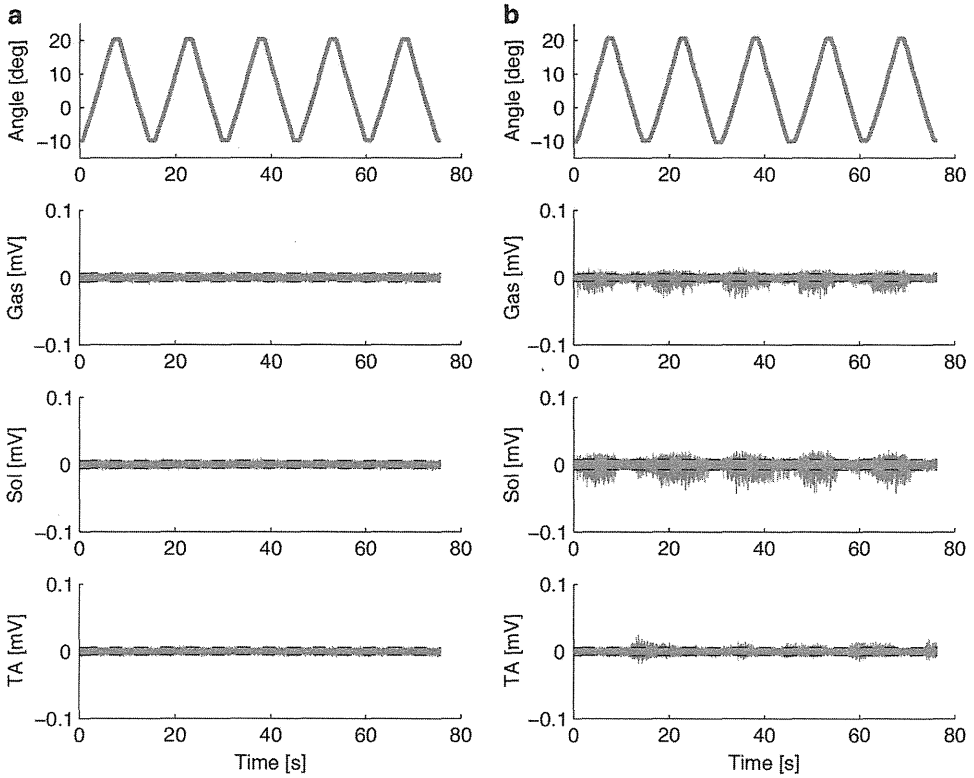

Figure 1 (**a**) Representative angle and electromyograms (EMG; Gas, Sol, TA) waveform during passive motion test. Broken lines indicate ±3 s.d. of baseline. (**b**) Angle and EMG waveform of an excluded participant (participant 3 in Table 1).

and five trials without muscle activity of the lower limb were selected and analyzed further. One participant (Table 1, participant 3) showed remarkable muscle activity exceeding ±3 s.d. of baseline (Figure 1b), therefore the data from this participant was excluded.

The passive motion test was then followed by a stretch reflex test in the SCI participants with spasticity and healthy control participants only. Stretch reflexes were elicited by quick 10° toe-up rotations of the footplate from 0° with participants at rest. Four different angular velocities (60, 90, 120 and 150 deg s^{-1}) were applied five times in each participant in a random order. The inter-stimulus intervals were not constant but were randomized within 4–9 s to avoid anticipation of forthcoming perturbations. After all trials, the MAS of the right plantar-flexor was assessed.

Analysis

Angle and torque data were averaged in five trials and digitally low-pass filtered using a fourth-order zero-lag Butterworth filter[13] with a 10-Hz cut-off frequency.[14–16] Three different mathematical models were fitted to the angle (θ)–torque (τ) data as previously described:[16–27]

(i) Second-order polynomial (SOP) model (Equation 1)

$$\tau(\theta) = a\theta^2, \tag{1}$$

where a is experimental constant;

(ii) Fourth-order polynomial (FOP) model (Equation 2)

$$\tau(\theta) = m\theta^4 + n\theta^3 + o\theta^2 + p\theta + q, \tag{2}$$

where m, n, o, p and q are experimental constants;

(iii) Exponential model similar to the Sten–Knudsen (SK) model (Equation (3))

$$\tau(\theta) = \frac{1}{\alpha}e^{\alpha(\theta-\theta_{slack})}, \tag{3}$$

where α and θ_{slack} are experimental constants.

Prior to fitting Equation 1, the angle (θ) and torque (τ) data reference points were set to zero. The plantar-flexor, peak torque (torque waveform peak), and energy (the area between the angle–torque curve and the line torque equaling zero) were calculated from the normalized angle–torque data. In Equations 2 and 3, the raw angle–torque data were used. Equation 3 fitting parameters were determined by the non-linear least square method (Levendberg-Marquardt algorithm[28]) using the optimization toolbox from MATLAB (The Mathworks, Natick, MA, USA). A total of 1200[16] data points were used in each fitting. The stiffness indices (SI_{SOP}, SI_{FOP}, SI_{SK}) and $Angle_{SLACK}$ were determined from these equations as follows:

$$SI_{SOP} = 2a \tag{4}$$

$$SI_{FOP} = \text{mean}_{-10\le\theta\le20}(4m\theta^3 + 3n\theta^2 + 2o\theta + p) \tag{5}$$

$$SI_{SK} = \alpha \tag{6}$$

$$Angle_{SLACK} = \theta_{slack} \tag{7}$$

SI_{FOP} is the average of the derivative of the fourth-order polynomial regression of angle–torque curve across all angle ranges (10° plantar-flexion to 20° dorsiflexion). The determination coefficient (R^2) of the angle–torque curve fitting using SOP, FOP and SK models was also calculated. The reflex responses were analyzed as the peak-to-peak amplitude for 35 ms after perturbation. The SR gain and offset were calculated as the slope and interception of the regression line in the plotted stretch response to angular velocity.

Statistics

One-way analysis of variance was used to compare the determination coefficients (R^2) between the three models, and *post hoc* analysis was performed using Tukey's HSD test. All variables were logarithmically transformed[29] except the Gas and Sol offsets, which have negative magnitudes. Then, the Spearman's rank-correlation coefficient for the MAS and the Pearson's product-moment correlation coefficient for the other variables were calculated to determine the relationship between the injury duration and mechanical parameters. In addition, the relationships between all variables and injury localization were

determined using the same procedure. Before logarithmic transformation, MAS values were corrected by designating the minimum data value as one. The partial correlation coefficient, excluding morphological parameters, was also calculated for the mechanical parameters. The statistical significances of the correlation coefficients were assessed for SCI participants with spasticity. Statistical significance was set at $P<0.05$.

RESULTS

Representative angle–torque data (raw and normalized) and fitting data using the three models are illustrated in Figure 2. In both examples (Figure 2a: participant 12 in Table 1; Figure 2b: participant 13 in Table 1), the FOP model fits the angle–torque data well. However, data fitting using the SOP and SK models is poor in some participants (Figure 2b: participant 13 in Table 1). Similarly, the collective determination coefficient data fitting using the FOP model was very good ($R^2=0.999\pm0.001$) and significantly higher than in both the SOP ($R^2=0.869\pm0.135$) and SK (0.904 ± 0.130) models in all the participants ($n=33$). This trend also occurred and was significant in SCI participants with spasticity (FOP model: $R^2=0.999\pm0.001$; SOP model: $R^2=0.850\pm0.120$; SK model: $R^2=0.883\pm0.114$, $n=15$). MAS negatively correlated with the injury duration in the SCI participants with spasticity (Figure 3). In addition, the calf circumference and MG thickness were negatively correlated to the injury duration ($n=13$ excluding the missing data described previously; Figure 3). However, there were no statistically significant relationships between the neural parameters (Gas gain and offset, Sol gain and offset) and injury duration in the group.

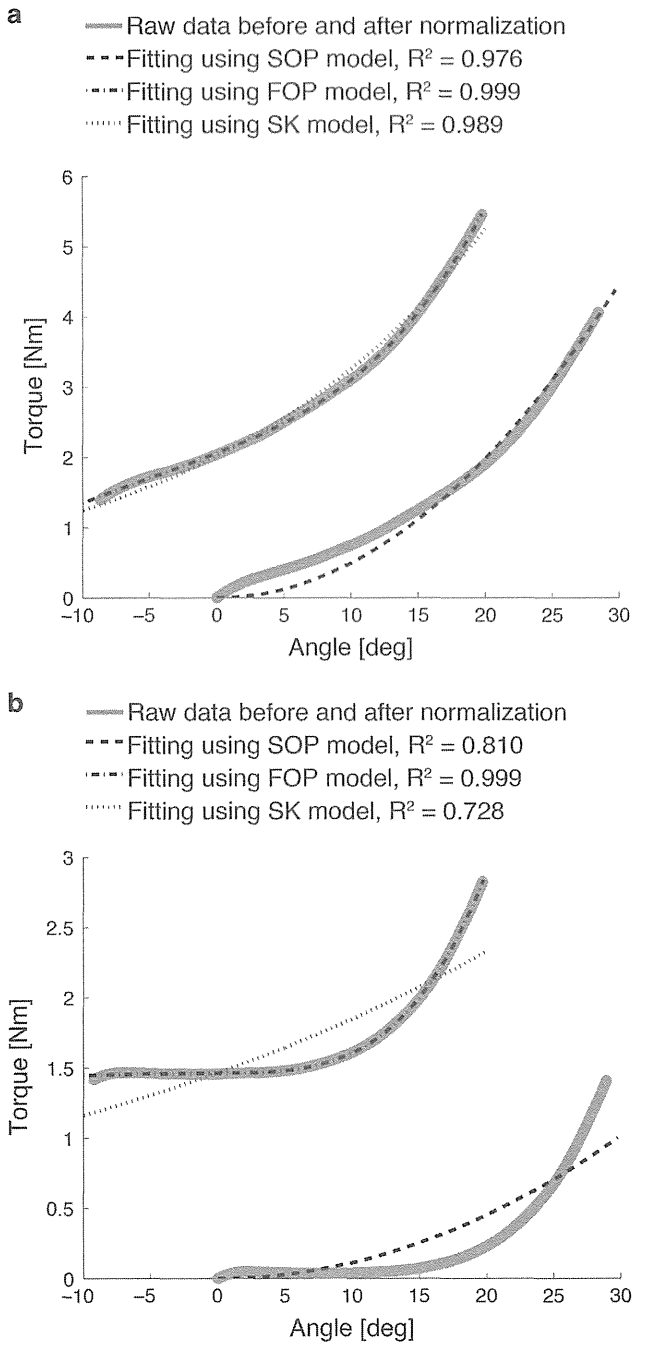

Figure 2 Representative angle–torque data fitting using three mathematical models (SOP, FOP and SK). (**a**) The fitting was good in all models, which were fitted to angle–torque data from participant 12 in Table 1. (**b**) The SOP and SK model fittings were inferior compared with the FOP model, based on angle–torque data from participant 13 in Table 1.

Angle–torque representative curves of all participants (excluding participant 3) are shown in Figure 4a. After the polynomial and exponential regression, the SI_{FOP}, SI_{SK} and $Angle_{SLACK}$ were calculated, and Figure 4b was generated by designating the angle and torque reference points in Figure 4a as zero. Using the data in Figure 4a, we calculated the peak torque, energy and SI_{SOP}, and found that the peak torque and SI_{FOP} (stiffness index calculated from the fourth-order polynomial regression) were inversely correlated with the injury duration ($n=15$).

Muscle morphology theoretically affects passive tension in the muscles and tendons. The effects of these morphologic variables (calf circumference and MG, lateral Gas, and Sol thicknesses) were excluded by calculating the partial correlation coefficients between the mechanical properties (peak torque, energy, SI_{SOP}, SI_{FOP}, SI_{SK} and $Angle_{SLACK}$) and the injury duration (Table 2). The peak torque and SI_{FOP} were negatively correlated, even after excluding the effects of the calf circumference and MG thickness, which significantly decreased with the injury duration. In addition, we confirmed that the injury severity was not correlated with these results (Figure 5).

DISCUSSION

We investigated the effect of injury duration on plantar-flexor elasticity in individuals with chronic SCI and found negative correlations between the clinical index of spasticity (MAS), morphologic parameters (calf circumference and MG thickness), mechanical parameters (peak torque and stiffness index) and injury duration in SCI participants with spasticity. In addition, there were significant partial correlation coefficients between the mechanical parameters and the injury duration excluding morphologic parameters.

Alteration of the ankle joint stiffness after SCI

As clearly shown in Figure 3, the decline in calf circumference and MG thickness with injury duration indicates that muscle atrophy continues in SCI even during the chronic stage. Castro *et al.*[2] measured the cross-sectional area of the lower musculature (Gas, Sol and tibialis anterior) during the 6 months following spinal injury and showed that the CSA of Gas decreased significantly. The present result showing a selectively decreased MG thickness is consistent with previous findings.[2] Notably, involuntary muscle activity resulting from clonus and spasm with spasticity might contribute to attenuate atrophy,[30] therefore the decreasing spasticity observed in this study, coupled with the immobilization, likely accelerated the muscle atrophy.

The Ashworth scale[31] remains a major clinical scale for evaluating spasticity,[32–34] but its validity and reliability are questioned by some

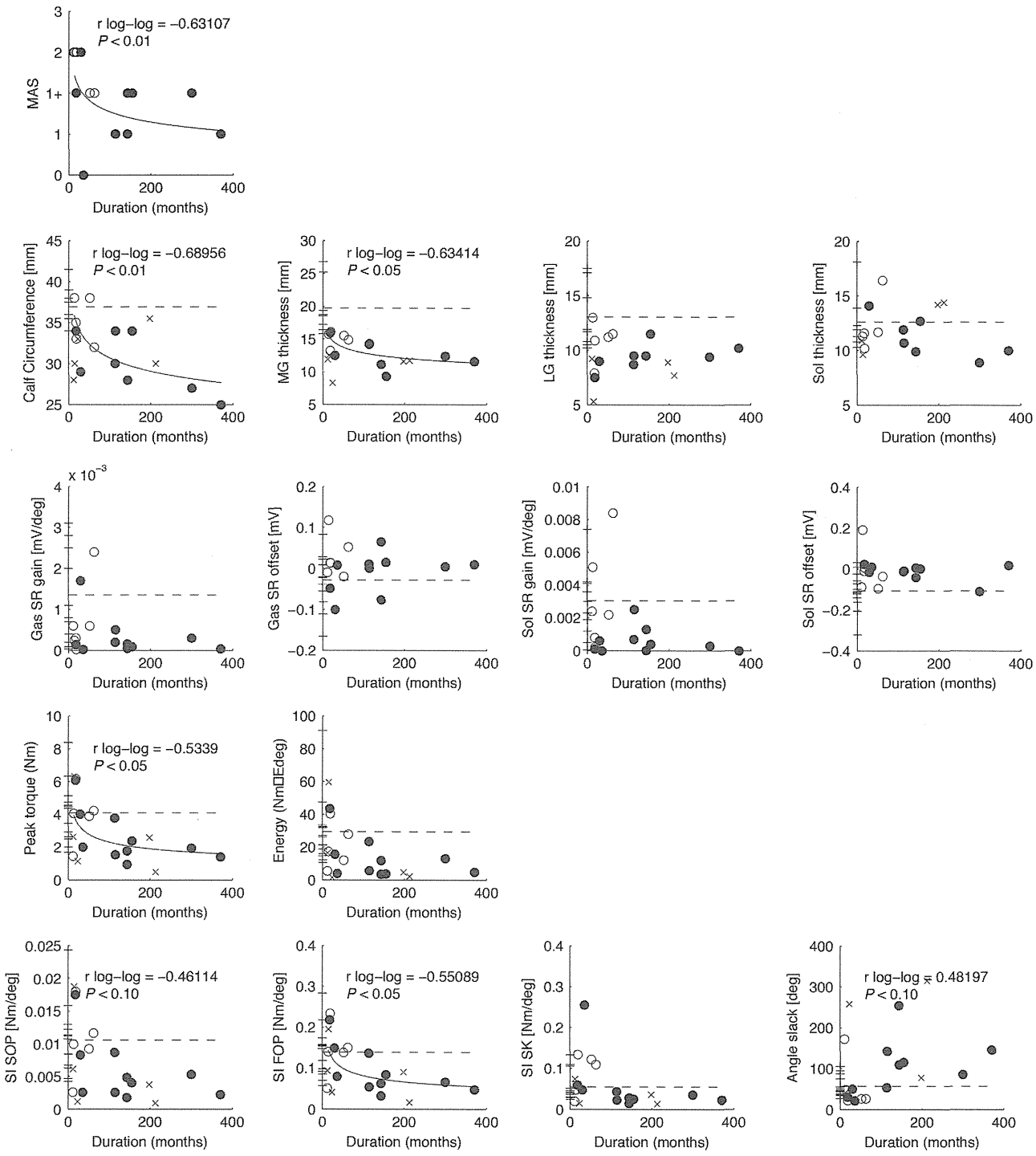

Figure 3 Relationship between mechanical variables and injury duration (months). Complete SCI participants with spasticity (filled circles), incomplete SCI participants with spasticity (open circles), complete SCI participants without spasticity (x), and healthy participants (horizontal bar) are shown. The horizontal dashed line indicates the mean value in healthy participants.

reports.[35,36] A positive correlation has been reported between the Ashworth scale and indices of clonus and spasm,[37] therefore the negative correlation between MAS and the injury duration observed in this study suggest that the frequency of involuntary muscle contraction because of spasm gradually decreases over time after SCI. In evaluating the extent of spasticity over time, we found no statistically significant correlation between the stretch reflex gain and offset in the lower muscles (MG, Sol) and injury duration. This result is consistent with our previous study[11] that found that the stretch reflex peak-to-peak amplitude and stretch reflex peak-to-peak amplitude/Mmax in complete and incomplete SCI participants were not correlated to the time post-injury, suggesting that spinal circuit excitability in SCI patients does not change during the chronic stage. However, several reports showed that Mmax was decreased more in the chronic SCI

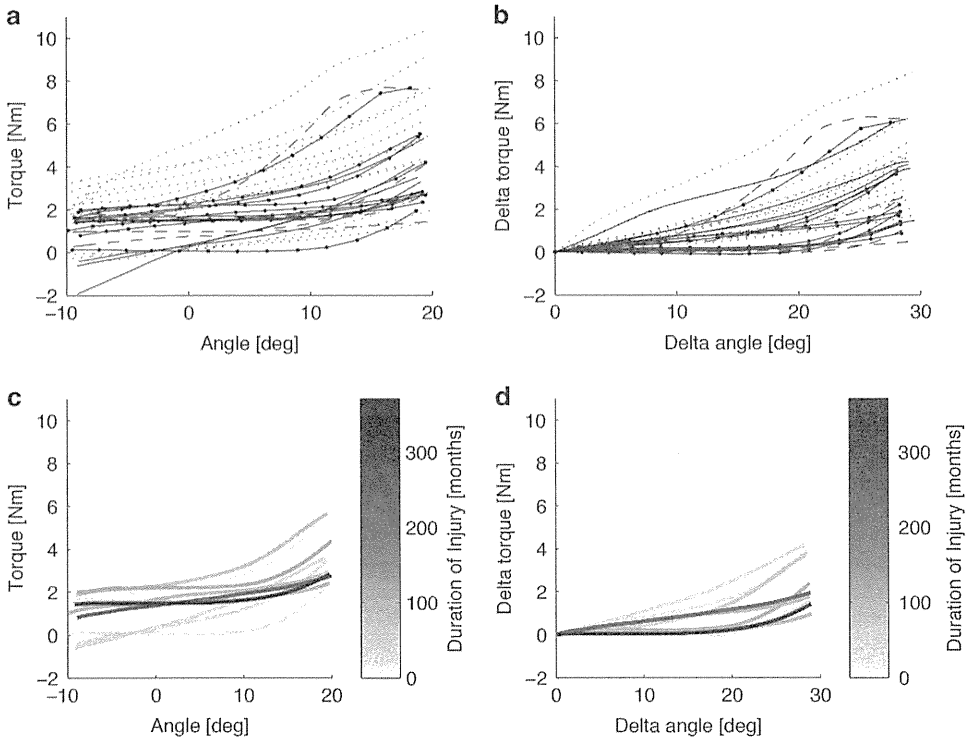

Figure 4 (**a**) Angle–torque curves of plantar-flexor muscles for all complete SCI participants with spasticity (chain), incomplete SCI participants with spasticity (solid), complete SCI participants without spasticity (dashed) and healthy control participants (dotted), excluding participant 3. (**b**) The angle and torque reference points in **a** were set at zero. (**c**) Angle–torque curve for SCI participants with spasticity (both complete and incomplete) from **a**, with grayscale intensities of lines indicating injury duration (months). (**d**) The angle and torque reference points in **c** were set at zero.

participants than in the age-matched healthy control participants,[38–44] presumably because of the changes in proprioceptor function or muscle atrophy. In these studies, the time-dependent decline in Mmax may be caused by muscle atrophy, but because we did not measure Mmax in our study, we cannot conclude that the spinal reflex excitability in chronic SCI is maintained. Further research is needed to clarify this relationship.

Characteristics of the mechanical properties of the paralyzed ankle joint in SCI

Previous research[16] indicates that the mathematical models used to calculate the mechanical parameters greatly impact the experimental result. Essentially, the change in a mechanical parameter (for example, stiffness) varied depending on the selected mathematical model applied to the angle–torque relation; thus, we evaluated several mathematical models (SOP, FOP and SK) to calculate each mechanical parameter. In this study, the peak torque and stiffness index (SI_{FOP}) calculated from the FOP model were inversely correlated with the injury duration. The determination coefficients for the angle–torque data fitted with the FOP model were significantly higher than the coefficients based on the alternative models (SOP, SK), therefore the result (SI_{FOP}) calculated using the FOP model was deemed to be the most reliable. Notably, SI_{SOP}, SI_{SK} and $Angle_{SLACK}$ calculated using the SOP and SK models were not significantly correlated with the injury duration. This observation likely reflects the inappropriate data fitting observed in some participants and the insufficient sample size; however, the underlying mechanism is unclear.

In addition, work was not significantly correlated with injury duration, which is consistent with a prior study asserting that work is not a spasticity indicator.[45] Furthermore, the decreased peak torque and SI_{FOP} over time suggest that plantar-flexor elasticity decreases with injury duration. However, several studies have found that the plantar-flexor passive-elasticity in the chronic SCI patients was higher than observed in the healthy age-matched participants.[24,26,46] We speculate that plantar-flexor elasticity in SCI patients increases with immobilization during the acute stage of injury (<1 year), and thereafter, elasticity gradually decreases over time due in part to the muscle atrophy observed in this study. In theory, muscle morphology influences passive tension. Thus, we calculated the partial correlation coefficient between mechanical parameters and injury duration to exclude effects from morphological parameters (Table 2); significant relationships persisted, therefore we concluded that tissue elasticity in the plantar-flexor was involved in the mechanical parameter changes.

We cannot determine whether the muscle or tendon caused the plantar-flexor elasticity change. Theoretically, both muscle and tendon arranged in series can affect the total tissue stiffness,[47] a relationship that has been experimentally confirmed in the plantar-flexors of healthy participants.[17] In contrast, Diong *et al.*[24] observed that the Gas muscle stiffness is increased in SCI participants, whereas Olson *et al.*[6] found that the muscle changes occur at the cell level and reflect muscle fiber transformation from Type I to Type II. In addition, Maganaris[48] showed that patellar tendon CSA, stiffness, and Young's modulus decreased significantly in chronic (18–288 months after injury) complete SCI patients. Overall, these studies suggest that the tendon rather than the muscle has a primary role in the decreasing plantar-flexor elasticity. Further research is needed to clarify the different changes in muscle and tendon function over time following a SCI.

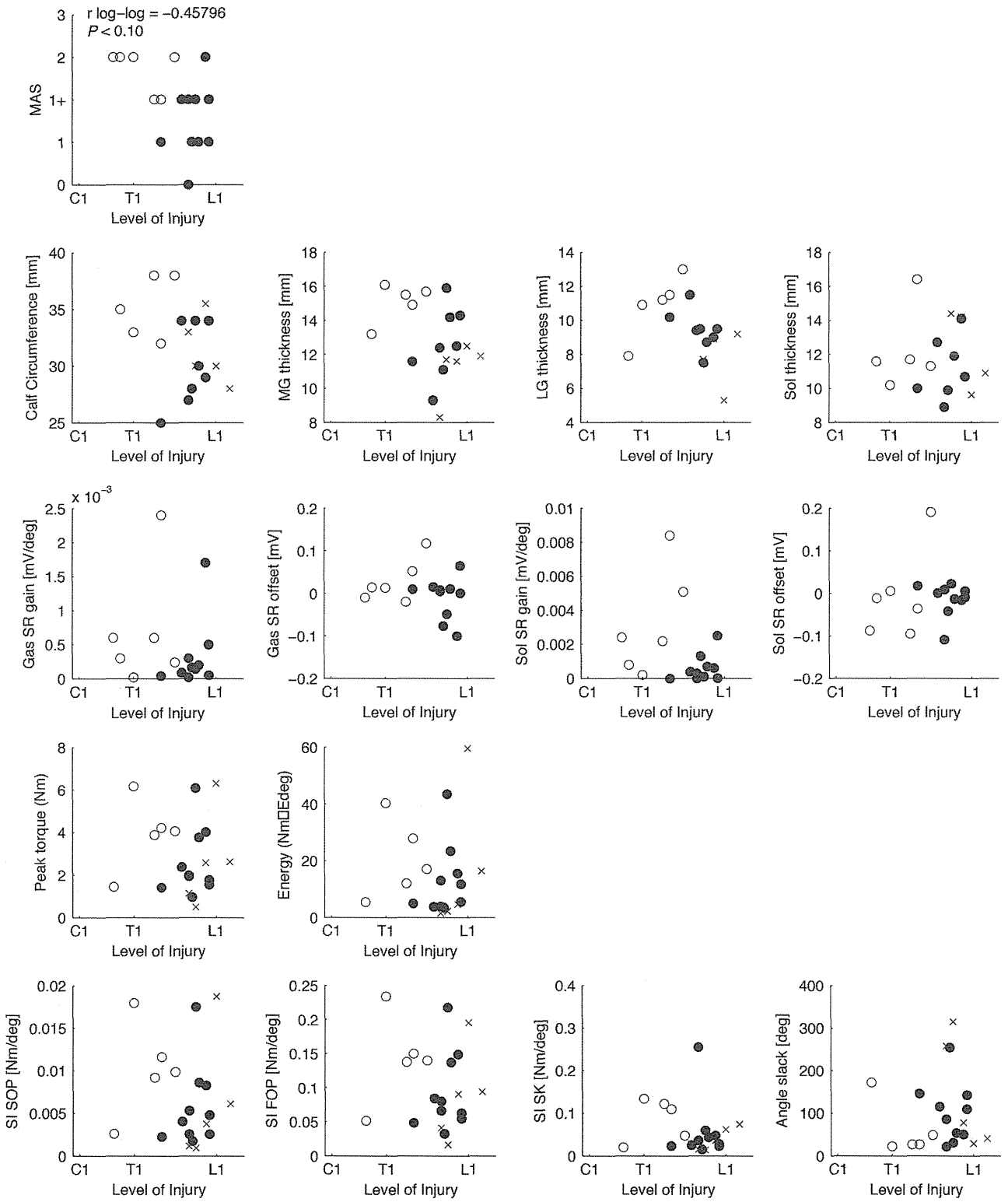

Figure 5 Relationships between mechanical parameters and injury location. Complete SCI participants with spasticity (filled circles), incomplete SCI participants with spasticity (open circles), complete SCI participants without spasticity (x).

Limitations

We did not measure the range of motion of plantar-dorsiflexion in the SCI participants. Therefore, it is unclear whether contracture occurred in SCI participants. However, we visually confirmed that all participants achieved a 20° dorsiflexion while keeping the heel in contact with the dynamometer footplate during the passive motion test. In addition, we confirmed that muscle activity in the calf muscles (MG, Sol) and antagonist (tibialis anterior) were low (within the

baseline mean ± 3 s.d.) during the passive motion test (representative participant in Figure 2a). Thus, the influence of articular alternation and muscle activity on the plantar-flexor torque was minimal, and the passive motion test was conducted appropriately.

In conclusion, the degree of spasticity (MAS), morphologic (circumference and MG muscle thickness) and mechanical (muscle-tendon elasticity) properties of the plantar-flexors in chronic SCI patients with spasticity decreased with the injury duration. Therapeutic intervention, such as FES or BWST, may be needed to prevent these sequelae in chronic SCI patients.

DATA ARCHIVING

There were no data to deposit.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

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ORIGINAL ARTICLE

Minocycline Does Not Decrease Intensity of Neuropathic Pain, but Improves Its Affective Dimension

Masahiko Sumitani,[1] Hiroshi Ueda,[2] Jun Hozumi,[3] Reo Inoue,[3] Takamichi Kogure,[3] Toru Ogata,[4] and Yoshitsugu Yamada[3]

ABSTRACT

Purpose: Recent understanding of the neuron–glia communication shed light on an important role of microglia to develop neuropathic pain The analgesic effect of minocycline on neuropathic pain is promising but it remains unclear in clinical settings. *Methods:* The study included 20 patients with neuropathic pain of varied etiologies. We administered 100 mg/day of minocycline for 1 week and then 200 mg/day for 3 weeks, as an open-label adjunct to conventional analgesics. An 11-point numerical rating scale (NRS) and the short-form McGill Pain Questionnaire (SF-MPQ) were used to evaluate pain severity. The data were collected at baseline and after 4 weeks of therapy and analyzed using the Wilcoxon signed-rank test. *Results:* All except two patients tolerated the full dose of minocycline. There was no significant improvement in the scoring of NRS (5.6 ± 1.2 at baseline vs. 5.3 ± 1.9 at 4 weeks; $p = .60$). The total score of the SF-MPQ decreased significantly (17.2 ± 7.4 vs. 13.9 ± 9.6; $p = .02$), particularly in the affective subscale (4.4 ± 2.7 vs. 3.3 ± 3.6; $p = .007$) but not so in the sensory subscale (12.8 ± 5.2 vs. 10.6 ± 6.2; $p = .06$). *Conclusion:* Minocycline failed to decrease pain intensity but succeeded in reducing the affective dimension associated with neuropathic pain.

KEYWORDS neuropathic pain, minocycline, microglia, SF-MPQ

INTRODUCTION

Chronic pain is characterized by enhanced sensory neurotransmission that underlies increased sensitivity to noxious stimuli (i.e., hyperalgesia) and the perception of non-noxious stimuli as painful (i.e., allodynia), and its related affective disorders. Neuropathic pain is one of the most debilitating chronic pain conditions that considerably affects the activities of daily living (ADL) and the quality of life (QoL) of patients.[1] Several strategies are used in the clinical treatment of neuropathic pain; however, it is often resistant to therapy and pain intensity remains severe. Therefore, the search for novel treatments continues.

Pharmacological treatments of neuropathic pain have primarily targeted neurons, including the axons and dendrites. And thereby, some adverse effects (e.g., somnolence), related to restraint of neuronal activity, cannot be avoided, although they have limited analgesic efficacy. Recent advances in the understanding of the neuron–glia communication shed light on an important role of microglia in the spinal dorsal horn for the development and persistence of neuropathic pain.[2] Microglia are primary immune sentinels of the central nervous system (CNS). Pain arising from neuronal injury enhances the release of pro-nociceptive mediators (e.g., ATP, glutamate, cytokines, and neurotrophic factors) and activates both neurons and microglia in the spinal dorsal horn. Following the nerve injury, activated microglia further produce pro-nociceptive neurotransmitters (e.g., NO, cytokines, and chemokines), all of which promote synaptic transmission. This process

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of neuron–glia communication ultimately leads to increased excitability of the spinal dorsal horn neurons and causes the sensation of persistent pain in the absence of continuous noxious stimuli to the tissue.[3] Once microglia are activated, the neuron–microglia communication maintains this vicious process and prolongs neuropathic pain.[4]

Numerous physiological and pharmacological studies have succeeded in attenuation of neuropathic pain by using a variety of molecules that target inhibition of microglial activation through suppression of divergent purinergic receptors (e.g., P2 X 4, P2Y12) on the microglial surface.[5] Among these, minocycline is a lipophilic molecule absorbed rapidly and readily across the blood–brain barrier. In addition to its actions as an antibiotic, minocycline has neuroprotective and anti-inflammatory effects in the CNS, probably due to its potent and preferential inhibitory action on the microglia rather than neurons. The mechanism of action of minocycline on the microglia is multifaceted;[6] but for example, previous studies have shown that its inhibitory effect on microglia activation attenuates the development of hypersensitivity in rat models of neuropathic pain.[4,7] Moreover, because of neuroprotective properties, minocycline can restore locomotor property in the model of spinal cord injury and reduce the lesion size after injury, indicating the ability to prevent neuronal apoptosis in the injury site.[8,9] Apoptosis of spinal dorsal horn neurons was reported in patients with post-herpetic neuralgia, even though it is a peripheral neuropathic pain condition.[10]

Considering the above data, the analgesic effect of minocycline is promising but it remains still unclear whether it will be clinically useful in the treatment of neuropathic pain. To elucidate this question, the present study was designed to investigate the analgesic effect of minocycline in patients with neuropathic pain.

MATERIALS AND METHODS

The study was approved by an institutional ethical review board and registered in the University Medical Information Network (UMIN), which is one of data center as public institution in Japan (trial ID: UMIN000008594).

Subjects

The study included 20 patients with the diagnosis of neuropathic pain recruited from our outpatient clinic. Diagnosis of neuropathic pain was established for each patient by their pain specialist based on the available diagnostic grading system.[11] The inclusion criteria were as follows: (1) diagnosis of neuropathic pain (irrespective of its origin); (2) mean pain intensity in the past week of >4 on an 11-point numerical rating scale (NRS) (where 0 stands for no pain, and 10 for worst possible pain); (3) pain duration >3 months; and (4) age between 20 and 80 years. Patients with slight or more clinically relevant cognitive dysfunction and those with a history of allergy to tetracycline antibiotics were excluded. All patients gave written informed consent to participate in the study.

Minocycline Treatment

All patients took a stable dose of analgesics, including gabapentinoids, tricyclic antidepressants, opioids and others, for at least 1 month before baseline screening. Next, they started an open-label treatment with minocycline. Participants were required to continue the same dose of analgesics throughout the study. After baseline assessment, minocycline treatment was initiated according to the following titration schedule: 50 mg orally twice a day (daily dose, 100 mg) for the first week, and 100 mg orally twice a day (daily dose, 200 mg) for 3 weeks. At the end of the first week, patients were screened for any side effects of minocycline.

Treatment was continued providing that the patients gave their consent and accepted potential complications.

Evaluation of Neuropathic Pain

We evaluated pain intensity using an 11-point NRS and the Japanese version of the short-form McGill Pain Questionnaire (SF-MPQ). The total score and both sensory and affective subscales of the SF-MPQ were used. ADL were assessed by the Japanese version of the Brief Pain Inventory (BPI). Because different characteristics of pain description can indicate different underlying mechanisms,[12] we evaluated seven pain characteristics (burning sensation, tingling or prickling sensation, tactile allodynia, electric shock-like pain attacks, thermal allodynia, numbness, and hyperpathia) on a 6-point scale (0 = never, 1 = hardly noticed, 2 = slightly, 3 = moderately, 4 = strongly, and 5 = very strongly) for speculating the analgesic mechanism(s) by minocycline. To evaluate pain characteristics, we took an example from the rating system of PainDETECT.[13] PainDETECT is a screening questionnaire that describes all the seven characteristics as specific for neuropathic pain and has been confirmed to screen for a neuropathic pain component from a variety of pain complaints.

All of the above measures were conducted twice: at baseline and after 4 weeks of minocycline therapy. The primary outcome measure of the present study was a decrease in the NRS score of more than 30%, because it is known that a decrease in pain intensity of at least 30% can lead to the improvement of general health status of patients with chronic pain.[14] All data were expressed as means ± standard deviation. We used the Wilcoxon signed-rank test to compare between parameters at baseline and those at 4 weeks. The level of statistical significance was set at a p-value of less than 0.05.

Since minocycline has been reported to reverse opioid-induced antinociceptive tolerance,[15] we divided patients into two groups (those who used opioid analgesics vs. those who did not). Using the Mann–Whitney test, we compared the two groups in terms of a percent decrease in the NRS score caused by minocycline.

RESULTS

Of 20 patients, 2 dropped out of the study. One patient was a 77-year-old woman with postherpetic neuralgia who suffered from severe allergic skin rash 3 days after starting minocycline. She was treated with oral steroids for 2 weeks and the rash disappeared completely. The other patient was a 76-year-old man with chronic inflammatory demyelinating polyneuropathy who complained of nonspecific hoarseness and showed poor medication compliance at the end of the first week. The remaining 18 patients tolerated the full dose of minocycline (100 mg/d for 1 week and 200 mg/d for 3 weeks) (6 patients had post-brachial plexus avulsion injury pain, 2 post herpetic neuralgia, 1 thalamic pain, 1 median nerve injury, 1 phantom limb pain after amputation, 1 spinal cord injury, 1 lumbar radicular nerve injury, 1 chemotherapy-induced polyneuropathy, 3 failed back surgery syndrome, and 1 caudal neurinoma). No adverse events were observed in these patients; thus, our final analysis included these 18 patients with neuropathic pain. Their demographical data as well as the results of the outcome measures are presented in Table 1.

There was no significant change in pain intensity after minocycline therapy (5.6 ± 1.2 at baseline vs. 5.3 ± 1.9 at 4 weeks; $p = 0.60$). The total scores of BPI were also comparable before and after treatment (31.3 ± 13.9 vs. 27.9 ± 18.0; $p = 0.23$). The total score of the SF-MPQ decreased significantly (17.2 ± 7.4 vs. 13.9 ± 9.6; $p = 0.02$), particularly in the affective subscale (4.4 ± 2.7 vs. 3.3 ± 3.6; $p = 0.007$) but not so in the sensory subscale (12.8 ± 5.2 vs. 10.6 ± 6.2; $p = 0.06$). Of 7 pain characteristics, only thermal allodynia improved significantly (1.6 ± 1.4 vs. 1.0 ± 1.1; $p = 0.02$). The remaining six characteristics did not improve after minocycline therapy (Table 1).

Simultaneous use of opioids and minocycline did not significantly relieve pain. In the group with opioid use ($n = 9$), we observed a decrease in the NRS of 5.5% ± 37.8%, and in that without opioid use ($n = 9$), it was 14.9% ± 22.2% ($p = 0.23$).

TABLE 1. Demographic and clinical data of participants with neuropathic pain

		Pre	Post	p-value**
No. of patients*		18	18	
Male: Female		14:4		
Age (y)		58.5 ± 13.9		
Duration of pain (wk)		109.6 ± 94.8		
Pain intensity (NRS)		5.6 ± 1.2	5.3 ± 1.9	0.60
BPI		31.3 ± 13.9	27.9 ± 18.0	0.23
SF-MPQ	Total	17.2 ± 7.4	13.9 ± 9.6	0.02
	Sensory	12.8 ± 5.2	10.6 ± 6.2	0.06
	Affective	4.4 ± 2.7	3.3 ± 3.6	0.007
Pain characteristics	Burning	2.2 ± 1.6	1.9 ± 1.1	0.22
	Tingling/prickling	2.7 ± 1.3	2.5 ± 1.2	0.53
	Tactile allodynia	1.8 ± 1.0	1.9 ± 1.1	0.85
	Electric shock-like	2.5 ± 1.4	2.3 ± 1.4	0.45
	Thermal allodynia	1.6 ± 11.4	1.0 ± 1.1	0.02
	Numbness	2.9 ± 1.9	2.8 ± 1.6	0.61
	Hyperpathia	1.9 ± 1.4	1.8 ± 1.4	0.93

*Initially, 20 patients were enrolled into the study; 2 patients dropped out during the study and their data were not included in the table.
**Statistical analyses were conducted by the Wilcoxon signed-rank test.
NRS = Numerical Rating Scale; BPI = Brief Pain Inventory; SF-MPQ = short-form McGill Pain Questionnaire

DISCUSSION

In the present study, minocycline did not reduce the intensity of neuropathic pain, as measured by the NRS. Likewise, a recent study found that minocycline improves neuropathic pain, which is naïve to analgesics other than nonsteroidal anti-inflammatory drugs and acetaminophen, compared to placebo but its effect size is very small, and concluded that minocycline is not likely to be clinically meaningful.[16] Several studies involving animal pain models indicated that minocycline could exert an analgesic effect on neuropathic pain when given preemptively or immediately after neural injury, but the same effect was not observed at longer times after injury.[4,7,8] This suggests that there may be a therapeutic time window for postinjury administration of minocycline. Prescribing minocycline at the initiation of neuropathic pain can prevent prolonged pain. In clinical practice, most patients with neuropathic pain, including those in our study, seek treatment only after the signs and symptoms of pain have developed. The time point when minocycline was administered to our patients was probably not within the therapeutic time window.

The majority of studies involving animal pain models focused on the measures of hypersensitivity and allodynia subsequent to tissue or nerve injury. Among these, both tactile and thermal allodynia have been reported to be attenuated by minocycline.[4,7,17] In our study, minocycline did not reduce tactile allodynia but improved thermal allodynia. Thus, our data from humans are not in line with the previous findings in animals. At the level of nerve conduction and neurotransmission, the underlying mechanisms of tactile and thermal allodynia are different, which may explain the differences in analgesic action of minocycline on neuropathic pain in human subjects. Another explanation might be that thermal allodynia was the least impaired of all seven characteristics evaluated in this study, and hence it might be most responsive to minocycline.

Minocycline has also been reported to reverse opioid-induced antinociceptive tolerance,[15] which is considered to share some common pathological mechanisms with neuropathic pain. These mechanisms possibly explain why opioid analgesics show the ceiling effect on neuropathic pain.[18] In our study, minocycline was less effective in patients who used opioid analgesics compared with those who did not. Our study did not show any clinical evidence that minocycline can reverse opioid-induced antinociceptive tolerance. The development of opioid-induced antinociceptive tolerance can be prevented if minocycline is administered at the adequate stage.[19] The time point when minocycline was administered to our patients was probably not within the therapeutic time window.

Pain is defined as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage" (by The International Association for the Study of Pain). Thus, pain always has two aspects: sensory and affective. In the present study, minocycline did not decrease pain intensity but improved neuropathic pain-associated affective disorders. In several studies on animal pain models, affective disorders, such as depression and anxiety, were improved by a pharmacological treatment, and the results were directly related to particular brain regions (e.g., anterior cingulate cortex and amygdala).[20] Our findings indicate that minocycline works mainly at the supraspinal level. Supporting evidence comes from several reports that minocycline has a potential to protect cerebral damage in multiple sclerosis and Parkinson's disease,[6] and that it can normalize neuronal dysfunction in schizophrenia. In particular, minocycline improved not only positive symptoms (i.e., hallucination, delusion, and catatonia) but also negative affective symptoms (i.e., depression and flattening of emotion) of schizophrenia.[21] Previous basic studies reported that neuropathic pain occurred after microglial activation in the spinal cord, and, what follows, minocycline attenuated neuropathic pain by inactivating spinal microglia. However, those studies did not investigate the microglia in the supra-spinal CNS, and our clinical findings require basic research to reveal the close associations between neuropathic pain, affective disorders, and microglia.

To adequately control chronic pain in the clinical setting, it is vital for healthcare professionals to educate patients on how to cope with chronic pain.[22] For example, cognitive behavioral therapy sometimes fails to decrease pain intensity but usually succeeds in reducing distress associated with chronic pain and improving ADL and QoL in the long term.[23] Thus, minocycline might become a novel treatment for neuropathic pain, in that it could be used as a pharmacological pain-coping strategy. Of note, some of our patients expressed a wish to continue minocycline at the end of the study, although they recognized that it did not satisfactorily decrease pain intensity.

Our findings have to be interpreted with caution owing to a number of limitations of the study, including an open-label design, lack of a control group, and a small sample size, in addition to no significant improvement in the ADL. However, our results may possibly lead to novel basic-science insight into the mechanisms of neuropathic pain and associated

affective disorders because pain is both sensory and affective experience. What follows, our findings may indicate the necessity to consider the sensory and affective aspects of pain separately in clinical settings.

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Conflict of Interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

Implication statement

Microglia in the spinal dorsal horn play an important role for the development of neuropathic pain. Minocycline modulates microglial activation and its inhibitory effect on microglia attenuates neuropathic pain. In neuropathic pain patients, minocycline failed to decrease pain intensity but succeeded in removing the distress associated with neuropathic pain.

Author contribution

M Sumitani directed the whole study, conducted experimental procedures, and drafted the manuscript. H. Ueda and T. Ogata provided advices to M. Sumitani about the potential analgesic and neuroprotective effect of minocycline on the basis of their basic investigations, and discussed the present findings. J. Hozumi, R. Inoue, and T. Kogure recruited the participants and they assisted data acquisition by M. Sumitani. Y. Yamada discussed the present findings and critically commented on the manuscript.

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