REVIEW
Animal models of spinal cord injury: a systematic review

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Study Design: PRISMA-guided systematic review.
Objectives: To provide a comprehensive framework of the current animal models for investigating spinal cord injury (SCI) and categorize them based on the aims, patterns and levels of injury, and outcome measurements as well as animal species.
Setting: Sina Trauma and Surgery Research Center, Tehran University of Medical Sciences, Tehran, Iran.
Methods: An electronic search of the Medline database for literature describing animal models of SCI was performed on 1 January 2016 using the following keywords: 'spinal cord injuries' and 'animal models'. The search retrieved 2870 articles. Reviews and non-original articles were excluded. Data extraction was independently performed by two reviewers.
Results: Among the 2209 included studies, testing the effects of drug's or growth factor's interventions was the most common aim (36.6%) followed by surveying pathophysiologic changes (30.2%). The most common spinal region involved was thoracic (81%). Contusion was the most common pattern of injury (41%) followed by transection (32.5%) and compression (19.4%). The most common species involved in animal models of SCI was the rat (72.4%). Two or more types of outcome assessments were used in the majority of the studies, and the most common assessment method was biological plus behavioral (50.8%).
Conclusions: Prior to choosing an animal model, the objectives of the proposed study must precisely be defined. Contusion and compression models better simulate the biomechanics and neuropathology of human injury, whereas transection models are valuable to study anatomic regeneration. Rodents are the most common and probably best-suited species for preliminary SCI studies.
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INTRODUCTION

Spinal cord injury (SCI), a fundamental medical problem, is associated with severe disability and high rates of mortality. Although there is no definite treatment for SCI, various research studies are ongoing including experimental models to understand the anatomical and biological events involved in SCI and repair, and to test the safety and efficacy of potential therapies. Animal models are *in vivo* investigational methods used to analyze events under controlled conditions. Therefore, to be insightful for a given human condition, a disease or injury model should not only be similar in terms of the causation and function to the human analog but also must have advantages over simple clinical observation.[1,2] Animal models also allow for in-depth investigation of physiological and pathological events. An astute researcher should be capable of choosing a proper model and outcome assessment given the specific objectives. To select an appropriate model and test a specific hypothesis, all existing SCI animal models and related outcome assessments must be considered. So far, some reviews focused on the paradigms[3,4] or outcomes[5–7] in animal models of SCI. However, there is a gap in the literature for a complete categorization of animal models of SCI studies. This systematic review provides a comprehensive framework of the current animal models of SCI and categorizes them based on the study aims, patterns and levels of injury, and outcome measurements as well as animal species.

MATERIALS AND METHODS

This systematic review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) statement.[8] An electronic search of the Medline literature for animal models of SCI was performed (1946 to 1 January 2016), using the following conditions: SCI (MeSH Terms) AND (Models, Animal (MeSH Terms) OR Behavior, Animal/Physiology (MeSH Terms) OR Animal Experimentation (MeSH Terms)). Next the titles and abstracts of studies of the resultant articles were independently screened by two reviewers. Reviews and non-original articles were excluded at this stage. Full-texts of the remaining manuscripts were retrieved and further screened by reviewers against the exclusion/inclusion criteria without language restriction. Reports were excluded based on the following: review articles, articles not relevant to the experimental SCI, *in vitro* spinal cord preparations and overlapping publications of larger studies. In the latter case, the original studies that had used animal models of SCI were included in the systematic review. Data extraction from the resulting studies meeting inclusion criteria was performed. The purpose of data extraction was to obtain the goals, methods and outcome measurements from each study in a consistent manner so that the findings could be interpreted and analyzed (Supplementary 1). The data extraction form was pre-tested by all investigators using five sample studies. Agreement among reviewers in this pilot test was achieved by discussion to expose any ambiguity of the instrument and

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to improve subsequent reliability. Extraction was executed by the reviewers in the following manner: a reviewer would first extract data into the form and a second reviewer would confirm the accuracy of data extracted by the first reviewer. Any disagreements were resolved by a third reviewer. Full-text of studies in languages other than English would be screened by one reviewer only. For this purpose, the corresponding authors of non-English articles were asked to send the English version of research method via email. In case that no reply was received, translations were done using Google Translate. Descriptive summaries were conducted using SPSS (IBM Corp., Endicott, NY, USA).

RESULTS

Initial records identified through Medline literature searching included 2870 articles (Supplementary 2). The study selection procedure is summarized in Figure 1.

In total, 661 articles were excluded because they were reviews, non-original articles, not related to the experimental SCI, or a part of larger included studies. A total of 74 non-English articles were included.

Among the 2209 included studies, testing the effects of drug's or growth factor's interventions was the most common aim of studies (36.6%) followed by evaluating pathophysiologic changes (30.2%). Other aims and their frequencies based on injury patterns are shown in Table 1.

The most common spinal region studied was thoracic (1790, 81%) followed by cervical (265, 12%), lumbar (113, 5.1%), sacral (16, 0.7%), other (16, 0.7%) and unknown (64, 2.9%). In 54 studies (2.4%), SCIs were induced in multiple regions.

On the basis of the mechanism of injury, SCI models were classified as mechanical traumatic injury (94.5%) and non-mechanical injury (5.5%). Contusion as a transient force to displace and damage the spinal cord was the most common pattern of injury. Transection and compression which were characterized by compression of the spinal cord over an extended period of time were the next most frequent injury patterns. In 25 studies (1.1%), more than one pattern was used. Figure 2 shows all the types and subtypes of mechanical traumatic SCI patterns in 2087 related studies.

The injury extent was not reported in 722 studies (33%). In 785 studies (35%), the injury was described as complete, and incomplete injury was used in another 702 studies (32%), which included transection.
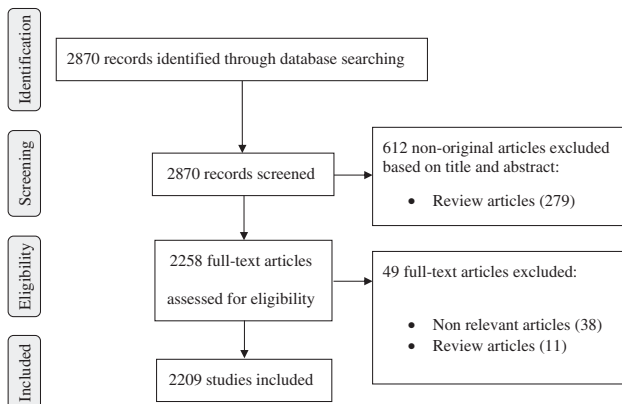

Figure 1 Flow diagram of summarized search procedure. A full color version of this figure is available at the *Spinal Cord* journal online.

Table 1 Study aims and injury patterns in 2209 animal model studies of SCI sorted by frequency

Aim of study, N (%)	Injury pattern, N(%)									
	Mechanical traumatic, 2087 (94.5)						Non-mechanical, 122 (5.5)			
	Contusion	Transection	Compression	Distraction/dislocation	Existent	Aspiration	Ischemic	Excitotoxic	Photochemical	Electrolytic
Effect of a drug or neurotrophic/growth factor interventions, 809 (36.6)	324	244	181	3	3	0	29	9	7	5
Pathophysiologic changes, 668 (30.2)	287	220	113	6	7	2	9	17	6	8
Effect of cell transplantation, 227 (10.3)	98	82	36	2	1	2	1	2	2	0
Characterization of a new model, 199 (9)	71	32	64	13	2	0	3	3	10	4
Behavior assessment, 86 (3.9)	30	46	12	0	0	0	0	0	0	1
Diagnosis, 64 (2.9)	34	16	11	0	1	0	1	1	0	1
Effect of scaffold, 46 (2.1)	9	33	2	0	0	2	0	0	0	0
Effect of electrical stimulation, 31 (1.4)	11	16	3	0	0	0	1	0	0	0
Effect of locomotor training therapies, 30 (1.4)	14	16	0	0	0	0	0	0	0	0
Effect of decompression/ column fixation, 15 (0.7)	7	4	4	0	0	0	0	0	0	0
Effect of other treatments, 12 (0.5)	6	5	1	0	0	0	0	0	0	0
Effects of hypothermia, 7 (0.3)	5	1	0	0	1	0	1	0	0	0
Effect of gene therapy, 7 (0.3)	4	1	1	0	0	0	0	0	0	0
Effect of x-irradiation, 5 (0.2)	4	1	0	0	0	0	0	0	0	0
Effect of cerebrospinal fluid drainage, 3 (0.1)	2	0	0	0	0	0	1	0	0	0
Total: 2209 (100)	906 (41)	717 (32.5)	428 (19.4)	24 (1.1)	15 (0.7)	6 (0.3)	46 (2.1)	32 (1.4)	25 (1.1)	19 (0.9)

Abbreviation: SCI, spinal cord injury.

Figure 1 flow diagram content:

- Identification: 2870 records identified through database searching
- Screening: 2870 records screened → 612 non-original articles excluded based on title and abstract: • Review articles (279)
- Eligibility: 2258 full-text articles assessed for eligibility → 49 full-text articles excluded: • Non relevant articles (38) • Review articles (11)
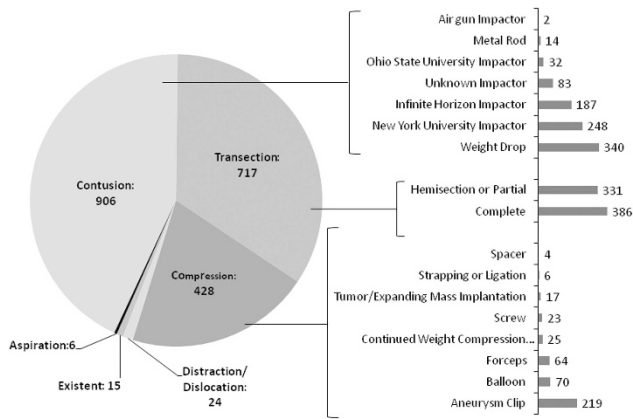- Included: 2209 studies included

Figure 2 Injury types and subtypes in 2087 animal model studies with mechanical traumatic SCI. A full color version of this figure is available at the *Spinal Cord* journal online.
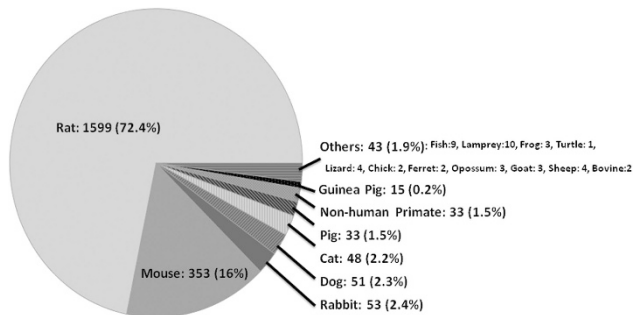

Figure 3 Species of animal used in 2209 animal model studies of SCI.

Rat was the most common species in the animal models of SCI. All species of animal used in studies are shown in Figure 3.

Outcome assessments were classified into seven categories which included biological, behavioral, neurophysiology, imaging, musculoskeletal, cardiovascular and respiratory evaluations (Table 2, Supplementary 3). Figure 4 is a schematic drawing of outcome assessments based on frequency and coincidence. With respect to the histological methods, chemistry techniques and behavioral methods used, these were expanded based on the specific tests, frequencies and features presented in Tables 3 and 4, separately.

Sixty-three percent of studies used two or more types of outcome assessments (Table 5). The most common combination of multiple outcome assessments was the usage of biology and behavior assessments.

DISCUSSION

Aim of studies

Testing the effects of drug's or growth factor's interventions was the most common aim of studies (36.6%) followed by evaluating pathophysiologic changes (30.2%). Because of the technical and ethical issues associated with human studies, most of the existing knowledge regarding SCI pathophysiology and related interventions is derived from animal studies.[9] Animal models provide opportunities to discern the cause and effect relationship between functional deficits and their essential biology. However, the heterogeneous nature of SCIs remains an obstacle to translating findings across different paradigms and severity of injuries as well as various species and to humans.[10] Table 1, showing the aims of studies based on injury patterns, could help

Table 2 Outcome assessments in 2209 animal model studies of SCI sorted by frequency[a]

Type, N (%)[a]	Subtype, N (% of type)[a]
Biology, 1827 (82.7)	Immunohistochemistry, 1154 (63.2)
	Staining and labeling[b], 1089 (59.6)
	Chemistry techniques[b], 644 (35.2)
	Genetic techniques, 371 (20.3)
	Flow cytometry, 42 (2.3)
	Blood–spinal cord barrier permeability, 34 (1.9)
	Cell Count, 30 (1.6)
Behavior[c], 1383 (62.6)	Locomotor tests, 1234 (89.2)
	Sensory tests, 225 (16.3)
	Sensory–motor tests, 184 (13.3)
	Autonomic tests, 92 (6.7)
	Reflex–response based tests, 78 (5.6)
Neurophysiology, 393 (17.8)	SSEPs, 162 (41.3)
	Motor-evoked potentials, 129 (32.8)
	Electromyography, 113 (28.8)
	Electroneurogram, 39 (9.9)
	Membrane potentials, 29 (7.4)
Imaging, 151 (6.8)	MRI, 108 (71.5)
	Computerized tomography, 19 (12.6)
	Functional MRI, 11 (7.3)
	X-ray, 8 (5.3)
	Ultrasonography, 6 (4)
	Positron emission tomography, 4 (2.6)
Cardiovascular evaluation, 61 (2.8)	
Musculoskeletal evaluation, 41 (1.9)	
Respiratory evaluation, 17 (0.8)	

Abbreviations: MRI, magnetic resonance imaging; SCI, spinal cord injury; SSEP, somatosensory evoked potentials.
[a]Some studies used more than one outcome assessment, simultaneously (See Table 5).
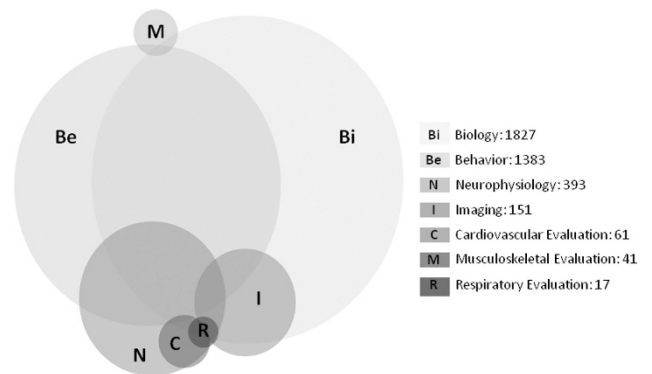[b]Expanded in Table 3.
[c]Expanded in Table 4.

Figure 4 Schematic drawing of outcome assessments based on frequency and coincidence in 2209 animal model studies of SCI.

researchers to select an appropriate model, especially about less common and non-mechanical models.

Region of injury

The most common region involved in SCIs in humans is the cervical.[11] However, these data indicate that thoracic SCIs are the most common location used in animal models (81%). On the basis of the literature, thoracic SCI models are apparently reliable and easy to reproduce.[12,13] As reduction of gray matter in this spinal region causes less identifiable functional loss, SCIs in thoracic levels permit the

Table 3 Staining and Labeling, and Chemistry Techniques in 2209 animal model studies of SCI, classified using MeSH database and sorted by frequency

Main technique, N (%)	Specific technique, N (% of main technique)	Reflects
Staining and labeling, 1089 (49.3)	H&E, 511 (46.9)	Spared tissue
	Luxol fast blue, 229 (21)	Myelin
	Cresyl violet, 223 (20.5)	Nissl bodies
	Tract tracing, 216 (19.9)	Neural pathways
	Osmium tetroxide, 210 (19.3)	Myelin, for electron microscopy
	Eriochrome (solochrome) cyanine, 75 (6.9)	Myelin
	Toluidine blue, 46 (4.2)	Nissl bodies
	Silver, 35 (3.2)	Axon distribution and vascularity
	Masson's Trichrome, Van Gieson or Sirius Red, 17 (1.6)	Collagen fibers
	Kluver–Barrera or Weil, 16 (1.5)	Myelin
	In Situ hybridization, 15 (1.4)	Nucleic acid
	Prussian blue, 4 (0.4)	Iron in the tissue
Chemistry techniques, 644(29.2)	Identification of proteins (western blotting, electrophoresis), 480 (74.5)	Protein interactions
	Peroxidase activity, 110 (17.1)	Leukocyte accumulation
	Biochemical analysis, 103 (16)	Chemical reactions or enzymatic activities
	High performance liquid chromatography, 23 (3.6)	Substance P
	Cerebrospinal fluid analysis, 18 (2.8)	Cerebrospinal fluid properties

Abbreviations: H&E, hematoxylin and eosin; SCI, spinal cord injury.

isolation and study of white matter deficits.[14] On the other hand, cervical SCIs can result in respiratory compromise and are associated with a high mortality rate due to interruption of the bulbospinal respiratory drive to phrenic motoneuron pools and diaphragm impairment.[14,15] However, cervical and thoracic spinal levels differ with respect to various anatomical aspects such as spinal cord diameter, distance between the cell bodies of injured axons and the injury site, relative dedication of the cord to specific ascending and descending pathways, degree of vascularization, size of the sensory and motor neuron populations and white/gray matter composition.[16] Therefore, thoracic SCI models may not be readily translatable to cervical injury patterns.[16] In the cervical region, white matter disruption leads to spastic paralysis below the level of injury, sensory loss and chronic pain, as well as gastrointestinal, cardiovascular and sexual dysfunction.[11] Furthermore, use of the hind limb paws and digits cannot be assessed as carefully as in the forelimb. Thus, forelimb evaluation could overestimate the efficacy of potential therapies, particularly when improvements are mild.[16,17] These differences and region-specific properties justify the development of cervical SCI models in recent years.[18] On the basis of these data, however, cervical SCI was done in only 12% of the studies considered.

Pattern of injury

In general, SCI models are selected based on the aims of the researchers. Every animal model and injury pattern is designed to focus on a specific question, and hence has associated consideration, advantages and disadvantages. Most human SCIs happen due to blunt trauma (for example, motor vehicle crash), where the spinal cord is damaged by an object or displaced bone or other tissue. On the basis of our data, 62% of included studies used a blunt trauma injury pattern such as contusion, compression, distraction, dislocation or existing traumatic SCI (for example, dogs injured in traffic accidents; Table 2). These models were developed in order to study post-traumatic lesional processes, and repair processes after spinal cord decompression or neuroprotective treatment.[19]

If the aim of study is investigation of pathophysiologic changes, a contusion and/or compression model is preferred, because most human SCIs involve contusion or compression.[20] Contusion is the

oldest and most commonly used for SCI models. The first reported SCI model was presented by Allen in 1911, where a mass was dropped from a prescribed height onto the dorsal surface of the canine dura. Most data available concerning induction of a contusive SCI are modifications of the injury model proposed by Allen.[21] The contusive models can make SCI in several severities which have been characterized by inflammation, ischemia, hemorrhagic necrosis and central cavitation. Our study showed that Weight Drop was the most common method of contusion (37.5%), followed by New York University (NYU) impactor (27.4%) and infinite horizon impactor (20.6%). Although the New York University impactor is rather easier to use, due to electromagnetic driving the infinite horizon and Ohio State University impactors have greater precision and more reliably produce SCIs.[22] Unilateral contusion or hemicontusion is typically used in cervical regions because life-threatening adverse effects could occur after complete cervical lesions. As motor dysfunction is more apparent in the forelimbs, pain-related behavior is difficult to estimate, and hence cervical contusion is frequently utilized only for motor functional analysis.[11]

Compression models are helpful to simulate the constant spinal canal occlusion that is common in human SCIs and study the effects of compression or the optimal timing of decompression. Appropriate devices for compressive SCI are shown in Table 2. On the basis of our data, more than half of the compression models use the aneurism clip that causes ischemia and mimics common clinical injuries.[23,24] The balloon-induction method is a simple technique that does not cause any damage to the surrounding structures.[25] Calibrated forceps can be used to produce a lateral compression injury by insertion on either side of the spinal cord.[1] A spacer can produce a precise degree of narrowing of the spinal canal and mimic an anteroposterior compression.[26]

Dislocation models cause injury by lateral displacement of vertebra, whereas distraction models apply opposing traction forces to stretch the spinal cord.[4]

Transection models are useful to study the effects of scaffolds, biomaterials and neurotrophic factors in SCI and may be more appropriate to investigate regeneration, degeneration, tissue engineering strategies or neuroplasticity.[27] Sometimes transection models are

Table 4 Behavior assessment tests in 2209 animal model studies of SCI sorted by frequency

Main test, N (%)	Specific test, N (% of main test)	Reflects
Locomotor, 1234 (55.9)	BBB, 609 (49.3)	Locomotion
	Open-field activity, 200 (16.2)	Locomotion
	Inclined plane, 145 (11.8)	Muscle strength
	Basso mouse scale, 115 (9.3)	Locomotion
	Automated walkway, 86 (7)	Locomotion
	Tarlov, 81 (6.6)	Locomotion
	Kinematic analysis during treadmill, 74 (6)	Locomotion
	Forelimb locomotor scale, 66 (5.3)	Locomotion
	Footprint, 55 (4.5)	Motor coordination
	Hind limb function, 41 (3.3)	Locomotion
	Swim, 36 (2.9)	Swimming ability
	Limb grip strength, 36 (2.9)	Muscle strength
	Food pellet reaching, 36 (2.9)	Motor coordination
	Forelimb asymmetry, 31 (2.5)	Paw preference
	Rotarod, 31 (2.5)	Locomotion
	Combined behavioral score, 29 (2.4)	Locomotion
	Rearing, 25 (2)	Paw preference
	Limb hanging, 14 (1.1)	Grasping
	Tail position, 4 (0.3)	Locomotion
	Eshkol–Wachmann notation, 1 (0.1)	Locomotion
Sensory, 225 (10.2)	Von Frey or Semmes–Weinstein, 128 (56.9)	Mechanical allodynia
	Pinch reflex, 109 (48.4)	Reflex
	Hot plate, 58 (25.8)	Temperature
	Cold tests, 17 (7.6)	Temperature
	Paw compression, 12 (5.3)	Pain
Sensory–motor, 184 (8.3)	Grid walking (foot fault), 80 (43.5)	Sensory–motor coordination
	Foot slip (horizontal ladder), 61 (33.2)	Sensory–motor coordination
	Grooming, 28 (15.2)	Sensory–motor connection
	Narrow beam walking, 28 (15.2)	Balance
	Rope walk testing, 10 (5.4)	Balance
Autonomic, 92 (4.2)	Urinary bladder function, 69 (75)	Bladder function
	Autonomic dysreflexia, 13 (14.1)	Autonomic dysreflexia
	Erection-based tests or mating, 6 (6.5)	Erection
	Telemetric monitoring, 6 (6.5)	Micturition erection
	Manometric or balloon Test, 4 (4.3)	Sphincter control
	Gastric motility, 3 (3.3)	Gastric function
Reflex–response, 78 (3.5)	Contact placing response, 43 (55.1)	Reflex
	Toe spread reflex, 28 (35.9)	Reflex
	Righting reflex (Static), 18 (23.1)	Reflex
	Hoffmann's reflex, 12 (15.4)	Reflex

Abbreviations: BBB, Basso, Beattie, Bresnahan scale; SCI, spinal cord injury.

Table 5 Applied types of outcome assessments in 2209 animal model studies of SCI

Types of outcome assessments, N (%)	The most common mode, N (%)
One type, 823 (37.3)	Biology, 574 (26)
Two types, 1122 (50.8)	Biology+behavior, 886 (40.1)
Three types, 245 (11.1)	Biology+behavior+neurophysiology, 148 (6.7)
Four types, 19 (0.9)	Biology+behavior+neurophysiology+imaging, 18 (0.8)

Abbreviation: SCI, spinal cord injury.

used to study the role of neuronal circuitries in locomotion.[28–31] On the other hand, transected spinal cords are rarely encountered in clinical practice, and thus the transection models do not truly reflect the pathophysiology of SCI and are not appropriate as a basis to test neuroprotective treatment.[32] Spinal cord transection is performed after laminectomy with fine surgical scissors (iridectomy scissors) to induce complete or targeted interruption of the spinal cord.[22] Therefore to maintain tissue continuity, a researcher can interrupt selective pathways via partial transections and preserve a tissue connection between the rostral and caudal ends of the spinal cord.[33] On the basis of our study, almost half of the transection models are incomplete or partial. (Table 2) Since a complete transection makes such a destructive tissue milieu, incomplete transection reduces the physical harm to the cord and the major cavitation and physical separation. Several studies have revealed different plasticity in the spinal cord after complete and incomplete transections.[31] Therefore, the researchers should be careful in the interpretation results focusing the extent of injury. A dorsal hemisection for selective interruption of the corticospinal tract can be performed with some feedback from the change in texture and color between the white and gray matter, giving an indication of the degree of hemisection.[1,34] However, dorsal hemisection cannot be used specifically to evaluate true axon regeneration.[33] Dorsolateral quadrant

Table 6 The three most commonly used outcome assessments in rat model studies with cervical and thoracic SCI

Level of Injury	Injury Pattern	Outcome assessments					
		Biology			Behavior		
		1	2	3	1	2	3
Cervical	Transection	IHC	Tract tracing	Cresyl violet	Food pellet reaching	Forelimb asymmetry	Kinematic analysis during treadmill locomotion
	Contusion	IHC	Cresyl violet	H&E	BBB	Limb grip strength	Forelimb asymmetry
	Compression	IHC	H&E	Tract tracing	BBB	Food pellet reaching	Forelimb asymmetry or Limb grip strength
Thoracic	Transection	IHC	Identification of proteins[a]	Tract tracing	BBB	Open-field activity	Inclined plane
	Contusion	IHC	Identification of proteins	H&E	BBB	Open-field activity	Inclined plane
	Compression	IHC	H&E	Identification of proteins	BBB	Open-field activity	Inclined plane

Abbreviations: BBB, Basso, Beattie, Bresnahan scale; H&E, hematoxylin and eosin; IHC, immunohistochemistry; SCI, spinal cord injury.
[a]Western blotting, electrophoresis.

lesions are used to disrupt the rubrospinal tract, and lateral hemisections interrupt all tracts on one side while sparing some or all tracts on the opposite side. The transection model is the most appropriately combined with neuroanatomical tract tracing and electrophysiological studies.[22,34]

Ischemic models are established using an anterior sternotomy with temporary aortic occlusion created by aneurysm clips. Spinal cord ischemia remains an unknown clinical dilemma which mostly occurs after aortic problems.[35–37] The present study showed that more ischemic models (63%) were performed to the test effects of drug's or growth factor's interventions after SCI.

Excitotoxic models replicate selected components of the secondary injury cascade. They are produced through intraspinal or intrathecal injection of some excitotoxins.[38]

Photochemical models are among the most reproducible ischemic models of SCI.[39] There is no need for laminectomy or mechanical trauma to the cord. After spinal column exposure and appropriate beam placement, a photosensitizing dye (for example, rose Bengal) is administrated systemically. The resultant photochemical reaction leads to vascular stasis and congestion, edematous pale-staining white matter tracts and hemorrhagic necrosis of the central gray matter.[7]

Electrolytic injury is used to study the neuronal pathways in the central nervous system. The technique involves the insertion of a fine metal electrode into the spinal cord. Restricted damage of cells in this region is accomplished by heating the electrode tip by passage of a high electric current.[40] On the basis of our review, 84% of electrolytic injury models were composed in the spinothalamic tract of rats to study behavioral outcomes, especially with central neuropathic pain.

Animal species
Our analysis demonstrated that rodents were the most commonly used species (92%; Figure 3). Although rodents are best suited for preliminary SCI studies,[41] large animal and non-human primate experiments better approximate human SCIs.[42] Nonetheless, due to expensive care and housing, rigorous regulatory requirements, and ethical considerations, larger mammals are rarely used.[27] Other vertebrates, such as fish and lamprey, have been studied to test novel strategies for human SCI due to their distinctive regenerative capacities.[43]

Rat models were the most widely used to study SCIs (72.4%). The rat is low cost and easy to care for with a well-understood anatomy, relatively uncommon surgical infections, and well-established functional analysis techniques.[7,34] Furthermore, similar to human pathology, rats often develop large cystic cavities at the site of injury. For these reasons, more rats are typically preferable to mimic the human pathology.[44] However, rats are quadrupeds not bipeds, and their corticospinal tract is mainly dorsal,[45] and these differences must be properly accounted for.

Mice models are being implemented increasingly to study the basic cellular and molecular biology of SCIs.[44] These data estimate that 16% of SCI models used mice. Advantages of mice models include the similar genomes to humans, ease of handling, high reproductive rates and low cost of use.[46] Conversely, the small working size prohibits many surgical maneuvres and device implantations.[7,34] The injury site in mice is densely packed with cells and actually decreases in size over time due to absence of the cyst. Knockout models as genetically modified animal studies mainly use mice. Using rats for knockout models is so difficult.[47]

Outcome assessments
Seven types of outcome assessments and the rate of use of each type of the included studies are presented in Tables 2,3,4 and 5. Nearly two-thirds of these studies used two or more types of outcome assessments with the most common assessment being biology plus behavior tests (1122 studies, 50.8%). Greater variety of outcome assessments improves the study significance and provides the possibility of observing changes from different perspectives. With respect to the testing methods used in 1528, rat models of mechanical traumatic SCI (as the most widespread models), the three most common outcome assessments, are presented in Table 6.

Biological analysis can yield helpful information on the extent of the primary injury, preservation of particular fiber tracts, cellular infiltration, secondary degeneration and molecular changes. At later stages it can be used to evaluate the scar and cyst formation.[22] Besides traditional histological stainings, there is a broad variety of immunohistochemistry methods that allow for the investigation of cells, structures and processes. The cell phenotype expressing a particular protein in the injured spinal cords could be visualized using immunohistochemical analysis. Also, the identification of proteins is done to evaluate differential gene expression involved in SCI and treatment models thereof. Neuroanatomical tract tracing gives a detailed visualization of specific neuronal connections. Tract tracing

demonstrates regeneration and plasticity after defined fiber lesions, and augments functional and physiological studies.[34] In traditional methods, histological outcomes, including sparing, quantification of myelin loss and motor neuron counts, are demonstrated via staining sections of the spinal cord.[11] Hematoxylin and eosin (H&E) is a well-known general structural stain used in many tissues. Cresyl violet stains both neurons and glia and connects to acidic parts of cells such as ribosomes, nuclei and nucleoli and also demonstrates the Nissl substance. The myelin could be displayed using different techniques such as Luxol Fast Blue, Osmium Tetroxide, Eriochrome Cyanine and Kluver–Barrera or Weil. Table 3 presents the frequency and utilization of histological and chemical techniques used in the animal models of SCI classified according to MeSH terms.

Behavioral outcomes in experimental SCI models are important for evaluating the extent of injury and treatment efficacy. Activity levels are related to the extent of neuronal destruction in the gray matter at the injury region, the loss of descending and ascending axons in the white matter and the reorganization of the remaining functional nervous system.[48] The locomotion could be assessed using different tests. Observing and evaluating animal activity in an open field, for example, by the Basso–Beattie–Bresnahan (BBB) score, is a simple way to assess locomotion. Table 4 presents the frequency and utilization of behavior assessment tests used in the animal models of SCI. To discern small changes in motor recovery requires more accurate and particular tests. Video recording-based kinematic analysis is one of the most sensitive behavioral evaluation tools and permits the perception of functional changes that are undetectable by visual observation alone.[22] The upper limb movements could be evaluated from home-cage activity[49] and reaching/grasping behavior including the food pellet reaching, the Montoya staircase and the isometric pull task.[50] The forelimb asymmetry test during vertical exploration (cylinder test) can measure the recovery of forelimb function following cervical SCI.[11]

Neurophysiology is a broader field that includes evoked potentials, electromyography, electroencephalography, electroneurography, membrane potentials and nerve conduction studies. These evoked potential assessments are useful for surveying the deficits and functional recovery in the neural substrates, and neural pathway integrity.[51,52] Somatosensory evoked potentials (SSEP) are helpful for the assessment of sensory spinal axon conduction.[53] Motor-evoked potentials offer valuable insight into the physiological status of motor tracts within the spinal cord and are appropriate for animal studies; these are a complementary for SSEP monitoring, not an alternative. All evoked potential methods take little time and cause only minimal distress or pain and can safely be done without anesthesia.[54] Electromyography can be achieved both by intramedullary manipulation and rapidly applied transaxial spinal cord compression, and can be used to study autonomic dysreflexia.[55] According to our data, neurophysiology was assessed in 17.8% of related studies.

Imaging. Magnetic resonance imaging (MRI) has been conveniently applied in rodent SCI models. MRI can discriminate between white and gray matter, and approximate the size of lesions and discern the formation of scars and cavities. MR images also can be used on living animals.[22] Functional MRI is a precise method used to distinguish between the recovery of sensory and motor function.[56]

Limitations

In general, a systematic review requires a comprehensive literature involving at least two databases. However, PubMed is the most complete bibliographic database of biomedicine.[57] Furthermore,

MeSH-based queries return more relevant articles compared with keyword searching and are generally recommended by librarians.[57] Although it is desirable to include the greatest possible number of applicable articles, using two databases to retrieve all articles with a high yield, low specificity approach could result in an unreasonably large number of irrelevant articles at tremendous burden to the investigators. Hence a crucial balance must be found, and it does not appear that a more widespread search leads to a significant difference in the overall results. In addition, a comprehensive survey involving even unpublished work is beyond the scope of this study.

Gender and transgenic animals were not addressed in this systematic review. On the basis of the checklist of Hassannejad et al.[9] about potential variables affecting the quality of animal SCI studies, gender was not included. Also, gender was not mentioned in many animal studies, especially about fish, reptiles and birds.

CONCLUSION

Animal models of SCI have been tremendously helpful for understanding of the pathophysiology, potentials for recovery, and implementations of new therapeutic strategies, and will continue to play an essential role in the investigation into such injuries. These models provide an opportunity for researchers to determine the characteristic pattern of cell death and sparing, and measurement of any demyelination, collateral sprouting, regeneration, neuroprotection and recovery of locomotor or other deficits. However, prior to choosing an animal model, the goals and objectives of the proposed study must precisely be defined. Various injury mechanisms and study designs can be useful depending on the information that is desired. Contusion and compression models better simulate the biomechanics and neuropathology of human injury. Transection models are valuable for studying anatomic regeneration. Newer SCI models require further improvement and validation. Rodents are the most commonly used and probably best-suited species for preliminary SCI studies. Controlling and monitoring injury mechanism techniques for evaluating outcomes should be continually improved as technology progresses.

DATA ARCHIVING

There were no data to deposit.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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