Spinal Cord Injury Causes Prominent Tau Pathology Associated with Brain Post-Injury Sequela

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Abstract

Spinal cord injury (SCI) can lead to neurological impairment with significant functional and cognitive deficits. It is obvious that SCI causes focal neurodegeneration that gradually expands to the other cord areas. On the other hand, it is clear that traumatic brain injuries result in tau protein pathology and profound neurodegeneration. Tau is a microtubule-associated protein, which is highly expressed in neurons, and its abnormalities result in neuronal cell death. Moreover, it is clear that tau pathology spreads in various brain areas upon trauma. Therefore, we herein examined tau pathology in the spinal cord as well as brain samples at various time-points in severe SCI mouse models. We examined the effects of severe SCI on locomotor function, spatial memory, and anxiety/risk-taking behavior. We found a gradual increased tau pathology in the spinal cord as well as brain areas; confirmed by immunostaining and immunoblotting. Moreover, we studied the brain samples with electron microscopy and observed disrupted mitochondria and microtubule structure upon SCI. SCI caused motor dysfunction, memory impairment, and abnormal risk-taking behavior. Importantly, pathogenic *cis* P-tau elimination with systemic administration of respective monoclonal antibody restored SCI-related pathological and functional consequences. Thus, our finding suggests that SCI results in profound tauopathy, which spreads to brain areas, reflecting brain dysfunction. Moreover, tau immunotherapy with anti-*cis* P-tau antibody could suppress the pathogenic outcomes in the SCI mouse models, which would have profound clinical implications in the SCI patients.

Introduction

The global prevalence of spinal cord injury (SCI) is approximately 500000 people each year due to motor vehicle crashes, falls, violence, and sporting accidents [1, 2], and can result in substantial neurological impairment along with significant emotional and psychological distress [3].

SCI can interrupt nerve impulses conduction, resulting in neurological dysfunction [4]. Primary injury affecting the spinal cord has been shown to promptly disrupt cell membranes, myelin and axons destruction within the longitudinal tracts. Moreover, the SCI damages microvessels, resulting in destructive secondary injury by releasing different harmful factors [5]. Different cellular and molecular mechanisms in the secondary injury process may lead to comprehensive neurodegeneration [6]. Such cascades as active biological processes offer a chance of treating SCI by selective inhibitors.

SCI is also able to change systemic immune functions, whereby affecting the brain [7]. Moreover, the released agents may reach to the brain via cerebrospinal fluid (CSF). The brain abnormalities, caused by SCI, are due to afferent and efferent routes modifications. However, there are remarkable neuropathological hallmarks triggered by SCI, such as decreased number of cortical neurons [4, 8–12]. Moreover, there is cognitive impairment in 60% of the SCI population [4, 10–12].

Despite extensive considerations, it remains uncertain how SCI results in the brain abnormalities. It is clear that tau protein abnormality is a major pathological hallmark upon traumatic brain injury (TBI) [13].

Tau is a microtubule-associated protein that promotes microtubules (MTs) formation and stabilization [14, 15]. Tau is commonly subjected to hyperphosphorylation in tauopathies on Ser/Thr residues, which in turn impairs the function of the MT as well as changes in protein integrity, resulting in its aggregation and tangle formation [16, 17]; particularly in chronic traumatic encephalopathy (CTE) [18–23], and Alzheimer's disease (AD) [16, 17].

It is clear that phosphorylated tau at Thr231 exists in the two distinct *cis* and *trans* conformation in which that *cis* pThr231-tau (*cis* P-tau) conformer is extremely neurotoxic and early driver of tauopathy process upon TBI. Peptidyl-prolyl *cis/trans* isomerase (Pin1) suppresses the tau pathology development as well as neurodegeneration in AD by changing the phosphorylated tau at Thr231-Pro motif from neurotoxic *cis* to the physiological *trans* conformation [24–32]. Moreover, tauopathy process could be blocked by *cis* P-tau monoclonal antibody (*cis* mAb); both *in vitro* and *in vivo* [33, 34]. Also, pathogenic *cis* P-tau shows a prion nature and spreads in brain areas as well as CSF in tauopathy mouse models [33–37].

A few studies have investigated total tau and P-tau concentrations in CSF, serum, and spinal cord tissue in patients and experimental animals with SCI. However, it remains to be fully understood the molecular mechanism of tau pathology in SCI. Also, the causative link between SCI and brain dysfunction remains elusive thus far. Thus, we herein examined tau pathology process in severe SCI (sSCI) mouse models; as proposed previously [38]. We induced sSCI and investigated various pathogenic tau species formation, and neurodegeneration in both cord and brain tissues at different time points to examine whether SCI injury can lead to the brain pathology.

Materials And Methods

Animals and study design. Male Balb/c mice (2–3 months old) weighting 22–26 g, were obtained and housed in clear plastic cages, under controlled temperature and humidity in a 12 h light/dark cycle with free access to water and food. All animals were allowed to acclimate to their new surroundings for 1 week before they undergo any experimental procedures. All protocols were approved by the ethics committee of Tabriz University of Medical Sciences (approval No. IR.TBZMED.VCR.REC.1398.067). All experiments were performed according to the Guide for the Care and Use of Laboratory Animals by the National Institutes of Health (NIH Publications).

-54 adult male mice were randomly divided into six groups ($n=9$): Sham group (laminectomy surgery without compression injury), sSCI (48h), sSCI (2W), and sSCI (1M) groups (severe compression injury at 8th thoracic segment 'T8' of the spinal cord and were sacrificed 48 h, 2 weeks, and 1 months after the SCI), sSCI (2M) + IgG group (severe compression injury at T8 and received IgG after the SCI for 2 months), and sSCI (2M) + *cis* mAb group (severe compression injury at T8 and received *cis* mAb after the SCI for 2 months).

Laminectomy and calibrated forceps model of spinal cord compression. All procedures were performed under sterile conditions. Mice were anesthetized with 4% isoflurane, and a laminectomy was done at T7-9 to expose the T8 segment of the spinal cord, without any damage to the dura. Pairs of forceps were

applied for laterally compressing the spinal cord to the corresponding thickness (0.25 mm) for 15 sec [39, 40]. Sham group received laminectomy and forceps placement around the spinal cord, without a compression. Muscles and skin were stitched after forceps removal followed by administration of saline solution for rehydration (1 ml), buprenorphine (0.05 mg/kg) to alleviate pain and ciprofloxacin (5 mg/kg) to treat/prevent bladder infection, all subcutaneously (SC) two times a day for 3 days. Animals were monitored in a temperature-controlled room until recovery and then transferred to their separate cage. Bladders of SCI mice were manually expressed two times a day until the establishing of the urinary reflex.

Locomotor analysis. Motor function was assessed in mice to ensure that an effective SCI or a successful laminectomy was done. Hind-limb function in groups was tested by the Basso mouse scale (BMS) one day after injury in the open-field (OF) [41]. In brief, animals were individually placed in the OF chamber (22.5 × 22.5 cm) and allowed to freely explore for 5 min. Two evaluators independently gave each animal a score of 0 to 9, with a score of 0 indicating a complete defect in locomotor function and a score of 9 indicating no locomotor deficits.

To determine the characteristics of *cis* P-tau, AT8 P-tau, and AT100 P-tau induction upon SCI, mice in the sSCI (48h) and sSCI (2W) groups were anesthetized with intraperitoneal (IP) injection of ketamine (60 mg/kg) and xylazine (10 mg/kg) 48 h and 2 weeks after the SCI, and the spinal cord tissue samples were collected for immunoblotting and immunofluorescence staining analyses.

Antibody treatment of mice. To evaluate the efficacy of *cis* mAb in treating SCI, we examined whether *cis* mAb could affect intracellular P-tau in sSCI (2M) + *cis* mAb, and sSCI (2M) + IgG groups. Undergoing sSCI, male mice were randomly treated with mouse *cis* mAb or mouse IgG. Animals received 1 dose of *cis* mAb/IgG IP pre-treatment (200 µg/per mouse) 3 days before the injury, single IP post-injury treatment (20 µg in 5 µl) 15 min after SCI, then IP post-treatment (200 µg) every 4 days for 2 weeks, followed by 200 µg weekly for the rest of the two month treatment (33).

Transmission electron microscopy (TEM). The ultrastructural assessment was carried out using the TEM method. The brain and spinal cord specimens, from sham and SCI mouse models treated with either control IgG or *cis* mAb, were cut into pieces of 2 × 2 mm. Briefly, the cells were fixed in glutaraldehyde 2.5%, buffered at 0.1 M phosphate (pH 7.4), osmium tetroxide 1% was used for post-fixation, and finally embedding was performed using resin. Ultrathin sections around 60–90 nanometer were cut and taken on a copper grid and stained with a mixture of uranyl acetate and lead citrate and examined under a ZIESS electron microscope (EM902A), and then viewed by a Leo 906 (Leo, Germany) transmission electron microscope.

Immunoblotting analysis. For immunoblotting, spinal cord and the brain samples were homogenized in RIPA buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 2 mM EDTA, 1% NP 40, 0.1% SDS, 0.5% Na-deoxycholate, 50 mM NaF) containing proteinase and phosphatase inhibitors and then mixed with the SDS sample buffer and loaded onto a gel after boiling. The proteins were resolved by polyacrylamide gel electrophoresis 12% and transferred to a PVDF membrane. Then the membranes were blocked with 2% milk in TBST (10 mM Tris-HCl pH 7.6, 150 mM NaCl, 0.1% Tween 20) for 1 h. Next, the membranes were

incubated with primary antibodies overnight at 4°C. Then, the immunoblots were incubated with HRP-conjugated secondary antibody in 2% milk in TBST. The signals were detected using chemiluminescence reagent (Perkin Elmer, San Jose, CA). The membranes were washed 6 times with TBST after each step. Immunoblotting results were quantified with imageJ. The band of interests were normalized against actin.

Immunostaining analysis. Mice were deeply anesthetized and perfused through the left ventricle with 10% neutral buffered formalin. The spinal cord and brain were removed immediately, and post-fixed in 10% neutral buffered formalin overnight. A 1.5 cm segment of spinal cord centered on the injury site and brain were embedded into paraffin, and then cut into 8 µm increments by a microtome.

Sections were dewaxed, and then dehydrated in a serial dilution of ethanol. A 5% ammonium chloride solution (Merck, 101145) were used to quench autofluorescence. The sections were placed in a steamer (0.01 M) Sodium citrate (Sigma-Aldrich, S4641) for 20 minutes for antigen enhancement. Then, the slides were permeabilized with 0.5% Triton X-100 (Sigma-Aldrich, T8532) for 15 min, and blocked with 10% anti-goat serum for one hour. Next, the slides were incubated with following primary antibodies overnight: *cis* P-tau mAb (gift from KP. Lu), AT8 P-tau (MN1020), and AT100 P-tau (MN1060). The samples were incubated with the anti-mouse secondary antibodies (Alexa Fluor 488 or 594) at 37°C for one hour. The nuclei were stained with Dapi (Invitrogen, D1306), and the images were visualized by a fluorescent microscope (BX71; Olympus equipped with DP72 digital camera).

Twenty ROIs (The 350×450 µm² dimension representative view) from the cortices of the mouse brains were randomly selected and quantified for each group. The immunofluorescence intensity were quantified with ImageJ ($n = 3$ /per group).

Open-field locomotion. On days 1, 30, and 60 post-injury, hind-limbs functions and spontaneous locomotor activity were evaluated in the OF test. We used the BMS and the related subscale to score locomotor performance. To determination of spontaneous locomotor activity, as mice freely explore the OF chamber for 5 min, a computer-based video-tracking system (Noldus Ethovision) recorded total traveled distance and speed. The OF test is not only used to measure motor activity, but also to measure other factors such as anxiety. Rodents usually spend more time in contact with the walls of the chamber than in the center area (thigmotaxis) [42, 43]. On day 60 after injury, the mouse freely explored the maze for 5 min, and the tracking software recorded movement. Mice that spend most of their time in the unprotected central area of the maze indicate anxiolytic-like behaviors [42, 43].

Elevated plus-maze (EPM) test. The EPM task is an experiment model to evaluate anxiety/risk-taking behavior; which examines cortical circuits [44–49]. The test is performed on a plus-shaped apparatus which is raised 50 cm above the ground, with two opposite open (aversive, without walls) and two opposite closed (safe, 15 cm high walls) arms (30 × 5 cm), and a central square. A mouse is placed on the central square of the apparatus, facing an open arm, and explore through the maze for 5 min. The number of open arm entries and total time exploring open arms were recorded (Noldus Ethovision). The

maze was thoroughly cleaned after each trial to avoid any odorant confounding the test. Mice with normal levels of anxiety/risk-taking behavior enter the open arms less often and spend less time in the open arms. Entering the open arms is being addressed to abnormal risk-taking and anxiety behaviour associated with a cognitive decline [33, 50].

Y-maze spontaneous alternation test. The Y-maze test is used for the evaluation of spatial working memory or to quantify cognitive deficits in rodents. The Y-maze consists of three identical black arms (30 cm long, 6 cm wide with 15 cm high walls) mounted in the shape of the letter 'Y' at a 120° angle from each other. Rodents usually show a tendency to explore a new arm rather than exploring the arms previously visited. One arm (arms A–C) was randomly selected as the 'start' arm, and the mouse is placed at the end of it and allowed to move through each arm. The number of arm entries (when all four paws enter the arm) was recorded for 10 min. Alternation is consecutive entries into each arm without any repeats. The alternation percentage is calculated by using the following formula: (Number of alternations / Number of arm entries) × 100. A mouse with intact working memory scored significantly $\geq 50\%$ [51–53].

At the end of the second month, and after the behavioral tests, mice were anesthetized by ketamine-xylazine IP injection. For electron microscopy, immunoblotting, and immunofluorescence staining analyses, samples were collected from the brain and spinal cord tissues.

Statistical analysis. All normally distributed data were evaluated using one-way analysis of variance (ANOVA) followed by Tukey's HSD multiple comparisons post hoc test to compare the groups mean differences. All data were analyzed with SPSS software version 22, and were reported as the means $\pm$ standard deviation (SD) with $P < 0.05$ considered significant.

Results

Compression severe SCI induced tau pathology in the cord neurons. We used SCI mouse models to study tau pathology triggered by the injury by performing immunoblotting and immunofluorescence staining on the spinal cord tissues. Severe SCI acutely and persistently induced pathogenic *cis* P-tau 48 h after the injury, and remained at high levels over 2 weeks. Robust *cis* P-tau signal (1.128 ± 0.03875) and (1.319 ± 0.06179) were detected in the cords at 48 hours and two weeks after injury (Fig. 1a, $P < 0.0001$, $P = 0.0035$). Immunoblotting also showed the progressive increase in AT8 P-tau [$F(2, 4) = 122.8$, $P = 0.0056$], (early tangle), and AT100 P-tau [$F(2, 4) = 255.8$, $P = 0.0034$], (late tangle) in the cord tissues upon trauma in a timely manner (Fig. 1b-e). It seems that the SCI engendered tau pathology in the cord tissue.

Immunotherapy with anti *cis* mAb improved tau pathology consequences triggered by sSCI. It is clear that *cis* P-tau is associated with neurodegenerative outcomes and progression of tauopathy triggered by TBI. As mentioned before, we showed that *cis* P-tau was induced 48 h of sSCI, and thus, there could be a relationship between *cis* P-tau and pathological changes after injury. To understand the brain complications triggered by SCI, and specially, the effectiveness of *cis* mAb on neurodegenerative pathologies triggered by sSCI, we treated the animal models with either *cis* mAb or control IgG isotype for 2 months. We have shown that pathogenic *cis* P-tau spread to various brain areas after sSCI, resulting in

brain tau pathogenicity. However, that *cis* mAb treatment effectively suppressed tau pathology (Fig. 2); consistent with previous findings [33, 34, 54–57].

Immunofluorescence staining of sSCI mouse brains showed profound *cis* P-tau signals one month after injury (11.70 ± 2.078 , $P = 0.0191$) (Fig. 2a-b). Immunoblotting assay also confirmed the presence of profound amount of *cis* P-tau (1.296 ± 0.068 , $P = 0.0228$), AT8 P-tau (1.386 ± 0.08493 , $P = 0.0102$), and AT100 P-tau (1.242 ± 0.088 , $P = 0.0132$) in the cortices of sSCI mice while there was no significant amount of the P-tau epitopes in sham animals (0.4958 ± 0.076 , 0.247 ± 0.050 , 0.334 ± 0.040 , Fig. 2c-f). Interestingly, while immunotherapy with control IgG did not suppress *cis* P-tau formation in the cortices of the sSCI animals compared to the sham groups (27.94 ± 3.554 , $P = 0.0234$), *cis* mAb administration effectively stopped tau pathology and *cistauosis* in the immunostained sSCI mouse's brains (1.611 ± 0.4818 , $P = 0.7424$, Fig. 2a-b). Similarly, the efficacy of *cis* mAb administration to hinder *cis* P-tau (0.3967 ± 0.1611), AT8 P-tau (0.4870 ± 0.02438), and AT100 P-tau formation (0.5332 ± 0.07576) was shown in the immunoblots of sSCI mouse brains; while IgG treatment did not change the amount of tau epitopes formation (Fig. 2c-f).

It seems that SCI could induce tau pathology in both cord and brain areas and *cis* mAb immunotherapy of sSCI animals can efficiently impeded the pathogenic P-tau formation in the cortices of mouse brains.

SCI caused motor function impairment, improved by *cis* mAb. Open-field test was performed on days 1, 30, and 60 of the sSCI to examine spontaneous locomotor activity, and hind-limbs functions using the BMS scores [41]. On every three days of the experiment, mice from both sSCI (1M) and sSCI (2M) + IgG groups displayed significant reduction in total traveled distance ($P < 0.001$) during the test, compared with sham mice. The reduction in total traveled distance was paralleled by reduction in walking speed in sSCI (1M) and sSCI (2M) + IgG mice ($P < 0.001$) versus sham mice. Importantly, on days 30, and 60 after injury, we observed significant improvement of spontaneous locomotor activity in *cis* mAb-treated sSCI group ($P < 0.001$) compared with sSCI and IgG-treated sSCI groups (Fig. 4a, b).

All mice indicated almost complete loss of motor function one day after injury (BMS score of 1). Hind-limb functional recovery, using the BMS score, significantly improved in *cis* mAb-treated mice compared with IgG-treated animals ($P < 0.001$) by day 60 (Fig. 4c). Therefore, *cis* P-tau elimination improved the recovery of spontaneous locomotor activity as well as hind-limb function in sSCI mice.

SCI caused cognitive dysfunction, restored by *cis* mAb. The Y-maze spontaneous alternation test was performed to measure spatial working memory. Sham group displayed functional working memory with ~ 65% spontaneous alteration. Severe SCI significantly reduced percentage of spontaneous alteration ($P < 0.001$) compared with the sham group. SCI mice treated with *cis* mAb showed an increased percentages of spontaneous alteration compared with the IgG-treated sSCI animals ($P = 0.002$ or $P < 0.01$) (Fig. 5a).

To examine the anxiolytic properties of *cis* mAb, we performed open-field and elevated-plus maze tests 60 days after SCI. The sham animals frequently entered the center portion of the maze while the sSCI mouse

treated with IgG spent limited time in the center of the maze in the open-field test. These findings demonstrated of high levels of thigmotaxis (tendency to stay close the walls), indicating an increased anxiety-like behavior. We observed that *cis* mAb-treated sSCI mouse spent more time in the center area compared to the IgG-treated sSCI animals (Fig. 5b). In the EPM test, sham animals explored significantly less time in the open arms than IgG-treated sSCI ($P = 0.009$ or $P < 0.01$) mice. Moreover, *cis* mAb-treated group, similar to the sham group, spent significantly less time in the open arms ($P < 0.001$) compared with IgG-treated sSCI groups (Fig. 5c). SCI (2M) + IgG mice displayed more total open arm entries than the sham and *cis* mAb-treated sSCI mice (Fig. 5d). As a result, all IgG-treated sSCI mice, exploring the two open arms, showed anxiety/risk-taking behavior by contrast, *cis* mAb-treated mice displayed minimal anxious behavior. Our data confirm this viewpoint that, rodents prefer protected areas such as the nest. Thus, *cis* mAb not only eliminates *cis* P-tau and *cistaurosis*, but also restores behavioral deficits triggered by SCI.

Discussion

It is clear that SCI impairs brain function and results in cognitive decline in the patients. However, the molecular mechanisms by which SCI affects the brain has remained elusive thus far. SCI can reorganize the cerebral cortex and thalamus by anterograde and retrograde [60]. For example, the thalamic nucleus can send cortical efferents to the hippocampal compartment [61]. Moreover, injured neurons in spinal cord produce cysteine-cysteine chemokine ligand 21, which activates microglia at distant spinal cord sections as well as thalamus [62, 63]. SCI also alters systemic immune functions [64]; affecting the brain. It is possible that neuronal cell injury or death releases intracellular MT binding proteins into the extracellular space, whereby the proteins travel to the brain through the CSF [65, 66]. Several studies have shown that tau level increases in the CSF during acute TBI [67] as well as SCI [68]. Despite extensive considerations, the underlying molecular mechanisms mediating brain pathology due to the SCI remain elusive [69].

It has been shown that single severe TBI (ssTBI) or repetitive mild TBI (rmTBI) induce pathogenic *cis* P-tau in axons few hours following injury, leading to neurodegeneration. *Cis* P-tau has a prion nature and spreads to various brain areas [33, 35]. Notably, *cis* mAb treatment can eliminate pathogenic *cis* P-tau and restore axonal pathologies, such as MT defects, organelle transport and long-term potentiation (LTP), and prevent developing many short- and long-term pathological and functional consequences after ssTBI or rmTBI [33–35, 59].

We herein explored tau pathologies triggered by SCI in order to determine and treat brain complications, including axonal pathology, functional deficits, and cognitive impairment; as proposed previously [38]. We found that *cis* P-tau is the initial and important mediator of neurodegeneration and functional defects following SCI in the mouse model. Moreover, we evaluated the *cis* mAb therapy effects on pathological and functional brain complications following injury. We observed: (1) Severe SCI had a robust and persistent effect on *cis* P-tau induction, and led to histological changes in the spinal cord and brain tissues; (2) Severe SCI caused locomotor impairment, cognitive deficits and anxiety-like behaviors; (3)

Treating SCI mice with *cis* mAb effectively prevented the development of extensive tauopathy, improved histopathological consequences, restored motor and cognitive function. Importantly, our histological results showed that *cis* P-tau level at the site of injury was significantly increased, and spread from the spinal cord to the brain within 2 months of the injury. These findings indicate that *cis* P-tau induction is essential to develop several pathological and functional outcomes following SCI.

Conclusion

We herein examined the relationship between SCI and tau pathology in the mouse models. We have shown that pathogenic tau induced focally in the spinal cord tissue early after a severe compression SCI, which spread to the brain areas; likely through CSF, and induced prominent tauopathy, resulting in motor dysfunction. Taken these together, we propose *cis* P-tau as a reliable biomarker to evaluate the SCI pathologic outcomes.

Additional studies in pre-clinical SCI models and patients with different degrees of SCI are needed to determine the value of *cis* P-tau as a biomarker. The biomarkers may propose novel SCI therapeutic targets as well as treatment approaches. In summary, our results suggest that pathogenic *cis* P-tau is a tauopathy driver in SCI, which is a potential diagnosis and therapeutic target for immunotherapy.

Abbreviations

SCI, Spinal cord injury; CSF, Cerebrospinal Fluid; TBI, Traumatic Brain Injury; MTs, Microtubules; CTE, Chronic Traumatic Encephalopathy; *cis* P-tau, *cis* pThr231-tau; AD, Alzheimer's disease; Pin1, Peptidyl-prolyl *cis/trans* isomerase; *Cis* mAb, *cis* P-tau monoclonal antibody; sSCI, Severe SCI; NIH, National Institute of Health; SC, Subcutaneously; BMS, the Basso Mouse Scale; OF, Open-field; IP, intraperitoneal; TEM, Transmission Electron Microscopy; EPM, Elevated plus-maze; ROI, Region Of Interest; ANOVA, Analysis of Variance; SD, Standard Deviation; ssTBI, Single Severe TBI; rmTBI, Repetitive Mild TBI; LTP, long-term potentiation.

Declarations

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Conflict of Interest. The authors have no conflicts of interest to declare that are relevant to the content of this article.

Ethics approval. This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of Tabriz University of Medical Sciences (Code of ethics: IR.TBZMED.VCR.REC.1398.067).

Consent to participate. Not applicable.

Consent for publication. All the authors approve content of the manuscript and agree with *Molecular Neurobiology* publication policies.

Availability of data and material. The data sets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions. All authors contributed to the study conception and design. Elnaz Nakhjiri expressed the idea of the article and performed the literature search. The first draft of the manuscript was written by Elnaz Nakhjiri, Koorosh Shahpasand and Parviz Shahabi. All authors edited the manuscript and approved the final manuscript.

References

1. Ackery A, Tator C, Krassioukov A (2004) A global perspective on spinal cord injury epidemiology. *J Neurotrauma* 21(10):1355–1370
2. van den Berg MEL, Castellote JM, Mahillo-Fernandez I, de Pedro-Cuesta J (2010) Incidence of Spinal Cord Injury Worldwide: A Systematic Review. *Neuroepidemiology* 34(3):184–192. <https://www.karger.com/Article/FullText/279335>
3. Fehlings M, Singh A, Tetreault L, Kalsi-Ryan S, Nouri A (2014) Global prevalence and incidence of traumatic spinal cord injury. *Clin Epidemiol* <http://www.dovepress.com/global-prevalence-and-incidence-of-traumatic-spinal-cord-injury-peer-reviewed-article-CLEP>
4. Collins-Praino LE, Corrigan F (2017) Does neuroinflammation drive the relationship between tau hyperphosphorylation and dementia development following traumatic brain injury? *Brain Behav Immun* 60:369–382. <https://linkinghub.elsevier.com/retrieve/pii/S0889159116304366>
5. Bethea JR, Dietrich WD (2002) Targeting the host inflammatory response in traumatic spinal cord injury. *Curr Opin Neurol* 15(3):355–360
6. Tator CH, Fehlings MG (1991) Review of the secondary injury theory of acute spinal cord trauma with emphasis on vascular mechanisms. *J Neurosurg* 75(1):15–26. <https://thejns.org/view/journals/j-neurosurg/75/1/article-p15.xml>

7. Nielson JL, Sears-Kraxberger I, Strong MK, Wong JK, Willenberg R, Steward O (2010) Unexpected Survival of Neurons of Origin of the Pyramidal Tract after Spinal Cord Injury. *J Neurosci* 30(34):11516–28. Available from: <https://www.jneurosci.org/lookup/doi/10.1523/JNEUROSCI.1433-10.2010>
8. Nielson JL, Strong MK, Steward O (2011) A reassessment of whether cortical motor neurons die following spinal cord injury. *J Comp Neurol* 519(14):2852–2869. <https://onlinelibrary.wiley.com/doi/10.1002/cne.22661>
9. Wannier T, Schmidlin E, Bloch J, Rouiller EM (2005) A Unilateral Section of the Corticospinal Tract at Cervical Level in Primate Does Not Lead to Measurable Cell Loss in Motor Cortex. *J Neurotrauma* 22(6):703–717. <http://www.liebertpub.com/doi/10.1089/neu.2005.22.703>
10. Coulthart MB, Jansen GH, Cashman NR (2016) Evidence for transmissibility of Alzheimer disease pathology: Cause for concern? *Can Med Assoc J* 188(10):E210–E212. <http://www.cmaj.ca/lookup/doi/10.1503/cmaj.151257>
11. Cohen ML, Tulskey DS, Holdnack JA, Carlozzi NE, Wong A, Magasi S et al (2017) Cognition among community-dwelling individuals with spinal cord injury. *Rehabil Psychol* 62(4):425–434
12. Craig A, Guest R, Tran Y, Middleton J (2017) Cognitive Impairment and Mood States after Spinal Cord Injury. *J Neurotrauma* 34(6):1156–1163. <http://www.liebertpub.com/doi/10.1089/neu.2016.4632>
13. Jeter CB, Hergenroeder GW, Hylin MJ, Redell JB, Moore AN, Dash PK (2013) Biomarkers for the Diagnosis and Prognosis of Mild Traumatic Brain Injury/Concussion. *J Neurotrauma* 30(8):657–670. <http://www.liebertpub.com/doi/10.1089/neu.2012.2439>
14. Gendron TF, Petrucelli L (2009) The role of tau in neurodegeneration. *Mol Neurodegener* 4(1):13. <http://molecularneurodegeneration.biomedcentral.com/articles/10.1186/1750-1326-4-13>
15. Alonso ADC, Zaidi T, Grundke-Iqbal I, Iqbal K (1994) Role of abnormally phosphorylated tau in the breakdown of microtubules in Alzheimer disease. *Proc Natl Acad Sci U S A* 91(12):5562–5566
16. Wang Y, Mandelkow E Tau in physiology and pathology. *Nat Rev Neurosci* [Internet] (2016) Jan 3;17(1):22–35. <http://www.nature.com/articles/nrn.2015.1>
17. Iqbal K, Liu F, Gong C-X (2016) Tau and neurodegenerative disease: the story so far. *Nat Rev Neurol* 12(1):15–27. Available from: <http://www.nature.com/articles/nrneurol.2015.225>
18. Goldstein LE, Fisher AM, Tagge CA, Zhang X-L, Velisek L, Sullivan JA et al (2012) Chronic Traumatic Encephalopathy in Blast-Exposed Military Veterans and a Blast Neurotrauma Mouse Model. *Sci Transl Med* 4(134):134ra60-134ra60. Available from: <https://stm.sciencemag.org/lookup/doi/10.1126/scitranslmed.3003716>
19. Blennow K, Hardy J, Zetterberg H (2012) The Neuropathology and Neurobiology of Traumatic Brain Injury. *Neuron* 76(5):886–99. Available from: <http://dx.doi.org/10.1016/j.neuron.2012.11.021>
20. DeKosky ST, Blennow K, Ikonomic MD, Gandy S (2013) Acute and chronic traumatic encephalopathies: pathogenesis and biomarkers. *Nat Rev Neurol* 9(4):192–200. <http://www.nature.com/articles/nrneurol.2013.36>

21. McKee AC, Stein TD, Nowinski CJ, Stern RA, Daneshvar DH, Alvarez VE et al (2013) The spectrum of disease in chronic traumatic encephalopathy. *Brain* 136(1):43–64.
<https://academic.oup.com/brain/article-lookup/doi/10.1093/brain/aws307>
22. Smith DH, Johnson VE, Stewart W (2013) Chronic neuropathologies of single and repetitive TBI: substrates of dementia? *Nat Rev Neurol* 9(4):211–21. Available from:
<http://www.nature.com/articles/nrneurol.2013.29>
23. Zare-shahabadi A, Masliah E, Johnson GVW, Rezaei N (2015) Autophagy in Alzheimer's disease. *Rev Neurosci* 26(4). Available from: <https://www.degruyter.com/document/doi/10.1515/revneuro-2014-0076/html>
24. Ping Lu K, Hanes SD, Hunter T (1996) A human peptidyl–prolyl isomerase essential for regulation of mitosis. *Nature* 380(6574):544–7. Available from: <http://www.nature.com/articles/380544a0>
25. Lu P-J, Wulf G, Zhou XZ, Davies P, Lu KP (1999) The prolyl isomerase Pin1 restores the function of Alzheimer-associated phosphorylated tau protein. *Nature* 399(6738):784–788.
<http://www.nature.com/articles/21650>
26. Pastorino L, Sun A, Lu P-J, Zhou XZ, Balastik M, Finn G et al (2006) The prolyl isomerase Pin1 regulates amyloid precursor protein processing and amyloid- β production. *Nature* 440(7083):528–534. <http://www.nature.com/articles/nature04543>
27. Sultana R, Boyd-Kimball D, Poon HF, Cai J, Pierce WM, Klein JB et al (2006) Oxidative modification and down-regulation of Pin1 in Alzheimer's disease hippocampus: A redox proteomics analysis. *Neurobiol Aging* 27(7):918–25. Available from:
<https://linkinghub.elsevier.com/retrieve/pii/S0197458005001181>
28. Lu KP, Zhou XZ (2007) The prolyl isomerase PIN1: a pivotal new twist in phosphorylation signalling and disease. *Nat Rev Mol Cell Biol* 8(11):904–16. Available from:
<http://www.nature.com/articles/nrm2261>
29. Liou Y-C, Sun A, Ryo A, Zhou XZ, Yu Z-X, Huang H-K et al (2003) Role of the prolyl isomerase Pin1 in protecting against age-dependent neurodegeneration. *Nature* 424(6948):556–561.
<http://www.nature.com/articles/nature01832>
30. Lim J, Balastik M, Lee TH, Nakamura K, Liou Y-C, Sun A et al (2008) Pin1 has opposite effects on wild-type and P301L tau stability and tauopathy. *J Clin Invest* Available from:
<http://www.jci.org/articles/view/34308>
31. Lee TH, Chen C-H, Suizu F, Huang P, Schiene-Fischer C, Daum S et al (2011) Death-Associated Protein Kinase 1 Phosphorylates Pin1 and Inhibits Its Prolyl Isomerase Activity and Cellular Function. *Mol Cell* 42(2):147–59. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S1097276511002036>
32. Chen CH, Li W, Sultana R, You MH, Kondo A, Shahpasand K et al (2015) Pin1 cysteine-113 oxidation inhibits its catalytic activity and cellular function in Alzheimer's disease. *Neurobiol Dis* 76:13–23
33. Kondo A, Shahpasand K, Mannix R, Qiu J, Moncaster J, Chen C-H et al (2015) Antibody against early driver of neurodegeneration cis P-tau blocks brain injury and tauopathy. *Nature* 523(7561):431–436.
<http://www.nature.com/articles/nature14658>

34. Albayram O, Kondo A, Mannix R, Smith C, Tsai CY, Li C et al (2017) Cis P-tau is induced in clinical and preclinical brain injury and contributes to post-injury sequelae. *Nat Commun* 8(1)
35. Albayram O, Herbert MK, Kondo A, Tsai C-Y, Baxley S, Lian X et al (2016) Function and regulation of tau conformations in the development and treatment of traumatic brain injury and neurodegeneration. *Cell Biosci* 6(1):59. Available from:
<https://cellandbioscience.biomedcentral.com/articles/10.1186/s13578-016-0124-4>
36. Nakamura K, Greenwood A, Binder L, Bigio EH, Denial S, Nicholson L et al (2012) Proline Isomer-Specific Antibodies Reveal the Early Pathogenic Tau Conformation in Alzheimer's Disease. *Cell* 149(1):232–44. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S0092867412002164>
37. Lu KP, Kondo A, Albayram O, Herbert MK, Liu H, Zhou XZ (2016) Potential of the Antibody Against cis-Phosphorylated Tau in the Early Diagnosis, Treatment, and Prevention of Alzheimer Disease and Brain Injury. *JAMA Neurol* 73(11):1356. Available from:
<http://archneur.jamanetwork.com/article.aspx?doi=10.1001/jamaneurol.2016.2027>
38. Nakhjiri E, Vafaei MS, Hojjati SMM, Shahabi P, Shahpasand K (2020) Tau Pathology Triggered by Spinal Cord Injury Can Play a Critical Role in the Neurotrauma Development. *Mol Neurobiol* 57(11):4845–4855. <https://link.springer.com/10.1007/s12035-020-02061-7>
39. Faulkner JR, Herrmann JE, Woo MJ, Tansey KE, Doan NBSM (2004) Reactive Astrocytes Protect Tissue and Preserve Function after Spinal Cord Injury. *J Neurosci* 24(9):2143–2155.
<https://www.jneurosci.org/lookup/doi/10.1523/JNEUROSCI.3547-03.2004>
40. Plemel JR, Duncan G, Chen K-WK, Shannon C, Park S, Sparling JS et al (2008) A Graded Forceps Crush Spinal Cord Injury Model in Mice. *J Neurotrauma* 25(4):350–70. Available from:
<http://www.liebertpub.com/doi/10.1089/neu.2007.0426>
41. Basso DM, Fisher LC, Anderson AJ, Jakeman LB, McTigue DM, Popovich PG. Basso (2006) mouse scale for locomotion detects differences in recovery after spinal cord injury in five common mouse strains. *J Neurotrauma* 23(5):635–659
42. Holmes A, Kinney JW, Wrenn CC, Li Q, Yang RJ, Ma L et al. Galanin GAL-R1 (2003) Receptor Null Mutant Mice Display Increased Anxiety-Like Behavior Specific to the Elevated Plus-Maze. *Neuropsychopharmacology* 28(6):1031–44. Available from:
<http://www.nature.com/articles/1300164>
43. Hefner K, Cameron HA, Karlsson R-M, Holmes A (2007) Short-term and long-term effects of postnatal exposure to an adult male in C57BL/6J mice. *Behav Brain Res* 182(2):344–348.
<https://linkinghub.elsevier.com/retrieve/pii/S0166432807001921>
44. Rodgers RJ, Dalvi A (1997) Anxiety, defence and the elevated plus-maze. *Neurosci Biobehav Rev* 21(6):801–10. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S0149763496000589>
45. Hogg S (1996) A review of the validity and variability of the Elevated Plus-Maze as an animal model of anxiety. *Pharmacol Biochem Behav* 54(1):21–30.
<https://linkinghub.elsevier.com/retrieve/pii/0091305795021264>

46. Adhikari A, Topiwala MA, Gordon JA (2011) Single Units in the Medial Prefrontal Cortex with Anxiety-Related Firing Patterns Are Preferentially Influenced by Ventral Hippocampal Activity. *Neuron* 71(5):898–910. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3624763/pdf/nihms412728.pdf>
47. Gholami M, Saboory E, Khalkhali HR (2014) Chronic morphine and tramadol re-exposure induced an anti-anxiety effect in prepubertal rats exposed neonatally to the same drugs. *Clin Exp Pharmacol Physiol* 41(10):838–43. Available from: <https://onlinelibrary.wiley.com/doi/10.1111/1440-1681.12274>
48. Mechiel Korte S, De Boer SF (2003) A robust animal model of state anxiety: fear-potentiated behaviour in the elevated plus-maze. *Eur J Pharmacol* 463(1–3):163–175. <https://linkinghub.elsevier.com/retrieve/pii/S0014299903012792>
49. Nakhjiri E, Saboory E, Roshan-Milani S, Rasmi Y, Khalafkhani D (2017) Effect of prenatal restraint stress and morphine co-administration on plasma vasopressin concentration and anxiety behaviors in adult rat offspring. *Stress* 20(2):205–211. <https://www.tandfonline.com/doi/full/10.1080/10253890.2017.1306053>
50. Schindowski K, Bretteville A, Leroy K, Bégard S, Brion JP, Hamdane M et al (2006) Alzheimer's disease-like tau neuropathology leads to memory deficits and loss of functional synapses in a novel mutated tau transgenic mouse without any motor deficits. *Am J Pathol* 169(2):599–616
51. Baratz R, Rubovitch V, Frenk H, Pick CG (2010) The influence of alcohol on behavioral recovery after mTBI in mice. *J Neurotrauma* 27(3):555–563
52. Wu J, Zhao Z, Sabirzhanov B, Stoica BA, Kumar A, Luo T et al (2014) Spinal Cord Injury Causes Brain Inflammation Associated with Cognitive and Affective Changes: Role of Cell Cycle Pathways. *J Neurosci* 34(33):10989–11006. <https://www.jneurosci.org/lookup/doi/10.1523/JNEUROSCI.5110-13.2014>
53. Sierksma ASR, van den Hove DLA, Pfau F, Philippens M, Bruno O, Fedele E et al (2014) Improvement of spatial memory function in APPswe/PS1dE9 mice after chronic inhibition of phosphodiesterase type 4D. *Neuropharmacology* 77:120–130. <https://linkinghub.elsevier.com/retrieve/pii/S002839081300422X>
54. Brody DL, Holtzman DM (2008) Active and Passive Immunotherapy for Neurodegenerative Disorders. *Annu Rev Neurosci* 31(1):175–193. <http://www.annualreviews.org/doi/10.1146/annurev.neuro.31.060407.125529>
55. Rosenmann H (2013) Immunotherapy for Targeting Tau Pathology in Alzheimer's Disease and Tauopathies. *Curr Alzheimer Res* 10(3):217–228. <http://www.eurekaselect.com/openurl/content.php?genre=article&issn=1567-2050&volume=10&issue=3&spage=217>
56. Pedersen JT, Sigurdsson EM (2015) Tau immunotherapy for Alzheimer's disease. *Trends Mol Med* 21(6):394–402. <https://linkinghub.elsevier.com/retrieve/pii/S1471491415000581>

57. Sevigny J, Chiao P, Bussière T, Weinreb PH, Williams L, Maier M et al (2016) The antibody aducanumab reduces A β plaques in Alzheimer's disease. *Nature* 537(7618):50–56.
<http://www.nature.com/articles/nature19323>
58. Shahpasand K, Sepehri Shamloo A, Nabavi SM, Ping Lu K, Zhen Zhou X (2018) "Tau immunotherapy: Hopes and hindrances." *Hum Vaccin Immunother* 14(2):277–284.:
<https://www.tandfonline.com/doi/full/10.1080/21645515.2017.1393594>
59. Qiu C, Albayram O, Kondo A, Wang B, Kim N, Arai K et al (2021) Cis P-tau underlies vascular contribution to cognitive impairment and dementia and can be effectively targeted by immunotherapy in mice. *Sci Transl Med* 13(596):eaaz7615.
<https://stm.sciencemag.org/lookup/doi/10.1126/scitranslmed.aaz7615>
60. Nardone R, Höller Y, Brigo F, Seidl M, Christova M, Bergmann J et al (2013) Functional brain reorganization after spinal cord injury: Systematic review of animal and human studies. *Brain Res* 1504:58–73. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S0006899312019695>
61. Çavdar S, Onat FY, Çakmak Y, Yananli HR, Gülçebi M, Aker R (2008) The pathways connecting the hippocampal formation, the thalamic reuniens nucleus and the thalamic reticular nucleus in the rat. *J Anat* 212(3):249–256
62. Zhao P, Waxman SG, Hains BC (2007) Modulation of Thalamic Nociceptive Processing after Spinal Cord Injury through Remote Activation of Thalamic Microglia by Cysteine Cysteine Chemokine Ligand 21. *J Neurosci* 27(33):8893–8902.
<https://www.jneurosci.org/lookup/doi/10.1523/JNEUROSCI.2209-07.2007>
63. Hulsebosch CE, Hains BC, Crown ED, Carlton SM (2009) Mechanisms of chronic central neuropathic pain after spinal cord injury. *Brain Res Rev* 60(1):202–213.
<https://linkinghub.elsevier.com/retrieve/pii/S0165017308001483>
64. Ankeny DP, Popovich PG (2009) Mechanisms and implications of adaptive immune responses after traumatic spinal cord injury. *Neuroscience* 158(3):1112–1121.
<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3624763/pdf/nihms412728.pdf>
65. Segal MB (1993) Extracellular and cerebrospinal fluids. *J Inherit Metab Dis* 16(4):617–638.
<http://doi.wiley.com/10.1007/BF00711896>
66. Zetterberg H (2017) Tau in biofluids - relation to pathology, imaging and clinical features. *Neuropathol Appl Neurobiol* 43(3):194–199. <https://onlinelibrary.wiley.com/doi/10.1111/nan.12378>
67. Shultz SR, Wright DK, Zheng P, Stuchbery R, Liu S-J, Sashindranath M et al (2015) Sodium selenate reduces hyperphosphorylated tau and improves outcomes after traumatic brain injury. *Brain* 138(5):1297–313. Available from: <https://academic.oup.com/brain/article-lookup/doi/10.1093/brain/awv053>
68. Yokobori S, Zhang Z, Moghieb A, Mondello S, Gajavelli S, Dietrich WD et al (2015) Acute Diagnostic Biomarkers for Spinal Cord Injury: Review of the Literature and Preliminary Research Report. *World Neurosurg* 83(5):867–78. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S1878875013004592>

Figures

Figure 1

Severe SCI had a robust and persistent effect on tau pathogenicity induction in the cord. Mice were subjected to SCI by calibrated forceps. a, The sham and sSCI mouse cords (48h, and 2w after injury), were stained with anti *cis* P-tau antibody followed by immunofluorescence staining. Cis, green; DNA, blue; Scale bar, 100 μ m; n=3. b-e, Immunoblots were stained with *cis* P-tau, AT8 P-tau, and AT100 P-tau antibodies, followed by quantification analysis . Protein bands were quantified with ImageJ software and were normalized against actin. Data statistically analyzed by one-way ANOVA by Tukey's post-hoc test (mean $\pm$ SD.). *P < 0.05, **P < 0.01. h, hours; w, weeks.

Figure 2

Treating sSCI mice with *cis* mAb blocked cistauosis and tau pathology in the brain. a,b, Severe SCI mice were treated with *cis* mAb or control IgG for 2 months. The cortices of sham and sSCI mouse brains were stained with *cis* P-tau followed by immunofluorescence intensity quantification. *Cis* P-tau, red; Dapi, blue; Scale bar, 100 μ m; n=3. c-f, Immunoblots were stained with *cis* P-tau, AT8 P-tau, AT100 P-tau, and actin antibodies, followed by quantification analysis. The protein bands were quantified with ImageJ and were

normalized against actin, n=3. Data statistically analyzed by one-way ANOVA by Tukey's post-hoc test (mean $\pm$ SD.). *P < 0.05, **P < 0.01. M, month; cis, cis mAb; ns: not significant.

Figure 3

Treating sSCI mice with cis mAb suppressed the spinal cord and the brain ultrastructure destruction. a, b, Electron micrographs of spinal cord and brain sSCI mouse models; either treated or untreated with cis p-tau mAb. Axonal MTs (blue open arrows) and MIs (red filled arrows). Scale bars, 100 nm. M, month; MT, microtubule; MI, mitochondria.

Figure 4

Cis P-tau elimination in sSCI mice improved motor function. a-c, sSCI mice were subjected to open-field test, and spontaneous locomotor activity (a, b), as well as hind-limb locomotor functions, using the BMS score (c). Data are expressed as mean $\pm$ SD. *** $P < 0.001$ versus sham, ### $P < 0.001$ vs sSCI (2M) + *cis* mAb. M, month; D, day; BMS, Basso mouse scale.

Figure 5

Cis P-tau Immunotherapy restores cognitive dysfunction in SCI mice. a, After 2 months treatment with IgG or cis mAb, sSCI mice were subjected to the Y-maze spontaneous alteration test to assess spatial working memory. b, sSCI mice were subjected to the OF test to assess anxiety-like behavior, and the path taken by a mouse over 5 min was recorded. c, d, sSCI mice were subjected to the EPM test to assess anxiety/risk-taking behavior, and time spent in the open arms and open arm entries were measured. Data are

expressed as mean $\pm$ SD. ***P < 0.001 vs sham, **P < 0.01 vs sham, ###P < 0.001 vs sSCI (2M) + cis mAb, ##P < 0.01 vs sSCI (2M) + cis mAb. M, month; OAT, open arm time; OAE, open arm entry.

Supplementary Files

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