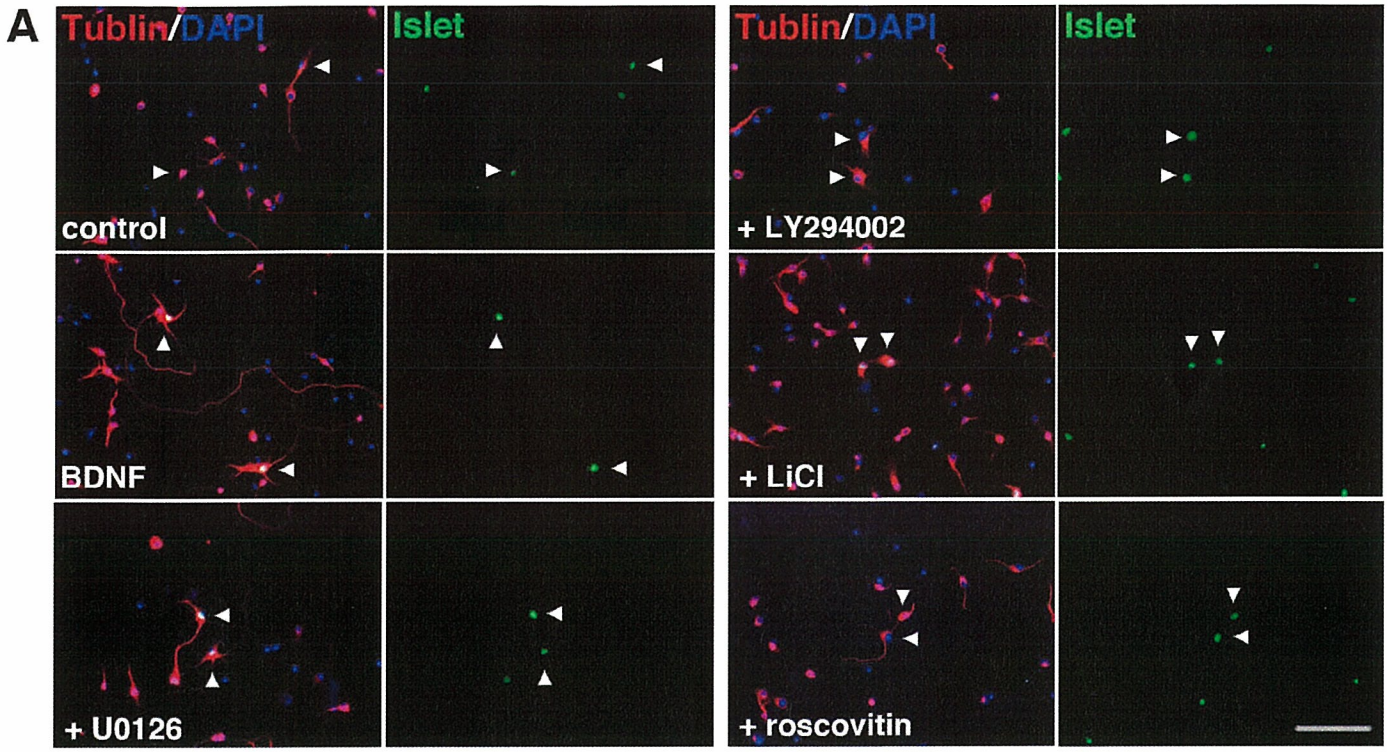

A
Tublin/DAPI
Islet
Tublin/DAPI
Islet
control
+ LY294002
BDNF
+ LiCl
+ U0126
+ roscovitin

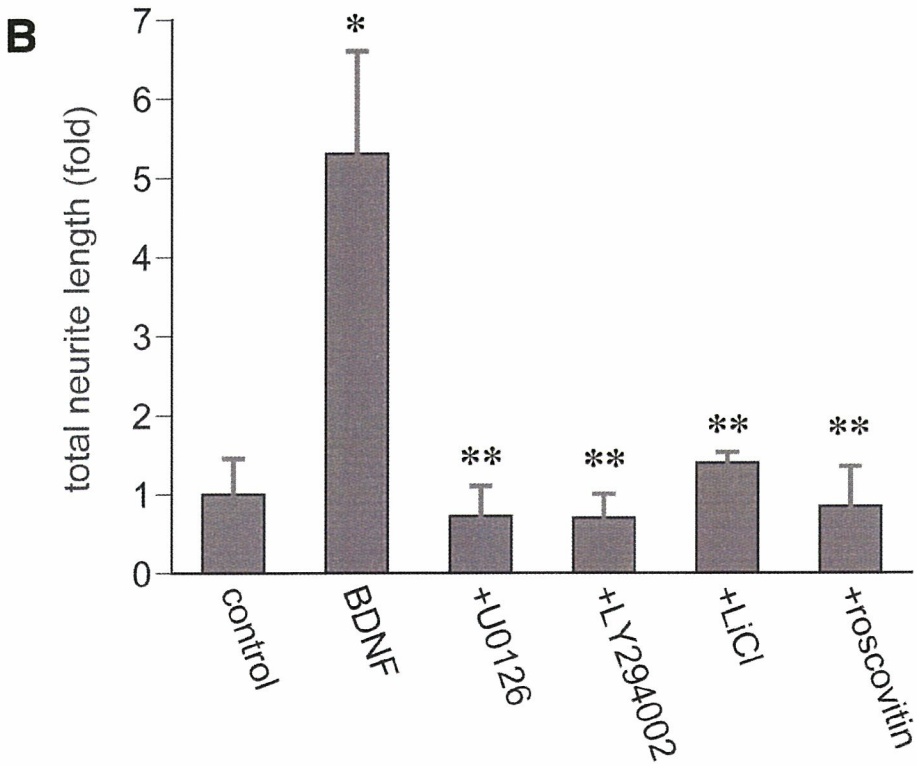

B
total neurite length (fold)
0
1
2
3
4
5
6
7
*
**
**
**
**
control
BDNF
+U0126
+LY294002
+LiCl
+roscovitin

図 2

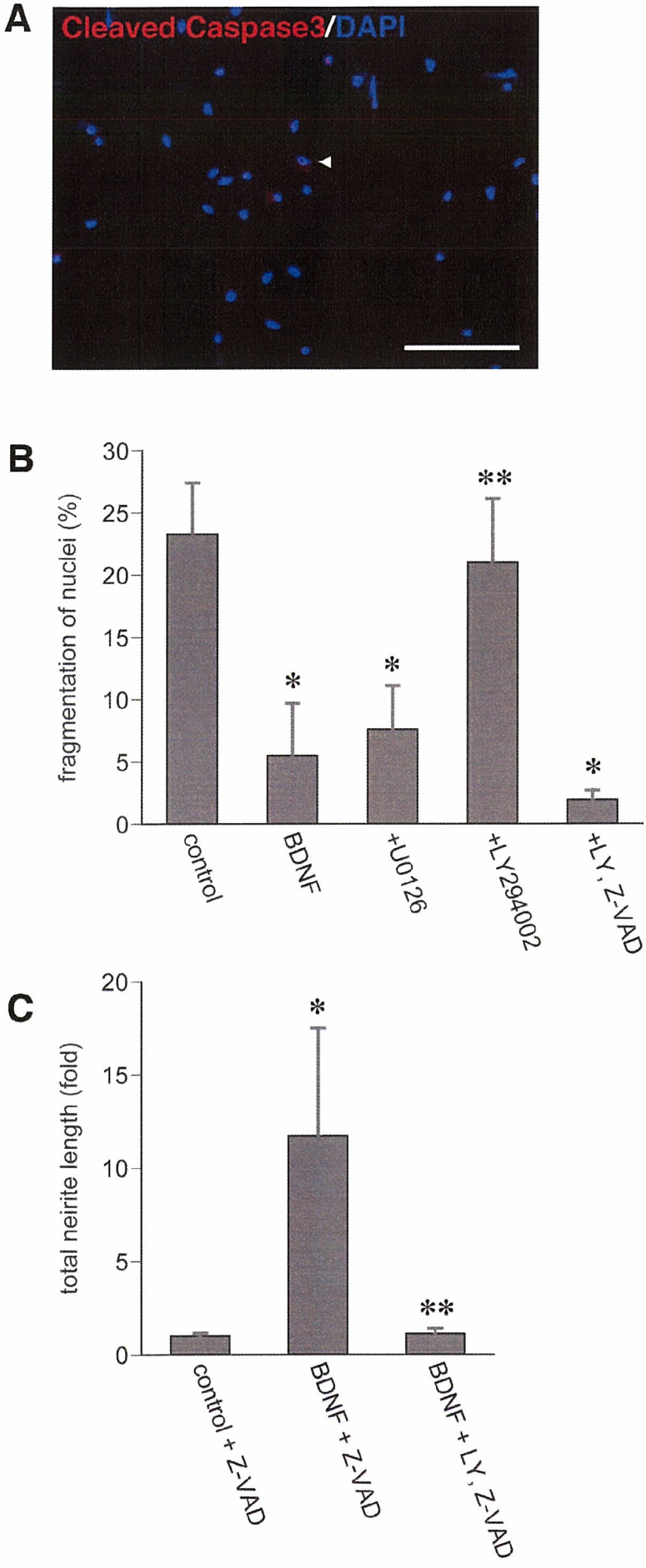
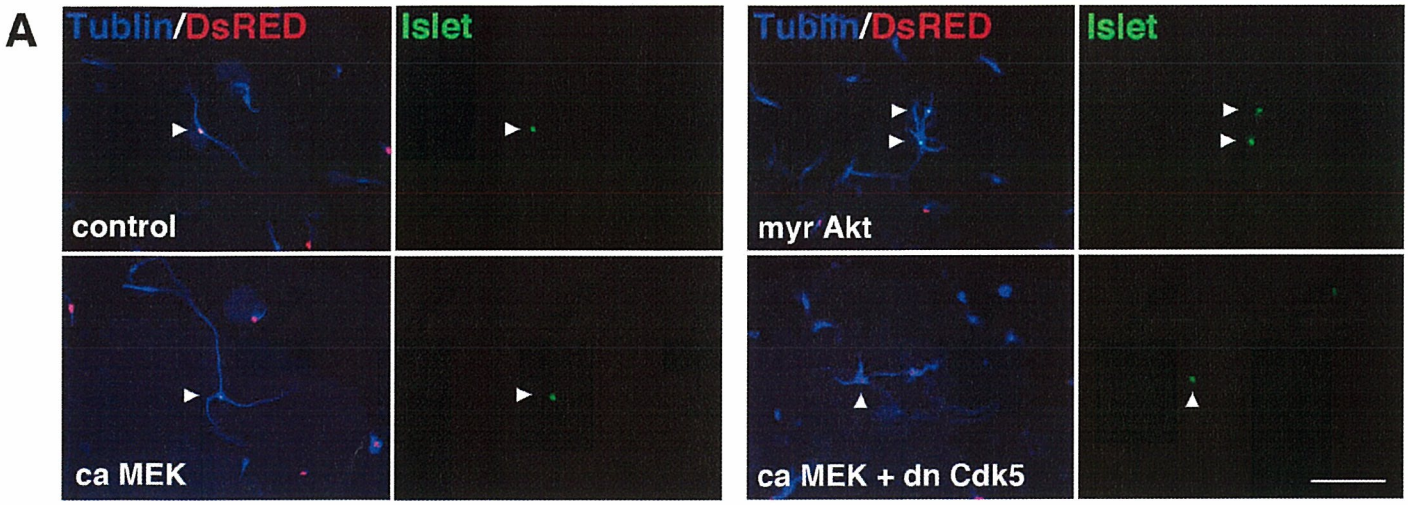
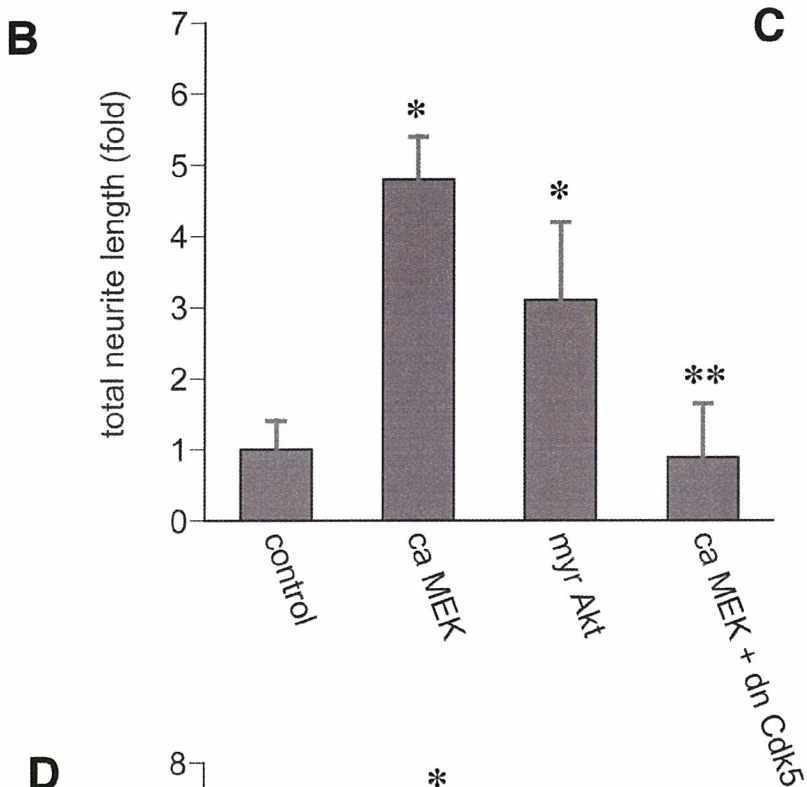
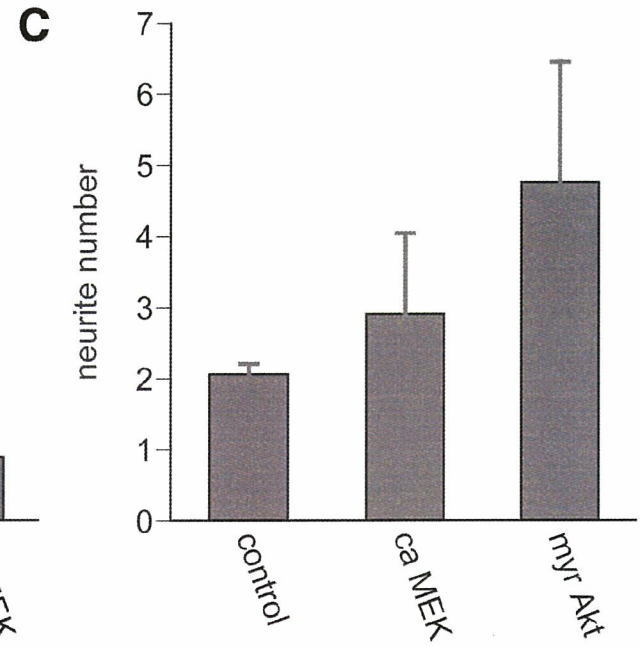

A
Cleaved Caspase3/DAPI
B
fragmentation of nuclei (%)
30
25
20
15
10
5
0
*
*
**
*
control
BDNF
+U0126
+LY294002
+LY, Z-VAD
C
total neirite length (fold)
20
15
10
5
0
*
**
control + Z-VAD
BDNF + Z-VAD
BDNF + LY, Z-VAD

図 3


A
Tublin/DsRED
Islet
control
myr Akt
ca MEK
ca MEK + dn Cdk5


B
total neurite length (fold)
0
1
2
3
4
5
6
7
*
*
**
control
ca MEK
myr Akt
ca MEK + dn Cdk5


C
neurite number
0
1
2
3
4
5
6
7
control
ca MEK
myr Akt

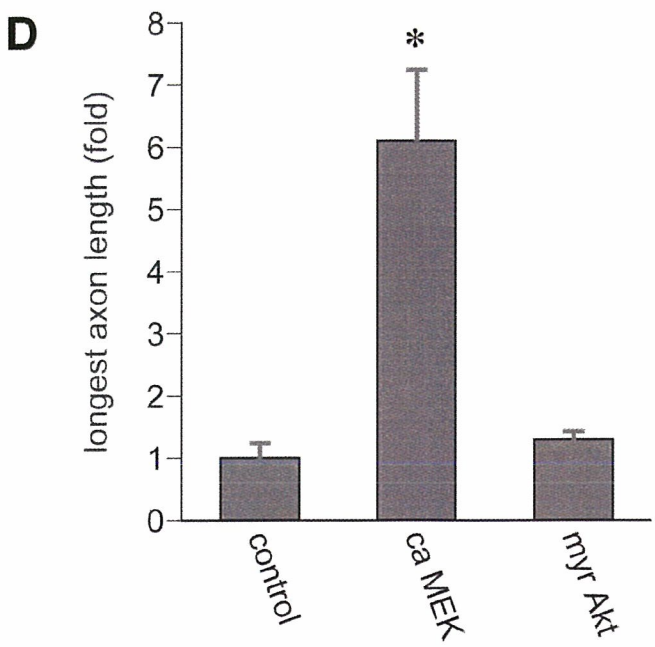

D
longest axon length (fold)
0
1
2
3
4
5
6
7
8
*
control
ca MEK
myr Akt

図 4

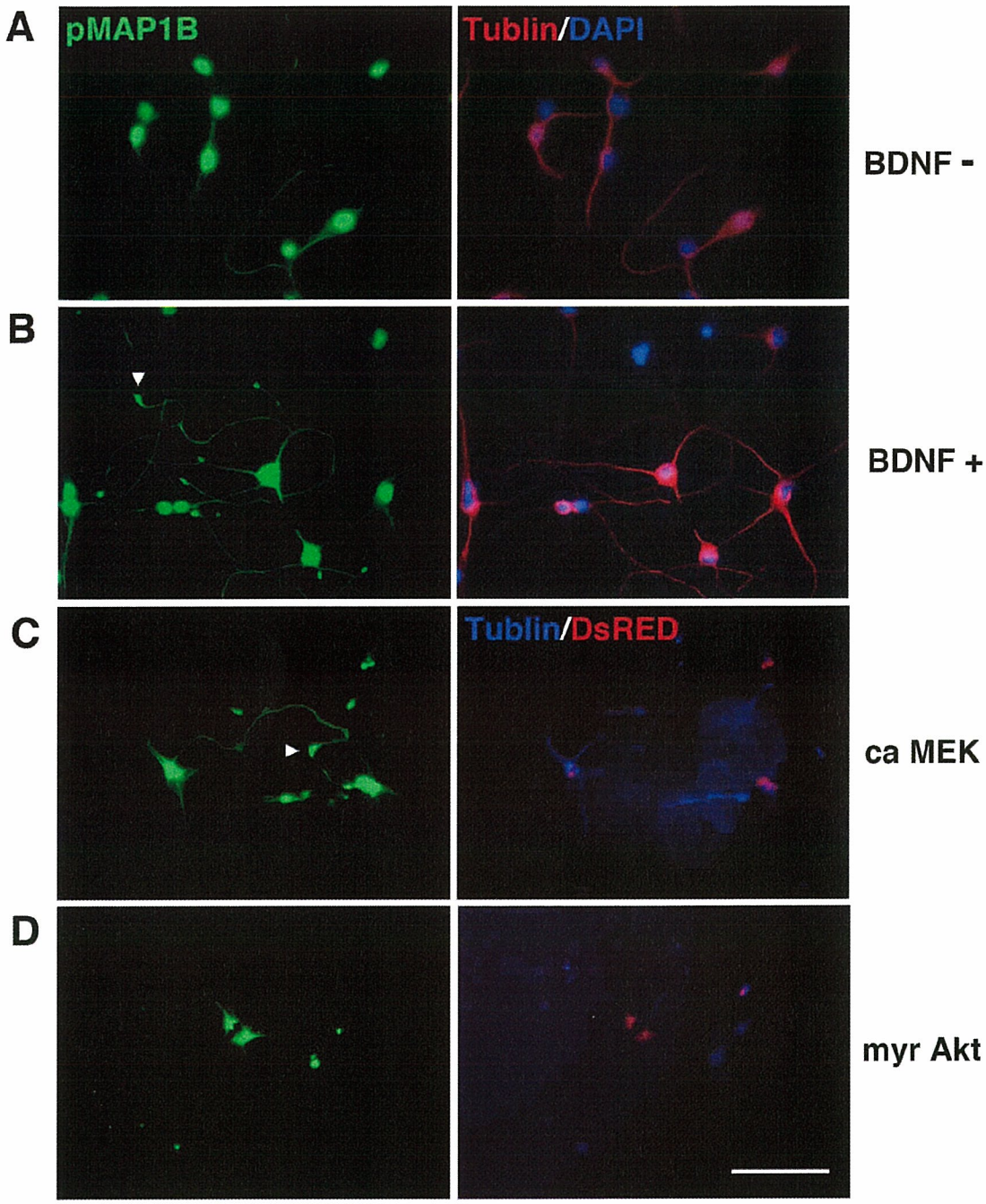

A
pMAP1B
Tublin/DAPI
BDNF -
B
BDNF +
C
Tublin/DsRED
ca MEK
D
myr Akt

図 5

研究成果の刊行物

研究成果の刊行に関する一覧表

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書　籍　名	出版社名	出版地	出版年	ページ
緒方徹・赤居正美	神経再生	岩崎洋	脊髄損傷理学療法マニュアル	文光堂	東京	2006	p. 341-343

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脊髄損傷理学療法マニュアル

編集
国立身体障害者リハビリテーションセンター
岩崎　洋

文光堂

10 神経再生

Open the door

●脳や脊髄といった中枢神経系はひとたび損傷を受けると再生しない，と考えられてきた．しかし，近年中枢神経系にも再生能力があることが明らかになり，さまざまな手法を用いた神経再生の試みが基礎研究の分野でなされている．

●一部の研究はすでに臨床への応用が検討されているが，実際にその有効性が検証され治療方法として確立するまでには相当の時間を要する．こうした現状はあるものの，さまざまな情報が脊損者をとり巻いているのが実状である．したがって，臨床の場面で脊損者からの新しい治療に関する質問に対し，適切に応対し本人の知識の整理を助けることが必要である．

●ポイントは次の4つである．

1. 脊髄損傷治療は急性期の神経保護，亜急性期神経再生，慢性期神経再生の3つに分類できる．
2. 現在研究されている新しい治療法のほとんどは，受傷後1ヵ月以内での治療を想定した亜急性期神経再生に関するものである．
3. いかなる脊髄再生治療も補完的な理学療法なしには成立しない．
4. 臨床現場における脊髄再生治療．

1 脊髄損傷治療は急性期の神経保護，亜急性期神経再生，慢性期神経再生の3つに分類できる（図1）

□ 損傷後の脊髄内では挫滅による物理的損傷に続いて炎症反応による2次的な障害が損傷範囲を拡大させる．これを最小限にとめる治療が神経保護治療とよばれる．

□ 現在，臨床で使用されているステロイド大量療法は神経保護治療に相当する．今後，炎症を抑制する薬剤，神経が細胞死に陥る過程を抑制する薬剤の利用が検討されている．

□ 亜急性期は組織の可塑性（変化する余地）が高いため，治療への反応性がはやいと考えられている．

□ 髄節レベルの運動機能回復には脊髄前角運動ニューロンの再生が必要であり，損傷髄節の上下をつなぐ索路（錐体路など）の回復には神経軸索の再生が必要となる．

□ 慢性期に対する治療は現時点では亜急性期の治療の延長上に捉えられている．しかしながら，すでにできあがった瘢痕組織の除去，神経組織の可塑性の再獲得といった課題は大きい．

2 現在研究されている新しい治療法のほとんどは受傷後1ヵ月以内での治療を想定した亜急性期神経再生に関するものである（図2）

□ 研究されている治療方法は大別して，細胞補充療法，神経軸索伸張促進治療に分けられる．

□ 損傷によって失われた組織を補うための細胞補充は，体外から補充する細胞移植治療と損傷脊髄内の細胞を賦活化する内在性細胞治療に分けられる．細胞移植に関してはさまざまな種類の細胞の補充が

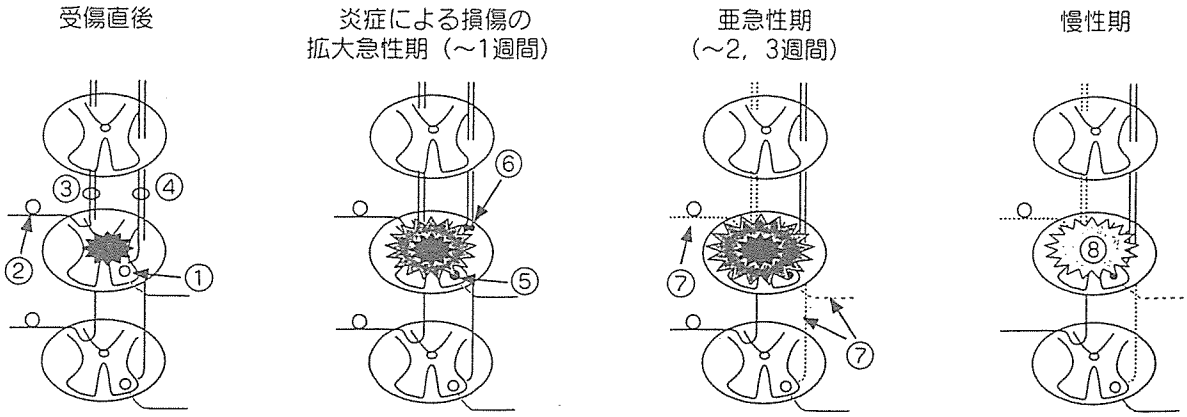

図１　脊髄損傷直後のプロセス

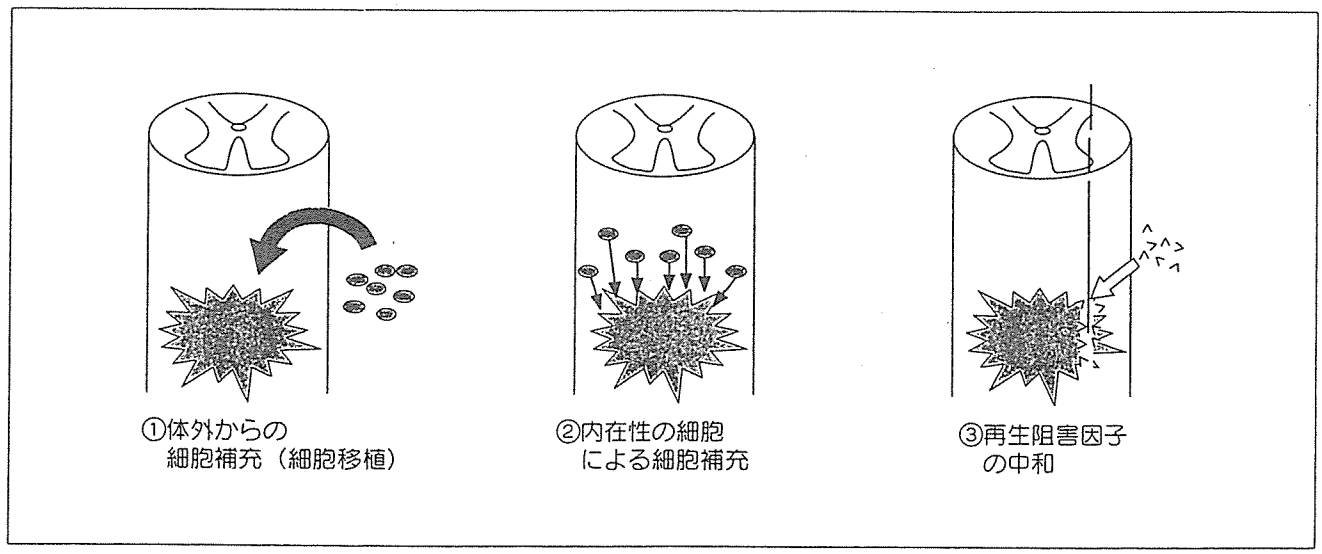

図２　亜急性期神経再生の構造

検討されているが，主には神経細胞またはグリア細胞である．

□ 細胞移植の方法としては局所注入，硬膜内投与，静脈内注入が検討されている．

□ 損傷脊髄内には軸索の再生を阻害する物質があるため，これを中和することで軸索再生を促進させることで索路の再生が試みられている．

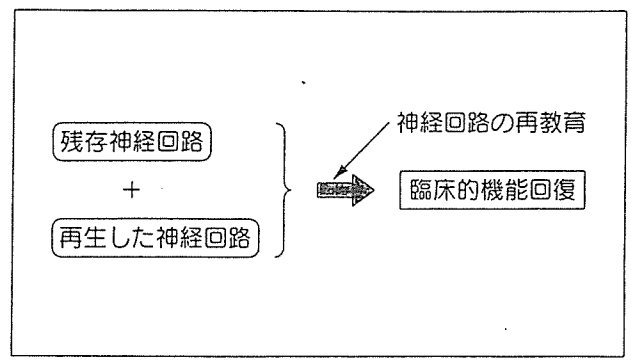

図３　神経回路の再構築

3 いかなる脊髄再生治療も補完的な理学療法なしには成立しない（図3）

□ 細胞補充も軸索伸張も損傷した神経回路を再構築することを目指している．しかしながら，回路が構築されても有効に機能しなければ臨床的な機能回復は得られない．

□ 神経が再生しても完全に元通りの回路になるとは考えにくい．したがって，残存神経回路と合わせ，理学療法などによる神経回路の再教育が必要である．

□ 一方で理学療法自体にも脊髄神経再生を促進する作用があるとする実験結果も報告されている．

□ 新たな治療体系の開発には脊髄再生治療とそれに対応した理学療法の確立が欠かせない．

4 臨床現場における脊髄再生治療

□ すでに海外においていくつかの再生治療が実際に行われており，今後日本国内も含め臨床治験が予定されている治療法がいくつか存在する．いずれも当面は限られた施設で行われる治療である．

□ 海外の場合，治療法によっては情報が限られているものもあり，一概に海外で行われているから効果が確立した治療とはいえない．

□ 脊損者から質問を受けた場合には，その治療の対象とする時期に当てはまるか（亜急性期か慢性期か），治療の目的に合っているか（髄節レベルの再生か索路の再生か）を確認した上，専門機関への問い合わせをすすめる．

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Exp Brain Res (2004) 157: 117–123
DOI 10.1007/s00221-003-1826-5

RESEARCH ARTICLES

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Induction of locomotor-like EMG activity in paraplegic persons by orthotic gait training

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Abstract This is, to our knowledge, the first report demonstrating the effects of orthotic gait training on the activity of the spinal locomotor neural networks. Three subjects with complete spinal cord injury (SCI) performed 1-h training with reciprocating gait orthosis 5 days/week for 12 weeks. The results showed that after 3 (n=1) or 6 weeks (n=2) of training, EMG activities synchronized with locomotor rhythm appeared in the soleus muscle (SOL) in all subjects, although very little EMG activity accompanied the orthotic gait at the early training stage. Our results suggest that the induced modulation in the SOL EMG waveforms might be attributable to changes in the orthotic gait movement pattern, and/or changes in the interneuronal activities of the spinal locomotor neural networks, as a result of orthotic gait training.

Keywords Spinal cord injury · Locomotion · Gait orthosis · Plasticity

Introduction

It has been well established that the human spinal cord has the potential to generate the basic locomotor pattern by interaction of the locomotor neural networks and peripheral sensory information concomitant with limb movements (Harkema et al. 2000). For example, several researchers have demonstrated that in severe spinal cord-injured (SCI) subjects, locomotor-like coordinated electromyographic (EMG) activity can be induced in paralyzed lower limb muscles by passive stepping on a moving treadmill with partial body-unloading (Dietz et al. 1994, 1995; Dobkin et al. 1994; Wernig et al. 1995). However, the nature of the neural networks involved in generation of locomotor EMG activities in SCI persons is not yet fully understood.

During a specific type of upright walking with gait orthosis called weight bearing control orthosis (WBCO) (Yano et al. 1997), modulation of lower leg-muscle activities that synchronize with that particular locomotor cycle can be induced (Kojima et al. 1998). Because the WBCO gait, like other reciprocating gait orthoses, is a "stiff-leg" gait, i.e., a gait with the knee locked in full extension and the ankle in a neutral position, the afferent information thought to primarily contribute to inducing the locomotor-like EMG activity would be associated with hip-joint movement and load on the leg (Dietz et al. 2002). This in turn might mean that use of the orthotic gait would allow us to investigate the contribution of the involvement of hip extension/flexion movement or load on the leg to generation of locomotor-like EMG modulation, specifically in the "lower leg" muscles, which are remote from the hip joint. However, in our experience, little EMG activity appears during the WBCO gait when the user is not well trained, whereas it has been demonstrated in a well-trained SCI subject that locomotor-like EMG is observed (Kojima et al. 1998). These empirical observations might be explained as follows: (1) afferent inputs concomitant with limb movements would not be sufficient to evoke locomotor EMGs during the untrained orthotic gait; and (2) the orthotic gait training induces an alteration in interneuronal activities in spinal neural networks, which would generate the locomotor EMG even when the pattern and amount of afferent inputs are the same. To test these possibilities, we first had to longitudinally evaluate changes in EMG activities in paralyzed muscles, and to relate these EMG changes with gait-motion changes during the time course of training. The purpose of this study, therefore, was to clarify: (1) whether alteration in the EMG activities in the lower leg muscles occurs during the time course of orthotic gait training, and (2) the relation between the EMG activities and kinetic/kinematic alteration of the gait motion due to the training.

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Materials and methods

Subjects and orthotic gait training

Three clinically complete paraplegic men (22–28 years; 60–68 kg; 173–177 cm) with traumatic spinal cord injuries (Th8–Th12) voluntarily participated in this study. The physical characteristics of the subjects are shown in Table 1. The American Spinal Injury Association (ASIA) impairment scale was used for the clinical assessment of subjects, and in each case the sensorimotor functions were classified as A, which means no motor or sensory function below the level of the lesion. The subjects gave their informed consent to the experimental procedures, which were conducted in accord with the Helsinki Declaration of 1975 and approved by the ethics committee of the National Rehabilitation Center for the Disabled, Tokorozawa, Japan. The long brace reciprocating gait orthosis, the WBCO, was used for the training. The subjects performed orthotic gait training with WBCO for 12 weeks, at 1 hour/d for 5 days/week. The mechanical features of the WBCO have been fully described elsewhere (Yano et al. 1997; Kojima et al. 1998). In short it has two specific features that the other existing gait orthoses do not have. The first one is a special gas-powered system to control the foot sole thickness. It can switch the sole thickness depending on the gait phase; when a leg is swinging forward, the sole of this particular leg is held at the thinner position; and just before the heel strikes, the sole gets changed to the thicker position. With this system a user can swing their legs easier without leaning the body sideward to make a clearance between a foot sole and floor. The second one is a special hip joint device. With this device a torque exerted by the right (left) hip joint is mechanically transmitted to the left (right) hip joint, resulting in the torque to the opposite direction exerted by the left (right) hip joint. This system assists each leg to reciprocally propel forward. As a whole these mechanical features enable a user ambulate at faster speed and with less energy expended (Kawashima et al. 2003).

Experiments

To evaluate the kinetic and kinematic changes in the orthotic gait motion during the course of training, the gait motion was measured with a three-dimensional motion-analysis system (VICON370, Oxford Metrics, UK). The motion-analysis system consists of a conventional video-analysis system with seven cameras and Kistler force plates. The force plates, sized 160×450 cm, consisted of two 80×200 cm plates and four 40×250 cm plates. These separate force plates enable us to measure ground reaction forces (GRF) under the feet and canes on both sides, separately. The orthotic gait motion was recorded along with electromyographic (EMG) activities in the right soleus (SOL) and tibialis anterior (TA) muscles. EMGs were recorded by two surface electrodes (Ag/AgCl, 0.8 cm diameter) attached along the muscle fibers over the belly of each muscle and set at an interelectrode distance of 0.5 cm. The EMG signal was detected by a bipolar differential amplifier with upper and lower cutoff frequencies of 50–3000 Hz. Very thin elastic nylon bandages were used to firmly hold both electrodes and lead lines to the body, preventing any small displacement of electrodes and lines that might cause artifacts. These measurements were carried out three times (after 1, 6, and 12 weeks) in subject A; six times (after 1, 2, 3, 4, 10, and 12 weeks of training) in subject B; and twice (after 1 and 6 weeks) in subject C during the training period. For the measurement, subjects ambulated along a 10-m walkway several times at comfortable cadences. They repeated the trials with short-time intermissions, usually a couple of minutes, until the minimum required number of data was obtained. We sampled at least six step cycles for the analysis. Many step cycles, for example more than ten cycles, could not be recorded in the measurements of this study, since high quality VICON data could be obtained only for one or two steps of around five steps in a trial. At the beginning of training, especially, the experimenter had to walk beside the subjects to prevent a fall. This disturbs the motion capture with the VICON system, and makes the space in which the motion capture is possible small. Due to this limitation it would have taken a relatively longer time for the subjects to record many step cycles. To reduce time for the experiment we decided to finish the measurement when at least six step cycles were obtained in good quality.

Changes in the following kinetic and kinematic variables were evaluated from the measured VICON data throughout the training period: kinetic variables, including the impulse and mean vertical GRF (mGRF) under the foot, and kinematic variables, including the stance time and swing time, velocity, cadence and step length, joint range of motion (ROM), and peak velocity during the stance phase of the hip and ankle joints. The digitized EMG signals were full wave-rectified after rejection of the DC component. Then, from the rectified EMG signals, mean values (mEMG) for the stance phase were calculated and normalized by those values at rest.

Stretch reflex test

The reflex EMG responses elicited by mechanical stretches at various velocities were tested to verify whether the stretch reflex mediated the induced EMG activity in the SOL during the orthotic gait. Stretch reflex responses were evoked by imposing a quick dorsiflexion with an amplitude of 10 deg to the SOL muscle, while the subjects were seated comfortably in a chair with the right leg fixed to a foot plate connected to a servo-controlled torque motor (Senoh Inc., Japan). The hip, knee and ankle joints were set at 80 deg, 60 deg flexed and 10 deg plantar-flexed positions (anatomical position is 0 deg), respectively. All of 25 perturbations, each consisting of various angular velocities (50–350 deg/s), were applied to the ankle joint in random order. In the present study, the short latency reflex component, M1, was evaluated, since only M1 component was induced in the three subjects. The onset of the first EMG response was defined as the moment when the rectified EMG activities reached levels higher than the average resting potential plus three times its standard deviation (BGA+3SD), and the response duration was defined as 30 ms from the response onset. The average rectified EMG value above the resting potential level over the response duration was considered as the M1 level, and the relation between the imposed stretch velocity and the M1 level was analyzed for each subject.

Additional experiments

Additional experiments were done for the subject A in order to ascertain whether: (1) the EMG activity was induced with another conventional gait orthosis (advanced reciprocating gait orthosis, ARGO), and (2) in order to compare how different gait velocities affected the induced EMG activity before and after the training. Because this subject continued the training for over half a year,

Table 1 Clinical characteristics of subjects studied

Subject	Sex	Age (years)	Height (cm)	Weight (kg)	Injury level (segment)	ASIA	Time postinjury (months)	Etiology
A	M	27	177	60	T10	A	10	Trauma
B	M	22	174	68	T12	A	8	Trauma
C	M	28	173	63	T8	A	12	Trauma

measurements could be taken at the 1st week (1-W), 4th week (4-W), and 20th week (20-W) of the training. In the measurements, the subject ambulated at various velocities, speeding up his pace on the basis of his comfort.

Statistics

The measured kinetic and kinematic variables during the training period were compared with the first measurement values using the Student's *t*-test. Data are presented as mean ± SD. Significance was accepted as $p<0.05$.

Results

All subjects could ambulate independently with the aid of canes in the 1st week of training. In subjects A and B, gait velocities were respectively increased from 7.7 to 13.2 m/min, and from 11.8 to 21.2 m/min after the 12-week training; these increases are concomitant with increases in step length, ROM, angular velocity of hip and ankle joints, and the mGRF during the stance phase. In subject C, however, both the hip and ankle-joint angular velocities were decreased; the hip joint's ROM was decreased; and the ankle joint's ROM was increased. These findings were likely due to the markedly faster gait velocity (22.4 m/min) of this subject, as compared to the other two subjects, at the beginning of the training. In none of the subjects was clear modulation in the EMG activities of either muscle observed at the beginning of the training. After three (subj. A) or six (subjs. B and C) weeks of training, however, synchronized EMG bursts with the stance phase commonly appeared in the SOL in all three subjects, whereas no clear modulation was observed in any of the TA EMG waveforms (Fig. 1A). Figure 2 shows changes in the walking velocities, hip and ankle joint angular velocities, mGRFs, and SOL EMGs during the time course of training in the three subjects. As mentioned above, the SOL EMG activities increased during the training period in all subjects, and this increasing time course was qualitatively most similar to the gait velocity and mGRF. The increasing patterns in the SOL EMGs were not necessarily in parallel with those in the hip and ankle joint velocities. In subject C, specifically, the hip joint and ankle joint velocities demonstrated a tendency to decrease, though the SOL EMG increased.

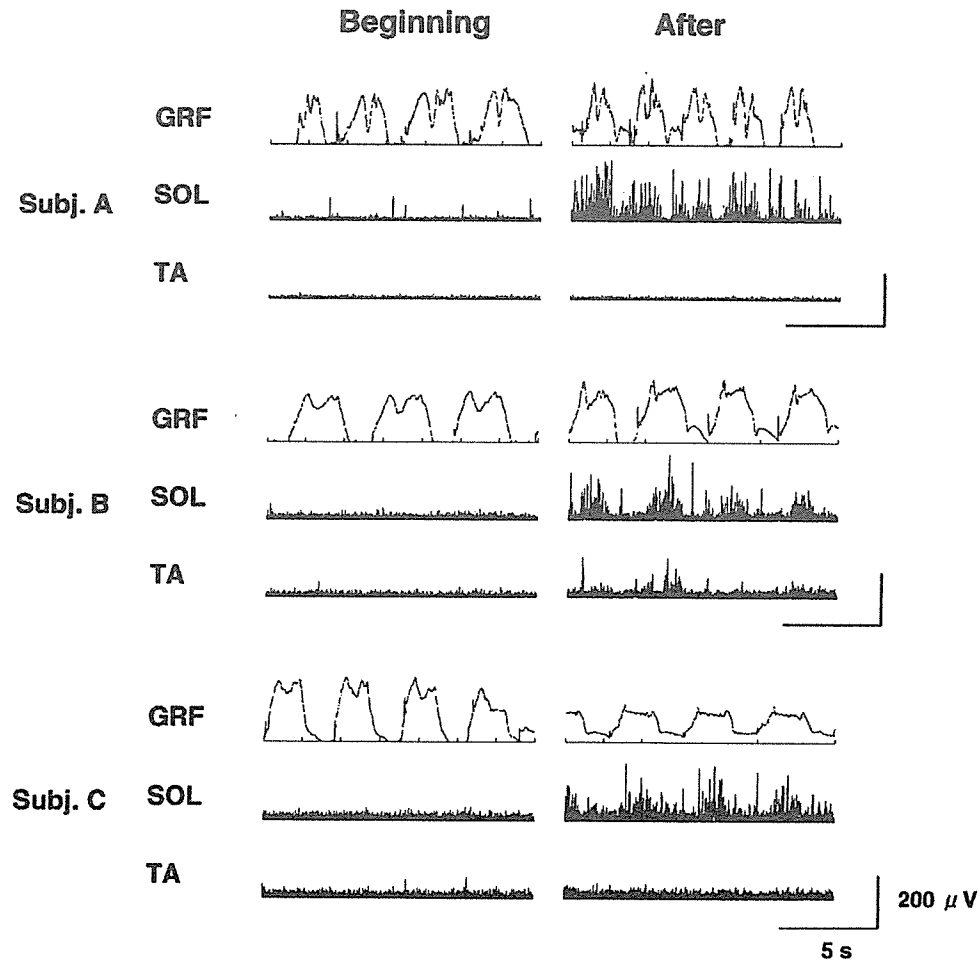

Fig. 1 Changes in EMG activities in the lower leg muscles during orthotic gait before and after the training. Results from the three subjects are shown (*GRF* the vertical ground reaction force, *SOL, TA* rectified EMGs from the soleus and tibialis anterior muscles, respectively)

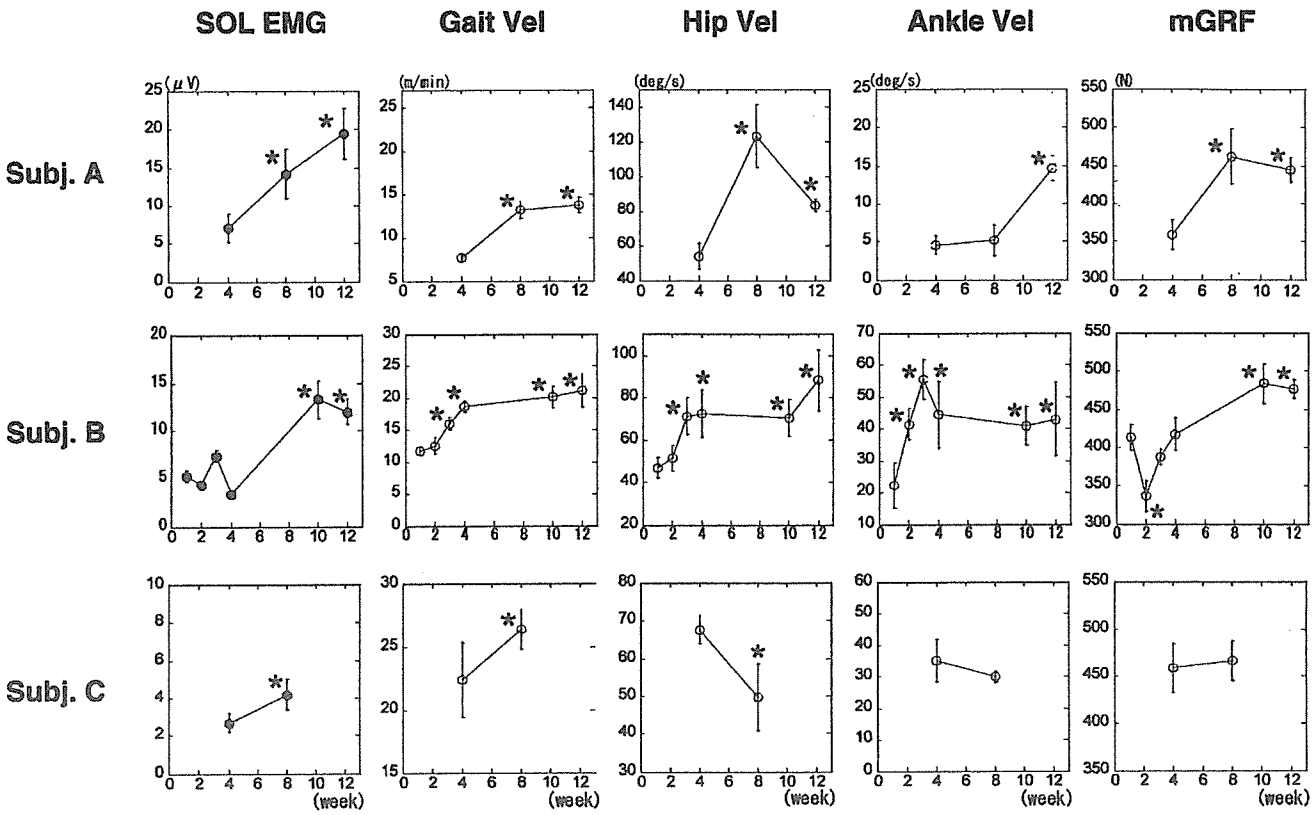

Fig. 2 Changes in the SOL EMGs, the gait velocities, the hip and ankle joint angular velocities, and the ground reaction forces over the training period for the three subjects

Additional experiment

Figure 3 shows the SOL EMG activities during the orthotic gait at three different gait velocities at the 1-W and 4-W measurements. It was demonstrated that the synchronized EMG burst with the stance phase increased drastically with the ambulation velocity after 4 weeks of training; no such clear modulation was observed at the 1st week measurement.

Relationships of the SOL EMGs during the stance phase with the gait velocity, hip velocity, ankle velocity, and ground reaction force, respectively, in each measurement

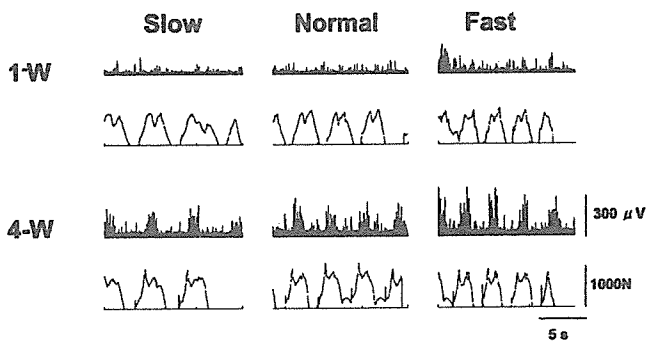

Fig. 3 A comparison of the velocity dependent changes in the SOL EMGs during orthotic gait before and after the 4-week training with the other gait orthosis

are demonstrated in Fig. 4. In the 1-W measurement, the SOL EMG did not clearly increase with the gait velocity or the other kinetic and kinematic variables; the measurement reflected no qualitatively clear modulation. In the 4-W and 20-W measurements, however, the SOL EMGs covaried with the gait velocity and the other variables. It should be noted that the levels of SOL EMGs in the 4-W and 20-W measurements were greater than those in the 1-W measurement, even though the kinetic and kinematic variables were in similar ranges. This result suggests that the observed increase in the SOL EMG during the time course of training was not merely dependent on the kinetic and kinematic factors; neurological factors were to some degree involved.

Stretch reflex test

Figure 5 demonstrates the relationships between stretch velocity and the reflex EMG responses in the SOL for the three subjects. It was indicated that in all three subjects, the stretch reflex EMG response was induced when the applied stretch velocity was faster than 100 deg/s, meaning the threshold velocity of the reflex was around 100 deg/s. These threshold velocities were well above the peak ankle dorsiflexion velocities observed during the orthotic gait in the three subjects, suggesting that the SOL EMGs

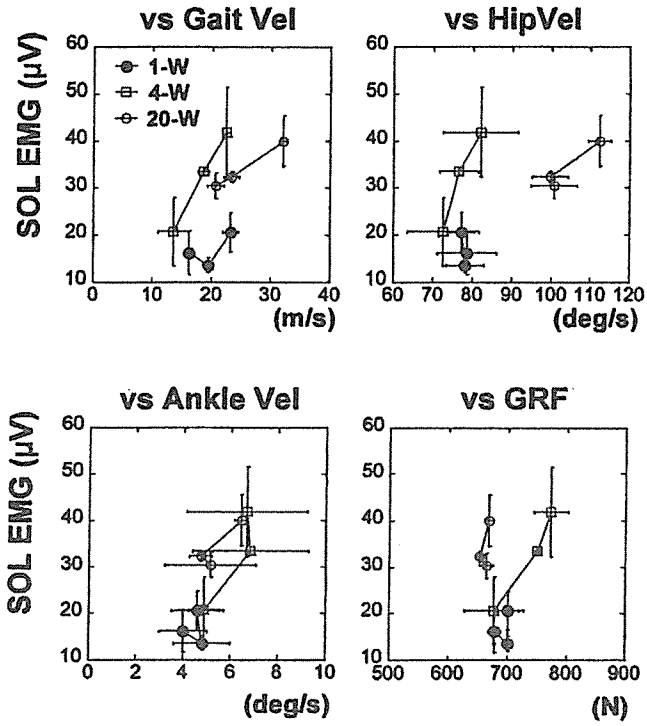

Fig. 4 Comparisons of the relationships of the SOL EMGs to the kinetic and kinematic variables before and after 1 month and 5 months of training. Abbreviations: *vs Gait Vel, vs Hip Vel, vs Ankle Vel,* and *vs GRF* refer to the relationships between the SOL EMG and the gait velocity, hip joint angular velocity, ankle joint angular velocity, and ground reaction force, respectively

observed were not merely mediated by the stretch reflex pathway.

Discussion

The results in the present study demonstrated that intense orthotic gait training induced modulation of EMG activities in the ankle extensor SOL muscle in individuals with clinically complete SCI. These results constitute neurologically significant indirect evidence that knee (and to some degree ankle) movements are far less important than hip movement and loading for the induction of locomotor EMG activity, at least in the SOL. Further, these results have great clinical significance especially in terms of gait rehabilitation of patients with incomplete spinal cord injury. It is worth noting that the results must be carefully interpreted, given that many factors may contribute the observed phenomena. Considering that possibility, we divided the various factors into two different types: (1) kinetic and/or kinematic, related to changes in the orthotic gait movement itself, and (2) neurological factors, namely, supposed changes in the interneuronal activities of the spinal locomotor networks as a result of training. We believe that neither the kinetic/kinematic nor the neurological factors alone can fully explain the observed results

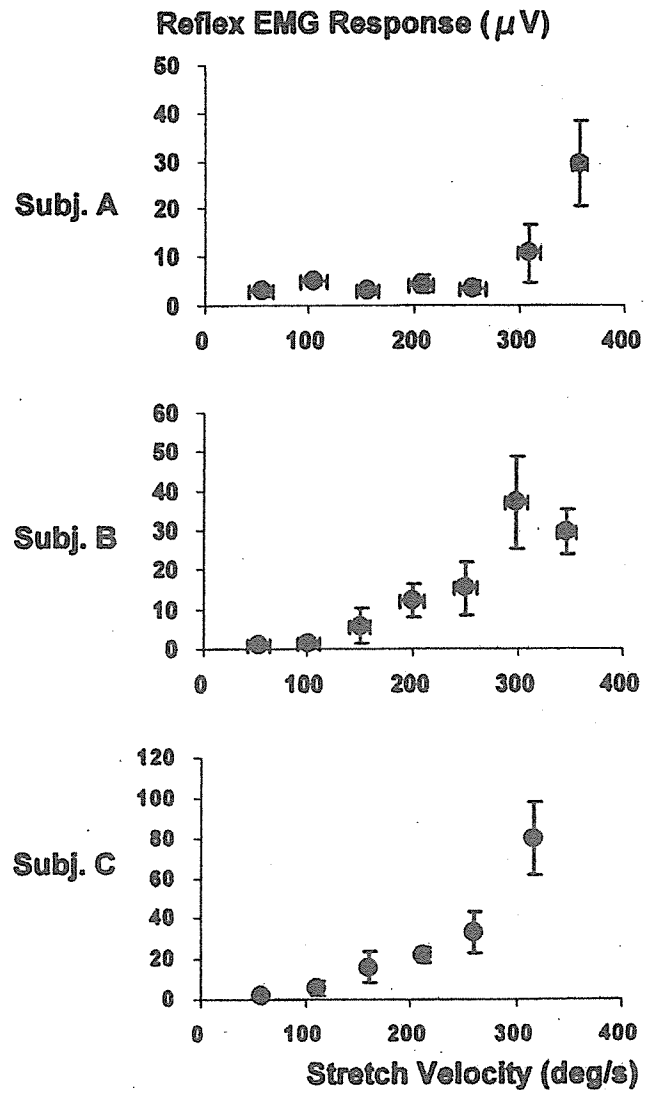

Fig. 5 Relationships between the stretch reflex EMG activities in the SOL and the applied stretch velocity for the three subjects. Note that the estimated threshold velocity to elicit the reflex is over 100 deg/s, which is far faster than the peak ankle joint velocity during the orthotic gait in any of the three subjects

Effects of the orthotic gait motion on modulation of EMG activity

The results of the stretch reflex test in the SOL indicated that the threshold velocities of stretch reflex in the SOL were far higher than the observed ankle dorsiflexion velocity during the orthotic gait. This result strongly suggests that the observed SOL EMGs during the orthotic gait were not induced merely by the spinal stretch reflex. Rather, in the present study, supposed spinal locomotor networks appeared to play a role in the induction of SOL EMGs during orthotic gait.

The observed modulation in the SOL EMG activities synchronized with gait phase confirmed our previous observation that even the knee-locked "stiff-legged" gait with a gait orthosis is effective to induce locomotor-like

EMG activity in lower limb muscles (Kojima et al. 1999). Because the stiff-legged gait is a gait with the knee locked in full extension and the ankle in a neutral position, the afferent information thought to primarily contribute to inducing the observed modulation in the EMG activity would be associated with the hip-joint movement and load on the leg. This result is consistent with recent observations from infant stepping experiments (Pang and Yang 2000) and experiments using a driven gait orthosis for paraplegic subjects (Dietz et al. 2002). Dietz et al. (2002), on the basis of their elegant series of studies on locomotor capacity of human spinal cord and relevant animal and human studies, concluded that "afferent input from hip joints, in combination with that from load receptors, plays a crucial role in the generation of locomotor activity in the isolated human spinal cord". The result in the current study would constitute indirect evidence to support this notion. With respect to afferent input from the hip joint, further, Schimit and Benz (2002) recently demonstrated that imposed hip joint extension/flexion movements in spinal cord-injured subjects induce a unique, stereotypical reflex response in hip, knee, and ankle joints, suggesting that hip movement would activate spinal interneuronal pathways associated with coordinated motor behaviors such as posture and locomotion. Given this notion, the results in the current study suggest that afferent information associated with hip extension during the orthotic gait might activate the spinal neural network responsible, at least in part, for the synchronized EMG activity in the SOL, which may be the common network generating the extensor reflex. With regard to the effect of limb loading on the locomotor activity in the paraplegic human, a growing body of indirect evidence from studies on humans has indicated that load-related afferent inputs play an essential role in the generation of locomotor-like efferent patterns by the human spinal cord (Harkema et al. 1997; Pang and Yang 2000, 2001; Dietz et al. 2002). The observed close relationship between the SOL EMG levels and mGRF during the training period (Fig. 2) and within a single experiment (Fig. 4) in the current study is consistent with that found in previous reports and our previous study (Kojima et al. 1999), in which we demonstrated that the levels of lower limb muscle EMGs during orthotic gait were well correlated to the level of limb loading.

Alteration of the intrinsic property of spinal neural networks due to training

The observed EMG alteration in the SOL might not solely depend on gait-motion changes due to training, but also on alterations in the intrinsic properties of neural networks. This hypothesis is supported by the result of an additional experiment, in which the EMG modulation occurred even under kinematic and kinetic profiles of orthotic gait similar to those of the pre-training gait after 4 weeks of training (Fig. 4). In addition, the effect of changing gait velocity was obviously different before and after several weeks of training, suggesting that the input and output properties in the spinal neural networks during orthotic gait were altered due to the training. Another observation that supports this hypothesis might be the observed EMG changes in subject C, whose orthotic gait motion was at a higher level (i.e., faster velocity and larger ROM, etc.) at the first stage of training and did not largely change during the training period. Nevertheless, modulation of the SOL EMG was induced in this subject, despite rather reduced angular velocities of hip and ankle joints after 8 weeks of training.

Use-dependent plasticity is now a well-known property of spinal neural networks (Hodgson et al. 1994; Muri and Steeves 1997; Raineteau and Schwab 2001). Repeated afferent input accompanying gait training might result in improvement in the transmission efficacy within the neural network responsible for the SOL EMG activity during orthotic gait. This possibility is extremely important with regard to rehabilitation strategy for SCI patients (Field-Fote 2001; Protas et al. 2001). If the act ambulation with an orthosis itself has the potential to improve neuronal activity in the spinal locomotor neuronal networks, a specific gait orthosis one could be designed and developed for locomotor training. Future studies should explore the optimal design for a gait orthosis that can effectively activate the spinal locomotor neural network, using the findings in the current study as the first step in such a series of investigations.

Finally, almost no EMG modulation appeared in the TA in the current study. This might be explained by the following: (1) because the correlation between the load on a limb and EMG activity is less in the TA than in the SOL (Harkema et al. 1997; Kojima et al. 1999), load-related afferent information during the orthotic gait in our study might not have been sufficient to evoke TA activity; and (2) although an imposed hip flexion can induce the ankle flexor response (Schmit and Benz 2002), the flexion amplitude or velocity during the orthotic gait was not sufficient to induce the TA activity. Further, the ankle joint was mechanically immobilized in the orthosis in our study, and the absence of ankle motion might have prevented the elicitation of TA activity. However, further training might induce modulation in TA EMG activity, based on the fact that we observed a reciprocal EMG activity pattern between the ankle extensor and flexor muscles during the orthotic gait in a well-trained SCI subject (Kojima et al. 1998). Further studies are needed to clarify this issue.

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Original Article

Potential impact of orthotic gait exercise on natural killer cell activities in thoracic level of spinal cord-injured patients

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Stydy design: Prospective before–after trial.
Objective: To examine the changes of natural killer (NK) cell activity in response to orthotic gait exercise in thoracic level of spinal cord-injured (SCI) patients.
Setting: National Rehabilitation Center for Persons with Disabilities, Japan.
Methods: In all, 10 thoracic level of SCI patients (ranging Th5–Th12), who experienced orthotic gait training, participated in this study. NK cell activity at an effector:target (E/T) ratio (20:1) was examined in a sample of peripheral blood taken before and just after orthotic gait exercise for 20 min. On a separate day, to evaluate the physical intensity of the orthotic gait exercise, cardiorespiratory responses at rest and during exercise were measured.
Results: The resting value of the NK cell activity in our SCI patients was remarkably lower than that in normal subjects reported in previous studies. The NK cell activity was significantly increased through a 20 min orthotic gait exercise (pre versus post; 12.7 ± 5.28 versus 17.76 ± 6.71, $P<0.05$). The steady-state value of oxygen (V_{O_2}) and heart rate (HR) were 18.13 ± 3.92 ml/kg and 142.53 ± 19.84 b/min, respectively. It was noteworthy that a patient who showed decrement of NK cell activity in response to exercise had the highest level of injury (Th5), and showed the higher energy cost of orthotic gait.
Conclusion: These findings suggested that the orthotic gait exercise has the potential to enhance the immune function for SCI persons, although patients with a higher level of SCI may have some difficulties.
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Keywords: natural killer cell activity; spinal cord injury; orthotic gait; secondary disorder

Introduction

Natural killer (NK) cells have been proposed as a major factor in the first-line defense system against viral infection.[1,2] Previous investigations demonstrated that spinal cord injury (SCI) brings depression of the immune system including decreased NK cell activities, and also reported restoration of the immune function through the rehabilitation therapy.[3]

Orthotic gait training is usually prescribed for paraplegic patients with SCI in the therapeutic phase to promote their general health. On the other hand, there are several obstacles to achieving locomotion for paraplegic patients, in particular the high-energy cost of orthotic gait leads to exhaustion within a few minutes of walking (for a review, see Nene *et al*[4]). Although some positive effects of orthotic gait have been reported,[5] it is still unclear whether the physical intensity of the orthotic gait is suitable for SCI patients to promote their health or not.

We previously examined the physiological characteristics of orthotic gait in thoracic level of SCI patients, and our findings suggested that the physical intensity during walking exercise is suitable to promote the aerobic capacity of SCI patients.[6] In the present study, we designed a direct approach to clarify the effect of orthotic gait exercise on the general health of SCI patients, particularly in terms of exercise-induced changes in NK cell activity. Previous investigations revealed that moderate intensity exercise can enhance NK cell activity.[1,7,8] Therefore, we focused on whether orthotic gait exercise in particular could enhance NK cell activity.

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Methods

Subjects

In all, 10 SCI patients who met the following criteria participated in this study: (i) injured at thoracic level, (ii) complete motor paralysis in the lower limb muscle (ASIA classification; grade A or B), (iii) no history of cardiorespiratory disease. All patients were at least 6 months since time of injury, with time since injury ranging from 8 to 32 months (Table 1). Each subject gave his or her informed consent to the experimental procedure, which was approved by the local biological ethics committee of the National Rehabilitation Center for the Persons with Disabilities (NRCD).

Orthotic gait training

All patients had undergone a standard rehabilitation program, consisting of muscle stretching, balance training, and transfer activity, and participated in orthotic gait training with a weight-bearing control orthosis (WBC) or advanced reciprocating gait orthosis (ARGO). Eight of 10 patients have kept the orthotic gait training for 15 weeks, and the other two patients have kept for 10 (patient E) and 4 weeks (patient G), respectively. Although there is individual variation, in many cases, lower thoracic level of paraplegic patients could walk after 10 weeks of gait training independently, while it needs more practice for higher thoracic level of patients. After the training period, each subject could perform the orthotic gait (patients F and G still required light support to avoid falling) independently, and were able to walk continuously for at least 20 min.

Apparatus

Appearance and sequential picture of walking with WBC and ARGO were shown in Figure 1. The mechanical features of the WBC have been fully described elsewhere.[11,12] This orthosis consists of a rigid frame that supports the user's body weight, a special hip joint device that reciprocally propels each leg forward, a gas-powered foot device that varies the sole thickness of the device for foot/floor clearance, and a control system of the orthosis. As a whole these mechanical features enable a user to ambulate at a faster speed and with less energy expended.[6] The ARGO also has a special hip joint device named 'hip driving cable' which connects both sides of the leg frame. With this device a torque exerted by the right (left) hip joint is mechanically transmitted to the left (right) hip joint, resulting in the torque to the opposite direction exerted by the left (right) hip joint.

Physical intensity during orthotic gait

On a separate day, cardiorespiratory responses at rest and during orthotic gait were measured. Subjects were asked to abstain from alcohol and caffeine for at least 12 h before the experiment. The temperature and humidity on the experiment were 23.5 ± 4.2°C and 68.3 ± 3.3%, respectively. The experimental procedure was as follows: 5 min at rest in the sitting position, 20 min of continuous walking at the most comfortable speed. The cardiorespiratory parameters at rest and during walking were measured continuously with a telemetric device (K4 Cosmed, Italy) and were analyzed in real time. The telemetric device consists of a transmitting unit, a face mask to sample the expired gas, a heart rate chest strip, a battery, and a receiving unit. The following cardiorespiratory parameters were

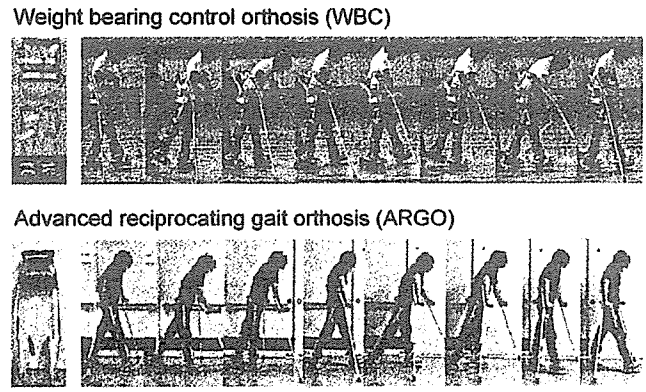

Figure 1 Appearance and sequential picture of walking with weight-bearing control orthosis (WBC; above) and advanced reciprocating gait orthosis (ARGO; below)

Table 1 Characteristics of the patients

Patient	*Sex*	*Age (years)*	*Height (cm)*	*Weight (kg)*	*Lesion level*	*Grade of ASIA*	*Duration of paraplegia (months)*	*Orthosis*
A	M	28	173	63	Th8	A	12	WBC
B	M	27	175	60	Th10	A	10	WBC
C	M	22	175	68	Th12	A	8	WBC
D	M	21	167	46	Th12	B	32	WBC
E	M	36	178	73	Th11	A	20	ARGO
F	M	19	175	53	Th5	B	24	ARGO
G	F	26	156	45	Th10	A	13	ARGO
H	M	30	178	67	Th12	A	13	ARGO
I	M	34	165	54	Th6	A	28	ARGO
J	M	23	168	65	Th8	A	26	ARGO