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Original Article

Pharmacological Inhibition of Soluble Tumor Necrosis Factor-Alpha Two Weeks after High Thoracic Spinal Cord Injury Does Not Affect Sympathetic Hyperreflexia

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Abstract

After a severe, high-level spinal cord injury (SCI), plasticity to intraspinal circuits below injury results in heightened spinal sympathetic reflex activity and detrimentally impacts peripheral organ systems. Such sympathetic hyperreflexia is immediately apparent as an episode of autonomic dysreflexia (AD), a life-threatening condition characterized by sudden hypertension and reflexive bradycardia following below-level sensory inputs; for example, pressure sores or impacted fecal matter. Over time, plasticity within the spinal sympathetic reflex (SSR) circuit contributes to the progressive intensification of AD events, as the frequency and severity of AD events increase greatly beginning ~2 weeks post-injury (wpi). The neuroimmune system has been implicated in driving sympathetic hyperreflexia, as inhibition of the cytokine soluble tumor necrosis factor-alpha (sTNFα) using the biological mimetic XPro1595 beginning within days post-SCI has been shown to attenuate the development of AD. Here, we sought to further understand the effective therapeutic time window of XPro1595 to diminish sympathetic hyperreflexia, as indicated by AD. We delayed the commencement of continuous intrathecal administration of XPro1595 until 2 weeks after a complete, thoracic level 3 injury in adult rats. We examined the severity of colorectal distension-induced AD biweekly. We found that initiation of sTNFα inhibition at 2 wpi does not attenuate the severity or intensification of sympathetic hyperreflexia compared with saline-treated controls. Coupled with previous data from our group, these findings suggest that central sTNFα signaling must be targeted prior to 2 weeks post-SCI in order to decrease sympathetic hyperreflexia.

Keywords: autonomic dysreflexia; neuroimmune plasticity; soluble TNFα; spinal cord injury; sympathetic hyperreflexia; XPro1595

Introduction

Cardiovascular disease is one of the leading causes of morbidity and mortality in individuals with high-level spinal cord injuries (SCI).[1] This is primarily due to the development of sympathetic hyperreflexia, which results in excess sympathetic output to effector organs, such as peripheral vasculature, in response to below-level noxious sensory stimuli, such as bladder or bowel distension. Sympathetic hyperreflexia immediately manifests as autonomic dysreflexia (AD), a condition occurring in up to 90% of individuals with SCI above thoracic segment 6 (T6), which is characterized by life-threatening hypertension and concomitant bradycardia, ultimately increasing the patients' risks for myocardial infarction and stroke.[2,3] Moreover, even milder symptoms of AD such as headaches, sweating, and sudden anxiety severely impair quality of life. Therefore, managing AD is a top priority for those with SCI.[4]

One of the major mechanisms that underlie sympathetic hyperreflexia development is damage to descending, supraspinal pathways, which regulate sympathetic pre-ganglionic neurons (SPNs) found within the intermediolateral cell column (IML) throughout the thoracolumbar spinal cord (T1-L2 spinal segments).[5] In the intact spinal cord, SPN activity is regulated via the integration of supraspinal and intraspinal inputs to modulate sympathetic output to peripheral vasculature. After a high-level SCI (i.e., at T6 or above), the loss of modulatory, inhibitory supraspinal inputs disrupts this spinal sympathetic reflex (SSR) circuit, resulting in a bias toward excitatory intraspinal input on SPNs.[6] Beginning ~2 weeks post-injury (wpi), a secondary phase develops in which the frequency and severity of AD episodes intensifies.[7–9] The development of this later phase is associated with maladaptive plasticity caudal to the SCI (i.e., sprouting of primary afferents and propriospinal axons, changes in intraspinal interneuron activity, and changes in input to SPNs).[8,10–15] As a

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result of this plasticity, a hyper-excitable SSR circuit develops in which a sensory stimulus below the injury leads to exaggerated sympathetic output.[6]

We recently found that the neuroimmune system is a key mechanism that drives plasticity within the SSR circuit. Specifically, the cytokine soluble tumor necrosis factor-alpha (sTNFα) has been shown to increase neuronal excitability, drive plasticity within the SSR circuit, and contribute to the development of sympathetic hyperreflexia following high-thoracic SCI.[8,9,16–18] sTNFα is upregulated within minutes post-SCI[19,20] and persists at heightened levels for weeks within the SSR circuit, as does its primary receptor TNFR1 and downstream NF-κB p65 activity.[8,9] Interestingly, pharmacological inhibition of central sTNFα signaling via intrathecal administration of the biologic mimetic XPro1595[21] beginning immediately or 3 days post-complete transection at the T3 spinal segment (T3Tx) has been shown to attenuate maladaptive plasticity within the SSR circuit and thereby mitigate the severity of sympathetic hyperreflexia.[8,9] Although it is known that blocking sTNFα/TNFR1 signaling relatively acutely post-SCI attenuates the development of sympathetic hyperreflexia, the critical period for injury-induced sTNFα activity on the development of sympathetic hyperreflexia is currently unknown. Moreover, as the exact mechanisms by which sympathetic hyperreflexia is initiated and sustained is a pressing question within the SCI field, we sought to further explore the temporal role of sTNFα in modulating sympathetic hyperreflexia. Here, we sought to determine if administering the dominant-negative biologic XPro1595 to inhibit sTNFα starting 2 weeks post-injury, when AD is already established and events begin to intensify, is sufficient to diminish and/or reverse sympathetic hyperreflexia and associated AD development.

Methods

Animals

Adult, female Wistar rats (~225–250 g; Charles River Laboratories) were used for all experiments. Animals were housed two to three per cage on a 12-h light/dark cycle with *ad libitum* access to food and water and were acclimated for at least 1 week after arrival, prior to any surgical procedures. All surgical procedures were performed with the animals under general isoflurane anesthesia (induction 5%, maintenance 2%, in 100% oxygen at 0.5–2 L/min) using aseptic technique, sterilized instruments, and a thermal heating pad. During all surgeries, animals were treated with ampicillin (Sandoz; 200 mg/kg, s.c.), meloxicam (Putney; 1 mg/kg, s.c.), and 3 mL Ringer's lactate solution (s.c.) and placed on a thermal heating pad to recover. Following SCI, animal bladders were manually expressed two to three times per day for the duration of the study. Animals were randomly divided into either saline ($n=11$) or XPro1595 ($n=15$) treatment groups. At the terminal time point, animals were euthanized with Euthasol™ (Virbac AH, Inc.) and transcardially perfused with 0.9% saline and 4% paraformaldehyde (PFA). All housing, surgical, and animal care procedures were in accordance with Drexel University Institutional Animal Care and Use Committee and Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC)/National Institutes of Health (NIH) guidelines.

Surgical procedures

Animals were implanted with radiotelemeter pressure transducers (HD-S10 or HD-S11; Data Sciences International, Inc.) into the descending aorta 1 week prior to a complete spinal transection at thoracic segment 3 (T3Tx) (Fig. 1). As previously described,[8,9,22] the telemeter catheter was threaded and secured to the femoral artery, and the transmitter body was placed in a subcutaneous pocket. Animals recovered for at least 1 week post-transplant, and baseline hemodynamic activity was recorded to verify catheter placement and pre-SCI cardiovascular parameters.

Following recovery and baseline recordings, all animals underwent a complete T3Tx-SCI, in which thoracic segment 3 was exposed following a T2 laminectomy, and ~2 mm of spinal cord was removed via vacuum aspiration. The cavity was visually examined to verify lesion completeness, gel foam was placed in the cavity, and the dura was sutured shut as previously described.[9]

Two weeks post-transection, all animals underwent baseline hemodynamic recordings and colorectal distension (CRD) prior to osmotic minipump implantation. Osmotic minipumps (Alzet, no. 2006) were used to intrathecally deliver either XPro1595 or saline continuously for 42 days at a rate of 60μg per day. As previously described,[9] minipumps were filled with either saline or XPro1595 (10 mg/mL; provided by INmune Bio Inc.) and an intrathecal catheter (ReCathCo) was attached to the minipump. Minipumps were prepared and incubated in sterile saline at 37°C 60 h prior to implantation.

To implant the minipumps, all animals underwent T9 laminectomies to expose spinal segment T8. The catheter was threaded in the subdural space to end just caudal to the T3Tx injury site and secured in place, as previously described.[8,9]

Hemodynamic parameter assessment of experimentally induced AD

Rats implanted with radiotelemeters were individually caged and placed on radiotelemeter receivers (RPC-1; Data Sciences International) to record baseline hemodynamic activity (i.e., heart rate [HR] and mean arterial pressure [MAP]) prior to SCI. This baseline activity was also used to ensure that HR and MAP values were within normal range. CRD, a well-established method for experimentally inducing an AD episode, was performed on weeks 2, 4, 6, and 8 post-T3Tx.[9,12,22] To provide a real-time readout of cardiovascular function during an AD event, HR and MAP measures were recorded every 2 sec in conscious animals using Dataquest A.R.T. or Ponemah v6.5 acquisition software (Data Sciences International). Similar to what has been previously described,[9,22] a silicone balloon-tipped catheter was carefully inserted 2 cm into the animals' rectum and secured with tape. Baseline hemodynamic activity was recorded for at least

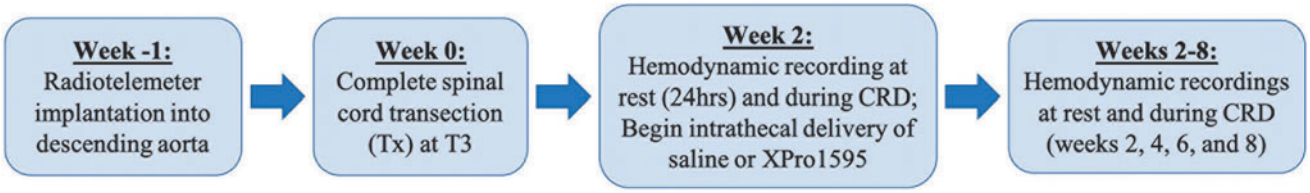

FIG. 1. Overview of experimental timeline. Color image is available online.

30 min during acclimation and established via a time-matched moving average of MAP and HR over a 6-min period. When animals consistently exhibited steady baseline activity, the balloon catheter was inflated with 2.0 mL of air over 10 sec and maintained for 1 min, thereby activating spinal viscero-sympathetic reflexes and inducing an AD episode.[8,9] CRD was performed for two or three trials per animal per time point, with at least 20 min between trials.

For each animal, the change in MAP and HR from baseline during CRD and time to return to baseline were calculated for each trial and averaged within and between groups. Differences in hemodynamic activity between treatment groups and over time were analyzed for significance via two-way mixed-analysis of variance (ANOVA) and post-hoc Fisher's Least Significant Difference (LSD) tests (GraphPad Prism 8, La Jolla, CA). All analyses were performed blinded to treatment group, and a p value <0.05 was considered significant.

Results

Virtually all animals with complete T3Tx-SCI displayed AD by 2 weeks after injury.[23,24] Moreover, AD began to intensify at this time point.[7–9,25,26] Here, we sought to determine if delaying administration of XPro1595 to inhibit sTNFα until 2 weeks post-SCI was sufficient to reverse AD or prevent the exacerbation of AD beyond 2 weeks. Using radiotelemetry, hemodynamic parameters were measured before injury in all animals and at biweekly time points throughout the duration of the study.

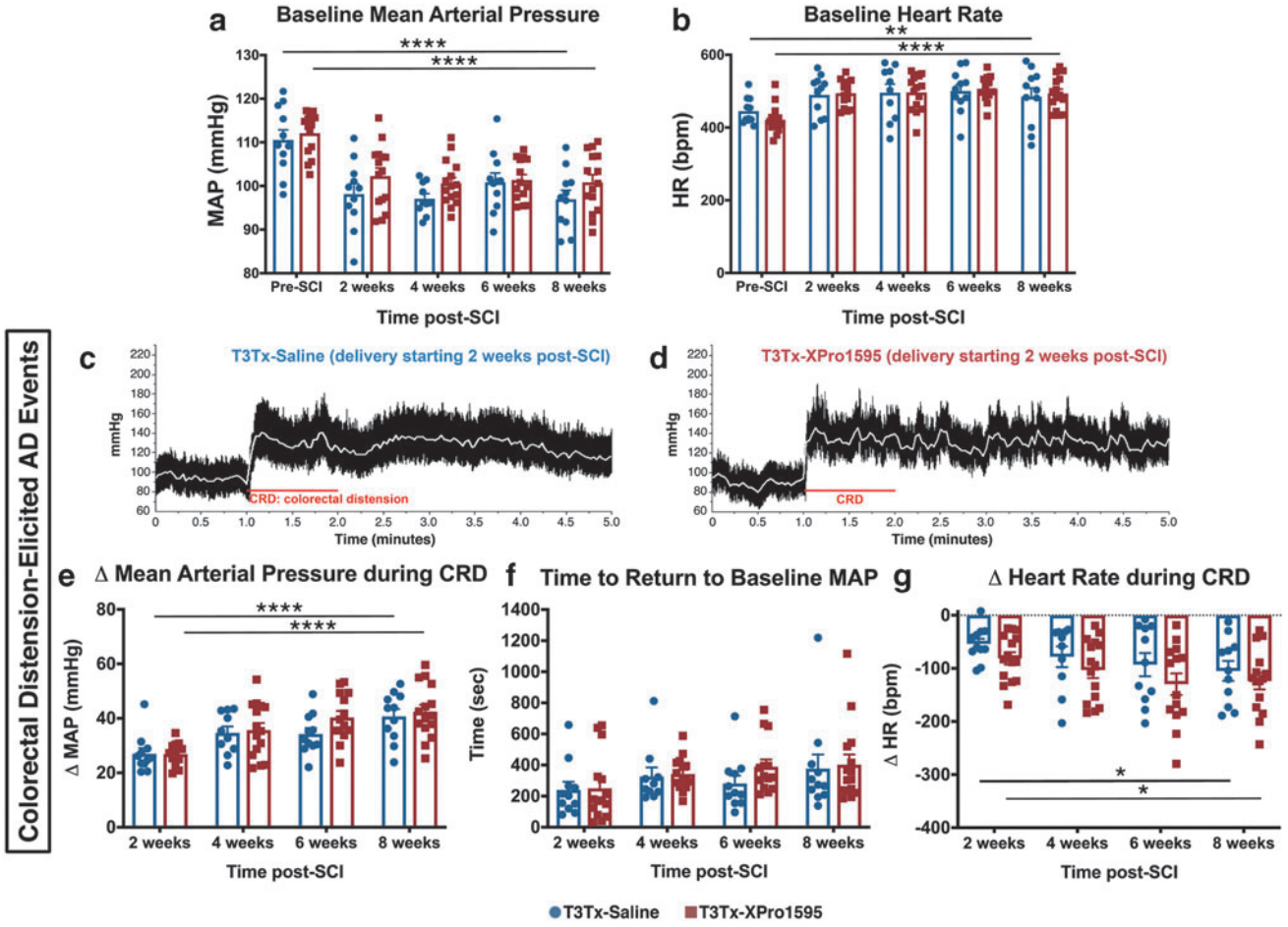

FIG. 2. Inhibition of central soluble tumor necrosis factor-alpha (sTNFα) signaling via intrathecal XPro1595 starting 2 weeks post-spinal cord injury (SCI) does not affect colorectal distension (CRD)-induced autonomic dysreflexia. (**a, b**) Continuous basal hemodynamic activity was measured prior to injury and at biweekly time points post-complete transection at the T3 spinal segment (T3Tx). In both groups, baseline mean arterial pressure (MAP) was significantly reduced following injury and persisted up to 8 weeks post-injury (wpi) (**a**). Injury also significantly increased heart rate (HR) and persisted in both groups (**b**). No differences between treatment groups were observed at any time point. (**c, d**) Representative traces of beat-to-beat arterial pressure (mm Hg) before, during, and after CRD in T3Tx-Saline ($n = 11$) and T3Tx-XPro1595 ($n = 15$) treated rats 8 wpi. The 1 min CRD event is indicated by the red line and MAP is indicated by the white line in the traces. Both T3Tx-Saline (**c**) and -XPro1595 (**d**) treated animals exhibit a sharp spike in MAP in response to CRD. (**e**) The severity of these CRD-induced MAP spikes significantly increases over 2–8 weeks post-SCI in both treatment groups. XPro1595 treatment initiated 2 wpi did not attenuate this sharp spike in MAP compared with saline-treated controls. (**f**) There was no difference within groups or across time in the duration of CRD-induced AD events, measured as time (seconds) to return to baseline MAP after the 1-min CRD period. (**g**) Inhibition of sTNFα beginning 2 wpi also had no significant effect on attenuating concomitant CRD-induced bradycardia, indicated as change (Δ) in HR beats per minute (bpm), compared with controls. In both treatment groups, such reflexive bradycardia significantly worsened over 2–8 weeks post-SCI, further demonstrating that AD worsens over time (**e, g**). Values represent mean ± standard error of the mean (S.E.M.). $*p < 0.05$; $**p < 0.01$; $****p < 0.0001$. P values were determined by two-way mixed-analysis of variance (ANOVA) and post-hoc Fisher's Least Significant Difference (LSD) tests. Color image is available online.

Baseline MAP was significantly reduced following SCI in both treatment groups and persisted up to 8 wpi (Fig 2a) ($F_{[4,94]} = 23.34$, $p < 0.0001$; post hoc, vs. 8 weeks T3Tx-Saline $p < 0.0001$; vs. 8 weeks T3Tx-XPro1595 $p < 0.0001$). SCI also resulted in significantly increased baseline HR activity up to 8 weeks in both groups (Fig. 2b) ($F_{[4,94]} = 19.92$, $p < 0.0001$; post hoc, vs. 8 weeks T3Tx-Saline $p = 0.0038$; vs. 8 weeks T3Tx-XPro1595 $p < 0.0001$), similar to what we and others have observed previously.[8,9,22,25,26] There were no differences in baseline MAP nor HR between treatment groups at any of the time points.

To trigger sympathetic hyperreflexia, all animals received CRD at 2, 4, 6, and 8 weeks post-T3Tx. CRD quickly and effectively induced hypertension and bradycardia, hallmarks of an AD event, in both T3Tx-Saline and T3Tx-XPro1595 animals (Fig. 2c,d). The magnitude of the CRD-induced MAP spike significantly increased between 2 and 8 weeks in T3Tx-Saline animals (Fig. 2e) ($F_{[3,70]} = 19.26$, $p < 0.0001$; post hoc, 2 weeks T3Tx-Saline vs. 8 weeks T3Tx-Saline $p < 0.0001$), consistent with previous studies demonstrating that AD intensifies over time.[7–9] T3Tx-XPro1595 animals exhibited a similar progression in the magnitude of CRD-induced hypertension over 2–8 weeks (Fig. 2e) ($F_{[3,70]} = 19.26$, $p < 0.0001$; post hoc, 2 weeks T3Tx-XPro1595 vs. 8 weeks T3Tx-XPro1595 $p < 0.0001$). There was no significant difference in the magnitude of the increase in MAP between treatment groups (Fig. 2e).

We previously found that starting inhibition of sTNFα early after T3Tx-SCI attenuates the duration of CRD-induced AD events.[8,9] However, initiation of XPro1595 treatment at 2 wpi did not shorten the duration of AD events compared with saline-treated animals (Fig. 2f).

We also assessed CRD-induced reflexive bradycardia. T3Tx-Saline animals had significantly more severe bradycardia during an AD episode over time (Fig. 2g) ($F_{[3,70]} = 4.797$, $p = 0.0043$; post hoc, 2 weeks T3Tx-Saline vs. 8 weeks T3Tx-Saline $p = 0.0192$). T3Tx-XPro1595 animals exhibited similarly heightened bradycardia between 2 and 8 weeks post-injury (Fig. 2g) ($F_{[3,70]} = 4.797$, $p = 0.0043$; post hoc, 2 weeks T3Tx-XPro1595 vs. 8 weeks T3Tx-XPro1595 $p = 0.0214$). There were no differences between treatment groups at any of the time points. Taken together, these data suggest that inhibition of central sTNFα beginning 2 weeks after high-thoracic SCI does not mitigate the severity or progressive intensification of sympathetic hyperreflexia, as indicated by AD.

Discussion

Dysregulation of sympathetic activity following SCI results in widespread central and systemic consequences that are detrimental to those who sustained a SCI. Therefore, there is a pressing need to identify therapeutic strategies to prevent or temper the development of sympathetic hyperreflexia. We previously showed that inhibition of central sTNFα signaling with XPro1595 beginning immediately or 3 days post-T3Tx-SCI significantly attenuates the development of sympathetic hyperreflexia and associated AD. However, neither the therapeutic window of XPro1595 nor the critical period of injury-induced sTNFα activity on driving plasticity is known. As the severity of AD events intensifies beginning 2 wpi,[8,25,27] we sought to determine whether inhibition of central sTNFα signaling beginning at this 2 wpi time point would attenuate, or even reverse, the development of sympathetic hyperreflexia, as indicated by AD.

We found that initiation of XPro1595 administration at 2 wpi did not attenuate the severity or intensification of AD events over time. We do not believe that this lack of an effect was the result of this particular lot of XPro1595 being ineffective, as we used the same batch of XPro1595 here as in our previously published study, in which we did observe attenuated sympathetic hyperreflexia when initiated 3 days post-injury (dpi).[8] Therefore, these findings indicate that sTNFα signaling within 3 days to 2 weeks after high-level SCI is crucial for the initial development of sympathetic hyperreflexia. However, once sympathetic hyperreflexia is established, sTNFα signaling is not needed for its maintenance. Thus, XPro1595 treatment needs to commence before 2 weeks post-injury in order to dampen sympathetic hyperreflexia.

Interestingly, even with early initiation of XPro1595 treatment,[8] we found that sympathetic hyperreflexia continues to intensify over time. These previous and present findings suggest that additional neuroimmune factors likely contribute to the continued intensification of AD over time. Indeed, injury-induced signaling cascades result in the dynamic, phasic infiltration of various leukocytes at the injury epicenter and below injury.[28,29] In particular, activation of central microglia and infiltration of peripherally derived macrophages are expressed at persistently elevated levels for several weeks post-SCI and are known to contribute to various forms of intraspinal plasticity.[30,31] Our laboratory previously demonstrated the persistent presence of reactive microglia/macrophages, astrocytes, and activated NF-κB in animals that started continuous treatment with XPro1595 shortly after SCI.[9] As glial cells are known to release a myriad of immune and inflammatory factors post-injury[29,32] that contribute to various forms of intraspinal plasticity,[33,34] exploring the role of these cells in sympathetic dysregulation post-injury will be an intriguing area of research. To elucidate the mechanisms underlying the ''second wave'' of AD intensification starting at 2 wpi, it would be important to characterize the different types of infiltrating leukocytes at various time points below the injury and how they affect SSR circuit plasticity.

In addition to characterizing the role of glial cells in sympathetic dysregulation, it would be interesting to explore the role of specific neuroimmune and inflammatory factors other than sTNFα. Specifically, interleukin (IL)-1β and IL-6 are intriguing potential mechanisms because of their known roles in modulating the expression of growth factors associated with afferent sprouting,[35] as well as in altering the balance of excitatory to inhibitory inputs in neural circuits.[36] Interestingly, injury-induced activation of NF-κB is known to be involved in driving the expression of proinflammatory cytokines and ensuing intraspinal plasticity. Moreover, persistent expression of such factors also contributes to the formation of a proinflammatory autocrine feedback loop via NF-κB, thereby forming a chronic, inflammatory microenvironment that drives such intraspinal plasticity.[37,38] For example, NF-κB signaling appears to contribute to sprouting of nociceptive calcitonin gene-related peptide $(CGRP)^+$ afferents post-SCI, which is associated with sympathetic hyperreflexia[9,11,12,39]; inhibition of NF-κB activity attenuates $CGRP^+$ neurite extension and growth.[9] Cytokine-mediated activation of NF-κB has also been implicated in shaping neuronal excitability, thereby contributing to an overall shift in excitability within intraspinal circuits.[40,41] Targeted manipulation of these cytokines and/or NF-κB may provide an improved understanding of the mechanisms underlying the development of sympathetic hyperreflexia, particularly during this secondary, intensifying phase. Moreover, dissecting the roles of such neuroimmune and inflammatory factors may elucidate a potential therapeutic target for attenuating sympathetic hyperreflexia and mitigating the development of secondary outcomes, such as AD and peripheral dysimmunity.

Conclusion

In conclusion, we demonstrated that inhibiting central sTNFα signaling after 2 weeks post-SCI does not affect the development of sympathetic hyperreflexia and associated AD. This furthers our understanding of the effective therapeutic window for XPro1595, which, to our knowledge, is the first pharmacological treatment shown to prophylactically treat sympathetic hyperreflexia in preclinical SCI studies.

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Author Disclosure Statement

No competing financial interests exist.

References

1. Garshick, E., Kelley, A., Cohen, S.A., Garrison, A., Tun, C.G., Gagnon, D., and Brown, R. (2005). A prospective assessment of mortality in chronic spinal cord injury. Spinal Cord 43, 408–416.
2. Curt, A., Nitsche, B., Rodic, B., Schurch, B., and Dietz, V. (1997). Assessment of autonomic dysreflexia in patients with spinal cord injury. J. Neurol. Neurosurg. Psychiatry 62, 473–477.
3. Eldahan, K.C., and Rabchevsky, A.G. (2018). Autonomic dysreflexia after spinal cord injury: Systemic pathophysiology and methods of management. Auton. Neurosci. 209, 59–70.
4. Anderson, K.D. (2004). Targeting recovery: priorities of the spinal cord-injured population. J. Neurotrauma 21, 1371–1383.
5. Karlsson, A.K. (1999). Autonomic dysreflexia. Spinal Cord 37, 383–391.
6. Rabchevsky, A.G. (2006). Segmental organization of spinal reflexes mediating autonomic dysreflexia after spinal cord injury. Prog. Brain Res.152, 265–274.
7. Zhang, Y., Guan, Z., Reader, B., Shawler, T., Mandrekar-Colucci, S., Huang, K., Weil, Z., Bratasz, A., Wells, J., Powell, N.D., Sheridan, J.F., Whitacre, C.C., Rabchevsky, A.G., Nash, M.S., and Popovich, P.G. (2013). Autonomic dysreflexia causes chronic immune suppression after spinal cord injury. J. Neurosci. 33, 12,970–12,981.
8. Mironets, E., Fischer, R., Bracchi-Ricard, V., Saltos, T., Truglio, T.S., O'Reilly, M.L., Swanson, K.A., Bethea, J.R., and Tom, V.J. (2020). Attenuating neurogenic sympathetic hyperreflexia robustly improves antibacterial immunity after chronic spinal cord injury J. Neurosci. 40, 478–492.
9. Mironets, E., Osei-Owusu, P., Bracchi-Ricard, V., Fischer, R., Owens, E.A., Ricard, J., Wu, D., Saltos, T., Collyer, E., Hou, S., Bethea, J.R., and Tom, V.J. (2018). Soluble TNFalpha signaling within the spinal cord contributes to the development of autonomic dysreflexia and ensuing vascular and immune dysfunction after spinal cord injury. J. Neurosci. 38, 4146–4162.
10. Krenz, N.R., and Weaver, L.C. (1998). Sprouting of primary afferent fibers after spinal cord transection in the rat. Neuroscience 85, 443–458.
11. Krenz, N.R., Meakin, S.O., Krassioukov, A.V., and Weaver, L.C. (1999). Neutralizing intraspinal nerve growth factor blocks autonomic dysreflexia caused by spinal cord injury. J. Neurosci. 19, 7405–7414.
12. Cameron, A.A., Smith, G.M., Randall, D.C., Brown, D.R., and Rabchevsky, A.G. (2006). Genetic manipulation of intraspinal plasticity after spinal cord injury alters the severity of autonomic dysreflexia. J. Neurosci. 26, 2923–2932.
13. Hou, S., Duale, H., and Rabchevsky, A.G. (2009). Intraspinal sprouting of unmyelinated pelvic afferents after complete spinal cord injury is correlated with autonomic dysreflexia induced by visceral pain. Neuroscience 159, 369–379.
14. Ueno, M., Ueno-Nakamura, Y., Niehaus, J., Popovich, P.G., and Yoshida, Y. (2016). Silencing spinal interneurons inhibits immune suppressive autonomic reflexes caused by spinal cord injury. Nat. Neurosci. 19, 784–787.
15. Llewellyn-Smith, I.J., and Weaver, L.C. (2001). Changes in synaptic inputs to sympathetic preganglionic neurons after spinal cord injury. J. Comp. Neurol. 435, 226–240.
16. Beattie, E.C., Stellwagen, D., Morishita, W., Bresnahan, J.C., Ha, B.K., Von Zastrow, M., Beattie, M.S., and Malenka, R.C. (2002). Control of synaptic strength by glial TNFa. Science 295, 2282–2285.
17. Spicarova, D., Nerandzic, V., and Palecek, J. (2011). Modulation of spinal cord synaptic activity by tumor necrosis factor alpha in a model of peripheral neuropathy. J Neuroinflammation 8, 177.
18. Stellwagen, D., Beattie, E.C., Seo, J.Y., and Malenka, R.C. (2005). Differential regulation of AMPA receptor and GABA receptor trafficking by tumor necrosis factor-alpha. J. Neurosci. 25, 3219–3228.
19. Bethea, J.R., Nagashima, H., Acosta, M.C., Briceno, C., Gomez, F., Marcillo, A.E., Loor, K., Green, J., and Dietrich, W.D. (1999). Systemically administered interleukin-10 reduces tumor necrosis factor-alpha production and significantly improves functional recovery following traumatic spinal cord injury in rats. J. Neurotrauma 16, 851–863.
20. Wang, C.X., Nuttin, B., Heremans, H., Dom, R., and Gybels, J. (1996). Production of tumor necrosis factor in spinal cord following traumatic injury in rats. J. Neuroimmunol. 69, 151–156.
21. Steed, P.M., Tansey, M.G., Zalevsky, J., Zhukovsky, E.A., Desjarlais, J.R., Szymkowski, D.E., Abbott, C., Carmichael, D., Chan, C., Cherry, L., Cheung, P., Chirino, A.J., Chung, H.H., Doberstein, S.K., Eivazi, A., Filikov, A.V., Gao, S.X., Hubert, R.S., Hwang, M., Hyun, L., Kashi, S., Kim, A., Kim, E., Kung, J., Martinez, S.P., Muchhal, U.S., Nguyen, D.H., O'Brien, C., O'Keefe, D., Singer, K., Vafa, O., Vielmetter, J., Yoder, S.C., and Dahiyat, B.I. (2003). Inactivation of TNF signaling by rationally designed dominant-negative TNF variants. Science 301, 1895–1898.
22. Hou, S., Tom, V.J., Graham, L., Lu, P., and Blesch, A. (2013). Partial restoration of cardiovascular function by embryonic neural stem cell grafts after complete spinal cord transection. J. Neurosci. 33, 17,138–17,149.
23. Krassioukov, A.V., and Weaver, L.C. (1995). Episodic hypertension due to autonomic dysreflexia in acute and chronic spinal cord-injured rats. Am. J. Physiol. 268, H2077–H2083.
24. Marsh, D.R., and Weaver, L.C. (2004). Autonomic dysreflexia, induced by noxious or innocuous stimulation, does not depend on changes in dorsal horn substance p. J. Neurotrauma 21, 817–828.
25. West, C.R., Popok, D., Crawford, M.A. and Krassioukov, A.V. (2015). Characterizing the temporal development of cardiovascular dysfunction in response to spinal cord injury. J Neurotrauma 32, 922–930.
26. Rabchevsky, A.G., Patel, S.P., Lyttle, T.S., Eldahan, K.C., O'Dell, C.R., Zhang, Y., Popovich, P.G., Kitzman, P.H., and Donohue, K.D. (2012). Effects of gabapentin on muscle spasticity and both induced as well as spontaneous autonomic dysreflexia after complete spinal cord injury. Front. Physiol. 3, 329.
27. West, C.R., Crawford, M.A., Laher, I., Ramer, M.S., and Krassioukov, A.V. (2016). Passive hind-limb cycling reduces the severity of autonomic dysreflexia after experimental spinal cord injury. Neurorehabil. Neural. Repair 30, 317–327.
28. Donnelly, D.J., and Popovich, P.G. (2008). Inflammation and its role in neuroprotection, axonal regeneration and functional recovery after spinal cord injury. Exp. Neurol. 209, 378–388.
29. Trivedi, A., Olivas, A.D., and Noble-Haeusslein, L.J. (2006). Inflammation and spinal cord injury: infiltrating leukocytes as determinants of injury and repair processes. Clin. Neurosci. Res. 6, 283–292.
30. Popovich, P.G., Wei, P., and Stokes, B.T. (1997). Cellular inflammatory response after spinal cord injury in Sprague-Dawley and Lewis rats. J. Comp. Neurol. 377, 443–464.
31. David, S., and Kroner, A. (2011). Repertoire of microglial and macrophage responses after spinal cord injury. Nat. Rev. Neurosci. 12, 388–399.
32. Grace, P.M., Hutchinson, M.R., Maier, S.F., and Watkins, L.R. (2014). Pathological pain and the neuroimmune interface. Nat. Rev. Immunol. 14, 217–231.

33. Gaudet, A.D., and Fonken, L.K. (2018). Glial cells shape pathology and repair after spinal cord injury. Neurotherapeutics 15, 554–577.
34. O'Reilly, M.L., and Tom, V.J. (2020). Neuroimmune system as a driving force for plasticity following CNS injury. Front. Cell. Neurosci. 14, 187.
35. Weaver, L.C., Marsh, D.R., Gris, D., Meakin, S.O., and Dekaban, G.A. (2002). Central mechanisms for autonomic dysreflexia after spinal cord injury. Prog. Brain Res. 137, 83–95.
36. Kawasaki, Y., Zhang, L., Cheng, J.K., and Ji, R.R. (2008). Cytokine mechanisms of central sensitization: distinct and overlapping role of interleukin-1beta, interleukin-6, and tumor necrosis factor-alpha in regulating synaptic and neuronal activity in the superficial spinal cord. J. Neurosci. 28, 5189–5194.
37. Liu, T., Zhang, L., Joo, D. and Sun, S.C. (2017). NF-kappaB signaling in inflammation. Signal Transduct. Target Ther. 2, 17023.
38. Jost, P.J., and Ruland, J. (2007). Aberrant NF-kappaB signaling in lymphoma: mechanisms, consequences, and therapeutic implications. Blood 109, 2700–2707.
39. Weaver, L.C., Verghese, P., Bruce, J.C., Fehlings, M.G., Krenz, N.R., and Marsh, D.R. (2001). Autonomic dysreflexia and primary afferent sprouting after clip-compression injury of the rat spinal cord. J. Neurotrauma 18, 1107–1119.
40. Shim, D.J., Yang, L., Reed, J.G., Noebels, J.L., Chiao, P.J., and Zheng, H. (2011). Disruption of the NF-kappaB/IkappaBalpha autoinhibitory loop improves cognitive performance and promotes hyperexcitability of hippocampal neurons. Mol. Neurodegener. 6, 42.
41. Yu, Z., Cheng, G., Wen, X., Wu, G.D., Lee, W.-T., and Pleasure, D. (2002). Tumor necrosis factor α increases neuronal vulnerability to excitotoxic necrosis by inducing expression of the AMPA–glutamate receptor subunit GluR1 via an acid sphingomyelinase- and NF-κB-Dependent mechanism. Neurobiol. Dis. 11, 199–213.

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