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

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Abstract

Spinal cord injury (SCI) can result in significant neurological impairment and functional and cognitive deficits. It is well established that SCI results in focal neurodegeneration that gradually spreads to other cord areas. On the other hand, traumatic brain injury (TBI) is strongly associated with tau protein pathology and neurodegeneration that can spread in areas throughout the brain. Tau is a microtubule-associated protein abundant in neurons and whose abnormalities result in neuronal cell death. While SCI and TBI have been extensively studied, there is limited research on the relationship between SCI and brain tau pathology. As a result, in this study, we examined tau pathology in spinal cord and brain samples obtained from severe SCI mouse models at various time points. The effects of severe SCI on locomotor function, spatial memory, anxiety/risk-taking behavior were investigated. Immunostaining and immunoblotting confirmed a progressive increase in tau pathology in the spinal cord and brain areas. Moreover, we used electron microscopy to examine brain samples and observed disrupted mitochondria and microtubule structure following SCI. SCI resulted in motor dysfunction, memory impairment, and abnormal risk-taking behavior. Notably, eliminating pathogenic *cis* P-tau via systemic administration of appropriate monoclonal antibodies restored SCI's pathological and functional consequences. Thus, our findings suggest that SCI causes severe tauopathy that spreads to brain areas, indicating brain dysfunction. Additionally, tau immunotherapy with an anti-*cis* P-tau antibody could suppress pathogenic outcomes in SCI mouse models, with significant clinical implications for SCI patients.

Keywords Spinal cord injury · *Cis* P-tau · Traumatic brain injury · Pin1 · Monoclonal antibody · Cognitive decline

Abbreviations

SCI	Spinal cord injury
CSF	Cerebrospinal fluid
TBI	Traumatic brain injury
MTs	Microtubules
CTE	Chronic traumatic encephalography
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

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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

Introduction

Each year, approximately 500,000 people worldwide experience a spinal cord injury (SCI) due to motor vehicle collisions, falls, violence, and sporting accidents [1, 2], resulting in significant neurological impairment and significant emotional and psychological distress [3].

SCI can disrupt nerve impulse conduction, resulting in neurological dysfunction [4]. Primary spinal cord injury has been shown to rapidly disrupt cell membranes, myelin, and axons within longitudinal tracts. Additionally, the SCI damages microvessels, causing destructive secondary injury by releasing various harmful factors [5]. Numerous cellular and molecular mechanisms may contribute to extensive neurodegeneration during the secondary injury process [6]. As active biological processes, such cascades offer the possibility of treating SCI with selective inhibitors.

SCI can also alter systemic immune functions, affecting the brain [7]. Moreover, the released agents may enter the brain through the cerebrospinal fluid (CSF). The brain abnormalities caused by SCI result from modified afferent and efferent routes. However, SCI induces distinct neuropathological changes, such as decreasing the number of cortical neurons [4, 8–12]. Additionally, cognitive impairment affects 60% of the SCI population [4, 8, 9, 12].

Despite extensive considerations, the mechanism by which SCI results in brain abnormalities remains unknown. Clearly, abnormal tau protein expression is a significant pathological hallmark of traumatic brain injury (TBI) [13]. Tau is a microtubule-associated protein that promotes the formation and stabilization of microtubules (MTs) [14, 15]. Tau is frequently hyperphosphorylated on Ser/Thr residues in tauopathies, impairing the MT's function and altering the protein's integrity, resulting in tau aggregation and tangle formation [16, 17], most notably in chronic traumatic encephalopathy (CTE) [18–22] and Alzheimer's disease (AD) [16, 17].

It is well established that phosphorylated tau at Thr231 exists in two distinct *cis* and *trans* conformations, with the *cis* pThr231-tau (*cis* P-tau) conformer being highly neurotoxic and acting as an early initiator of the tauopathy process following TBI. Peptidyl-prolyl *cis*/*trans* isomerase (Pin1) inhibits the development of tau pathology and neurodegeneration in AD by converting the neurotoxic *cis* conformation of phosphorylated tau at the Thr231-Pro motif to the physiological trans conformation [23–31]. Additionally, the tauopathy process can be inhibited in vitro and in vivo using a *cis* P-tau monoclonal antibody (*cis* mAb) [32–34]. Also, pathogenic *cis* P-tau is prion-like and spreads throughout the brain and CSF in tauopathy mouse models [33–37].

Several studies have examined the concentrations of total tau and P-tau in CSF, serum, and spinal cord tissue from patients and experimental animals with SCI. However, the molecular mechanism underlying tau pathology in SCI is not entirely understood. Furthermore, the causal relationship between SCI and brain dysfunction has remained elusive. Thus, as previously proposed, we examined the tau pathology process in severe SCI (sSCI) mouse models [38]. We induced sSCI and investigated the formation and degeneration of various pathogenic tau species in cord and brain tissues at various time points to determine whether SCI injury can result in brain pathology.

Materials and Methods

Animals and study design Male Balb/c mice (2–3 months old) weighing 22–26 g were obtained and housed in clear plastic cages with a 12-h light/dark cycle and free access to water and food under controlled temperature and humidity. All animals were given one week to acclimate to their new environment before undergoing experimental procedures. The ethics committee of Tabriz University of Medical Sciences approved all protocols (approval No. IR.TBZMED.VCR.REC.1398.067). Experiments were conducted following the National Institutes of Health's Guide for the Care and Use of Laboratory Animals (NIH Publications).

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

Laminectomy and spinal cord compression model using calibrated forceps All procedures were conducted in a sterile environment. Mice were anesthetized with 4% isoflurane, and a laminectomy was performed at T7-9 to expose the

T8 segment of the spinal cord without damaging the dura. Pairs of forceps were applied for laterally compressing the spinal cord to the corresponding thickness (0.25 mm) for 15 s [39, 40]. The sham group received laminectomy and forceps placement around the spinal cord without compression. After forceps removal, muscles and skin were stitched, followed by the administration of saline solution (1 ml) for rehydration, buprenorphine (0.05 mg/kg) for pain relief, and ciprofloxacin (5 mg/kg) for bladder infection treatment/prevention, all subcutaneously (SC) twice daily for three days. The animals were monitored in a temperature-controlled room until they recovered, at which point they were transferred to their own cage. SCI mice bladders were manually expressed twice daily until the urinary reflex was established.

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

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

Antibody treatment of mice To assess *cis* mAb's efficacy in treating SCI, we examined whether *cis* mAb could affect intracellular P-tau in sSCI (2 M) + *cis* mAb and sSCI (2 M) + IgG groups. Antibody-treated sSCI animals were given either mouse *cis* mAb or IgG at random. Three days prior to the injury, animals received one dose of *cis* mAb/IgG IP (200 µg/per mouse), a single IP post-injury treatment (20 µg in 5 µl) 15 min after SCI, and then IP (200 µg) every four days for two weeks, followed by 200 µg weekly for the remainder of the two months of treatment [34].

Transmission electron microscopy (TEM) The ultrastructural examination was performed using the TEM method. Brain and spinal cord specimens were cut into 2 × 2 mm pieces from the sham and SCI mouse models treated with either control IgG or *cis* mAb. The cells were fixed in glutaraldehyde 2.5%, buffered with 0.1 M phosphate (pH 7.4), post-fixed with 1% osmium tetroxide, and finally embedded in resin. Ultrathin sections measuring 60–90 nm were cut and placed on a copper grid, stained with a solution of uranyl acetate and lead citrate, and examined using a ZEISS electron microscope (EM902A) and a Leo 906 transmission electron microscope (Leo, Germany).

Immunoblotting analysis Immunoblotting was performed on the spinal cord, and brain samples 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 SDS sample buffer and loaded onto a gel after boiling. Polyacrylamide gel electrophoresis at 12% resolved the proteins and transferred them to a PVDF membrane. The membranes were then blocked for 1 h with 2% milk in TBST (10 mM Tris–HCl pH 7.6, 150 mM NaCl, 0.1% Tween 20). The membranes were then incubated overnight at 4ºC with the following primary antibodies: *cis* P-tau mAb (gift from KP. Lu), AT8 P-tau (MN1020), AT100 P-tau (MN1060), and Tau-5 (MAB361). The immunoblots were then incubated with a secondary antibody conjugated to HRP in 2% milk in TBST. Chemiluminescence was used to detect the signals (Perkin Elmer, San Jose, CA). Following each step, the membranes were washed six times with TBST. ImageJ was used to quantify the immunoblotting results.

Subsequently, two-stage normalization was conducted. Initially, the band of interests was normalized against actin. The relative intensity of P-tau markers (*cis* P-tau, AT8 P-tau, and AT100 P-tau) was normalized to total tau (Tau-5). Afterward, normalized data were visualized as expressions in test samples compared to sham groups.

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

Dewaxed sections were then dehydrated in a series of ethanol dilutions. A 5% ammonium chloride solution (Merck, 101,145) was used to quench autofluorescence. For antigen enhancement, the sections were placed in a steamer (0.01 M) sodium citrate (Sigma-Aldrich, S4641) for 20 min for antigen enhancement. The slides were then permeabilized for 15 min with 0.5% Triton X-100 (Sigma-Aldrich, T8532) and blocked for one hour with 10% goat serum. Following that, the slides were incubated with *cis* P-tau mAb (gift from KP. Lu). The samples were incubated with the anti-mouse secondary antibodies (Alexa Fluor 488 or 594) at 37 °C for 1 h. DAPI (Invitrogen, D1306) was used to stain the nuclei, and the images were captured using a fluorescent microscope (BX71; Olympus equipped with DP72 digital camera).

Twenty ROIs (350 × 450 µm2 dimension representative view) were randomly selected and quantified from the

cerebral cortices. ImageJ was used to quantify the immunofluorescence intensity (n = 3 per group).

Open-field locomotion The OF test was used to assess hind-limb functions and spontaneous locomotor activity on days 1, 30, and 60 following injury. We scored locomotor performance using the BMS and a related subscale [42]. We used a computer-based video tracking system (Noldus Ethovision) to record mice's total traveled distance, speed, and walking pattern for 5 min to determine spontaneous locomotor activity [42–46].

Elevated plus-maze (EPM) test The EPM task is an experimental model used to examine cortical circuits to assess anxiety/risk-taking behavior [47–52]. The test is typically conducted on a plus-shaped apparatus raised 50 cm above the ground and equipped with two opposite open (aversive, no walls) and two opposite closed (safe, 15 cm high walls) arms (30 × 5 cm), as well as a central square. A mouse is placed on the apparatus's central square, facing an open arm, and is allowed 5 min to explore the maze. We kept track of the number of open arm entries and the amount of time spent exploring open arms (Noldus Ethovision). The maze was thoroughly cleaned following each trial to ensure that no odorant interfered with the test. Mice with normal levels of anxiety/risk-taking behavior enter and stay in the open arms less frequently and for a shorter time. The open arms approach addresses the abnormal risk-taking and anxiety behaviors associated with cognitive decline [34, 53].

Y-maze spontaneous alternation test The Y-maze test assesses spatial working memory in rodents and quantifies cognitive deficits. The Y-maze is composed of three identical black arms (30 cm long, 6 cm wide, and 15 cm high walls) mounted at a 120° angle to one another in the shape of the letter 'Y.' Generally, rodents will explore a new arm rather than revisiting previously visited arms. A random arm (arms A–C) was chosen as the 'start' arm, and the mouse was placed at its end 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 defined as non-repeating entries into each arm. The percentage of alternations is calculated as follows: (Number of alternations / Number of arm entries) × 100. A mouse with intact working memory scored significantly > 50% [46, 54, 55].

Mice were anesthetized with ketamine–xylazine IP injections at the end of the second month and following behavioral tests. Brain and spinal cord tissue samples were collected for electron microscopy, immunoblotting, and immunofluorescence staining analyses.

Statistical analysis To compare the groups' mean differences, all normally distributed data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's HSD multiple comparisons post hoc test. Additionally, we used one-way multivariate covariance analysis (MANCOVA) to eliminate the effect of controlling variables or covariates on the relationship between independent and dependent variables. All data were analyzed using SPSS software (v. 22) and reported as mean ± standard deviation (SD), with $P < 0.05$ considered significant. All graphs were obtained by GraphPad Prism version 8.4.3. F represents the degree of freedom; n denotes the number of different animals.

Results

Compression severe SCI induced tau pathology in cord neurons We studied tau pathology induced by SCI in SCI mouse models using immunoblotting and immunofluorescence staining of spinal cord tissues. Severe SCI induced pathogenic *cis* P-tau acutely and persistently 48 h after the injury and maintained it at elevated levels for two weeks. At 48 h and two weeks after injury, robust *cis* P-tau signals (3.638 ± 0.0442) and (4.975 ± 0.753) were detected in the cords (Fig. 1a–c, P = 0.136, $P = 0.0167$). Immunoblotting also demonstrated a progressive increase in AT8 P-tau (early tangle) [F (2, 4) = 31.98, $P = 0.0229$] and AT100 P-tau (late tangle) [F (2, 4) = 57.77, $P = 0.0018$] in cord tissues following timely trauma (Fig. 1b-e). Evidently, the SCI resulted in the development of tau pathology in the cord tissue.

Improved tau pathology effects triggered by sSCI via *cis* mAb It is well established that *cis* P-tau is associated with neurodegenerative outcomes and the progression of TBI-induced tauopathy. As previously stated, we demonstrated that *cis* P-tau was induced 48 h after sSCI, implying that there may be a link between *cis* P-tau and pathological changes following injury. To better understand the brain complications caused by SCI, particularly the efficacy of *cis* mAb in treating neurodegenerative pathologies caused by sSCI, we treated animal models for two months with either *cis* mAb or a control IgG isotype. Following sSCI, this study demonstrates that pathogenic *cis* P-tau spreads to the brain and induces tau pathology [34] in various areas, including the cortices (Fig. 2a–d). Moreover, as previously observed, *cis* mAb treatment effectively suppressed tau pathology (Fig. 2a–d) [34, 56–59].

One month after injury, immunofluorescence staining of sSCI mouse brains revealed strong *cis* P-tau signals (11.70 ± 2.078, $P = 0.0191$) (Fig. 2a, b). Additionally, immunoblotting confirmed the presence of a significant amount of *cis* P-tau (2.511 ± 0.071, $P = 0.0272$), AT8 P-tau (5.473 ± 0.432, $P = 0.0111$), and AT100 P-tau (3.358 ± 0.323, $P = 0.0124$) in the cortices of sSCI mice,

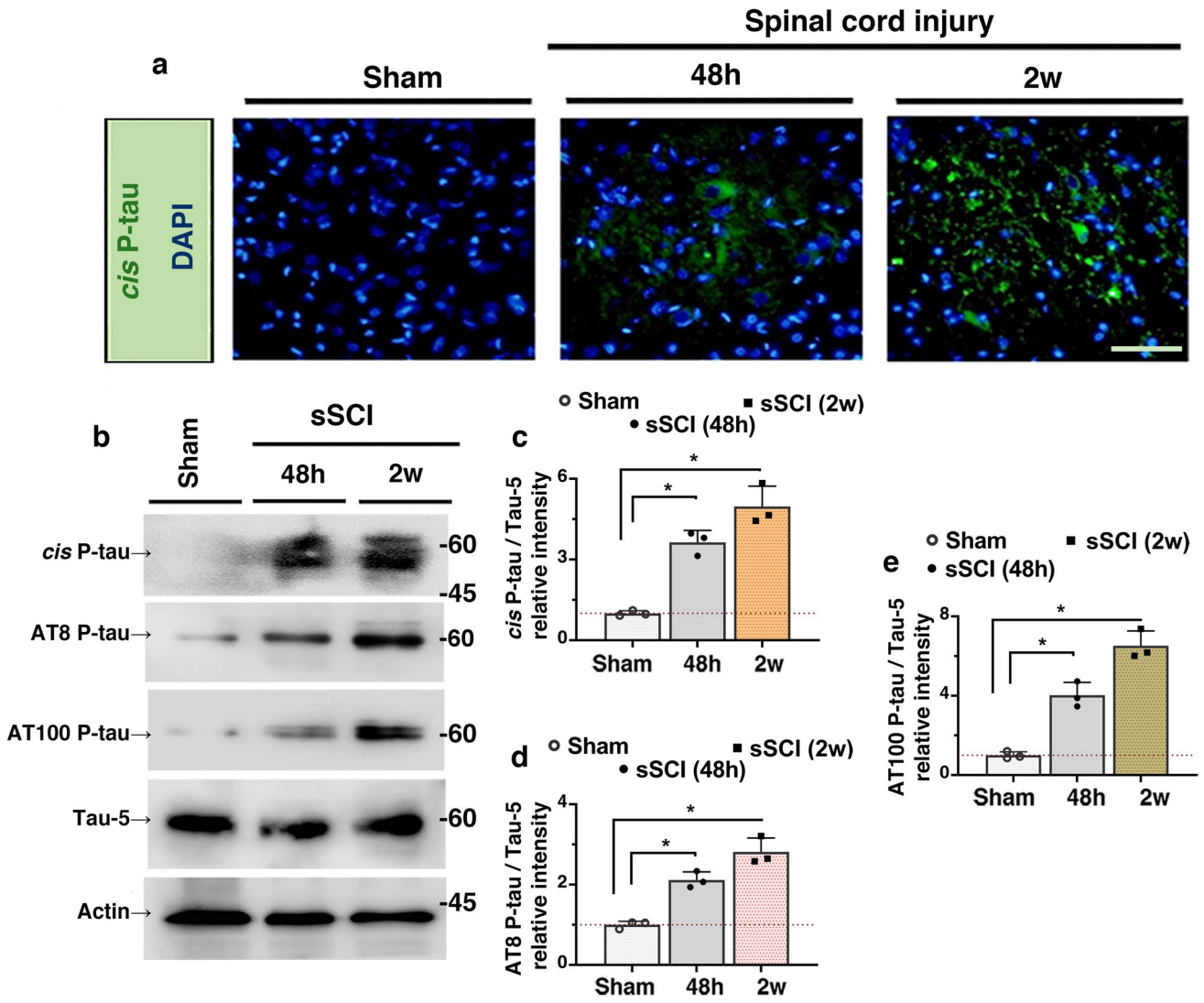

Fig. 1 Severe SCI had a robust and persistent effect on tau pathogenicity induction in the cord. Mice were subjected to SCI via calibrated forceps. (**a**) The sham and sSCI mouse cords (48 h and 2w after injury) were stained with anti *cis* P-tau antibody followed by immunofluorescence staining. *Cis*, green; DNA, blue; scale bar, 100 μm; 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 normalized against actin and *cis* P-tau, AT8 P-tau, and AT100 P-tau relative intensities against total tau (Tau-5). Normalized data were depicted as expressions in test samples versus sham groups, n = 3. One-way ANOVA statistically analyzed data by Tukey's post hoc test (mean ± SD.). $^{*}P < 0.05$, $^{**}P < 0.01$. h, hours; w, weeks

whereas no significant amount of P-tau epitopes was detected in animals in the sham group (Fig. 2c–f). While immunotherapy with control IgG did not affect the formation of *cis* P-tau in the cortices of sSCI animals (27.94 ± 3.554, $P = 0.0234$) compared to the sham group, *cis* mAb administration effectively prevented tau pathology and *cistauosis* in immunostained sSCI mouse brains (1.611 ± 0.4818, $P = 0.7424$) (Fig. 2a, b). Similarly, *cis* mAb administration effectively inhibited the formation of *cis* P-tau, AT8 P-tau, and AT100 P-tau in sSCI mouse brain immunoblots, when compared to two-month sSCI IgG treated mice ($P = 0.0492$, $P = 0.0219$, $P = 0.0293$, respectively); however, IgG treatment did not affect the number of tau epitopes formed (Fig. 2c–f).

Evidently, sSCI can cause tau pathology in both the spinal cord and brain and immunotherapy with *cis* mAb can effectively inhibit the formation of pathogenic *cis* P-tau in the brains of sSCI animals.

Improved SCI-related ultrastructural impairment via *cis* mAb According to previous research, SCI causes axonal injury and intracellular Ca^{2+} and tau hyperphosphorylation. Ca^{2+} uptake by the mitochondria results in mitochondrial dysfunction and an increase in reactive oxygen species

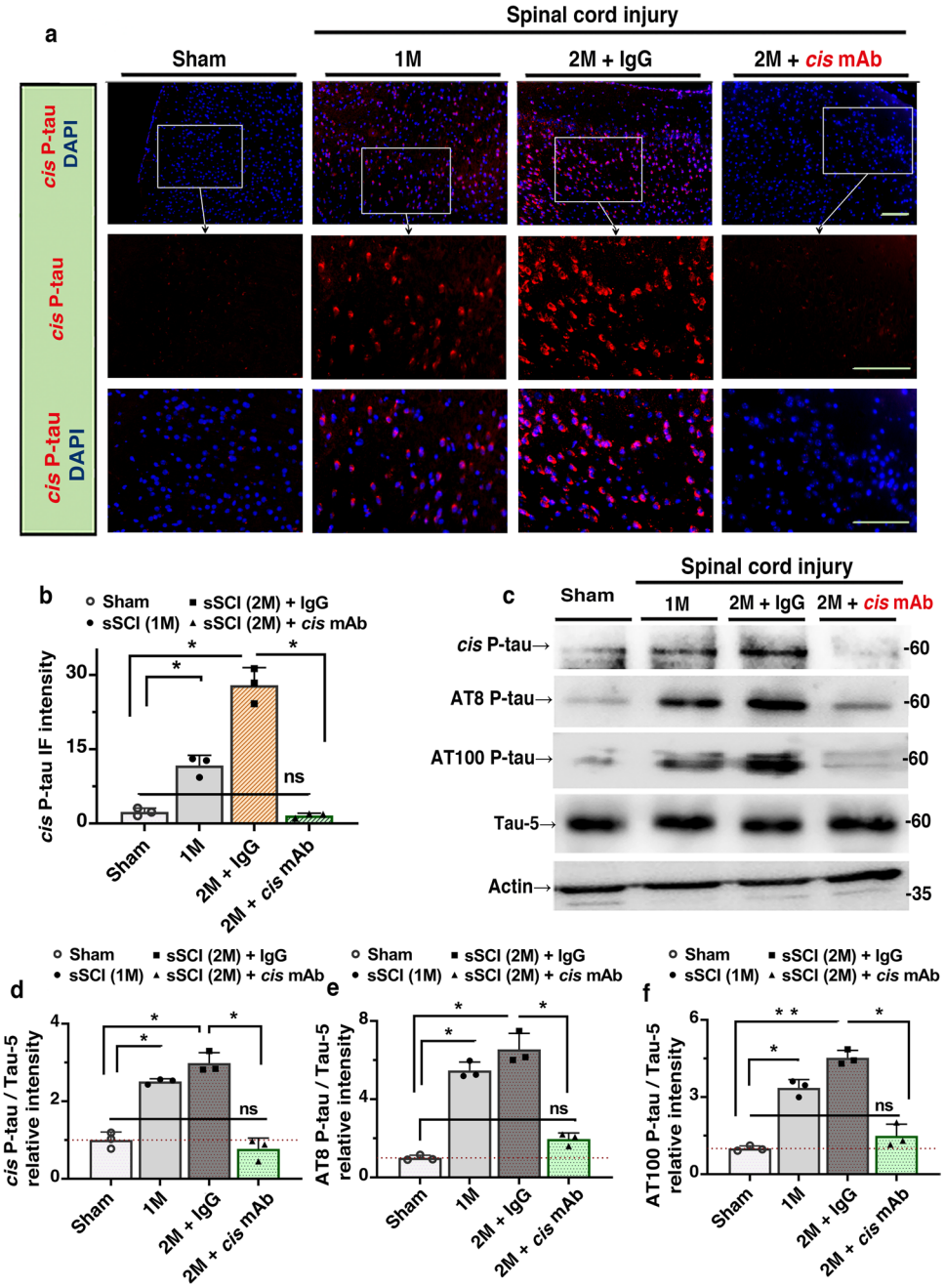

Fig. 2 Treating sSCI mice with *cis* mAb blocked *cistauosis* and tau pathology in the brain. (**a**, **b**) Mice with severe SCI were treated with *cis* mAb or control IgG for two months. The cortices of sham and sSCI mouse brains were stained with *cis* P-tau followed by immunofluorescence intensity quantification. *Cis* P-tau, red; DAPI, blue; scale bar, 100 μm; 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 normalized against actin and *cis* P-tau, AT8 P-tau, and AT100 P-tau relative intensities against total tau (Tau-5). Normalized data were depicted as expressions in test samples versus sham groups, n = 3. One-way ANOVA statistically analyzed data by Tukey's post-hoc test (mean ± SD.). $^{*}P < 0.05$, $^{**}P < 0.01$. M, month; *cis*, *cis* mAb; ns: not significant

(ROS). Furthermore, hyperphosphorylated tau promotes neuroinflammation, ROS generation, mitochondrial dysfunction (disrupted mitochondrial membrane), microtubule disruption (distributed microtubules), and tangle formation [16, 38, 46]. TBI has previously been shown to cause significant microtubule and mitochondrial disruption [34]. Given that the disruption is caused by pathogenic *cis* P-tau, *cis* mAb treatment may restore the impairment [33, 34, 60, 61]. To this end, we examined brain ultrastructural changes employing the respective monoclonal antibody with or without pathogenic *cis* P-tau elimination. We observed that SCI resulted in pathogenic *cis* P-tau and structural damage to the spinal cord and brain. Additionally, *cis* mAb restored the abnormalities effectively (Fig. 3).

Improved SCI-related motor function impairment via *cis* mAb On days 1, 30, and 60 following sSCI, an open field test was used to assess spontaneous locomotor activity and hind-limb functions using BMS scores [41]. On each of the three days of the experiment, mice in the sSCI (1 M) and sSCI (2 M) + IgG groups demonstrated a significant decrease in the total traveled distance ($P < 0.001$) when compared to mice in the shame group. The decrease in the total traveled distance was mirrored by a decrease in walking speed in

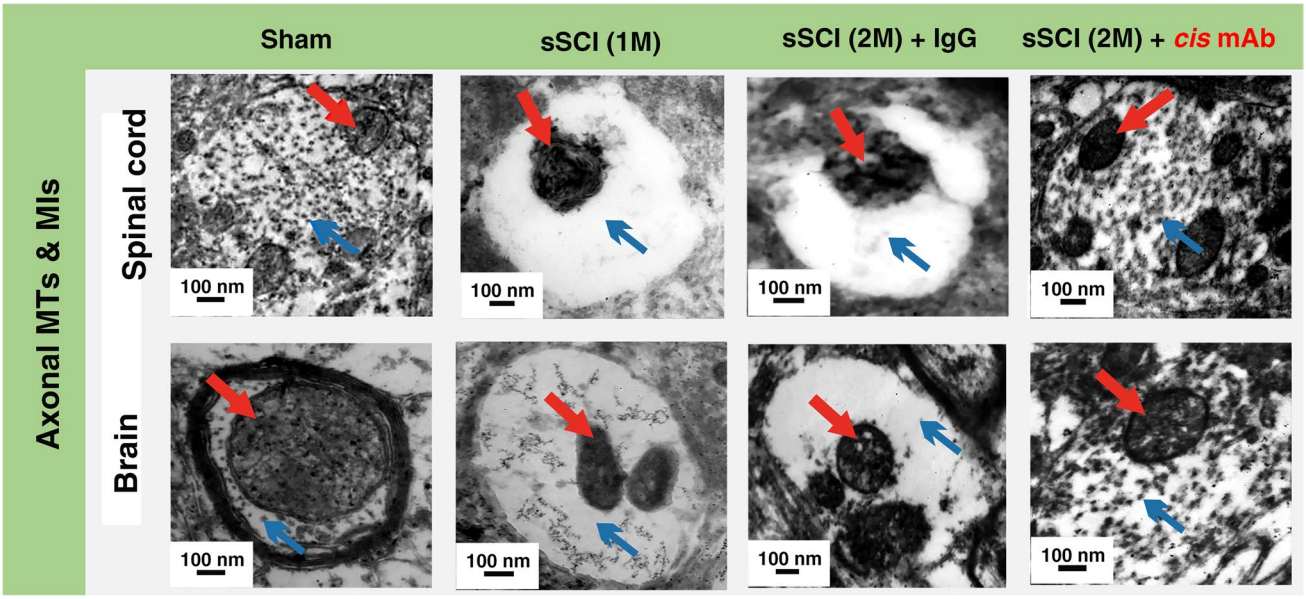

Fig. 3 Treatment of sSCI mice with *cis* mAb inhibited the destruction of the spinal cord and brain ultrastructure. Electron micrographs of spinal cord and brain sSCI mouse models; either treated or untreated with *cis* P-tau mAb. Axonal MTs (blue open arrows) and MIs (red filled arrows). Scale bars, 100 nm. M, month; MT, microtubule; MI, mitochondria

sSCI (1 M) and sSCI (2 M) + IgG mice ($P < 0.001$) compared to the sham group. Notably, on days 30 and 60 following injury, we observed a significant improvement in spontaneous locomotor activity in the sSCI group treated with *cis* mAb ($P < 0.001$) compared to the sSCI and IgG-treated sSCI groups (Fig. 4a, b, d).

One day after the injury, all mice exhibited a near-complete loss of motor function (BMS score of 1). By day 60, hind-limb functional recovery was significantly improved in *cis* mAb-treated mice compared to IgG-treated mice ($P < 0.001$) (Fig. 4c). As a result, *cis* P-tau elimination improved spontaneous locomotor activity and hind-limb function recovery in sSCI mice.

Restored SCI-related cognitive dysfunction via *cis* mAb The Y-maze spontaneous alternation test was performed to measure spatial working memory. The sham group exhibited functional working memory with a spontaneous alteration rate of ~ 65%. Compared to the sham group, severe SCI significantly decreased the percentage of spontaneous alteration ($P < 0.001$). SCI mice treated with *cis* mAb had a significantly higher rate of spontaneous alteration than sSCI mice treated with IgG ($P = 0.002$ or $P < 0.01$) (Fig. 5a).

To assess *cis* mAb's anxiolytic efficacy, we used an elevated-plus maze test 60 days after sSCI. In the EPM test, the sham group animals explored the open arms significantly less than IgG-treated sSCI mice ($P = 0.009$ or $P < 0.01$). Additionally, similar to the sham group, the *cis* mAb-treated group spent significantly less time in the open arms ($P < 0.001$) than the IgG-treated sSCI groups (Fig. 5b). Total open arm entries were increased in SCI (2 M) + IgG mice compared to the sham group and *cis* mAb-treated sSCI mice (Fig. 5c). As a result, all IgG-treated sSCI mice exhibited anxiety/risk-taking behavior when exploring the two open arms; in comparison, *cis* mAb-treated mice exhibited minimal anxiety. Our data support the notion that rodents prefer protected environments, such as the nest.

The data for cognitive functions (alternation percentage and OAT) were then normalized against locomotor activity on day 60 (speed and total traveled distance; see Fig. 4a, b) to eliminate the effect of locomotor activity on the relationship between IgG/*cis* mAb treatment and cognitive functions. The data indicated that *cis* mAb restores cognitive deficits independently and significantly ($P < 0.01$) (Fig. 5d, e). As a result, *cis* mAb abolishes *cis* P-tau and *cistauosis* and reverses behavioral deficits triggered by SCI.

Discussion

It is self-evident that SCI impairs brain function and contributes to patients' cognitive decline. However, the molecular mechanisms by which SCI affects the brain remain unknown. SCI can disrupt the cerebral cortex and neural circuits retrogradely, such as thalamic afferents to the hippocampal compartment [62, 63].

Moreover, injured neurons in the spinal cord produce the chemokine cysteine-cysteine ligand 21, which activates microglia in distant spinal cord sections and the thalamus

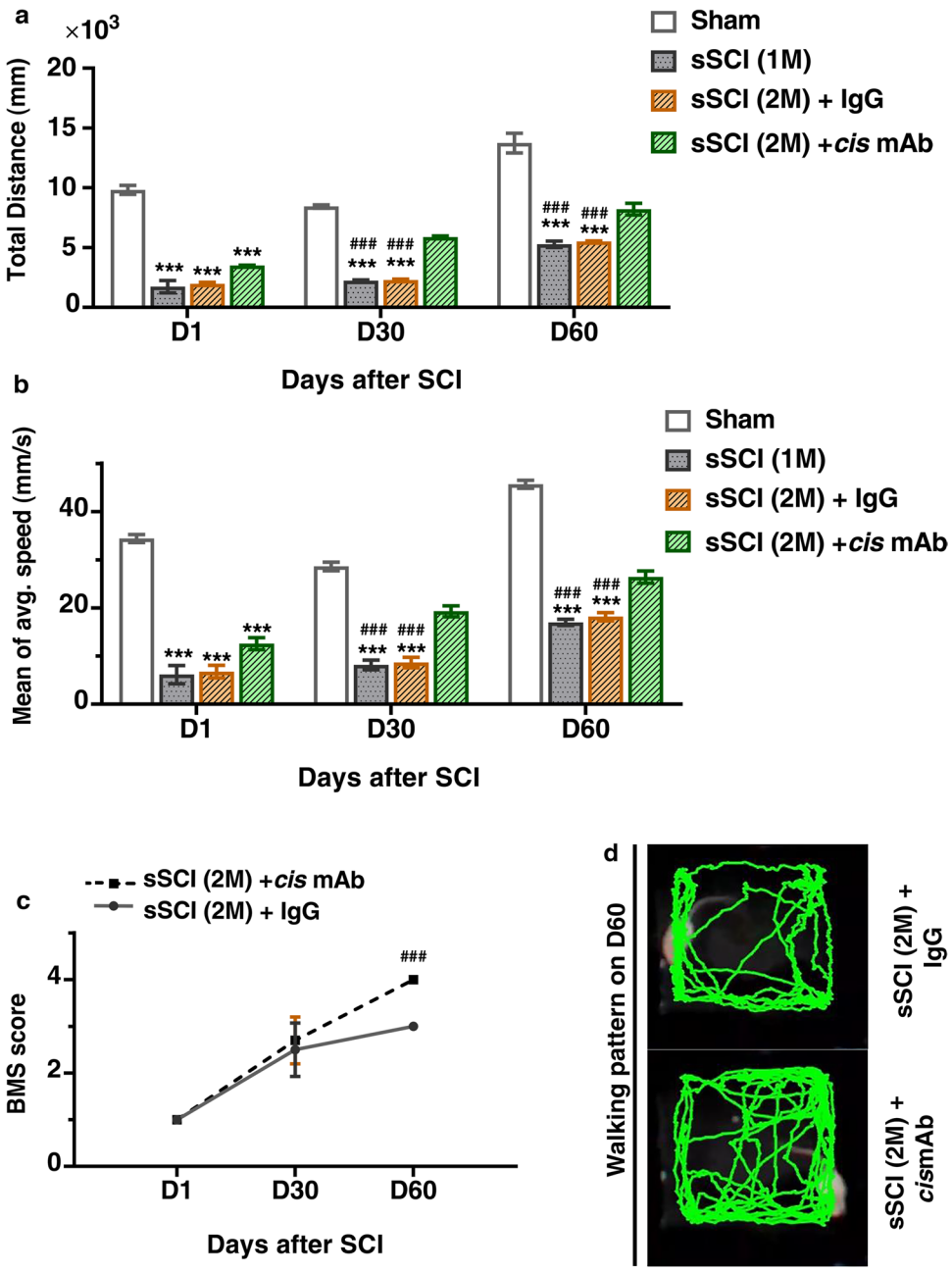

Fig. 4 *Cis* P-tau elimination in sSCI mice improved motor function. Severe SCI mice were subjected to an open field test to examine spontaneous locomotor activity (**a**, **b**), hind-limb locomotor functions, using the BMS score (**c**), and walking pattern (**d**). Data expressed as mean ± SD. ***$P < 0.001$ versus sham, ###$P < 0.001$ vs sSCI (2 M) + *cis* mAb. M, month; D, day; BMS, Basso mouse scale

[64, 65]. Furthermore, SCI impairs systemic immune functions [66], which affects the brain. Neuronal cell injury or death can release intracellular MT binding proteins into the extracellular space, traveling to the brain via the CSF [67, 68]. Several studies have demonstrated an increase in tau levels in the CSF following acute TBI [69] and SCI [70]. Despite extensive consideration, the molecular mechanisms underlying the brain pathology caused by SCI remain unknown [71].

It has been evidenced that single severe TBI (ssTBI) or repetitive mild TBI (rmTBI) induces pathogenic *cis* P-tau in axons a few hours after injury, resulting in neurodegeneration [34]. *Cis* P-tau is prion-like and spreads throughout the brain, typically accompanied by neuron pathogenicity [34, 37]. Notably, *cis* mAb treatment can eliminate pathogenic *cis* P-tau, restore axonal pathologies such as MT defects, organelle transport, and long-term potentiation (LTP), and prevent the development of numerous short- and long-term pathological and functional consequences following ssTBI or rmTBI [33, 34, 37, 61]. TBI induces oxidative stress and Pin1 oxidation at Cys113 and Ser71, resulting in Pin1 inactivation and *cis* P-tau accumulation [34]. SCI, on the other hand, results in severe oxidative stress [39, 64, 65]. Similarly, we observed that SCI stress promotes the accumulation

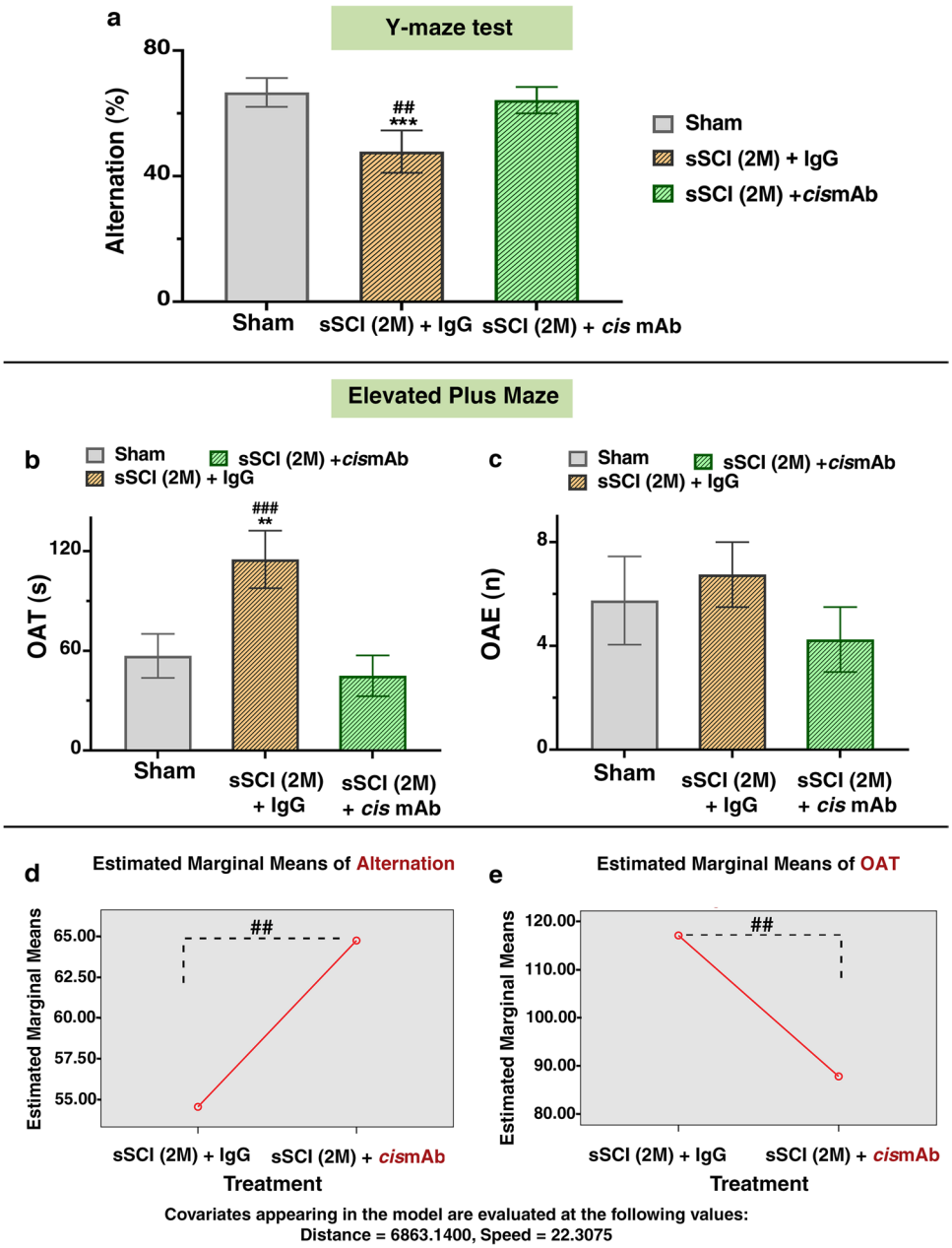

Fig. 5 *Cis* P-tau immunotherapy restores cognitive dysfunction in mice with SCI. (**a**) After two months of treatment with IgG or *cis* mAb, sSCI mice were subjected to the Y-maze spontaneous alteration test to assess spatial working memory. (**b**, **c**) sSCI mice were subjected to the EPM test to assess anxiety/risk-taking behavior, and time spent in the open arms and open arm entries were measured. (**d**, **e**) Cognitive functions data (alternation percentage and OAT) were normalized against the locomotor activity (speed and total traveled distance) in IgG/*cis* mAb treated groups to control locomotor activity as a covariate. The MANCOVA analyses showed that *cis* mAb independently and significantly restored cognitive deficits ($P < 0.01$). Data expressed as mean ± SD. ***$P < 0.001$ vs sham, **$P < 0.01$ vs sham, ###$P < 0.001$ vs sSCI (2 M) + *cis* mAb, ##$P < 0.01$ vs sSCI (2 M) + *cis* mAb. M, month; OAT, open arm time; OAE, open arm entry

of pathogenic *cis* P-tau, most likely via oxidative stress and Pin1 suppression. We hypothesized that SCI-induced oxidative stress would result in Pin1 inactivation and *cis* P-tau accumulation.

We investigated tau pathologies induced by SCI in order to identify and treat brain complications such as axonal pathology, functional deficits, and cognitive impairment; as previously proposed [38], we discovered that *cis* P-tau is the initial and critical mediator of *cis* P-tau neurodegeneration and functional defects following SCI in the mouse model. Additionally, we assessed the effects of *cis* mAb therapy on pathological and functional brain complications associated with injury. We observed the following: (1) Severe SCI had a robust and persistent effect on *cis* P-tau induction and resulted in histological changes in the spinal cord and brain tissues; (2) severe SCI resulted in locomotor impairment, cognitive deficits, and anxiety-like behaviors; (3) treatment with *cis* mAb effectively prevented the development of extensive tauopathy, improved histopathological consequences, and restored motor and cognitive function in SCI mice. Notably, our histological findings demonstrated that the *cis* P-tau level was significantly increased at the injury site and spread from the spinal cord to the brain within two months of the injury. These findings suggest that induction of *cis*

P-tau is required for the development of several pathological and functional outcomes following SCI.

Conclusion

We examined the relationship between SCI and tau pathology in the mouse models. We demonstrated that pathogenic tau was induced focally in spinal cord tissue early after a severe compression SCI, spread to brain areas via CSF, and induced prominent tauopathy, resulting in motor dysfunction. Overall, we propose *cis* P-tau as a reliable biomarker for assessing the pathologic outcomes of SCI.

While we demonstrated that SCI increases *cis* P-tau accumulation, additional experiments are necessary to determine whether tau pathology is caused by Pin1 inactivation.

Additional research in preclinical SCI models and patients with varying degrees of SCI is required to determine the biomarker value of *cis* P-tau. The biomarkers may lead to identifying novel SCI therapeutic targets and treatment strategies. In summary, our findings suggest that pathogenic *cis* P-tau is a tauopathy driver in SCI, making it a potential target for immunotherapy diagnosis and treatment.

Authors' contributions All authors contributed to the study conception and design. Elnaz Nakhjiri expressed the idea of the article and performed the literature search. Material preparation, data collection and analysis were performed by Selva Zamanzadeh, Ehsan Ehsani, Hamid Soltani Zangbar, Shaqayeq Roqanian, Daryoush Mohammadnejad and Shahin Ahmadian. 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.

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

Declarations

Ethics approval This study was conducted following the Declaration of Helsinki's principles. The Tabriz University of Medical Sciences Ethics Committee approved this study (Code of ethics: IR.TBZMED.VCR.REC.1398.067).

Consent to participate Not applicable.

Consent to publish Not applicable.

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