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Current Spinal Cord Injury Animal Models are Too Simplistic for Clinical Translation

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Background

Spinal cord injury (SCI) is a devastating disease that has a global impact on individuals and society. The number of SCI cases in 2016 was 27 million worldwide, which was predominantly due to falls and road traffic collisions [1]. Alarmingly, the number of new SCI cases in most countries has risen over the last few decades [2]. Patients often experience multiple sequalae to injury, such as loss of sensory and motor function below the site of spinal injury, and in the long term develop complex conditions, including chronic pain [3,4]. As mortality is rare and SCI often occurs at a young age, it can cost up to $5.4 million USD in lifetime care per patient [5]. To date, there are no effective treatments available for SCI and the understanding of the pathophysiology of SCI remains limited. Therefore, animal research models, mainly in the form of rodents have been developed and used [6,7].

There are many rodent models of SCI, ranging from hemisection injury in relation to gunshot and stab wounds, to contusion and compression injuries in close imitation of injuries from road traffic collisions and falls [6,7]. The most clinically relevant SCI model is contusion injury induced by force or spinal cord displacement [8]. Many of these preclinical studies have given rise to promising therapies that have transitioned into the clinical trials stage, but sadly their success has halted there. To date, there have been over 1,100 clinical trials since 1986 aiming to improve outcomes for SCI patients [5]. Several studies have focused on drug therapies in the form of neuroprotection [9], neuroregeneration [10], and cell-based therapy [11]. Although the pre-clinical trials have demonstrated success and reproducibility in various SCI models, all have struggled to reproduce these results in large human trials.

Clinicians and researchers have suggested that the failure of SCI clinical trials is due to a variety of reasons within the clinical trials such as small sample sizes, heterogeneity in SCI severity (i.e. ASIA A vs ASIA D), and variations in spinal level studied (e.g. cervical vs lumbar) [9]. However, one factor that has not been fully addressed is the suitability and simplicity of animal SCI models for the clinical setting. The creation of traumatic SCI animal models has been designed to represent human patients as much as possible [12]. For example, in contusion injury, a transient force is applied onto the spinal cord. Not only is the mechanism of injury similar between rats and humans, but also the majority of the pathophysiological responses such as neurodegeneration, neuroinflammation and cyst formation are also similar [13]. Interestingly, this does not hold true for mice, who have several neuropathological differences after SCI when compared to rats. Mice have limited cyst formation, reduced glial scar formation and diminished blood-spinal cord barrier disruption after SCI [14]. Other sources of error may result from the frequent use of young, female adults that are healthy, unintoxicated and injured at the thoracic, rather than cervical level. This does not represent the average human SCI and may make the results clinically irrelevant.

Age

Many preclinical animal studies focus on SCI in young adult rodents. This neglects to account for the increasing number of older SCI patients, often owing to fall-induced tetraplegic injury [15]. It should not be assumed that young adults respond

similarly to older adults after SCI. One rodent study focusing on SCI-induced chronic central pain syndrome found that spontaneous locomotor recovery, completion of behavioural test training and development of neuropathic behaviour differed in the young adult. Recovery in the younger rodent (2 months rat age, equivalent to approximately 15 years human age) progressed faster than middle aged rats (12 months rat age, equivalent to approximately 33 years human age) [16-18]. Interestingly, it was shown that aged rats (15 months age, equivalent to approximately 41 years human age) with SCI had a higher vulnerability to mortality after SCI surgery and cell transplantation interventions [17]. However, in SCI clinical trials, the age of participants often ranges from 18 to 65 years old, and, in some studies, the maximum age reaches 80 years old [5]. This large age range in clinical trials could introduce heterogeneity in the cohort of SCI patients, with peaks of SCI incidence occurring at 16-30 years and again at over 65 years. A potential solution is to limit the age range in the clinical trials, but this would decrease the sample size and lengthen the duration of the study.

Sex

Although there are more male SCI patients, with a ratio of approximately 3.6 : 1 (males : females) [15], much of the pre-clinical rodent research focuses on females. This is mostly due to their relative practical benefits, including manual bladder emptying following SCI. Additionally, their smaller size and less aggressive nature facilitates ease of handling and group housing [17]. However, it has recently been shown that recovery of motor function and preservation of grey and white matter after SCI was greater in females than males [19]. It was hypothesised that oestrogen may be a contributing neuroprotective factor, but a study with young adult male and ovariectomized female rats with SCI disproved this [20]. The effects of age and sex in pre-clinical SCI studies have been recently reviewed in detail [21]. Therefore, it is important to consider the inclusion of males in a program of preclinical studies for the development of a therapy.

Substance Abuse

Approximately 25% of SCI incidents involve patients that have consumed alcohol. Of these, 51% had a persistent drinking problem and/or had driven under the influence of alcohol [22]. Furthermore, one or more illicit drugs, such as marijuana, cocaine, and amphetamines, were found in the system of 44% of SCI patients [22]. This is unsurprising given alcohol and illicit drugs can impair vision, balance, reaction time, and judgment. They additionally alter behavioural responses, leading to aggression and neglect, all of which dramatically increases the risk of SCI [23,24]. Interestingly, 45% of SCI patients after injury onset exhibit some form of alcohol dependency, which is a far larger proportion than the 13% affected in the general population. Notably, this percentage amongst SCI patients decreases 17 months after injury [25].

Acute alcohol intoxication has been shown to exacerbate injury following trauma in an animal spinal contusion model by altering the biochemical response to injury and potentially worsening the secondary injury [26]. Furthermore, SCI patients with chronic pain may become heavily reliant on opioids, which can result in further misuse [27]. Substance abuse may also worsen pain and pressure ulcers, thereby increasing mortality [28]. Alcohol abuse can interfere with rehabilitation, lengthen hospital stay, and cause or exacerbate mental health disorders such as depression and anxiety [29]. Alcohol and illicit drugs if not declared or identified by SCI clinical trial investigators may interact with treatment and alter the drug treatment's effect. For example, alcohol has serious side effects in isolation, yet it can additionally enhance the side effects of other medications such as opioids, leading to respiratory depression [30]. Therefore, drug screening and questionnaires may be required in SCI patient recruitment to clinical trials to ensure the therapy under investigation is not compromised or alternatively, investigate in preclinical studies whether SCI treatment is affected by alcohol and/or illicit drugs.

Spinal Level

Even though injury to the cervical spinal cord accounts for approximately 50% of clinical SCI cases, over 80% of pre-clinical rodent SCI studies focus on thoracic level injury [31]. Experimenting with thoracic level injury decreases the risk of accidental rodent mortality and reduces the burden of post-operative care as only the hindlimbs are affected [8]. Furthermore, many behavioural tests, such as the universally used Basso, Beattie, and Bresnahan (BBB) locomotor score, predominantly focus on the hindlimbs. Importantly, there are a few rodent studies that focus on cervical level injury [32,33]. Behavioural testing for the forelimb is performed via the Montoya staircase and/or single pellet reaching test. While the training is difficult, it provides a reliable indication of the dexterity and sensorimotor function of the forelimbs and minimises false positive results, hence it has been recommended for use in the stroke field [34]. However, if researchers are interested in cervical injury, it is important to consider various ethical concerns given the greater impact of higher spinal level injuries [7]. As most clinical trials do not discriminate between spinal levels of injuries in patient recruitment, it is important to consider the differences in motor, autonomic and cardiovascular roles of the cervical and thoracic spinal cord. Notably, the thoracic spinal level has comparatively scarce grey matter and the presence of the sympathetic preganglionic neurones within the intermediate lateral horn of the grey matter [35-37]. Therefore, it is important to consider the inclusion of cervical level injury in preclinical animal studies.

Diet

Rats in the laboratory setting are often fed on a diet that provides optimum energy and nutrients for healthy living

[38]. It is currently not known whether a healthy diet would reduce injury severity and hasten recovery after SCI compared with a high fat, high carbohydrate Western diet. However, dietary therapies including a diet enriched with omega-3 fatty acids, such as docosahexaenoic acid (DHA), have shown to be neuroprotective after spinal hemisection, contusion, and compression injuries [33,39]. By contrast, treatment with omega-6 fatty acids, such as arachidonic acid, can exacerbate hemisection injury [40,41]. Initiating the ketogenic diet (high fat, low carbohydrate), 4 hours post cervical unilateral contusion demonstrated significant improvement in forelimb function and reduced lesion size, suggesting that SCI patients should limit high carbohydrate content [42]. Therefore, more preclinical studies are required to understand how certain diets of high risk SCI patients may influence injury severity and recovery.

Traumatic Brain Injury

Often SCI patients suffer from trauma to other regions due to road traffic collisions and falls. Recent studies have shown that 40-47% of SCI patients additionally have a clinical concomitant traumatic brain injury (TBI) [43,44]. Unsurprisingly, current SCI clinical trials omit patients that have a concurrent TBI since this co-presentation generally requires a longer hospital stay and adversely impacts functional improvement measures [45]. However, if SCI patients with TBI remain excluded from clinical trials, then treatment options for this group will remain sparse. The clinical attitude to this is mirrored in the paucity of preclinical studies, which show limited data. One study has addressed this gap by administering a unilateral cervical contusion alongside an ipsilateral or contralateral unilateral TBI [46]. They demonstrated a complex recovery dependent on the laterality of the SCI and TBI lesions. Therefore, unless more preclinical studies are conducted to understand the pathophysiology of concomitant SCI with TBI, a large proportion of SCI patients will be excluded from SCI clinical trials.

Conclusion

Given that the role of pre-clinical studies is to prepare for the clinical eventuality, it is important that all controllable variables are mimicked as closely as possible to ensure effective therapeutic translation. If this is not achieved, then any significant progress made in pre-clinical trials will be difficult to replicate successfully in SCI patients. To date, many of the factors that could affect the response to spinal cord trauma such as age, sex, drug intake prior and after injury, and head trauma are not included in the majority of pre-clinical SCI models. Unless the pre-clinical animal models represent the clinical setting, there will be more failures in SCI clinical trials.

References

1. Ahmed RU, Alam M, Zheng YP. Experimental spinal cord injury and behavioral tests in laboratory rats. Heliyon. 2019; 5 (3): e01324.

2. Ashammakhi N, Kim HJ, Ehsanipour A, Bierman RD, Kaarela O, Xue C, et al. Regenerative therapies for spinal cord injury. Tissue Engineering Part B: Reviews. 2019 Dec 1;25(6):471-91.

3. Badhiwala JH, Wilson JR, Kwon BK, Casha S, Fehlings MG. A review of clinical trials in spinal cord injury including biomarkers. Journal of Neurotrauma. 2018 Aug 15;35(16):1906-17.

4. Blandino A, Cotroneo R, Tambuzzi S, Di Candia D, Genovese U, Zoja R. Driving under the influence of drugs: Correlation between blood psychoactive drug concentrations and cognitive impairment. A narrative review taking into account forensic issues. Forensic science international: Synergy. 2022 Jan 1;4:100224.

5. Bryce TN, Biering-Sørensen F, Finnerup NB, Cardenas DD, Defrin R, Lundeberg T, et al. International spinal cord injury pain classification: part I. Background and description. Spinal Cord. 2012 Jun;50(6):413-7.

6. Budisin B, Bradbury CC, Sharma B, Hitzig SL, Mikulis D, Craven C, et al. Traumatic brain injury in spinal cord injury: frequency and risk factors. Journal of Head Trauma Rehabilitation. 2016 Jul 1;31(4):E33-42.

7. Burnside ER, De Winter F, Didangelos A, James ND, Andreica EC, Layard-Horsfall H, et al. Immune-evasive gene switch enables regulated delivery of chondroitinase after spinal cord injury. Brain. 2018 Aug 1;141(8):2362-81.

8. Byrnes KR, Fricke ST, Faden AI. Neuropathological differences between rats and mice after spinal cord injury. Journal of Magnetic Resonance Imaging. 2010 Oct;32(4):836-46.

9. Cheriyan T, Ryan DJ, Weinreb JH, Cheriyan J, Paul JC, Lafage V, et al. Spinal cord injury models: a review. Spinal cord. 2014 Aug;52(8):588-95.

10. Chikritzhs T, Livingston M. Alcohol and the Risk of Injury. Nutrients. 2021 Aug 13;13(8):2777.

11. GBD 2016 Neurology Collaborators. Global, regional, and national burden of neurological disorders, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet Neurol. 2019 May;18(5):459-480.

12. Cui LL, Golubczyk D, Jolkkonen J. Top 3 behavioral tests in cell therapy studies after stroke: difficult to stop a moving train. Stroke. 2017 Nov;48(11):3165-3167.

13. Datto JP, Bastidas JC, Miller NL, Shah AK, Arheart KL, Marcillo AE, et al. Female rats demonstrate improved locomotor recovery and greater preservation of white and gray matter after traumatic spinal cord injury compared to males. Journal of Neurotrauma. 2015 Aug 1;32(15):1146-57.

14. Davis JF, Cao Y, Krause JS. Changes in alcohol use after the onset of spinal cord injury. The Journal of Spinal Cord Medicine. 2018 Mar 4;41(2):230-7.

15. Dietz VA, Roberts N, Knox K, Moore S, Pitonak M, Barr C, et al. Fighting for recovery on multiple fronts: The past, present, and

future of clinical trials for spinal cord injury. Frontiers in Cellular Neuroscience. 2022 Sep 7;16:977679.

16. Eldridge LA, Piatt JA, Agley J, Gerke S. Relationship between substance use and the onset of spinal cord injuries: A medical chart review. Topics in Spinal Cord Injury Rehabilitation. 2019;25(4):316-21.

17. Frostell A, Hakim R, Thelin EP, Mattsson P, Svensson M. A review of the segmental diameter of the healthy human spinal cord. Frontiers in Neurology. 2016 Dec 23;7:238.

18. Furlan JC, Sakakibara BM, Miller WC, Krassioukov AV. Global incidence and prevalence of traumatic spinal cord injury. Canadian Journal of Neurological Sciences. 2013 Jul;40(4):456-64.

19. Gomes T, Juurlink DN, Mamdani MM, Paterson JM, van den Brink W. Prevalence and characteristics of opioid-related deaths involving alcohol in Ontario, Canada. Drug and Alcohol Dependence. 2017 Oct 1;179:416-23.

20. Griffin JM, Bradke F. Therapeutic repair for spinal cord injury: combinatory approaches to address a multifaceted problem. EMBO Mol. Med. 12, e11505.

21. Gwak YS, Hains BC, Johnson KM, Hulsebosch CE. Effect of age at time of spinal cord injury on behavioral outcomes in rat. Journal of Neurotrauma. 2004 Aug 1;21(8):983-93.

22. Hagen EM, Eide GE, Rekand T, Gilhus NE, Gronning M. Traumatic spinal cord injury and concomitant brain injury: a cohort study. Acta Neurologica Scandinavica. 2010 Jul;122:51-7.

23. Halt PS, Swanson RA, Faden AI. Alcohol exacerbates behavioral and neurochemical effects of rat spinal cord trauma. Arch Neurol. 1992 Nov;49(11):1178-84.

24. Huang WL, King VR, Curran OE, Dyall SC, Ward RE, Lal N, et al. A combination of intravenous and dietary docosahexaenoic acid significantly improves outcome after spinal cord injury. Brain. 2007 Nov 1;130(11):3004-19.

25. Inoue T, Lin A, Ma X, McKenna SL, Creasey GH, Manley GT, et al. Combined SCI and TBI: recovery of forelimb function after unilateral cervical spinal cord injury (SCI) is retarded by contralateral traumatic brain injury (TBI), and ipsilateral TBI balances the effects of SCI on paw placement. Experimental neurology. 2013 Oct 1;248:136-47.

26. King VR, Huang WL, Dyall SC, Curran OE, Priestley JV, Michael-Titus AT. Omega-3 fatty acids improve recovery, whereas omega-6 fatty acids worsen outcome, after spinal cord injury in the adult rat. Journal of Neuroscience. 2006 Apr 26;26(17):4672-80.

27. Krassioukov AV, Bunge RP, Pucket WR, Bygrave MA. The changes in human spinal sympathetic preganglionic neurons after spinal cord injury. Spinal Cord. 1999 Jan;37(1):6-13.

28. Lilley E, Andrews MR, Bradbury EJ, Elliott H, Hawkins P, Ichiyama RM, et al. Refining rodent models of spinal cord injury. Experimental Neurology. 2020 Jun 1;328:113273.

29. Liu ZH, Yip PK, Adams L, Davies M, Lee JW, Michael GJ, et al. A single bolus of docosahexaenoic acid promotes neuroplastic changes in the innervation of spinal cord interneurons and motor neurons and improves functional recovery after spinal cord injury. Journal of Neuroscience. 2015 Sep 16;35(37):12733-52.

30. Lujan HL, DiCarlo SE. Direct comparison of cervical and high thoracic spinal cord injury reveals distinct autonomic and cardiovascular consequences. Journal of Applied Physiology. 2020 Mar 1;128(3):554-64.

31. Martín-López M, González-Muñoz E, Gómez-González E, Sánchez-Pernaute R, Márquez-Rivas J, Fernández-Muñoz B. Modeling chronic cervical spinal cord injury in aged rats for cell therapy studies. Journal of Clinical Neuroscience. 2021 Dec 1;94:76-85.

32. Motiei-Langroudi R, Sadeghian H. Traumatic spinal cord injury: long-term motor, sensory, and urinary outcomes. Asian Spine Journal. 2017 Jun;11(3):412-18.

33. Pellizzon MA, Ricci MR. Choice of laboratory rodent diet may confound data interpretation and reproducibility. Current Developments in Nutrition. 2020 Apr;4(4):nzaa031.

34. Quinn R. Comparing rat's to human's age: how old is my rat in people years?. Nutrition. 2005 Jun 1;21(6):775-7.

35. Saunders LL, Krause JS. Psychological factors affecting alcohol use after spinal cord injury. Spinal Cord. 2011 May;49(5):637-42.

36. Shang Z, Wang M, Zhang B, Wang X, Wanyan P. Clinical translation of stem cell therapy for spinal cord injury still premature: results from a single-arm meta-analysis based on 62 clinical trials. BMC Medicine. 2022 Sep 5;20(1):284.

37. Sharif-Alhoseini M, Khormali M, Rezaei M, Safdarian M, Hajighadery A, Khalatbari MM, et al. Animal models of spinal cord injury: a systematic review. Spinal Cord. 2017 Aug;55(8):714-21.

38. Stewart AN, Jones LA, Gensel JC. Improving translatability of spinal cord injury research by including age as a demographic variable.Front Cell Neurosci . 2022 Nov 17;16:1017153.

39. Streijger F, Plunet WT, Lee JH, Liu J, Lam CK, Park S, et al. Ketogenic diet improves forelimb motor function after spinal cord injury in rodents. PloS One. 2013 Nov 4;8(11):e78765.

40. Stroud MW, Bombardier CH, Dyer JR, Rimmele CT, Esselman PC. Preinjury alcohol and drug use among persons with spinal cord injury: Implications for rehabilitation. The Journal of Spinal Cord Medicine. 2011 Sep 1;34(5):461-72.

41. Swartz KR, Fee DB, Joy KM, Roberts KN, Sun S, Scheff NN, et al. Gender differences in spinal cord injury are not estrogen-dependent. Journal of Neurotrauma. 2007 Mar 1;24(3):473-80.

42. Tate DG, Forchheimer MB, Krause JS, Meade MA, Bombardier CH. Patterns of alcohol and substance use and abuse in persons with spinal cord injury: risk factors and correlates. Archives of Physical Medicine and Rehabilitation. 2004 Nov 1;85(11):1837-47.

43. Thompson C, Mutch J, Parent S, Mac-Thiong JM. The changing demographics of traumatic spinal cord injury: An 11-year study of 831 patients. The Journal of spinal cord medicine. 2015 Mar 1;38(2):214-23.

44. Valbuena Valecillos AD, Gater Jr DR, Alvarez G. Concomitant brain injury and spinal cord injury management strategies: A narrative review. Journal of Personalized Medicine. 2022 Jul 6;12(7):1108.

45. Yip PK, Bowes AL, Hall JC, Burguillos MA, Ip TR, Baskerville T, et al. Docosahexaenoic acid reduces microglia phagocytic activity via miR-124 and induces neuroprotection in rodent models of spinal cord contusion injury. Human Molecular Genetics. 2019 Jul 15;28(14):2427-48.

46. Zhang Z, Zhang YP, Shields LB, Shields CB. Technical comments on rodent spinal cord injuries models. Neural Regeneration Research. 2014 Mar 3;9(5):453-5.