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Original Article

Tidal volume and diaphragm muscle activity in rats with cervical spinal cord injury

Hidetaka Imagita, PT, PhD[1)*], Akira Nishikawa, MSc[1, 2)], Susumu Sakata, PhD[1)], Yasue Nishii[1)], Akira Minematsu, PT, PhD[1)], Hideki Moriyama, PT, PhD[3)], Naohiko Kanemura, PT, PhD[4)], Hanae Shindo, PT, MSc[1, 5)]

[1)] *Graduate School of Health Sciences, Kio University: 4-2-2 Umaminaka, Koryo-cho, Kitakatsuragi-gun, Nara 635-0832, Japan*
[2)] *Department of Sports Health Management, Faculty of Business Information, Jobu University, Japan*
[3)] *Department of Physical Therapy, Faculty of Health Sciences, Kobe University, Japan*
[4)] *Department of Physical Therapy, Faculty of Health Sciences, Saitama Prefectural University, Japan*
[5)] *Department of Physical Therapy, Keihan Life Support Company, Japan*

Abstract. [Purpose] The purpose of this study was to make an experimental model of cervical spinal cord injury (CSCI) using Wistar rats, in order to analyze the influence of CSCI on the respiratory function. [Subjects] Thirty-two male 12-week-old Wistar rats were used. [Methods] The CSCI was made at the levels from C3 to C7, and we performed pneumotachography and electromyography (EMG) on the diaphragm. Computed tomography was used to determine the level of spinal cord damage. [Results] After the operation, the tidal volume of the rats with a C3 level injury decreased to approximately 22.3% of its pre-injury value. In addition, in the same rats, the diaphragmatic electromyogram activity decreased remarkably. Compared with before CSCI, the tidal volume decreased to 78.6% of its pre-injury value in CSCI at the C5 level, and it decreased to 94.1% of its pre-injury value in CSCI at the C7 level. [Conclusion] In the rats that sustained a CSCI in this study, the group of respiratory muscles that receive innervation from the thoracic spinal cord was paralyzed. Therefore, the EMG signal of the diaphragm increased. These results demonstrate that there is a relationship between respiratory function and the level of CSCI.
Key words: Cervical spinal cord injury, Respiratory function, Electromyography of diaphragm muscle

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INTRODUCTION

According to an epidemiological study of spinal cord injuries in Japan, there are 100,000 patients with spinal cord injury and there are approximately 5,000 new patients every year. Approximately 75% of these patients have cervical spinal cord injury (CSCI)[1)]. The causes of spinal injuries are traffic accidents in 43.7% of cases and falling in 41.8%, and the age incidence is bimodal, with peaks in subjects aged in their twenties and in adults older than 55 years of age[2)].

Pneumonia is the leading cause of death in CSCI patients, and is responsible for 20% of the overall mortality rate[3, 4)]. Eighty-seven percent of CSCI patients are expected to die from pneumonia, with 75% of these patients aged older than 60 years. Respiratory disorders are the most common cause of death in approximately 40% of CSCI patients, and this rate has remained virtually unchanged since the 1950s.

Kim and Hwangbo reported the lung capacities of cervical, thoracic and lumbar spinal cord injury patients in differential positions, and they indicated more attention should be paid to the effect of the injury level on the measured lung capacity[5)]. Patients with CSCI are unable to maintain adequate ventilation due to paralysis of the diaphragm. Due to medical progress, CSCI patients can become long-term survivors through the use of mechanical ventilators. Therefore, respiratory care of CSCI patients is vitally important, not only during the acute phase, but also during the chronic phase. Furthermore, CSCI patients feel the need for social participation. Therefore, we must devise detailed respiratory care to enable the enrichment of their lives and to prevent complications.

The respiratory function of CSCI patients not only influences their activities of daily living, but also has a considerable influence on life support. The purpose of the present study was to establish a CSCI model using Wistar rats, and to use this model to analyze the influence of CSCI on respiratory function[6)].

SUBJECTS AND METHODS

Subjects

We used thirty-two to 10–12-week-old male Wistar rats (body weight = 256.8±12.5 g) in this study. The rats were randomly divided into 5 groups: C3 (n = 7), C4 (n = 6), C5

*Corresponding author. Hidetaka Imagita (E-mail: h.imagita@kio.ac.jp)

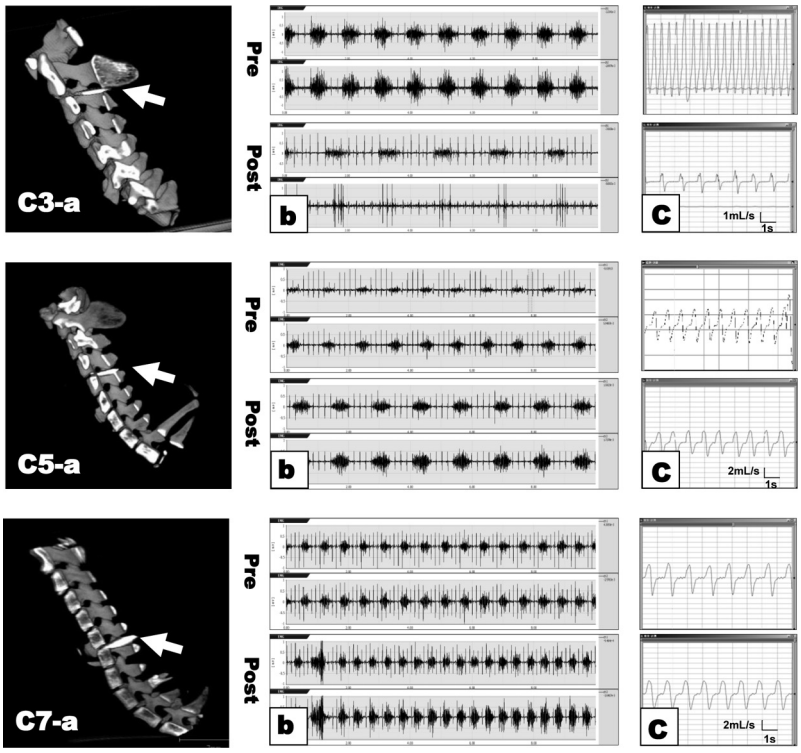

Fig. 1. Electromyograms and pneumotachograms of pre- and post-CSCI We determined the injury level using computed tomography imaging. The arrows indicate the site of the injury (a). Electromyograms of the diaphragm were measured for 10 s using an electromyograph (b). The tidal volume (VT) and minute ventilation volume (VE) were calculated from 10 respiratory waveforms displayed on a storage oscilloscope (C).

(n = 6), C6 (n = 7), and C7 (n = 6); however, 2 rats were excluded (1 each from the C3 and C6 groups) because they died after intubation and surgery, respectively. The rats were housed at 23 ± 2 °C, under a 12:12-h light-dark cycle, and were provided with rat chow and water ad libitum until performance of the CSCI procedure. All procedures used in this study were approved by the Animal Use Committee of Kio University, which adheres to the Japanese Physiological Society Animal Care Guidelines (authorization number 21-1-I-210413).

Methods

To analyze respiratory function according to the level of CSCI, we made experimental models of CSCI from the C3 to C7 levels. Surgical procedures were performed while the animals were anesthetized with ketamine (60 mg/kg) and xylazine (2.5 mg/kg). Briefly, the procedure was as follows: 1) tracheotomy and cannula insertion for measurement of respiratory functions; 2) measurement of respiratory functions before experimental cervical cord injury; 3) electrode placement on the diaphragm for measurement of its activity before experimantal CSCI; 4) performance of experimental CSCI; 5) measurement of respiratory functions and electromyography recording of the diaphragm after experimental CSCI; and 6) image capture of the injured site by computed tomography.

After confirmation of adequate anesthesia, the rats were fastened in a prone position and an incision was made at the cervical level. We ensured that the scapula and C2 spinous process were visible and palpable. Care was taken to ensure that only the spinal cord was transected and that the blood vessels underneath the spinal cord were avoided. We carefully interrupted virtually all pathways of the cord including the bilateral bulbospinal respiratory pathways to the phrenic motor neurons innervating the diaphragm. Using the C2 spinous process as a reference point, we determined the segmental levels and transected the cervical spinal cord using a sharp blade. A thin metallic plate was inserted at the injury level after measuring respiratory function and EMG. Subsequently, the cervical vertebrae were scanned using computed tomography, and the level of the injury was determined from the images (CBSTER MCT-100CB; Hitachi). Computed tomography images of the cervical vertebrae from C3 to C7 are shown in Fig. 1-a.

Under anesthesia, a midline incision was made in the neck skin and the cervical portion of the trachea was exposed to measure the tidal volume. A cannula was immediately inserted into the trachea after the tracheotomy for measurement, because cannulation for an extended period can decrease tidal volume. The respiratory waveform was displayed on a storage oscilloscope (Nihon Kohden, LEG-100), and the data were simultaneously recorded on a

Table 1. %VT, %VE, and %EMG of CSCI at C3 to C7

	C3	C4	C5	C6	C7
%VT	22.3±7.3	54.0±16.7	78.6±10.4*	80.4±16.1*	94.1±12.3†
%VE	9.2±4.6	48.6±15.3*	68.5±10.3*	76.0±20.1*	84.7±15.9†
%EMG	35.2±6.1	90.9±25.6*	132.4±28.2*	136.6±14.0*	114.6±19.9*

The average %VT, %VE, and %EMG, the post-CSCI values relative to the pre-CSCI values. All data are shown as the mean ± SD. * and †indicate significantly different levels ($p < 0.05$).
*: vs C3 ($p < 0.05$), †: vs C3 and C4 ($p < 0.05$)

computer for subsequent analysis. Tidal volume (VT) and minute ventilation volume (VE) were calculated from the 10 respiratory waveforms (Fig. 1). The data were averaged and %VT and %VE were calculated as the percentages of their pre-injury values.

Electrodes were placed on the diaphragm to measure its activity[7]. The animals were placed in a supine position on the operating table, and the diaphragm was exposed by cutting along the central line of the abdomen, 3–4 cm from the xiphoid process of the sternum. Pairs of insulated platinum wire electrodes (0.003 mm diameter; Cooner Wire), with 1 mm of the wire exposed at the tip, were implanted into the midcostal region of both sides of the diaphragm, and the electromyographic (EMG) activity was recorded using the Trias EMG system (SX230; Biometrics Co.). The root mean square was calculated for each level of CSCI, and the post-CSCI value was expressed as a percentage of the pre-CSCI value, %EMG.

All data are shown as the mean ± standard deviation (SD). We used the Kruskal Wallis test to analyze the differences in the injury level values of %VT, %VE and %EMG, and the Steel-Dwass method for multiple comparisons. In all analyses, values of $p<0.05$ were considered significant.

RESULTS

In C3 CSCI, VT decreased to 22.3% of its pre-injury value (Table 1), and VE decreased to 9.2% of its pre-injury value. In C4 CSCI, VT decreased to 54.0%, and VE to 48.6% of their pre-injury values; in C5 CSCI, VT decreased to 78.6%, and VE to 68.5% of their pre-injury values; in C6 CSCI, VT decreased to 80.4%, and VE to 68.5% of their pre-injury values; and in C7 CSCI, VT decreased to 94.1%, and VE to 84.8% of their pre-injury values.

%VT showed significant differences between C3 CSCI and those of all the other

CSCI levels except C4, and between C4 and C7 (Table 1). %VE showed significant differences between C3 CSCI and those of all the other CSCI levels, and between C4 and C7 (Table 1).

In C3 CSCI, EMG of the diaphragm decreased to 35.2% of its pre-injury value, and in C4 CSCI, it decreased to 90.9%. Conversely, EMG in C5 CSCI increased to 132.4%, in C6 CSCI to 136.6%, and in C7 CSCI to 114.6% of its pre-injury value (Table 1). There were statistically significant differences between %EMG of C3 CSCI and those of all the other CSCI levels.

DISCUSSION

In the CSCI sustained by the rats in this study, the group of respiratory muscles that receive innervation from the thoracic spinal cord, i.e., the external intercostal muscle, internal intercostal muscle, innermost intercostal muscle, and abdominal muscle, was paralyzed. The group of respiratory muscles that receive innervation from the cervical spinal cord has a functional role that partially corresponds with survival.

We found that the %VT of C3 CSCI was approximately 20%, and %VE was approximately 10%, and %VT and %VE of C4 CSCI were approximately 50%, suggesting that damage at these levels causes a remarkable decrease in ventilatory volume and is life-threatening, because it causes severe paralysis of the diaphragm. A previous study reported that the diaphragm is innervated by the phrenic nerve, which is derived from the myelomere, from C3 to C5, and consists of 71% motor fibers and 29% sensory fibers[8]. In addition, 74% motor fibers are found at the C4 level, and 68% sensory fibers are found at the C5 level. Therefore, the function of the phrenic nerve seems to be inactivated or impaired CSCI.

CSCI at C3 or C4 interrupts the bulbospinal respiratory pathways innervating the phrenic motoneurons; however, it was shown that the diaphragm is spontaneously and partially reactivated at 1 or 2 weeks after CSCI by the contralateral descending pathways[9–11]. In the present study, we concluded that the diaphragm appeared not to have been reinnervated by the contralateral descending pathways, because we measured some items immediately, and that ventilatory volume was decreased, because the respiratory muscle group that the subordinate cervical and thoracic spinal cord levels control had been impaired.

%VE after CSCI at the C5, C6, and C7 levels was 68.5%, 76.0%, and 84.8%, respectively, indicating that as the level of CSCI descended the spine, %VE increased. That is, as the level of CSCI became relatively lower, the function of the diaphragm was less impaired. In addition, we consider that some accessory respiratory muscles were activated by the increased ventilatory volume.

The phrenic nerve that innervates the diaphragm in humans mainly originates at the C4 level, and there is also supplementary innervation from C3 and C5. In this study, the activity of the diaphragm was remarkably decreased by upper level CSCI. It is possible that the activity of the diaphragm is increased to maintain breathing, and this is thought to be the underlying cause of overload weakness in human CSCI patients. In the present model, we cut the lateral and dorsal funiculus at the cervical spinal cord, i.e.,

the respiratory route. During surgery, we proceeded carefully to ensure that the nerve root was intact at each level; however, we might have inadvertently caused slight damage to the nerve root, and this might account for the large SD of %EMG in C4 and C5 CSCI. However, our findings clearly indicate that the activity of the diaphragm contributed greatly to tidal volume. Moreover, it is necessary to understand the state of the diaphragm carefully because it can fall into an overloaded state following the inactivation of other respiratory muscles. The present study clarified the relationship between the diaphragm and respiratory function in a model of CSCI.

%EMG after CSCI at C3 and C4 was approximately 65% and 10%, respectively. A previous study reported that 74% of motor fibers are concentrated at the C4 level, and 68% of sensory fibers are concentrated at the C5 level[4]. We speculate that if the sensory fibers of the phrenic nerve gather at the C5 level, CSCI at the C4 level is not susceptible to the effect of the afferent nerve. Road et al.[12] reported that electrical stimulation of the proximal end of the phrenic nerve triggers overventilation in dogs, and there is a positive feedback loop from diaphragmatic contraction. On the basis of this, we consider that it becomes difficult to induce overventilation at this level as the afferent input decreases.

EMG of the diaphragm muscle increased by 32.4% following CSCI at the C5 level compared with the control, and it increased by 36.6% at the C6 level. We consider that this increase in activity accompanies the dysfunction of the respiratory muscles that are innervated at the lower cervical and thoracic spinal cord levels, while innervation of the diaphragm is at such a level that it can function sufficiently after CSCI at levels below C5. Therefore, we postulate that this is a response that is activated strongly to maintain ventilation volume. Conversely, it was lower than after CSCI at C5 and C6, even though EMG of the diaphragm increased by 14.6% following CSCI at the C7 level. We considered this indicates that the subjects were able to maintain ventilatory volume after CSCI at C7, even when the diaphragm was not overactive. As many respiratory muscles that are innervated from the cervical spinal cord can function following CSCI at the C7 level, it is possible that the contribution of the diaphragm to ventilatory volume maintenance is lowered.

Based on our results, we conclude that the decrease of ventilatory volume caused by CSCI induced an alteration of the oxygen and carbon dioxide levels in the blood, thereby increasing the activity of the diaphragm. However, when CSCI occurs in the upper levels, the diaphragm is unable to increase its activity, cyanosis will develop, and the need for life support may become severe. To understand the effects of CSCI further, it may be necessary to utilize findings from studies in a wide range of disciplines, e.g., female sex hormones[13], theophylline treatment[14], the neuroanatomical basis underlying spontaneous recovery[10], and respiratory neuroplasticity related to nervous control from the spinal cord[15]. The activity of the diaphragm contributes to improvements in the sensation of dyspnea and ventilation efficiency, increases tidal volume and vital capacity, decreases oxygen consumption, and improves in exercise tolerance and activities of daily living.

In conclusion, we consider that it is important to study abdominal respiration with a functioning diaphragm to find the maximum possible ventilatory function of patients with CSCI. In the future, these evaluations may be necessary for the administration of respiratory support for CSCI patients.

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REFERENCES

1) Shingu H: Epidemiology of spinal cord injury in Japan from 1990 to 1992. (The third report). J Jap Med Soc Paraplegia, 1995, 8: 26–27 (in Japanese).
2) Ando N, Hasegawa Y, Mizuochi K, et al.: Elderly spinal cord injury patient. Study of Rehabil, 1992, 73: 19–14 (in Japanese).
3) Ideta R, Ueta T: The Japanease standard model database for spinal cord injury toward databank and EBM. Jap Soc Occup Trauma, 2009, 57: 168–172 (in Japanese).
4) Sumida M: Epidemiological study of spinal cord injury due to traffic accidents in Japan–using sekisonn database. Jap Soc Occup Trauma, 2007, 55: 33–38 (in Japanese).
5) Kim MK, Hwangbo G: The effect of position on measured lung capacity of patients with spinal cord injury. J Phys Ther Sci, 2012, 24: 655–657. [CrossRef]
6) Yoon J, Park J, Lee D, et al.: Comparisons of respiratory function and activities of daily living between spinal cord injury and stroke patients and normal elderly people. J Phys Ther Sci, 2012, 24: 465–469. [CrossRef]
7) Mantilla CB, Seven YB, Zhan WZ, et al.: Diaphragm motor unit recruitment in rats. Respir Physiol Neurobiol, 2010, 173: 101–106. [Medline] [CrossRef]
8) Liu M: Kinesiology of respiration. Sogo Rehabil, 1990, 18: 377–383 (in Japanese).
9) Mantilla CB, Greising SM, Zhan WZ, et al.: Prolonged C2 spinal hemisection-induced inactivity reduces diaphragm muscle specific force with modest, selective atrophy of type IIx and/or IIb fibers. J Appl Physiol 1985, 2013, 114: 380–386. [Medline] [CrossRef]
10) Lane MA, Lee KZ, Fuller DD, et al.: Spinal circuitry and respiratory recovery following spinal cord injury. Respir Physiol Neurobiol, 2009, 169: 123–132. [Medline] [CrossRef]
11) Vinit S, Darlot F, Stamegna JC, et al.: Long-term reorganization of respiratory pathways after partial cervical spinal cord injury. Eur J Neurosci, 2008, 27: 897–908. [Medline] [CrossRef]
12) Road JD, West NH, Van Vliet BN: Ventilatory effects of stimulation of phrenic afferents. J Appl Physiol 1985, 1987, 63: 1063–1069. [Medline]
13) Doperalski NJ, Sandhu MS, Bavis RW, et al.: Ventilation and phrenic output following high cervical spinal hemisection in male vs. female rats. Respir Physiol Neurobiol, 2008, 162: 160–167. [Medline] [CrossRef]
14) Hüttemann M, Nantwi KD, Lee I, et al.: Theophylline treatment improves mitochondrial function after upper cervical spinal cord hemisection. Exp Neurol, 2010, 223: 523–528. [Medline] [CrossRef]
15) Lane MA, Fuller DD, White TE, et al.: Respiratory neuroplasticity and cervical spinal cord injury: translational perspectives. Trends Neurosci, 2008, 31: 538–547. [Medline] [CrossRef]