厚生労働科学研究費補助金
障害保健福祉総合研究事業

再生医療による脊髄の歩行パターン発生能力と脊髄損傷者の歩行再獲得可能性に関する研究

平成17年度　総括・分担研究報告書

主任研究者　赤居　正美

平成18（2006）年４月

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厚生労働科学研究費補助金（障害保健福祉総合研究事業）
総括研究報告書

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主任研究者　赤居　正美　国立身体障害者リハビリテーションセンター病院　部長

研究要旨
本研究は、再生医療による損傷脊髄部分での軸索再生と末梢の残存脊髄機能の可塑性を研究することにより、脊髄損傷者の歩行再獲得を最終目的としている。その実現に向け、概ね以下の３つを目標におく。Ａ：神経生理学的研究により、信号の受け手側である損傷脊髄以下に残った機能回復能を調べる。Ｂ：再生医療に基づく細胞工学的手法により、上位信号の送り手側である損傷部位での軸索再生を計る。Ｃ：上記により得られた理論に立脚した、歩行能再獲得に向けた新たなリハビリテーション方法を開発する。

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研究所　室長）
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（国立身体障害者リハビリテーションセンター
研究所　主任研究官）

Ａ．研究目的

本研究は、再生医療による損傷脊髄部分での軸索再生と末梢の残存脊髄機能の可塑性を研究することにより、脊髄損傷者の歩行再獲得を最終目的としている。

目標としては、人間の脊髄に基本的な歩行パターンを生み出す能力および学習能力がどの程度あるのかを探求し、近年進歩がめざましい再生医学による脊髄の軸索延長と組み合わせて、治療モデルを作る。それを基に、対麻痺患者のための新たなリハビリテーションにつなげる。

近年のめざましい再生医学の進歩は、損傷後脊髄に従来考えられていた以上の回復能力があることを示している。しかし先行研究からは、動物にて少しずつ報告例が蓄積されつつある軸索再生をもってしても、損傷部位を越えて元のような点対点投射は困難と考えられる。他方不全損傷患者での臨床経験からは、歩行様トレーニングによる繰り返し刺激入力が脊髄歩行中枢の改善に結びつく可能性を示しているので、ごくわずかであっても中枢からの情報伝達を再建出来れば、いいかえれば完全損傷を不全損傷に変える事が出来れば、臨床への発展性はあると考えた。

交通事故等の外傷性脊髄損傷によって毎年多くの若者が四肢麻痺や対麻痺となり、その後の長い人生を車椅子生活でおくることを余儀なくされている。近年のめざましい再生医学の進歩は、従来再生能力はないとされた脊髄組織にも実際には軸索の伸長能があるものの、周囲組織の阻害によって再生が阻まれている事実を明らかにしつつある。加えて、近年の神経生理学を中心とした基礎医学の進歩は、損傷後脊髄に従来考えられていた以上の回復能力・可塑性があることを示している。

人間を対象とした研究は端緒についたばかりであり、まだまだ検証すべき点が多い。上記再生医学の知見と神経生理学的な脊髄の可塑性、学習能力を結びつけ、実際の患者における機能再建につながる一歩とする。

Ｂ．研究方法

Ａ：再生医学に基づく細胞工学的手法により、上位信号の送り手側である損傷部位での軸索再生を計る。

① 神経細胞の再生シグナルを神経軸索断端からの遺伝子導入によって活性化し、軸索伸長を促進させる。
② 再生軸索の足場として最適な表現形を持つ細胞を損傷部に誘導する。具体的には局在のグリア細胞への遺伝子導入を検討する。

Ｂ：神経生理学的研究により、信号の受け手側である損傷脊髄以下に残った機能回復能を定量的に把握する。

① ヒトの脊髄に歩行パターンを自立的に生み出す能力がどの程度あるのかを調べる。
② それは末梢からの感覚刺激に対してどの程度適応的に変化する能力があるのかを調べる。
③ その能力は上位中枢とどのような結合状態を取っているかを調べる。

平成16年度は次の二つの側面から研究を行った。

・脊髄損傷者を対象とし、歩行トレーニングに伴い脊髄神経回路と大脳運動野からの下行性司令がいか

に変容するのかを解明するための実験環境の整備と予備実験を開始した。

・脊髄神経再生能力に関する細胞・動物を用いた基礎実験を行うための遺伝子操作を含む実験環境を整えた。

平成１７年度では以下の内容を行った。

・立位歩行トレーニングの経時変化追跡をおこなう。不全および完全対麻痺者を対象として、トレッドミル上での歩行トレーニング中及び安静時に経頭蓋磁気刺激（TMS）を行い、それに対する下肢麻痺領域の応答を評価する。さらに下肢麻痺領域の脊髄反射を誘発し、これらがトレーニングによりどう変化するかを縦断的に調べた。

・齧歯類胎児中枢神経からの髄鞘を形成するオリゴデンドロサイト前駆細胞（再生軸索周囲の環境の最適化）の初代培養系を確立する。遺伝子導入を行うことで表現形を変化させ、増殖に対する影響を評価する。またラット胎児からの脊髄運動ニューロン（神経細胞の再生力の賦活化）の初代培養系を確立する。軸索伸長シグナルに関連する遺伝子を導入し、軸索長を評価した。

・損傷脊髄の組織学的検討のため、ラット脊髄圧挫モデルを確立し、オリゴデンドロサイト前駆細胞の挙動を解析した。

平成18年度は

・17年度同様の実験を継続し、結果をまとめる。すなわち、大脳運動野との結合が残存するか否かの違いが、脊髄歩行中枢の出力改善にどの程度影響するのかを明らかにする。

・前年度に得られたニューロンあるいは損傷部細胞への遺伝子導入の知見を脊髄神経損傷モデルに応用する。

（倫理面への配慮）

この研究において、人間を対象として行われる種々の検査、実験に対麻痺者あるいは健常者が参加することに関する倫理上の問題点については、国立身体障害者リハビリテーションセンター倫理審査委員会で審査を受け、その許可を得た。各実験、検査においては、事前に被検者に内容を十分説明し、インフォームドコンセントを得ると共に、実験、検査の中止は被検者の意志が最優先であり、いかなる場合においてもそれらを即時中止できることを徹底する。

C．研究結果

平成１６年度として

完全対麻痺患者、不全対麻痺患者および健常者を比較することで、上位中枢からの下行性入力の喪失が脊髄神経機能に及ぼす影響を電気生理学的に測定し、下腿おける伸張反射とH-反射にみられる３者間の違いを定量的に計測した。また、脊髄損傷者での実験プロトコルを作成し、倫理審査委員会の審査を受けた。しかし、ロボット型トレーニング機購入のための事務手続きが遅れ、次年度にずれ込んだ結果、体性感覚入力を用いた介入実験の開始が遅れた。

脊髄・神経再生能に関する遺伝子操作を含む細胞・動物を用いた基礎的実験を行う実験環境の整備が概ね整った。

平成１７年度として

脊髄損傷者の麻痺下肢に発現する歩行様筋活動とその血流動態に関する実験結果について報告をまとめた。

10月にロボット型歩行トレーニング機が導入され、同機を用いたトレーニング実験の実験系を整備した。受動歩行中の筋電図の記録と経頭蓋磁気刺激を行う環境を整え、健常者での実験を開始した。脊髄損傷患者を対象としたトレーニング介入実験を行うための倫理審査を終えた。ロボット型歩行トレーニング機の運用規定も制定し、８週間にわたるトレーニングを順次開始し、まず完全脊髄損傷者１名を対象としたトレーニングを行っている。

再生医療分野では、オリゴデンドロサイト・脊髄運動ニューロンの初代培養系を確立し、in vitro実験を開始している。また、齧歯類主にラット脊髄圧挫損傷モデルを確立し、in vivoでの前駆細胞の挙動を解析している。

D．考察

1) 健常者を対象とした受動歩行時のMEPの結果から、受動的歩行において、TAの皮質脊髄路興奮性が促通するという新たな知見が得られた。今後脊髄損傷者のトレーニング中に同様な検査を行う予定であるが、その基盤となるきわめて重要な結果を得ることができた。

2) この現象は、さらなる検証が必要ではあるものの、ヒラメ筋（SOL）には認められなかったことから、大脳皮質との結合が強いとされる前脛骨筋（TA）に固有の特徴である可能性が高い。最近、TAの静的および動的随意収縮時のMEPが脊髄損傷の程度をよく反映することが報告された（Diehl et al. 2006, J Neurol）。

3) 脊髄運動ニューロンにおいては、BDNFの下流でMEK-ERK経路が軸索伸長に関与している可能性が考えられた。

4) オリゴデンドロサイト前駆細胞においても、増殖因子下流でMEK-ERK経路が機能していると考えられた。

5) 内在するオリゴデンドロサイト前駆細胞の治療応用のためには細胞数の維持と損傷部への誘導が必要であると考えられた。

E．結論

平成17年度は

1) 新たな歩行トレーニング機（Lokomat）を導入し、脊髄損傷者の歩行トレーニング実験を開始するための環境整備を完了した。

2) 同時に健常者での基礎実験を行い、脊髄損傷後の回復程度を定量する基礎データを得ることができた。

3) 脊髄運動ニューロンの初代培養系を確立し、BDNFの軸索伸長効果を確認した。

4) オリゴデンドロサイト前駆細胞の初代培養系を確立し、増殖因子に対する反応が観察された。

5) ラット脊髄圧挫モデルにおいて、Olig2/BrdU二重陽性細胞の挙動を観察した。

F．健康危険情報

特になし

G．研究発表

1. 論文発表

Kawashima N, Akai M, Nakazawa K. Muscle oxygenation of the paralyzed lower limb in spinal cord-injured persons. Medicine & Science in Sports & Exercise 37:915-921, 2005

Nakazawa K, Kawashima N, Akai M. Enhanced stretch reflex excitability of the soleus muscle in incomplete rather than persons with complete chronic spinal cord injury. Archives of Physical Medicine and Rehabilitation 87:71-75, 2006.

2. 学会発表

H．知的財産権の出願・登録状況
（予定を含む。）

1. 特許取得
   特になし
2. 実用新案登録
   特になし
3. その他

厚生労働科学研究費補助金（障害保健福祉総合研究事業）
分担研究報告書

損傷脊髄の機能回復可能性に関する研究

分担研究者　中澤　公孝　国立身体障害者リハビリテーションセンター研究所　室長

研究要旨
神経生理学的研究により、中枢からの下行性指令信号の受け手側である損傷脊髄以下に残った機能回復能を調べる。具体的には
①ヒトの脊髄に歩行パターンを自律的に生み出す能力がどの程度あるのか、
②それは末梢からの感覚刺激に対してどの程度適応的に変化する能力があるのか、
③その能力は上位中枢とどのような結合状態を取っているか、
を明らかにする正常者での研究と、実際の脊髄損傷患者の損傷部位以下で、こうした能力はどの程度残存しているか、を定量的に把握する臨床研究を並行して行う。

A．研究目的

研究の分担として
神経生理学的研究により、中枢からの下行性指令信号の受け手側である損傷脊髄以下に残った機能回復能を調べる。

１7年度目標として
立位歩行トレーニングの経時変化追跡をおこなう。不全および完全対麻痺者を対象として、トレッドミル上での歩行トレーニング中及び安静時に経頭蓋磁気刺激（TMS）を行い、それに対する下肢麻痺領域の応答を評価する。さらに下肢麻痺領域の脊髄反射を誘発し、これらがトレーニングによりどう変化するかを縦断的に調べる。

B．研究方法

平成17年度はロボット型歩行トレーニング機（Lokomat、写真１）が導入され、次の二つの実験を開始した。

実験１：受動歩行時の皮質脊髄路興奮性の定量化
　ロボット型歩行トレーニング機（Lokomat）を用いた受動歩行中に経頭蓋磁気刺激（TMS）による誘発筋電位（MEP）を前脛骨筋（TA）から記録した。現在のところ、健常者８名、脊髄損傷者1名（C7、ASIA　B）の結果を得ている。

実験2：受動歩行トレーニング実験
　上記の脊髄損傷者1名を対象としトレーニング前後およびトレーニング中の各種検査、実験を行う体制と環境を整えトレーニングを開始した。

（倫理面への配慮）
ロボット型トレーニング機を用いた臨床実験のプロトコルを作成し、倫理委員会の承認後に試験を開始した。

C．研究結果

図１にLokomatを用いた受動歩行中のMEPの典型例を示した。受動歩行中にTAに誘発されるMEPが歩行周期の局面に応じて変調すること、安静時や静止立位時に比べて増大することが明らかとなった。すなわち、受動的歩行において、下肢をステッピングさせられることによりTAの皮質脊髄路が促通することが示された。
写真２は完全脊髄損傷者がLokomat歩行トレーニングを行っている様子である。図２は下肢の筋群から記録した受動歩行中の筋電図である。完全対麻痺者であっても受動歩行時に下肢麻痺領域に筋活動が誘発されること、しかもその振幅が運動開始時に比べて徐々に減少する、という先行研究と同様な結果が確認された。

D．考察

　健常者を対象とした受動歩行時のMEPの結果から、受動的歩行において、TAの皮質脊髄路興奮性が促通するという新たな知見が得られた。今後脊髄損傷者のトレーニング中に同様な検査を行う予定であるが、その基盤となるきわめて重要な結果を得ることができた。この現象は、さらなる検証が必要ではあるもののSOLには認められなかったことから、大脳皮質との結合が強いとされる前脛骨筋（TA）に固有の特徴である可能性が高い。最近、TAの静的および動的随意収縮時のMEPが脊髄損傷の程度をよく反映することが報告された（Diehl et al. 2006, J Neurol）。

今回の受動歩行中のMEPの変調についてもさらに追

跡することで、脊髄損傷後の歩行回復との関係を明らかにしたい。

今後の計画として、本年度はLokomat導入が平成17年10月であったため、脊髄損傷者でのトレーニングは1名にとどまったが、Lokomatの運用規定や検査項目、方法を整備することができたことから、来年度から本格的なトレーニング実験を行う予定である。

E．結論

平成17年度は新たな歩行トレーニング機（Lokomat）を導入し、脊髄損傷者の歩行トレーニング実験を開始するための環境整備を完了した。同時に健常者での基礎実験を行い、脊髄損傷後の回復程度を定量する基礎データを得ることができた。

F．健康危険情報

特になし

G．研究発表

1.　論文発表

Nakazawa K, Kawashima N, Akai M. Enhanced stretch reflex excitability of the soleus muscle in incomplete rather than persons with complete chronic spinal cord injury. Archives of Physical Medicine and Rehabilitation 87(1):71-75,2006

中澤公孝. 歩行の中枢とCPG. 老年医学, 43(1):93-98、2005

2.　学会発表

H．知的財産権の出願・登録状況
（予定を含む。）

1.　特許取得
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3.　その他

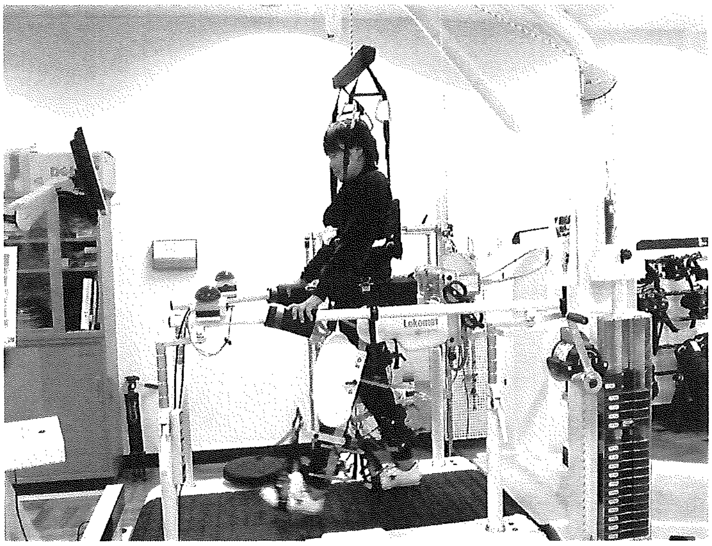

写真１　ロボット型歩行トレーニング機

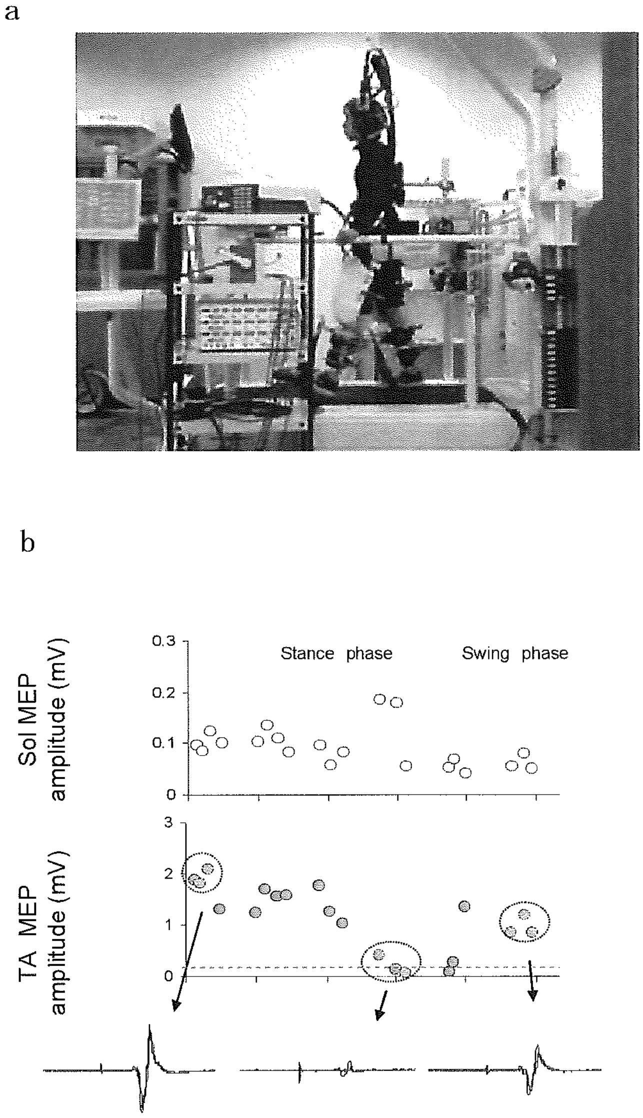

図１a:Lokomat歩行中にTMSを与えている実験の様子。b:TMSによって誘発したSOLとTAの筋電位の典型例。

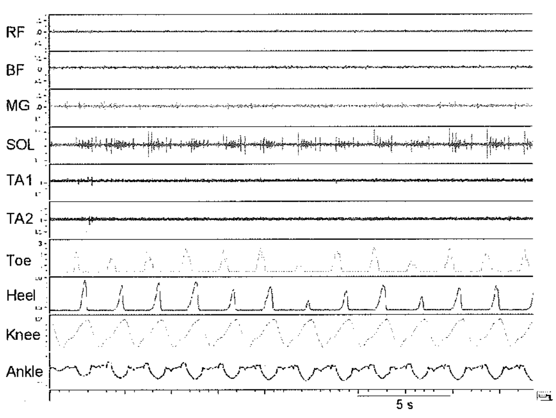

図２　Lokomat歩行中の筋電図と足部フットスイッチ、膝・股関節角度変化の例

厚生労働科学研究費補助金（障害保健福祉総合研究事業）
分担研究報告書

損傷脊髄神経における軸索伸張と髄鞘形成の分子メカニズムに関する研究

分担研究者　山本　真一　国立身体障害者リハビリテーションセンター研究所　主任研究官

研究要旨
再生医療に基づく細胞工学的手法により、上位信号の送り手側である損傷部位での軸索再生を計る。
現在脊髄での神経軸索再生には神経細胞の再生力の賦活化と再生軸索周囲の環境の最適化が必要と考えられている。そこで細胞培養、動物実験モデルを用い、以下の課題を検討する。
①神経細胞の再生シグナルを神経軸索断端からの遺伝子導入によって活性化し、軸索伸長を促進させる。
②再生軸索の足場として最適な表現形を持つ細胞を損傷部に誘導する。具体的には局在のグリア細胞への遺伝子導入を検討する。

A．研究目的

研究の分担として
脊髄・神経に対する細胞生物学的実験に基づく細胞工学的手法により、損傷部位での軸索再生促進を計る。
１7年度目標として
脊髄運動ニューロン（神経細胞の再生力の賦活化）(1)と髄鞘を形成するオリゴデンドロサイト前駆細胞（再生軸索周囲の環境の最適化）(2)に注目し、初代培養系を確立する。損傷脊髄の組織学的検討のため、ラット脊髄圧挫モデルを確立する (3)。

B．研究方法

1) ラット胎生14日の脊髄腹側細胞を選択的に採取し、脊髄運動ニューロンの初代培養を行った。BDNFによる軸索伸長効果と細胞内シグナル伝達経路に関して各種阻害剤を用いて検討した。
2) マウス胎生16日の大脳由来オリゴデンドロサイト前駆細胞の初代培養を行った。
3) 米国PSI社のInfinite Horizon Impactorを購入し、コンピュータ制御下にラット脊髄圧挫損傷モデルを作成した。Olig2/BrdU二重陽性細胞の挙動を解析した。
（倫理面への配慮）
本研究所の「動物実験委員会」の承認を得ている。

C．研究結果

1) 脊髄運動ニューロン(Islet1発現細胞)は全細胞中約20%の濃度で選択的に培養可能であった。対照群に対し、BDNFを加えた場合約2.5倍軸索が伸長した。またMEK,PI3Kなどの各種阻害剤を加えた場合、BDNFの軸索伸長効果はいずれもコントロール群と同程度またはそれ以下に阻害された。
2) オリゴデンドロサイト前駆細胞が増殖因子(FGF2/PDGF)存在下に約85%の純度で培養可能となった。増殖因子下流のMEKの機能が確認された。
3) Olig2/BrdU二重陽性を呈する前駆細胞の増殖は脊髄損傷部近傍で顕著に見られるものの、損傷後1週間の間にその6割が失われていることが明らかとなった。また、損傷部位へ向けての細胞の遊走は確認されなかった。
１８年度目標：
1) 脊髄運動ニューロンに恒常活性型MEKなど関連シグナルを強制発現させ、その効果を検討する。
2) オリゴデンドロサイト前駆細胞の増殖におけるMEK経路や関連分子の機能評価を試みる。
3) Olig2陽性細胞の分化過程を、NG2・APC発現などから検討する。

D．考察

1) 脊髄運動ニューロンにおいては、BDNFの下流でMEK-ERK経路が軸索伸長に関与している可能性が考えられた。
2) オリゴデンドロサイト前駆細胞においても、増殖因子下流でMEK-ERK経路が機能していると考えられた。
3) 内在するオリゴデンドロサイト前駆細胞の治療応用のためには細胞数の維持と損傷部への誘導が必要であると考えられた。

E．結論

1) 脊髄運動ニューロンの初代培養系を確立し、BDNFの軸索伸長効果を確認した。
2) オリゴデンドロサイト前駆細胞の初代培養系を確立し、増殖因子に対する反応が観察された。
3) ラット脊髄圧挫モデルにおいて、Olig2/BrdU二重陽性細胞の挙動を観察した。

F．健康危険情報

特になし

G．研究発表

1.　論文発表

2.　学会発表

緒方徹、田中栄、中福雅人、中村耕三、赤居正美、山本真一：損傷脊髄における内在性オリゴデンドロサイト前駆細胞の経時的局在変化の解析　第20回日本整形外科学会基礎学術集会、三重、2005.10.20-21.（日本整形外科学会雑誌79：8, S857, 2005）

山本真一、大堀靖夫、緒方徹、荒居聖子、伊藤順一、山本直哉、赤居正美、中福雅人、中村耕三：内在性神経前駆細胞を用いた脊髄再生誘導（シンポジウム：脊髄損傷研究に関する最新知見）　第40回日本脊髄障害医学会、東京、2005.11.11-12.

H．知的財産権の出願・登録状況（予定を含む。）

1.　特許取得
特になし

2.　実用新案登録
特になし

3.　その他

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

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Kawashima N, Akai M, Nakazawa K.	Muscle oxygenation of the paralyzed lower limb in spinal cord-injured persons	Medicine & Science in Sports & Exercise	37	p.915-921	2005
中澤公孝	歩行の中枢とCPG	老年医学	43(1)	p.93-98	2005
Nakazawa K, Kawashima N, Akai M.	Enhanced stretch reflex excitability of the soleus muscle in incomplete rather than persons with complete chronic spinal cord injury	Archives of Physical Medicine and Rehabilitation	87	p.71-75	2006
Kawashima N, Taguchi D, Nakazawa K, Akai M.	Effect of lesion level on the orthotic gait performance in individuals with spinal cord injuries.	Spinal Cord			In press

Muscle Oxygenation of the Paralyzed Lower Limb in Spinal Cord–Injured Persons

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ABSTRACT

KAWASHIMA, N., K. NAKAZAWA, and M. AKAI. Muscle Oxygenation of the Paralyzed Lower Limb in Spinal Cord–Injured Persons. *Med. Sci. Sports Exerc.*, Vol. 37, No. 6, pp. 915–921, 2005. **Purpose:** Even in the paralyzed lower limb muscle, EMG activity can be induced by imposing passive leg movement in standing posture in persons with spinal cord injury (SCI). The purpose of the present study was to ascertain whether the oxygenation level of the paralyzed lower limb muscle covaried with the muscle EMG activity during imposed passive leg movement. **Methods:** Six motor-complete SCI subjects and four neurologically normal controls were placed on a gait-training apparatus that enabled the SCI subjects to stand and move their legs passively. After a 1-min resting stage, consecutive passive alternate leg movements were performed at different frequencies (0.8, 1, 1.2, and 1 Hz, for 3 min at each stage). To obtain postexercise data, subjects were kept in a standing posture for 5 min after passive movement ceased. The EMG activity and concentration changes in the oxygenated (oxy-) and deoxygenated hemoglobin (Hb) (deoxy-Hb) were continuously measured using near-infrared spectroscopy (NIRS) from the gastrocnemius muscle. **Results:** In all SCI subjects, muscle EMG activity was observed during passive leg movement. The oxy-Hb level gradually increased, whereas the deoxy-Hb decreased, and these changes were independent of the total Hb changes. In the recovery stage, the total Hb level was found to exceed the preexercise level. In contrast to the SCI patients, the normal subjects showed neither EMG activity nor changes in oxy- or deoxy-Hb. **Conclusion:** The present results demonstrate that passive leg movement can induce not only muscular activity but also alteration of muscle oxygenation level in the paralyzed lower leg. Particularly, induced muscular activity seems to correlate with increased perfusion of the muscle. **Key Words:** SPINAL CORD INJURY, PARALYZED MUSCLE, OXYGENATION LEVEL, NEAR INFRARED SPECTROSCOPY, REFLEXIVE MUSCLE CONTRACTION, PASSIVE MOVEMENT

Previous studies have indicated that spinal cord injury (SCI) leads to extreme muscle atrophy (7,19), fiber type transformation toward fast-fatigable fibers (13,20), and lower bone mineral density (BMD) (11,33). This musculoskeletal degeneration can be attributed largely to the dramatic reduction of muscular activity and mechanical stress in the paralyzed limbs, which is due primarily to the motor paralysis following SCI. Furthermore, long-term immobilization of the paralyzed limb may bring about vascular effects such as reduction in vessel diameter (4,27), and changes in muscle blood flow (24) and vascular compliance (17,26,27). Because chronic inactivity and hypocirculation of the paralyzed area are especially crucial factors in cardiovascular-related complications such as pressure sores and deep venous thrombosis (5), enhancement of the metabolism and circulation in the paralyzed area is particularly important in preventing these problems.

It is now well recognized that, even in the paralyzed muscles of SCI patients, locomotion-like muscle activity can be induced by imposing stepping movement on a treadmill (8–10). Induced muscle activity is believed to have the potential to prevent degeneration of the musculoskeletal system in SCI patients. From the perspective of muscle metabolism, an important issue is whether the muscular activity induced by imposed passive leg movement is accompanied by alterations in the oxygenation level and/or circulation in the paralyzed area. The present study was designed to address this question by simultaneously recording the EMG activity and the muscle oxygenation using near-infrared spectroscopy (NIRS). NIRS, a noninvasive and reliable technique for measuring oxygenation and hemodynamics in tissue, is based on the principle that the near-infrared light absorption properties of hemoglobin (Hb) and myoglobin (Mb) depend on their O_2 saturations. Recently, NIRS has been applied in clinical fields to measure metabolic and circulatory patterns in a variety of diseases, and is recognized to be a useful method for identifying impairment of muscle metabolism (for a review, see Boushel et al. (6)).

The purpose of the present study was to ascertain whether the oxygenation level of the paralyzed lower limb muscle changed with muscle EMG activity during imposed passive leg movements. If muscle oxygenation and circulation can be facilitated by imposing passive movement, it may have significant ramifications for rehabilitation in cases of SCI,

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and especially in the prevention of secondary impairment following SCI. In the present study, we hypothesized that the muscle oxygenation level should change with the appearance of EMG activity in the paralyzed lower limb muscle.

METHODS

Participants

Six men with SCI (26.4 ± 4.4 yr) and four neurologically normal subjects (25.3 ± 2.4 yr) participated in the present study. All SCI patients had traumatic SCI at the thoracic level (between T4 and T12) and had complete paralysis of their lower limb muscles (American Spinal Injury Association (ASIA) Class A or B) (22) with moderate spasticity. Their postinjury time was longer than 6 months. The physical characteristics of the subjects are summarized in Table 1. The subjects gave their written informed consent for 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.

Experimental Procedure

Passive leg movement. To impose locomotion-like movement on the legs, we used an apparatus (Fig. 1A) developed for the physical exercise of persons with disabilities (Easy Stand Glider 6000, Altimate Medical, Inc., Morton, MN). This apparatus enables SCI subjects to stand securely by immobilizing their trunk and pelvis using front and back pads, and by preventing hyperextension of the knee joint using a kneepad. It also enables them to swing their legs by moving a handle connected to a foot plate. In the present study, the experimenter manually moved the handle back and forth in a sinusoidal manner.

Protocol. Subjects were asked to abstain from alcohol and caffeine for at least 12 h before the experiment. The subjects were placed in the device and held in standing posture. We verified that the standing posture was stable and that there was no hypotension. We had initially planned to measure postexercise data for 10 min, but some subjects showed orthostatic hypotension for 8 or 9 min after the cessation of the exercise in the preliminary experiment. We therefore set the duration of postexercise measurement at 5 min. After a 1-min resting stage, consecutive passive movements were performed for 3 min at each of the following frequencies: 0.8, 1, 1.2, and 1 Hz. This protocol was used to examine whether EMG activity and muscle oxygenation are dependent on the frequency of passive movement. The 1-Hz movement was repeated to examine time-dependent changes of muscle activity and oxygenation. During the movement, the hip joint range of motion was set at 40. The experimenter manipulated the lever, keeping pace with the rhythm of a metronome. The experimenter had conducted a sufficient number of practices before the resting session so that they could adjust the leg motion to the predetermined pattern (i.e., the range of motion and swing frequency) by monitoring the angle data from an electrogoniometer displayed on an oscilloscope. Since our aim was to estimate the muscle oxygenation due to EMG activity in the paralyzed muscle, the subjects were asked to relax their upper limbs.

TABLE 1. Characteristics of the SCI subjects.

Group	Subject	Age (yr)	Weight (kg)	Lesion Level	ASIA Grade	Duration of Paraplegia (months)
SCI	S1	24	75	T12	A	26
	S2	21	60	T12	B	25
	S3	30	74	T8	A	14
	S4	19	53	T5	B	26
	S5	39	67	T12	A	15
	S6	32	68	T12	A	34
	Mean	27.5	66.2			23.8
	SD	7.56	8.42			7.58
Normal	Mean	26.4	64.2			
	SD	4.54	5.43			

Near-infrared spectroscopy. During passive leg movement, the oxygenation levels of the medial head of the gastrocnemius (MG) muscle were continuously measured by a NIRO-300 (Hamamatsu Photonics, Inc., Hamamatsu, Shizuoka, Japan) with dual-channel near-infrared laser diodes. The NIRS signal has been assumed to reflect the combined absorption of the oxygenation level of Hb and Mb. Though it is impossible to distinguish between Hb and Mb because of identical spectral characteristics, contribution from myoglobin to the overall signal is quite small. Changes in oxygenated- (oxy-) and deoxygenated Hb (deoxy-Hb) were calculated by measuring light attenuation at 775-, 813-, 850-, and 913-nm wavelengths, and were then analyzed with an algorithm incorporating the modified Beer–Lambert law. The NIRS probe was placed on the upper portion of the bellies of the MG muscle, and a calibration procedure was carried out to ascertain whether the range of measurement was within the optimal range. Before the beginning of the passive leg movement, subjects were kept in standing posture on the apparatus until the total Hb value reached a constant level, that is, until the pooling of venous blood was completed. At that time, the concentrations of each Hb value were set at zero. Changes in the Hb values were calculated relative to the resting level, and are represented in micrometers.

Electromyography. The surface EMG signal was recorded from the MG muscle using bipolar electrodes. Because it is impossible to place both the NIRS sensor and the EMG electrode at the same place, they were placed proximally and distally on the medial side of the muscle (Fig. 1B). The electrode (DE-2.3, DelSys, Inc., Boston, MA) was placed at least 2 cm proximal to the end point of the MG muscle. This electrode has parallel bars (1 cm long and 1 mm wide) spaced 1 cm apart, and is designed with a built-in filter from 20 to 450 Hz. The common mode rejection ratio at 60 Hz is greater than 80 dB. SCI patients tend to have larger impedance in their paralyzed legs, and special care was thus taken to eliminate any artifacts of the EMG recording. The electrodes were attached using double-sided adhesive tape after careful preparation of the skin. The EMG

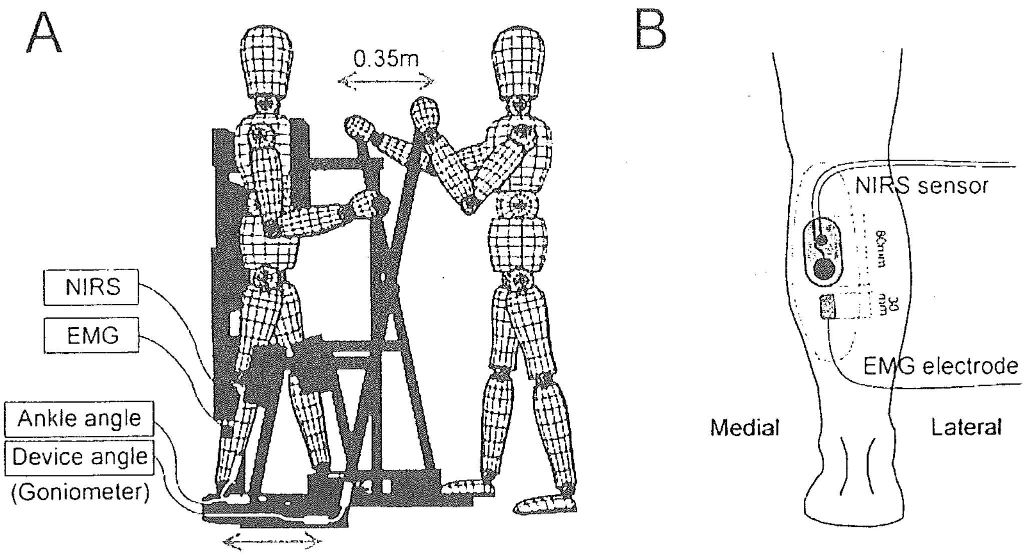

FIGURE 1—A. Experimental setup. This apparatus enables SCI patients to stand securely by immobilizing their trunk and pelvis using front and back pads, and by preventing hyperextension of the knee joint using a kneepad. It also enables them to swing their legs by moving a handle connected to a foot plate. In this study, the experimenter manually moved the handle back and forth in a sinusoidal manner by matching the movement frequency with the sound of a metronome. B. Location of the EMG electrode and the near-infrared spectroscopy (NIRS) sensor. Because it was impossible to place both the NIRS sensor and the EMG electrode at the same place, they were placed proximally and distally on the medial side of the muscle.

signal was amplified (Bagnoli-8 EMG System, DelSys, Inc.).

Electrogoniometer. In order to ascertain the similarity of the leg motion throughout the exercise session, the angle of the device was recorded by an electrogoniometer (Goniometer System, Biometrics Ltd., Ladysmith, VA) with sensors placed on the lateral aspect of the apparatus.

Heart rate. To confirm whether central circulation is enhanced by imposing the passive leg movement, HR was continuously measured by using an integrated telemetric monitor (HR meter, Polar, Vantage, Finland) in two SCI patients and two normal subjects.

Data Analysis

During the experiment, all data were continuously monitored by PowerLab software (Chart ver. 4, AD Instruments Inc., Milford, MA) and were digitized at 1 kHz for later analysis. For NIRS data, the average value in the last 30 s at each stage and those at 1, 3, and 5 min postexercise were evaluated for each parameter. The EMG signals were full-wave rectified after subtraction of the DC component. The magnitude of the EMG activity was quantified by the mean amplitude and integrated area of the EMG activity during the last 1 min of each stage.

Statistical Analysis

Values are given as means ± SD. Statistical differences in the size of EMG value and each Hb value were tested by ANOVA with repeated measures. Tukey's *post hoc* test was applied to identify differences between the conditions. The statistical software SPSS 11.0 was used to carry out all analyses. Significance was accepted at $P < 0.05$.

RESULTS

Figure 2 shows a typical example of the EMG activity, NIRS values, and leg motion during an experiment in a SCI patient (Fig. 2A) and a normal subject (Fig. 2B). As clearly shown in this figure, there are remarkable differences in both EMG activity and NIRS parameters between the two groups. It was confirmed that the leg motion was maintained within similar range throughout the exercise in both SCI and normal subjects.

EMG activity. During passive movement, all SCI patients showed EMG activity in the gastrocnemius muscle. The active phase of the EMG activity corresponded to the backward phase of leg movement. Despite the fact that the total (integrated) response area increased with the frequency of the movement, there was no remarkable change in amplitude (Fig. 3). Although the movement frequency in both the second and fourth stages was set at 1 Hz, the EMG amplitude in the fourth stage was significantly lower than that in the second stage (second vs fourth: 42.08 ± 3.73 vs 29.66 ± 6.19 μV, $P < 0.05$). In contrast to the SCI patients, normal subjects showed no visible EMG activity in the gastrocnemius muscle at any time during the exercise.

NIRS parameters. In both the SCI and normal groups, the concentrations of total Hb and deoxy-Hb showed rapid decrements following the onset of the exercise and remained at lower levels compared to the resting value while the legs were passively moved. The degree of the decrease of total Hb in the first stage was much smaller in the SCI group than in the normal group (SCI vs normal: 2.79 ± 0.99 vs 7.04 ± 2.18 μm). During the exercise period, an increase in oxy-Hb and a decrease in deoxy-Hb, which were independent of the changes in total Hb, were observed in the SCI group but not in the normal group (Figs. 2 and 4). In the recovery stage,

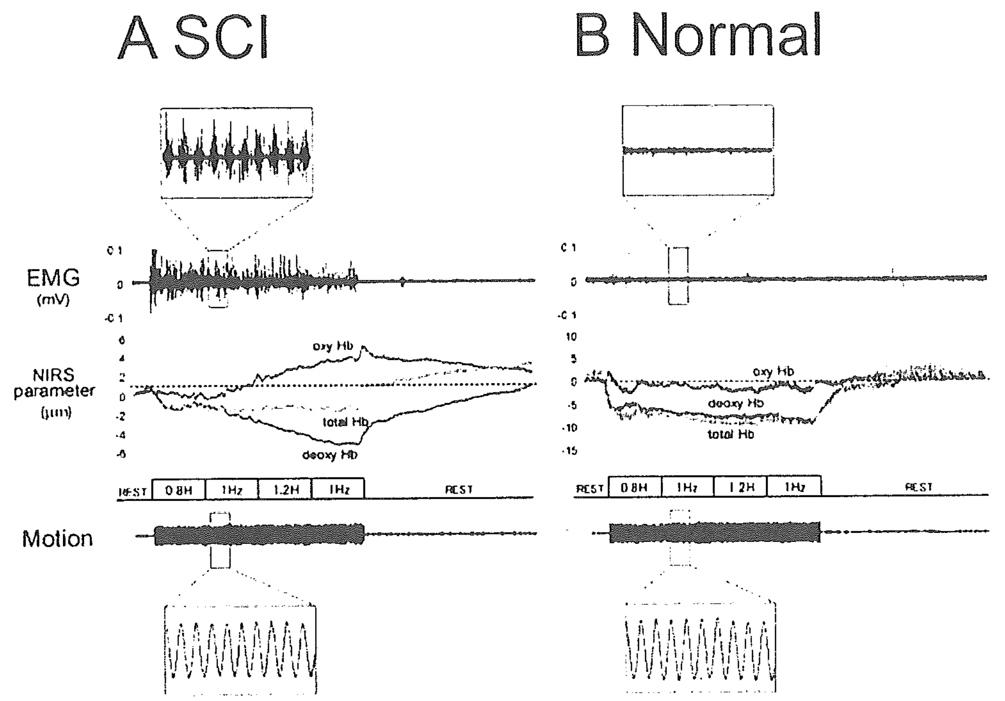

FIGURE 2—Typical example of the EMG activity and concentration changes in each hemoglobin parameter in a patient with motor-complete spinal cord injury (SCI) (A) and a neurologically normal subject (B). Note that there are remarkable differences in both EMG activity and near-infrared spectroscopy (NIRS) parameters between the two subjects. The motion of the apparatus was maintained within a similar range throughout the exercise for both subject groups.

the total Hb level exceeded the resting value in the SCI group, whereas it merely recovered to the pretest level in the normal group.

Heart rate. Figure 5 shows the change of the HR at rest and during passive leg movement and the recovery period obtained from two SCI patients and two normal subjects. As shown in this figure, HR increased just after the onset of passive leg movement in all subjects.

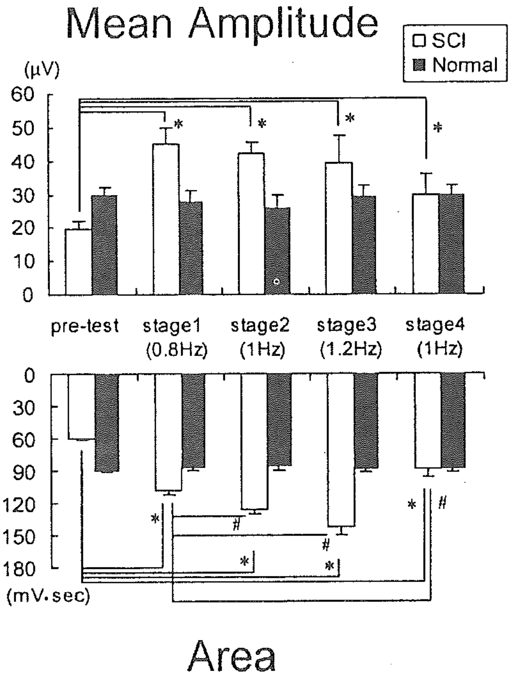

FIGURE 3—Mean amplitude and area of muscle EMG activity in the gastrocnemius muscle. The error bars indicate the SEM value. * Significant difference ($P < 0.05$) compared with the resting value. # Significant difference to the first set value. SCI, spinal cord injury.

DISCUSSION

The present study was designed to examine whether the oxygenation level of the paralyzed muscle is altered with the EMG activity induced by imposed passive leg movement. Our primary observations are the following: (i) during passive movement, all SCI patients showed EMG activity in the gastrocnemius muscle, whereas none of the normal subjects showed such activity; (ii) during the exercise period, an increase in oxy-Hb and a decrease in deoxy-Hb, both of which were independent of changes in total Hb, were observed in the SCI group; and (iii) in the recovery stage, total Hb exceeded the preexercise value in the SCI group. A possible mechanism for these changes in oxygenation level in the SCI patients and its implications for rehabilitation are discussed below.

Muscle activity during passive movement. Despite the motor paralysis in their lower legs, all six SCI subjects showed EMG activity in the paralyzed gastrocnemius muscle during passive movement. On the other hand, no normal subjects showed any EMG activity in the gastrocnemius muscle, even though the applied leg movements were identical to those applied to the SCI patients. In our previous data, it was found that the passive leg movement can also induce EMG activity in other lower leg muscles, for instance, the soleus and biceps femoris muscles (18). It is possible that the observed muscle activity consisted of complex spinal reflexes rather than simple stretch reflex responses induced by rhythmical stretching of the muscle tendon (14,18), and that the lack of EMG activity in normal subjects can be partly explained by the inhibitory neural input from a higher center to the spinal motor neurons (8). We do not discuss any further details of the neural mechanism of this EMG activity here because this article is con-

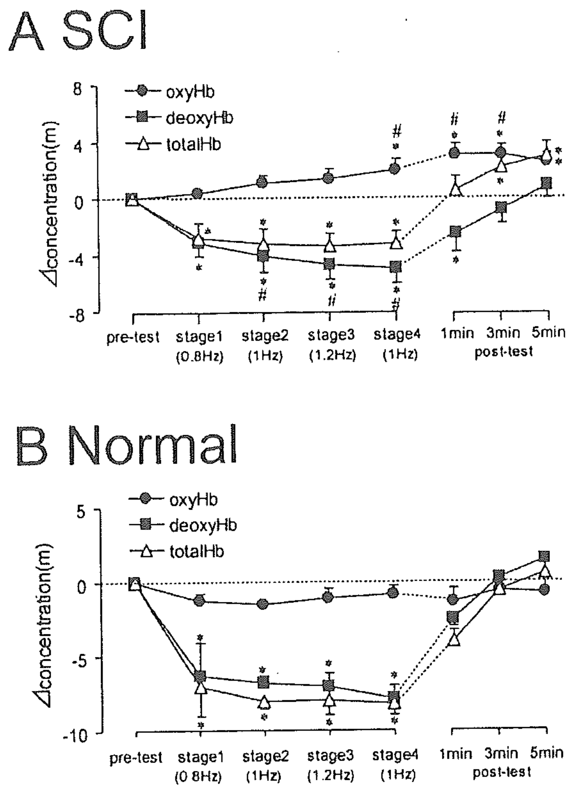

FIGURE 4—Concentration changes in total, oxygenated hemoglobin (oxyHb), and deoxygenated hemoglobin (deoxyHb) throughout the experiment for patients with spinal cord injury (SCI) (A) and normal subjects (B). The error bars indicate the SEM value. * Significant difference ($P < 0.05$) compared with the resting value. # Significant difference to the first set value.

cerned primarily with the relationship between the magnitude of muscular activity and the degree of the Hb value. The neural mechanism underlying this EMG activity has been described in detail in the previous research (for a review, see Harkema (14)).

The degree of changes in the NIRS signals should strongly depend on the muscle contraction level. It is therefore important to know how much the muscle activity occurs during passive leg movement. However, it is difficult to evaluate the muscle contraction level using the percentage of the maximal voluntary contraction (%MVC), which is commonly used to normalize and evaluate the muscle contraction level because SCI patients cannot accomplish voluntary contraction. When the EMG activity of the paralyzed muscle is expressed with size relative to the MVC obtained by normal subjects (average: 417.7 ± 43.24 μV), it corresponds with approximately 10% MVC. Given the muscle atrophy of the paralyzed muscle (7,19), it can be assumed that the SCI patients have an MVC lower than that in the normal subjects. Therefore, we estimate the contraction level observed in the SCI patients as no less than 10% MVC.

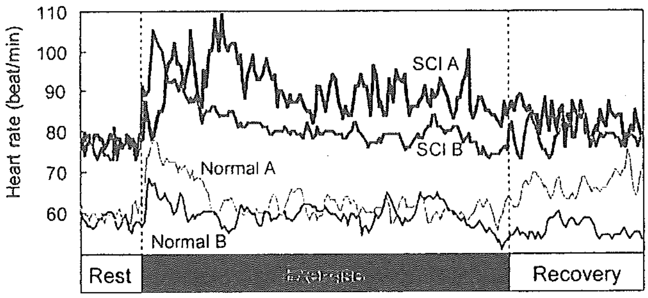

FIGURE 5—HR changes at rest, during passive leg movement, and in the recovery period obtained by two patients with spinal cord injury (SCI) and two normal subjects.

Changes in Hb concentration during exercise. In the present study, both the SCI and normal groups showed a rapid decrease in the total Hb concentration following the onset of exercise and maintained the lower value while the legs were passively moved. We considered venous blood in the calf to be complete, that is, to have reached plateau level, at the beginning of the passive leg movement because subjects were kept in standing posture until the total Hb value stabilized. By imposing passive leg movement, the pooling venous blood might be expelled from the calf because intramuscular pressure is increased due to the imposed length changes in the muscle (28,30), irrespective of the appearance of EMG activity. Therefore, it seems reasonable to assume that the decreased total Hb observed in this study during movement is explained by this expulsion of the pooling venous blood in the calf.

In addition, the degree of the concentration changes in the total Hb was much larger in the normal group than in the SCI group. This result is consistent with the report of van Beekvelt et al. (36) that muscle pump activity induced by imposing electrical stimulation is reduced in SCI subjects compared with healthy subjects. It has been suggested that this reduced muscle pump activity might be explained by the muscle atrophy and low venous capacity found in SCI subjects (17). A possible explanation for this result is that SCI patients have more fat because larger amounts of fat result in lower NIRS signals (37). However, as described later, our results which the oxy- and deoxy-Hb showed in the opposite concentration changes would not be expected from fat for the same reason.

In the present study, a gradual increase in oxy-Hb and decrease in deoxy-Hb that were independent of changes in total Hb were observed in the SCI subjects during the exercise period. On the contrary, there is no obvious concentration change of the oxy-Hb in the normal subjects who showed no EMG activity during passive leg movement. If no muscle oxygen consumption and/or supply was induced by the imposed movement, both the oxy-Hb and the deoxy-Hb should vary in a manner related to the concentration changes in the total Hb. Taken together with the occurrence of EMG activity in the SCI group, this change in the muscle oxygenation level can be attributed to the muscle activity produced by imposing passive leg movements. These results are in good agreement with a recent report by Bhambhani et al. (3), who suggest that changes in the oxygenation level in the paralyzed rectus femoris muscle during cycling movement are generated by functional electrical stimulation.

With respect to muscle oxygenation during exercise, previous studies have reported that continuous muscle contraction at moderate intensity follows the increments of deoxy-Hb because of the oxygen consumption in the acting muscle (6,34). Although we hypothesized that muscle was "active" during passive leg movement, the present results did not show increments of the deoxy-Hb. According to the general principle, concentration changes in oxy- and deoxy-Hb are dependent on the dynamics of the equilibrium between tissue oxygen demand and supply (2,16). Therefore, a possible reason for our result is that oxygen delivery far exceeds the oxygen extraction in the acting muscle. The enhancement of HR during passive leg motion (Fig. 5) provides evidence to support this notion.

Changes in Hb concentration after exercise. After the cessation of the passive leg movement, the concentration of total Hb in the SCI group exceeded the preexercise level, whereas that in the normal group simply recovered to the preexercise level. Because the total Hb reflects the degree of muscle blood flow (6), these changes may suggest that enhancement of the muscle blood flow occurred in the SCI group, possibly resulting from the muscle contraction and oxygenation during the exercise period. It is likely that the excess total Hb following exercise resulted from the pooling of blood in the calf. Nevertheless, in this study, the subjects were kept in a standing posture on the apparatus before the initiation of the exercise period until the total Hb value reached a constant level; therefore, the above total Hb changes during the recovery stage cannot be explained solely by blood pooling in the calf. These total Hb changes may be due to postexercise hyperemia (35).

HR changes by imposing passive leg movement. As shown in the Figure 5, the HR increased after the onset of the passive leg movement in both SCI and normal subjects. These results provide evidence of the enhancement of central circulation by imposing passive leg motion even in the SCI patients. The simplest explanation is that increments of the venous return due to the muscle pump activity result in the central circulation (29). However, taken together with the results of differences in the EMG activity, there would be different mechanisms underlying the enhanced HR between two groups. In the case of the normal subjects, it is plausible that the enhancement of the HR is induced by the neuronal factor, which is an afferent neural signal from the mechanoreceptor by inducing muscle stretching (12). On the other hand, this neuronal factor is not a suitable explanation for SCI results because of the sensory paralysis. Rather, our results, the appearance of muscular activity and an alteration of the NIRS signals, imply that a metabolic change accompanied by muscle contraction seems to play a primary role in the enhancement of the central circulation. Because we do not still have any direct evidence, further investigations are needed to clarify this point.

Implications for rehabilitation. As mentioned at the beginning, chronic inactivity and hypocirculation of the paralyzed area are crucial factors in secondary impairment in SCI subjects (25). The present results provide indirect evidence that passive leg movement performed in a standing posture could alter the oxygenation level of the paralyzed muscle and has the potential to facilitate circulation of the paralyzed area. Given that the muscle contraction level during normal walking is about 15% MVC (21), it is considered that the muscle contraction level observed in this study is adequate to facilitate neural activity and circulation of the paralyzed area.

On a practical level, the subjects in the present study did not move their upper limbs and trunk voluntarily, because our aim was to examine whether the oxygenation level of the paralyzed muscle was altered by imposing passive leg movement. In a nonexperimental situation, however, patients would commonly operate the device themselves by manipulating the lever with their upper limbs. It is possible that the additional voluntary upper limb movement could enhance circulation not only in the voluntarily acting area, but also in the paralyzed area.

Although muscular activity in the paralyzed area can also be induced by applying electrical stimulation, as is the case in functional electrical stimulation (FES) (1,23,31), there are essential differences between our method and the FES technique. Previous investigations pointed out that one of major disadvantages of the FES technique is that it is difficult to generate FES-induced continuous muscle contractions without fatigue (for a review, see Stein et al. (32)). The muscle fatigue can be attributed to the fact that the fatigable motor unit is preferentially recruited by imposing electrical stimulation, in that large motor nerves are more easily activated than smaller ones. In contrast, in the case of the passive leg movement produced in the present study, the motor units are presumably recruited according to the size principle (15), because the afferent input was offered from proprioceptors by imposing muscle stretch and body load. Furthermore, passive leg movement is simpler and more practical than FES, and has a lower risk of misuse. Therefore, this type of passive leg movement might be a useful and efficient method for rehabilitation following SCI.

CONCLUSION

The present results demonstrate that passive leg movement can induce not only muscular activity, but also alteration of the muscle oxygenation level in the paralyzed lower limb. There may be increased oxygen consumption, but this could not be ascertained from the measurements in this study. Further study will be needed to clarify this issue.

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