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Original Article

Comparison of muscular and articular factors in the progression of contractures after spinal cord injury in rats

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Study design: Experimental, controlled trial.
Objectives: To identify the relationship between the muscular and articular factors in the progression of contractures after spinal cord injury (SCI).
Setting: Hiroshima University, Hiroshima, Japan.
Methods: In total, 48 female Wistar rats were used. The 24 experimental rats that underwent a spinal cord transection and the other 24 control rats that underwent a sham-operation were assessed at 2, 4, 8, 12, 16, or 24 weeks postsurgery. Knee joint motion was measured for flexion and extension. Myotomy of the transarticular muscles was then performed and range of motion was measured again. The degree of contractures was assessed by goniometry measuring the femorotibial angle before and after the myotomies.
Results: The spinal cord-injured rats demonstrated flaccid paralysis during the first few days postsurgery and thereafter spastic paralysis. Intra- and inter-rater reliabilities for all measurements were >0.814. Knee flexion contractures developed in the all experimental rats, and progressed for the first 12 weeks and plateaued thereafter. Both the muscular ($48\pm5\%$) and articular ($52\pm5\%$) factors contributed almost equally to the overall progression of the contracture.
Conclusion: The present findings may shed light on the underlying pathophysiology of contractures and should help guide research towards finding the elucidation of contracture development after SCI.
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Keywords: spinal cord injuries; contracture; range of motion; articular; rats

Introduction

Joint contractures are a common complication of spinal cord injury (SCI),[1–3] and are characterized by limitations in the passive range of motion (ROM) of affected joints.[4,5] Contractures limit the function and independence for functional activities of SCI patients, and predispose patients to other complications such as pressure sores.[1,2] Advances in prevention and early intervention have improved the management of contractures,[6] and preventive measures have been established.[7] Nevertheless, we are often confronted with patients who have joints with contractures and no functional use of the limbs.[6,8] Contractures after SCI are especially difficult to treat and often progress despite optimal treatment. Possible factors involved in their progression are the availability of and compliance with treatment, and the presence of poor muscle control, weakness, impaired balance, hypertonicity, and spasticity or flaccidity.

Many studies in the literature have documented the factors responsible for contractures regarding the muscles and the periarticular structures. Most information obtained on contracture formation over time is based on animal models with contractures produced by immobilizing joints with internal fixation (with plate or splint),[5,6,9–11] external fixation (with cast, bandage, or splint),[12–14] pinning,[15] wiring,[16,17] or a combination of these procedures. Contractures after immobilization mainly result from stress deprivation of joints.[11,18] In spastic conditions, the consequent motion loss of the muscular imbalance resulting from spasticity is the basis for the development of contractures.[3,4,7,19] On the other hand, Enneking and Horowitz[20] observed histologically the articular changes in a human knee with traumatic paraplegia of three and one-half years' duration and

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identified the articular changes for contracture development. They found that the anterior portion of joint with flexion contractures was filled with fibrofatty connective tissue from the infrapattellar fat pad. Therefore, the relationship between the muscular and articular changes for contracture development after SCI remains unclear. We hypothesized that the factors most responsible for the limitation in ROM was originated from the muscular changes at the early stage of the contracture process and the articular changes at the later stage.

We used a rat model of SCI, and examined the knee joints, which have been the focus of frequent studies for the production of contractures by immobilization. Our objective was to identify the relationship between the muscular and articular factors leading to contractures at each phase of the contracture process after SCI.

Methods

Experimental design

In total, 48 female Wistar rats (CLEA Japan Inc., Tokyo, Japan), 8 weeks old, weighing 155–198 g, were included in this study. Use of female animals facilitated manual expression of bladders after SCI.[21] We randomized four experimental and four control animals to each of the six study groups that were examined respectively at 2, 4, 8, 12, 16, or 24 weeks postsurgery. The subgroup sample sizes were calculated by a power analysis based on pilot results for detecting a 10° difference in ROM 19 times out of 20. Rats were housed in sterilized cages with bedding (cedar shavings), and were maintained under artificial conditions at 23 ± 1°C, with a constant humidity of 55 ± 5%, and a cycle of 12 h of light and 12 h dark. The animals had free and easy access to food and tap water, and unlimited activity. This study was carried out in accordance with the Guide for Animal Experimentation, Hiroshima University and the Committee of Research Facilities for Laboratory Animal Science, Hiroshima University School of Medicine.

Surgical procedures

The 24 experimental animals were anesthetized by intraperitoneal administration of 0.8 ml/kg Nembutal (Abott Laboratories, North Chicago, IL, USA), and fixed in the prone position. A dorsal median incision was made over the distal thoracic vertebral column. The vertebral arch and the spinous processes of the T7 to T11 were exposed by cutting the latissimus dorsi muscle and the underlying paravertebral muscles from the origin on the spinous processes. The medial part of the paravertebral muscles was retracted to the side over two segments from the spinous processes and the arches of the vertebra. The spinal cord was exposed by a laminectomy of the T8 vertebra. The cord was then transected including the dura mater and the whole circumference of the cord at the level of T8 vertebra with a no. 11 scalpel blade in one movement from the right to the left. The cut was redone a second time from the left to the right taking great care to slide with the tip of the scalpel over the osseous surface of the spinal canal to ensure complete transection. The paravertebral muscles and the latissimus dorsi muscle of both sides were repositioned and gently opposed by 2-0 silk sutures. With the same suture, the skin was closed.

The 24 rats in the control group were also anesthetized with the same quantity of intraperitoneal Nembutal administration as the experimental group. A laminectomy of the T8 vertebra without spinal cord transaction was carried out as a sham-operation.

Postoperative care

All animals were monitored for sign of pain or distress every day throughout the experimental period. Further, the experimental animals' bladders were compressed manually twice daily. To prevent urine scald, the skin was wiped twice daily with diluted 0.1% chlorhexidine diacetate (Sigma, St Louis, MO, USA).[21] During surgery and throughout the experimental period, the knee joint was not violated.

ROM measurements

The animals were anesthetized with 0.8 ml/kg intraperitoneal Nembutal and placed in the supine position on a warm pad to prevent hypothermia. ROM measurements in flexion and extension were performed using a weight, which is a modification of that described by Wilson and Dahners.[16] Based on pilot studies, the maximal torque was determined such that it led to a tearing of the soft tissues and a drop in resistance (extension surpassing 0° and approximately 160° of flexion) after a myotomy.[11] In the absence of clinically interpretable data on rats,[6] the less torque (14.60 N mm) than the maximal torque was used, which the knee joint was stretched closely to its physiologic limits. The lateral femoral condyle was the pivot point from which the angle between the femur and tibia was measured. The femoral shaft was held by an examiner while the tibial shaft accommodated the gliding motion at knee joint during flexion-extension. Then, 14.60 N mm flexion and extension moments were applied to the tibia. The maximal extension ROM when the extension moment was applied and the maximal flexion ROM when the flexion moment applied were assessed by measuring with a goniometer the femorotibial angle of hindlimbs. The landmarks used three points (proximal femur, distal tibia, and lateral femoral condyle). Full extension was assigned 0° and full flexion 160°, so a restricted femorotibial angle would be the result of contractures. The right and the left knee joint served as different samples. Two examiners conducted the ROM measurements and repeated them 10 times for each leg for both flexion and extension. The examiners were blinded to each others scores. Values were the mean of the 20 measurements, the combined measurements taken by both examiners. The total ROM was the

sum, in absolute values, of the angular displacement of flexion and extension.

The animals were killed by exsanguination. Myotomy of the transarticular muscles were then performed and ROM was measured again in flexion and in extension. Measurements, after myotomies, were completed within 15 min of the animals' deaths, in order to minimize the possibility of postmortem rigidity. The same measurements were obtained for the knee joints of the control animals.

Calculation of myogenic and arthrogenic components of contractures

We defined myogenic contractures as contractures caused by the muscles including tendon and fascia, and defined arthrogenic contractures as contractures caused by the articular structures (bone, cartilage, synovium/subsynovium, capsule, and ligament); these were calculated ROM following the methods of Trudel and Uhthoff.[6] The formulas allowed isolation of the muscular and articular factors of contractures are briefly as follows: (1) Total contracture = ROM no myotomy (control group)−ROM no myotomy (experimental group). (2) Arthrogenic contracture = ROM after myotomy (control group)−ROM after myotomy (experimental group). (3) Myogenic contracture = (ROM after myotomy (experimental group)−ROM no myotomy (experimental group))−(ROM after myotomy (control group)−ROM no myotomy (control group)).

Statistical analyses

The statistical analyses were conducted using SPSS 11.5J for Windows (SPSS Japan Inc., Tokyo, Japan) and Microsoft Excel 2002 (Microsoft Co., Redmond, WA, USA). All values in the text, tables, and figures are presented as mean ± standard deviation (SD). To evaluate the contribution by the muscles and the articular structures to contracture development, we averaged for each group the ROM measurements obtained before and after the myotomies at different time points. These group means were used to calculate the myogenic and arthrogenic components, according to the formulas mentioned above. The SDs on the estimates of the myogenic and arthrogenic contractures were derived according to the procedure for calculating the SD for the mean differences between the groups.[22] Estimates are given with 95% confidence interval (CI). Using a length of the 95% CI not overlapping zero, we identified muscular and articular factors that were significantly responsible for contracture development, and evaluated the time effect on each contracture (total, myogenic, and arthrogenic contractures).[6] To assess the reliability of the ROM measurements, intraclass correlation coefficient (ICC) was used to analyze the results obtained from the two examiners. The intrarater reliability coefficient was calculated with the ICC (1, 1), and the inter-rater reliability coefficient with the ICC (3, 1).[23]

Results

Operative and functional outcome

No decubitus was observed in any of the rat. Three experimental rats with SCI died from suspected pyelonephritis and were replaced. All of the other rats survived throughout the experimental period, gained weight, were active, and appeared healthy except for hindlimbs functional deficits associated with SCI such that the hindlimbs were dragged behind the animal. The spinal cord-injured animals demonstrated a complete flaccid paralysis during the first few days postsurgery and thereafter spastic paralysis, as reported in humans[19] and animals[21,24] with SCI. After spasticity developed, hindlimb extension with rapid myospasm and toe abduction was observed at beginning of advancement after the slight movement of hip and/or knee joints. All animals before surgery and the control animals at any time during the experimental period after laminectomy had no observable deficits.

Intra- and inter-rater reliability coefficient

A highly statistical positive correlation was observed in the intra- and inter-rater reliability coefficients for the ROM measurements. The intrarater reliability coefficient was calculated for the following four ROM measurements: (1) flexion ROM under anesthesia (ie no myotomy); (2) extension ROM with no myotomy; (3) flexion ROM after myotomy; (4) extension ROM after myotomy, which, respectively, was (1) 0.814 and 0.814, (2) 0.996 and 0.994, (3) 0.975 and 0.942, and (4) 0.991 and 0.987 for the two examiners. The inter-rater reliability coefficient was also calculated for the four measurements: (1) 0.906; (2) 0.999; (3) 0.936; and (4) 0.996.

Relationship between myogenic and arthrogenic components of contractures

Knee flexion contractures developed in all the experimental rats with SCI. The total contractures indicated that the loss in total ROM was measurable after only 2 weeks postsurgery and progressed significantly for the first 12 weeks (95% CI; between the 2nd and 4th week, 4.27–9.73; 4th and 8th week, 4.27–9.73; 8th and 12th week, 12.27–17.73) (Table 1). The myogenic component progressed significantly between the 4th and 8th week (95% CI, 2.21–9.79). The arthrogenic component hardly changed for the first 8 weeks, but progressed sharply between the 8th and 12th week (95% CI, 6.91–17.09). Along with the progression of the total contractures, the myogenic component progressed similarly between the 2nd and 8th week, as did the arthrogenic component between the 8th and 12th week. Both myogenic and arthrogenic components progressed as well as the total contractures between the 16th and 24th week. Between the 12th and 16th week, the progression of both components stabilized toward the subsequent period (between the 16th and 24th week) (Figure 1a). The

Table 1 Myogenic and arthrogenic components of contractures in total ROM after spinal cord injury

Contractures	*2 weeks*	*4 weeks*	*8 weeks*	*12 weeks*	*16 weeks*	*24 weeks*
Total contracture	17±2[a]	24±3[a]	31±2[a]	46±3[a]	47±2	43±5
Myogenic component	7±4	11±4	17±3	20±5[b]	23±4	22±4
Arthrogenic component	10±4	13±2	14±3	26±6[b]	23±2	22±2

Values are given as mean±SD. Total contractures, and myogenic and arthrogenic components were calculated according to the formulas in the Methods section
[a]Significant difference between the beginning of the time interval and the end of the interval, using a length of the 95% CI not overlapping zero
[b]Significant difference between the myogenic and arthrogenic components at each time, using a length of the 95% CI not overlapping zero

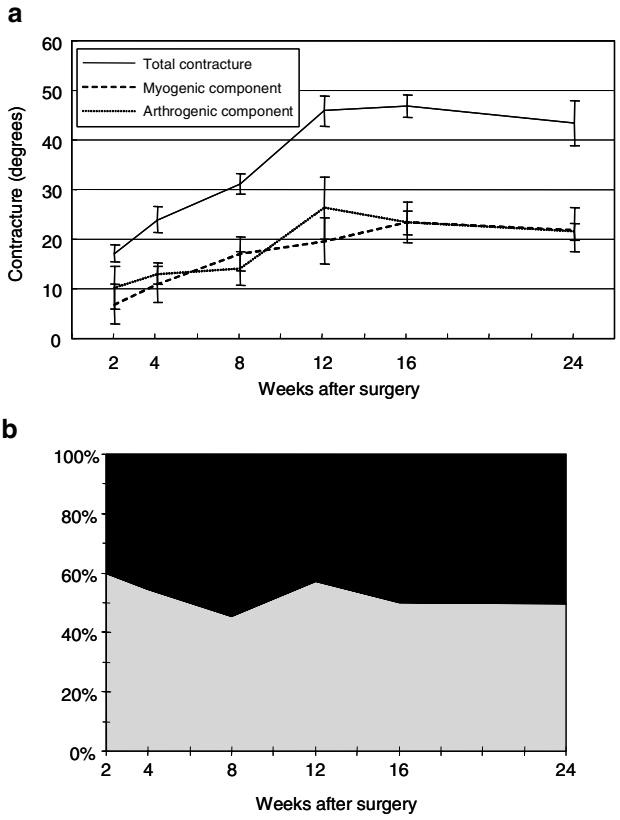

Figure 1 Components of contractures in total ROM over time. (**a**) Results in degrees. With the changing total contracture, the myogenic component had similar changes between the 2nd and 8th week, the arthrogenic component between the 8th and 12th week, and both components between the 16th and 24th week. The arthrogenic component increased sharply between the 8th and 12th week whereas the myogenic component progressed gradually. Error bars represent ±1 SD. (**b**) Results in percentages: myogenic component in black; arthrogenic component in grey. Both the muscles and the articular structures contributed to contracture development

arthrogenic component contributed more to contracture development at 12 weeks compared with the myogenic component (95% CI, 0.08–11.92) (Table 1). If the myogenic and arthrogenic proportions, when the total contractures were 100% at each time, were expressed as percentages, both myogenic and arthrogenic components would be shown to contribute to the overall loss in ROM throughout the experimental period. The arthrogenic proportion was 59% at 2 weeks, diminished from 54% (4 weeks) to 45% (8 weeks) of the total contracture with increasing myogenic proportion, and then increased to 57% at 12 weeks. Between the 16th and 24th week, both myogenic and arthrogenic proportions were almost 50% (Figure 1b).

Components of limitation in extension angular displacement

The results on extension corroborated the time course and the magnitude of contractures. The limitation in extension also progressed significantly for the first 12 weeks (95% CI; between the 2nd and 4th week, 2.86–7.15; 4th and 8th week, 5.30–8.70; 8th and 12th week, 12.30–15.70) and plateaued thereafter (95% CI; between the 12th and 16th week, −3.15 to 1.15; 16th and 24th week, −1.15 to 3.15). The arthrogenic limitations at 2 and 16 weeks were greater than myogenic limitations (95% CI, 4.86–9.15 and 2.27–7.73, respectively) (Table 2, Figure 2a). If expressed in percentages, both the myogenic and arthrogenic limitations contributed to contracture development following SCI (Figure 2b).

Components of limitation in flexion angular displacement

Both the myogenic and arthrogenic components in flexion contributed to contracture development (Figure 3). However, because the contracture was caused almost exclusively by limitation in extension, the results on flexion slightly affected the overall progression of the contracture. The myogenic limitations contributed significantly to contracture development at 8 and 16 weeks compared with the arthrogenic component (95% CI, 1.21–8.79 and 3.86–8.15, respectively). At 2, 8, and 16 weeks, the contribution of the arthrogenic limitation in extension was somewhat counteracted by the myogenic limitation in flexion such that no statistically significant differences were found between the myogenic and arthrogenic components in the total ROM at these time points.

Table 2 Myogenic and arthrogenic limitations in extension angular displacement

Contractures	*2 weeks*	*4 weeks*	*8 weeks*	*12 weeks*	*16 weeks*	*24 weeks*
Limitation in extension	22 ± 2^{a}	27 ± 2^{a}	34 ± 1^{a}	48 ± 2^{a}	47 ± 2	48 ± 2
Myogenic limitation	8 ± 2^{b}	13 ± 4	16 ± 2	23 ± 3	21 ± 3^{b}	24 ± 2
Arthrogenic limitation	15 ± 2^{b}	14 ± 3	18 ± 2	25 ± 4	26 ± 2^{b}	24 ± 1

Values are given as mean $\pm$ SD. When dealing with extension angular displacement, the formulas in the Methods section have to be modified (reversal of + and − signs) to be consistent with the results in total, flexion, and extension ROM[6]
[a]Significant difference between the beginning of the time interval and the end of the interval, using a length of the 95% CI not overlapping zero
[b]Significant difference between the myogenic and arthrogenic components at each time, using a length of the 95% CI not overlapping zero

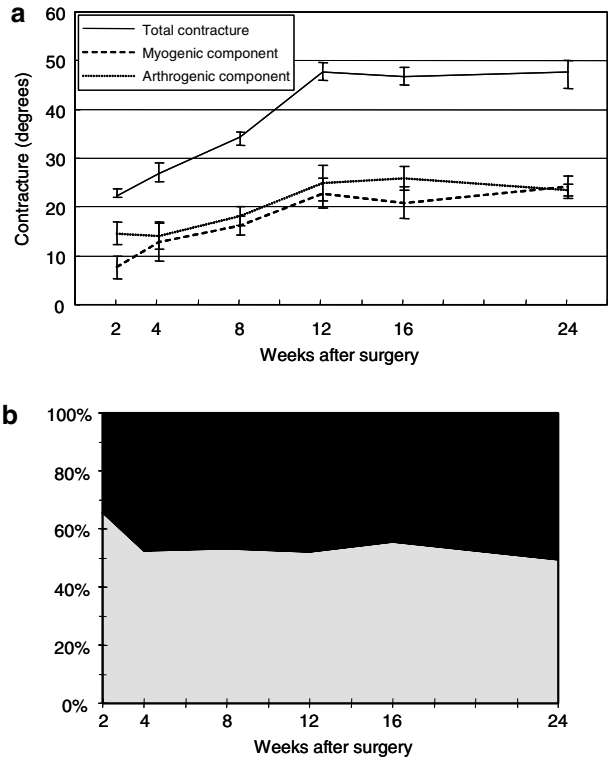

Figure 2 The contribution of the muscles and the articular structures to the limitation in extension angular displacement. (**a**) Results in degrees. Contractures progressed for the first 12 weeks and plateaued thereafter. The arthrogenic limitation contributed slightly to contracture development rather than the myogenic limitation. (**b**) Results in percentages: myogenic component in black; arthrogenic component in grey. Both the muscles and the articular structures contributed to the loss in extension ROM

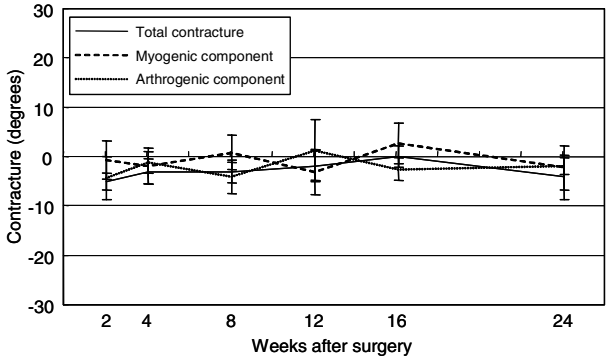

Figure 3 The components of the limitation in flexion angular displacement. The magnitude of contractures in flexion changed only slightly when compared with that of extension. In flexion, negative numbers were obtained in situations where the spinal cord-injured animals gained more angular displacement than the control. Error bars represent $\pm$1 SD

Discussion

Our results demonstrate that, different from the original hypothesis generated from the current literature, both the muscles and the articular structures contributed almost equally to the overall progression of the contracture, and these components are factors in the promotion of contracture development. The myogenic and arthrogenic contracture process stabilized with reaching the plateau of the total contracture process and then reached equilibrium. We also found that the arthrogenic restriction progressed sharply between the 8th and 12th week. Although this mechanism remains unclear, deterioration within the other knee joint structures except for the muscles is one of the factors responsible for the limitation in ROM and attention should be directed to the changes within arthrogenic constituents during this time.

Trudel and Uhthoff[6] have discussed the role of myogenic *versus* arthrogenic impediments in the progression of contractures after immobilization with internal fixation. They found that the arthrogenic proportion rose from 38.5% at 2 weeks to 98.5% of the total restriction in ROM at 32 weeks without plateauing, whereas the myogenic proportion decreased progressively from 2 to 32 weeks of immobilization and muscular changes resulted in an adaptive shortening. In our previous report,[25] the contracture process after SCI was similar to that of rigid internal immobilization, and the period when the restriction in ROM reached a plateau was similar.[11] However, the relationship

between the myogenic and arthrogenic components that we found differed greatly from those found by Trudel and Uhthoff.[6] We can propose four reasons to explain why the proportion of arthrogenic and myogenic components would be equal in this model whereas the arthrogenic component worsened with time in a model of contractures secondary to immobilization.

First, the subsequent conditions following SCI may have affected both the muscle and the articular structures. Sharrard[26] reported that based on clinical observations, contractures associated with central nervous system injuries always start in the soft tissues, and loss of elasticity and shortening may develop in tendons, fasciae, intermuscular septa and joint capsules and ligaments in which collagen is deposited. Finsterbush and Friedman[27] compared articular changes after peripheral denervation with those changes after immobilization, and they found specific changes caused by neurectomy; progressive atrophy of cells in all structures of knee joint, and degeneration in the middle layers of the articular cartilage suggesting that nutritional deficiency was involved. This findings raise the possibility that spinal cord transection also might affect partially the articular structures.

The second possible explanation is that the myogenic component after SCI may have changed greatly in the overall progression of the contracture when compared to the muscles around immobilized joints, and that specific changes in the paralyzed muscles may have progressed gradually and reached equilibrium. In immobilized joints, intact muscles are allowed voluntary isometric contraction and reach equilibrium rapidly, as explained by an adaptive shortening through the loss of sarcomeres.[6] Following SCI, it is well documented in humans[28–31] and animals[24,32,33] that skeletal muscles below the level of an upper motor neuron lesion undergo marked changes in their morphological, metabolic, and contractile properties.[34] Changes in spastic skeletal muscle generally include atrophy, loss of elasticity and relative increase in the connective tissue within the muscles, ingravescent accumulation of lipid, interstitial fibrosis and microcirculatory alteration.[7,24,29,34] Spastic muscles develop involuntary tetanic contraction, which produced a marked loss of sarcomeres in this muscles, which was greater than that from immobilization alone.[7,35] In SCI patients, slow-to-fast conversion that is the major effect of a spinal cord transection[36] occurred in early stages (<4 weeks) postinjury, and reached gradually the new steady state characterized by a predominance of fibers expressing only the fast by 73 months.[34]

The third reason is that the mechanical forces (involuntary joint movements and co-contraction of the muscles) related to spasticity may have prevented the changes in the articular structures. The capsular cellular elements mainly react to the lack of tension forces and commend extracellular matrix changes, which contribute to capsular stiffness.[11] The agonist–antagonist co-contraction related to spasticity may transmit forces through the joint and protect against capsular shortening and fibrosis. As a result of these forces, the arthrogenic component would be less in our model compared to the joint immobilization model.

Finally, the progression of myogenic and arthrogenic contractures might have repeated either remission or exacerbation, and these contracture progressions have stabilized toward equilibrium (between the 16th and 24th week). Hildebrand *et al*[17] have studied the recovery process of post-traumatic contractures with immobilized rabbit knees for 8 weeks followed by remobilization. They have shown that contracture severity decreases with time although it does stabilize in the long term and that processes involved are somewhat altered depending on the underlying etiology of the contracture process. The remission might have occurred because we observed hindlimb extension and slight movement of knee joints in the animals with SCI.

A potential weakness of the study is that the accuracy of the joint motion measures was not verified, although the reproducibility of the measurements was evaluated using ICC. Trudel *et al*[37] first built the arthrometer with acceptable reproducibility and accuracy, which allows for measures in full range of rat knee joint motion, while the animals are under anesthesia. When their arthrometer was used, the proximal thigh and distal leg had to be dissected to measure without altering the attachment to the bony landmarks. In their testing, the myogenic component should be damaged, influencing the results of ROM measurements. Therefore, we used three landmarks identified from body surface and then measured the angle created by the longitudinal axes of the greater trochanter and lateral malleolus with the vertex at the femoral condyles. Although our testing was appropriate for the present study investigating the relationship between muscular and articular factors in contracture development, this cannot be ruled out the obscurity of the underlying femur in animal limbs where the thigh musculature is well-developed. Measurements of rat knee angular displacement still must be developed.

The spinal cord-injured rats dragged the hindlimbs, and their knee joints were not treated throughout the experimental period. In a clinical setting, most patients with SCI are treated with early mobilization and then seated quickly so they are not generally positioned in bed for long periods of time. We have developed a unique animal model of contractures after SCI that mimics the natural and longitudinal process in humans with SCI as opposed to the clinical management. Contracture produced experimentally by immobilizing joints does not model specific spasticity-related contractures in humans with neuromuscular disease.[19] Therefore, a rat with SCI is unequivocally a more appropriate model than other animals with immobilized joints for the elucidation of these contractures. Although the rat model with SCI appears to closely reflect the outcome in humans in terms of histopathology and function,[38] whether our results can be applied to humans with SCI remains controversial. Further studies are needed to determine which tissues within the articular structures are most responsible for the findings.

Conclusion

The present findings provide evidence that both the muscles and the articular structures contribute to contracture development after SCI. Based on our results, in addition to the treatment of the muscles, more attention should be directed to treating the articular structures in the overall progression of the contracture. This animal model will allow investigations in the elucidation of contracture development that may shed light on the underlying pathophysiology of contractures after SCI. The results may encourage more reasonable treatment approaches and may help prevent irreversible change.

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