in severe injuries	No data
2- Number of myelinated axons	↓↓	No data	No data	No data	No data
3- Occurrence of dieback	↑↑	Dieback in crush model of SCI is significantly higher	Level of injury has not any effect	No data	Dieback in mice is lower than rat
4- Myelin sheet thickness	↓↓↓↓??	No data	No data	No data	No data
5- Internode length of myelin	???	No data	No data	No data	No data
6- Axonal conduction velocity	↓↓↓↓??	No data	No data	No data	No data
Subacute phase					
1- Total number of axons	↓↓↓	Total number of axons is lower in hemisection and transection models	Total number of axons is lower in thoracic and thoracolumbar injuries	No data	The animal species has not any effect axons after SCI
2- Number of myelinated axons	↓↓↓	No data	Total number of myelinated axons did not differ in thoracic and thoracolumbar injuries. There is not data for cervical injuries	No data	No data
3- Occurrence of dieback	↑↑↑	No data	Level of injury has not any effect	No data	No data
4- Myelin sheet thickness	↓↓↓	No data	No data	No data	No data

5-	Internode length of myelin	????	No data	No data	No data	No data
6-	Axonal conduction velocity	↓↓↓??	No data	No data	No data	No data
Chronic phase						
1-	Total number of axons	↓↓↓	Injury model has not any effect	Total number of axons is lower in thoracic injuries	Severity of injury has not any effect	The animal species has not any effect
2-	Number of myelinated axons	↓↓↓	Injury model has not any effect	No data	Severity of injury has not any effect	No data
3-	Occurrence of dieback	↑↑↑	Injury model has not any effect	Mean dieback is higher in thoracic injuries	No data	No data
4-	Myelin sheet thickness	↓	No data	No data	Myelin sheet is thinner in severe injuries	No data
5-	Internode length of myelin	↓↓↓	No data	No data	No data	No data
6-	Axonal conduction velocity	↓	No data	No data	No data	No data

↓, Decrease.

↓?: Decrease but there is not enough data.

↑: Increase.

???: There is not enough data.

The point that was determined in subgroup analysis was the role of SCI model on the number of axons and retrograde axonal degeneration. In the immediate-acute phase, the rate of neuron degeneration in the injuries caused by compression-contusion (crash model for retrograde axonal degeneration) was more than other models. However, in the chronic phase, the injury model did not affect the number of axons and dieback. In injuries caused by transection or hemisection, only focal tissue damage with less apoptosis, demyelination, and extension of injury were reported as well as less inflammation process (63, 64). However, inflammation, apoptosis and cellular damage are more severe in the contusion/compression models than the hemisection/transection models. In the compression/contusion model, a larger area would be affected due to the width of the clip or diameter of the weight drop apparatus. Also, an initial compression force lead to the immediate necrosis. However, in the transection model, there is no compression force, only the incision of the cord disturb the integrity of the axons and vessels in the transected area triggering the secondary injury at a later time. Thus, greater axonal loss in the contusion/compression model is expected. Therefore, some researchers believe that transection is not a proper model for evaluating tissue damage following SCI (63).

Among other factors affecting the axons number and degeneration following SCI is the level of injury. The rate of axon degeneration in thoracic injuries was reported to be higher than cervical injuries. In addition, it was found that mean retrograde degeneration of axons in the thoracic region was up to 3.5 times more than the cervical region injuries. The reason for this difference is not known and further studies are needed. However, studies show that in cervical region injuries, more recovery is observed compared to thoracic region injuries (65-69), which might be due to the number of live and active axons being present in cervical regions following injury.

Demyelination following SCI is a result of the rapid death of oligodendrocytes. Numerous studies are available to show that express apoptosis of oligodendrocytes following SCI is closely associated with demyelination (24, 25, 34, 38). In the chronic phase of SCI, oligodendrogenesis is seen when remyelination occurs (31, 52, 54). This compensation mechanism leads to a decrease in myelin debris and reduction in the number of degenerated myelin but the measures of these pathologies do not ever return to the normal level (25, 50). It is likely that myelin synthesized after SCI has both shorter length and smaller thickness compared to intact myelin, probably due to the difference in the mechanism of myelin synthesis between the periods of prenatal development and adulthood. Myelin synthesized at fetal life is able to become up to 40 times thicker and longer, while myelin synthesized in adulthood is shorter and thinner. In fetal life, the ratio of the oligodendrocytes to axons number is 1:1, while in adulthood this ratio reaches about 1:60. Therefore, it is likely that the decrease of oligodendrocytes in adulthood may explain the demyelination following SCI (70). This shortening of the space between two Ranvier nodes as well as myelin diameter becoming thinner is associated with changes in myelin function. Therefore, the conduction velocity in remyelinated axons is slower than intact

axons. There is evidence that shows axon conduction velocity decreases progressively with an increase of remyelinated segment numbers in the axon (71) and this decrease is even greater than the rate estimated in theoretical models.

There is still controversy regarding the extent to which pathologic changes are in line with the recovery observed after SCI. For example, Li et al. showed that nerve regeneration at the site of injury did not have an effect on recovery following SCI, the recovery observed was mostly due to the changes occurring in the caudal regions of injury (72). In addition, Jack et al. showed that the locomotion outcome of the animals under treatment becomes worse after electrical stimulation of the corticospinal tract, which was reported to promote significant axonal collateralization (73). However, two other studies show that axonal outgrow, following the use of electrical stimulation, is associated with improvement in locomotion after SCI (74, 75). It is likely that treatment interventions that don't independently result in axonal regeneration cannot lead to a significant improvement in motor function recovery and might even make it worse; because without the use of rehabilitation training program, proper synapse connections are not formed and this will lead to delay in recovery (73). These axonal collaterals may even intensify pain pathways in incomplete injuries and lead to neuropathic pain (76, 77).

The thoracic spinal cord and the cervical spinal cord may respond to the injury differently. In this systematic review, we found that the level of injury could affect the pathophysiology of SCI. one reason may be the distance of the axotomized location to the cell soma. For example, the nucleus of the supraspinal neurons are found in the brainstem and the axons pass the cord to the thoracic level. In addition, propriospinal neurons, which are intrinsic neurons of the spinal cord, could be divided into two groups: a) the short thoracic propriospinal (TPS) neurons are located in the thoracic level and their axons project rostrally or caudally for a few levels. b) The long projection propriospinal neurons include long ascending propriospinal tract (LAPT) neurons and long descending propriospinal tract (LDPT) neurons. The LAPT neurons are found in the lumbosacral enlargement that projects rostrally to the cervical enlargement, whereas the LDPT neurons are found in the cervical enlargement projecting mainly caudally to the lumbosacral enlargement. Therefore, there is a heterogeneity along the cord based on the type of the neurons and the length of the axons. Based on our previous systematic review on the fate of neurons after traumatic spinal cord injury in the rats (78), the propriospinal neurons have differential vulnerabilities to the contusion injury. The TPS neurons present an apoptotic response during the acute phase, but the LDPT neurons do not undergo apoptosis for at least one month. Therefore, the difference in the extent of axon degeneration after the cervical and the thoracic injury may be attributed to the cord heterogeneity.

One of the most common methods of measuring the status of myelin in the injured spinal cord is evaluating the volume of the myelinated area using myelin-specific staining such as Luxol fast blue staining or evaluating the expression of myelin sheath proteins such as basic myelin protein (56, 80). In the present study, the volume of the myelinated area was not evaluated since it would not provide the opportunity for assessing the rate of normal and regenerated myelin. Another limitation of the present study is the limited number of studies included in the section evaluating internode length and conduction velocity, which prevented subgroup analysis in this section. In addition, the risk of bias classification based on the quality of the studies was not feasible when there is no defined cut-off point. Moreover, interpretation of the findings in pre-clinical studies was not performed since there is no accurate definition of the level of evidence.

4.1 Conclusion:

Findings of the present meta-analysis indicated the difference in the pathophysiology of axons and myelin sheath in various phases of SCI. This difference in the structure of axons and myelin also leads to functional changes such as the decrease in conduction velocity. There is a higher rate of degeneration of axon and myelin in compression-contusion model, severe injuries, and thoracic injuries. In addition, there are still significant disagreements on the correlation between motor function recovery following SCI and axon regeneration. Further studies are needed to address whether the structural changes in the axons or other factors following SCI lead to motor and sensory functional recovery.

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Figure legends

Figure 1: PRISMA flow diagram of present meta-analysis

Figure 2: The risk of bias in assessed outcomes. Item 1. Species; Item 2. Using appropriate tests; Item 3. Severity of injury; Item 4. Level of injury; Item 5. Age/weight; Item 6. Number of animals per group; Item 7. Designation of strain; Item 8. Definition of control; Item 9. Description of statistical analysis; Item 10. Regulation and ethics; Item 11. Bladder expression; Item 12. Blindness of assessor; Item 13. Genetic background; Item 14. Method of allocation to treatments; Item 15. Description of the reasons to exclude animals from the experiment during the study (attrition).

Figure 3: Pathophysiological changes of axons after spinal cord injury. Mean retrograde axonal degeneration is presented in rat. CI: Confidence interval; SMD: Standardized mean difference.

Figure 4: Myelin Pathophysiological changes after spinal cord injury. CI: Confidence interval; SMD: Standardized mean difference.