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Experimental spinal cord trauma: a review of mechanically induced spinal cord injury in rat models

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Abstract: It has been shown that animal spinal cord compression (using methods such as clips, balloons, spinal cord strapping, or calibrated forceps) mimics the persistent spinal canal occlusion that is common in human spinal cord injury (SCI). These methods can be used to investigate the effects of compression or to know the optimal timing of decompression (as duration of compression can affect the outcome of pathology) in acute SCI. Compression models involve prolonged cord compression and are distinct from contusion models, which apply only transient force to inflict an acute injury to the spinal cord. While the use of forceps to compress the spinal cord is a common choice due to it being inexpensive, it has not been critically assessed against the other methods to determine whether it is the best method to use. To date, there is no available review specifically focused on the current compression methods of inducing SCI in rats; thus, we performed a systematic and comprehensive publication search to identify studies on experimental spinalization in rat models, and this review discusses the advantages and limitations of each method.

Keywords: balloon compression of spinal cord; clips compression of spinal cord; forceps compression of spinal cord; spinal cord strapping; spinal cord injury; Sprague-Dawley rat models.

Introduction

The number of new cases of people affected by spinal cord injury (SCI) worldwide based on the incidence since 1995 ranges from 10.4 to 83 people per million per year (Witiw and Fehlings, 2015). About 27% of human SCIs are lacerations caused by penetrating objects that tear the spinal cord tissue (open injuries), resulting in a discontinuity of the cord. The majority of the clinical cases (73%) are due to temporary compression of the cord that leaves the cord surface intact, as in the case of intervertebral disc herniation, for example. As SCI causes profound disability and lowers the quality of life for the patients, studies into SCI using animal models have been invaluable in understanding how to treat this condition better. Taking that the eventual goal is to provide a clinical intervention for humans, animal model of choice should reflect the clinical pathological process and pattern of human SCI and meet the requirements of availability and reproducibility (Bresnahan et al., 1991; Behrmann et al., 1992, 1994).

SCI models are categorized based on the mechanism of injury, including contusion, compression, dislocation, transection, or chemical models (Schmidt and Leach, 2003; Erschbamer, 2007; Cherian et al., 2014). Mechanical spinal cord trauma is the surgical compression of the spinal cord in experimental animals or the result of motor vehicle accidents, falls, and sporting injuries involving a sudden compression of the spinal cord, resulting in vertebral damage that allows the bone or intervertebral disc to encroach (herniate) onto the spinal cord in humans. In humans, the 1-cm-thick and 42-cm-long spinal cord has four anatomical divisions: the cervical, thoracic, lumbar, and sacral regions, but comparatively, the entire spinal cord of rats is only approximately 9 cm (Watson et al., 2009). SCI models have evolved significantly over the past century since Allen A. developed the first weight-drop contusion model in 1911 (Allen, 1911). Currently, rats and mice are the most popular animals used in such models because of both cost and accessibility (Jakeman et al., 2000; Plemel et al., 2008; Vijayaprakash and Sridharan, 2013; Aziz et al., 2014; McDonough et al., 2015). Rat models are most widely used to study SCI as they are
inexpensive, are easy to care for, and can be studied in large numbers. They have a well-understood anatomy and minimal potential to develop surgical infections (Sharif-Alhoseini and Rahimi-Movaghar, 2014). In addition, rats develop large fluid-filled cystic cavities at the injury site, similar to the human pathology. Therefore, they are preferable for studies where mimicking the human pathology is important, including preclinical studies that focus on the efficacy of novel cellular and/or pharmacological therapies (Lee and Lee, 2013). The corticospinal tract of rat is mostly dorsal and the corticospinal tract lesions would not significantly create profound disability (Sharif-Alhoseini and Rahimi-Movaghar, 2014).

A broad spectrum of injury paradigms ranging from sharp transections to blunt contusions have been applied (Kwon et al., 2002). Compression models are used to create the persistent spinal canal occlusion that is commonly seen in human SCIs and to investigate the effects of compression or the optimal timing of decompression (as duration of compression can affect the outcome of pathology) in acute SCI (Sharif-Alhoseini and Rahimi-Movaghar, 2014). Compression models involve prolonged cord compression and are distinct from contusion models, which apply only transient force. Key to these SCI models is recreating features of human SCI as closely as possible. Similarly, the need for a better understanding of the underlying pathophysiology of acute spinal cord trauma (Young, 2000) and for a setting in which to evaluate potential therapies has led to the development and refinement of a number of experimental animal models of SCI. The purpose of this review is to provide an overview of the current experimental paradigms used to mechanically induce SCI in rat models effectively and reproducibly.

Search strategy

A systematic search of MEDLINE, Embase, and ISI Web of Science database was performed to identify studies in rat models with a focus on mechanically induced spinal cord trauma (compression). The general search term used was ‘experimental spinal cord compression,’ whereas the specific search term ‘spinal cord injury’ was used in combination with either ‘forceps,’ ‘clip compression,’ ‘balloon compression,’ and ‘strapping’ to search for words in titles, abstracts, Medical Subject Headings, or all fields. Besides the database searches, manual searches were performed using reference lists of original articles and previous reviews.

The search term ‘experimental spinal cord compression’ generated 19 articles from MEDLINE, 17 articles from Embase, and 46 articles from ISI Web of Science up to 2016. A total of 82 publications were identified by our search strategy. At full-text level, 20 articles were carefully studied. Out of these 20 articles, 8 were on forceps-induced spinal cord compression, 5 were on clip compression, 6 were on balloon compression, and 1 was on strapping for spinal cord compression in rats. Articles were excluded as they were either studies not in rat models or the SCI not mechanically induced to initiate compression or lacked a reproducible comparison (except the spinal cord strapping model/method, which is yet to be validated). From our database and conventional manual searches, only original research articles using compression methods to induce SCI serve as the fulcrum around which our discussion of findings oscillate — with a view to standardize rat SCI models as well as in assessments of their outcomes so that investigators testing various interventions can directly compare their results and correlate them with the molecular, biochemical, and histological alterations.

Tools for the injury paradigm

We studied various forms of compression-induced SCI, which includes a clip, balloon, or spinal cord strapper (SC-strapper) and forceps. Compressive injury may be induced with clips calibrated to exert a convinced force to induce mild, moderate, or severe injury (Rahimi-Movaghar et al., 2008; Jazayeri et al., 2012). Compression could be induced with balloon in experimental animals when determining a sufficient amount of injury to inflict (Lim et al., 2007); similarly, a SC-strapper may also be used to induce compression to produce a precise degree of narrowing of the spinal canal diameter (da Costa et al., 2008). The advantages and disadvantages of the different tools of injury paradigm (compression) are adumbrated in Table 1. In all our reviews on calibrated forceps, standardized compression injury was induced using forceps by applying a constant mechanical force that can produce a lateral compression injury by inserting on either side of the spinal cord and closing together to induce a central hemorrhagic necrosis (Blight, 2000; Plemel et al., 2008; Vaughn et al., 2013; Aziz et al., 2014; McDonough et al., 2015).

Clip compression method

The application of a modified aneurysm clip to generate SCI was first used in rats in 1978 (Rivlin and Tator, 1978) which is calibrated to exert a convinced force to induce...
Table 1: Overview of the SCI (compression) models that are used in experimental research to mimic the clinical human SCI.

<table>
<thead>
<tr>
<th>Model/Paradigm</th>
<th>Mechanism</th>
<th>Strength</th>
<th>Weakness</th>
</tr>
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<tbody>
<tr>
<td>Calibrated forceps</td>
<td>The arms of a pair of forceps is inserted in the lateral side of the spinal cord and pressure is applied to a specific width using a spacer for a specified duration to produce precise SCI. The width and duration of compression can be modified to generate desired injury severity (Blight, 1991, 2000; Gruner et al., 1996; Kaynar et al., 1998; Plemel et al., 2008; Vaughan et al., 2013; Aziz et al., 2014; McDonough et al., 2015).</td>
<td>The technique is simple, inexpensive, consistent, and highly reproducible.</td>
<td>The amount/unit of force applied cannot be recorded.</td>
</tr>
<tr>
<td>Clip compression</td>
<td>Induced with vascular/aneurysm clip to exert graded force of moderate to severe injury (35–50 g) around the spinal cord to produce an acute injury of varying severity. The clip is closed over the entire cord for a few minutes (Rivlin and Tator, 1978; Bruce et al., 2002; Poon et al., 2007; Rahimi-Movaghar et al., 2008; Jazayeri et al., 2012).</td>
<td>The technique is widely used and allows control of injury induction.</td>
<td>Velocity of the force delivered and the extent of compression are not recordable.</td>
</tr>
<tr>
<td>Balloon compression</td>
<td>Involves the insertion of a Fogarty catheter with a small balloon inflated with saline affixed to the end of the catheter and passed through a laminectomy area to induce the injury. Injury can be made by varying the inflation volume, duration of compression, or both (Tarlov et al., 1953; Vanický et al., 2001; Fukuda et al., 2005; Nesathurai et al., 2006; Lim et al., 2007; Aslan et al., 2009).</td>
<td>The technique is less invasive and does not cause radical damage to the surrounding structures.</td>
<td>Less commonly performed; the injuries induced are inconsistent and the parameters cannot be recorded.</td>
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<tr>
<td>SC-strapping</td>
<td>A curved needle is inserted through the intervertebral foramen and the epidural space to pass anterior to the spinal cord. One end of the suture is affixed to the wall of the experimental device (SC-strapper) and the other end is attached to a suspended mass via a simple pulley. In this model, spinal cord compression is induced by the weight drop (da Costa et al., 2008).</td>
<td>The technique is less invasive.</td>
<td>Consistent reproducibility of injury at precise site and extent of injury due to impact are yet to be validated.</td>
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mild, moderate, or severe injury (Rahimi-Movaghar et al., 2008; Jazayeri et al., 2012). The techniques involve performing a laminectomy at a specific level of the spine. The vascular clip is dorsoventrally closed over the entire cord and adjusted to a desired force around the spinal cord, producing an acute injury, and is then left to compress the cord for a specific amount of time, usually for a few minutes to produce graded injuries in the thoracic spine in terms of both functional and histological outcomes (Rivlin and Tator, 1978; Bruce et al., 2002; Poon et al., 2007; Rahimi-Movaghar et al., 2008; Jazayeri et al., 2012). A wide range of closing forces (35–50 g) is available and adjusted to produce moderate to severe injuries as desired. With this method, the spinal cord becomes ischemic and mimics common clinical injuries and outcomes.

The advantage of clip compression is that it allows control of injury induction, which is adaptable in all regions of the spine. However, the only injury parameters that are recordable are the calibrated closing force induced by the clip and the duration of compression. The limitation of the clip method is that, as in other methods, the momentum of the clip upon contact with the cord is not recordable. A specific limitation of this method is that the extent of the segment of cord compression is not recordable because the pressure applied by aneurysm clip results in contusion-compression that may present.

### Balloon compression method

The application of balloon compression was first described in 1953 (Tarlov et al., 1953). The techniques involve performing a laminectomy at a specific level of the spine. A Fogarty catheter with a small balloon inflated with saline is affixed to the end of the catheter and passed through a laminectomized area and inserted into the epidural or subdural space (through a small hole made in the vertebral arch, advanced cranially to one or two higher spinal levels) to induce the injury. Rat models have been used extensively (Vanický et al., 2001; Lim et al., 2007), but the procedure has been refined in dogs, rabbits, and primates (Fukuda et al., 2005; Nesathurai et al., 2006; Aslan et al., 2009). The balloon-induced method has been used because it is a simple method that causes minimal damage to the surrounding structures. The limitation is that it is difficult to standardize the initial impact because the momentum cannot be accounted since the velocity or force delivered is not recordable.
Spinal cord strapping method

Spinal cord strapping is a novel SCI technique that was developed in 2008 (da Costa et al., 2008). The method involves the use of SC-strapper, where a curved surgical needle is inserted through the intervertebral foramen and the epidural space to pass anterior to the spinal cord. The needle is then maneuvered to pass through the contralateral intervertebral foramen and back to the skin so that the suture wraps around the spinal cord, compressing it against the ligamentum flavum. One end of the suture is affixed to the wall of the experimental device and the other end is attached to a suspended mass (weight) via a simple pulley, whereby compression is induced by the weight drop. Three different masses may be utilized to inflict mild, moderate, and severe injuries in the rats.

The advantage of spinal cord strapping is that it is the only model of spinal cord compression that does not require laminectomy and is therefore the least invasive approach to SCI. Furthermore, the force of compression can easily be computed based on the weight of the mass dropped at one end of the pulley. Despite the strength of this technique, spinal cord strapping cannot be considered as a 100% objective approach since the curve needle may actually pierce through the substance of the spinal cord rather than anterior to the cord as the investigator is blinded when the needle passes through the contents of the spinal canal. This challenge adds to the fact that the consistent reproducibility of injury at precise site and extent of injury impact are yet to be validated.

Calibrated forceps-induced compression method

The first recorded experimental study using forceps in rats dates back to 1996 (Gruner et al., 1996). Like other compression methods (with the exception of spinal cord strapping), this method requires laminectomy. A pair of modified forceps are used to compress the cord to a thickness of 0.8, 1.0, 1.2, 1.4, 1.6, or 1.8 mm as desired. Forceps are modified in such a way that calibrated ignition gauge is inserted between the arms of the forceps just above the cord surface, and a very precise compression is initiated by inserting the blades of the forceps between the lateral sides of the cord and the vertebral walls until the forceps slowly contacted the gauge (Blight, 1991, 2000; Gruner et al., 1996; Kaynar et al., 1998; Plemel et al., 2008; Vaughn et al., 2013; Aziz et al., 2014; McDonough et al., 2015). The forceps are usually held closed for some seconds and then gently removed.

The use of forceps to compress the spinal cord has been demonstrated to be an inexpensive, efficient, and reproducible alternative to costly contusion devices commonly used to induce SCI (Gruner et al., 1996). The experimental protocol for successful forceps-induced spinal crush has been investigated and has proved to be simple, inexpensive, consistent, and highly reproducible, which involves a simple experimental design to generate data that mimic clinical human SCI. However, the forceps-induced compression, like other methods, cannot monitor or compute the velocity or force delivered at the injury site.

Furthermore, forceps compression is the best choice of method to use when precision in compression to specific segment of the spinal cord is desired. This method was also developed to circumvent some of the biomechanical issues associated with contusion models causing variability due to bouncing of the impactor rod on the spinal cord after initial drop ‘weight bounce,’ resulting in multiple impacts.

Outcomes of spinal cord compression model approach

Compression models are distinct from contusion models in that they involve prolonged cord compression. In all the different methods of compression models, the spinal cord undergoes a sequence of pathological changes after a traumatic injury that causes the appearance of edema, hemorrhage, neuronal necrosis, gliosis, demyelination, cyst formation, and also cavitation (Gruner et al., 1996; Bruce et al., 2002; Lim et al., 2007; da Costa et al., 2008; Rahimi-Movaghar et al., 2008; Jazayeri et al., 2012; Vaughn et al., 2013; Aziz et al., 2014; McDonough et al., 2015). These outcomes appear to be consistent across the methods, with results showing the injuries to be graded in terms of both functional and histological outcomes. Despite the near uniformity in outcomes, the forceps method both in rats (Gruner et al., 1996; Vaughn et al., 2013; Aziz et al., 2014; McDonough et al., 2015) and mice (Faulkner et al., 2004; Plemel et al., 2008) has been shown to be more precise in inducing a central hemorrhagic necrosis and displacement of tissue in cranial and caudal directions.

Our systematic study therefore appreciates the complexity of human SCI and no one compression model can encompass 100% all aspects of injury. Even though each model aims to generate a consistent, easily reproducible and graded injury that mimics clinical humans SCI, there
is no single model of SCI that can reflect an exact representative of clinical phenotype. We, however, revealed that forceps-induced SCI may emerge as the ultimate option of SCI compression model, given the precision in injury induction to specific spinal cord segment and simplicity.

Conclusion

All injury paradigms are useful but differ in the simplicity and mechanism employed to induce the trauma. Taken together, it is clear that controlling and monitoring the injury mechanism within the surgical field have to be enhanced by technological improvement. For example, employment of extrafine laparascopy to the SC-strapper will overcome its inevitable challenges. Studies have shown that graded forceps crush is an attractive and viable alternative to the other rat models of SCI currently available (Gruner et al., 1996; Vaughn et al., 2013; Aziz et al., 2014; McDonough et al., 2015), which is also nicely demonstrated in mice model (Plemel et al., 2008). It must be emphasized that the choice of SCI model is important in designing experiments to determine the efficacy of treatments for human cases of SCI. Such experiments require an animal model with high throughput that is highly reproducible to limit variability and should also be of clinical relevance to accurately evaluate the human condition they are modeling. The results discussed here have shown that by using forceps with different separation distances (calibrated ignition gauge or spacers) at closure point, it is possible to achieve mild, moderate, and severe injuries that can be distinguished histologically or otherwise.

We therefore recommend the calibrated forceps-induced compression method to researchers who aim to circumvent biomechanical issues and desire to achieve precision in compression to specific segment of the spinal cord. The spinal cord strapping method would be best choice to researchers who desire the least invasive approach to SCI without the need of laminectomy.

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