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障害者対策総合研究事業

移植治療後の慢性期完全脊髄損傷患者のリハビリテーションと脳機能再構成および脊髄再生との関連性についての評価法の開発に関する研究

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研究代表者　岩月　幸一

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目次

別紙３

厚生労働科学研究費補助金（障害者対策総合研究事業）
（総合）研究報告書

移植治療後の慢性期完全脊髄損傷患者のリハビリテーションと脳機能再構成および脊髄再生との関連性についての評価法の開発に関する研究

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[研究要旨]
脊髄損傷に対する有効な神経再生療法は未だなく、残存機能の強化リハビリテーションが現在の唯一の治療法である。当グループは損傷後半年以上経過した慢性期完全脊髄損傷患者に対して自家嗅粘膜移植を行い、一定の機能回復を見た。しかし慢性期では下肢筋肉の委縮による神経栄養因子の枯渇から脊髄前角細胞の変性・下位運動神経の不全が起こり、脊髄(上位)神経軸索再生のみでは十分な機能回復は得られないことが示唆される。また効果的なリハビリテーションプログラム開発には、脊髄の組織的再生や脳の神経活動の機能的回復を継時的に評価する必要がある。本研究では、①術前にもリハビリテーションを行い、筋肉由来神経栄養因子の産生と下位運動神経の維持を図る、②自家嗅粘膜移植による脊髄神経軸索の再生、③術後のバイオフィードバックを用いた随意的筋放電の誘発、④長下肢装具およびロボットスーツ HAL 装着による積極的歩行訓練、の一連のプログラムにより、効率的機能再建を目標とする。さらに DTI(Diffusion Tensor Imaging)による損傷脊髄移植部位の組織的再生の可視化、および脳 fMRI による脳神経活動の再構築により機能回復プロセスの客観的指標の開発を目指す。

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A. 研究目的

脊髄損傷に対する有効な神経再生療法は未だなく、完全脊髄損傷患者においては残存機能の強化リハビリテーションが唯一の治療法である。当グループは損傷後半年以上経過した慢性期完全脊髄損傷患者に対して自家嗅粘膜移植を行い、一定の機能回復を見ているが、慢性期では下肢筋肉の委縮による神経栄養因子の枯渇から脊髄前角細胞の変性・下位運動神経の不全が起こり、脊髄(上位)神経軸索再生のみでは十分な機能回復は得られないことが示唆される。また効果的なリハビリテーションプログラム開発には、脊髄の組織的再生や脳の神経活動の機能的回復を継時的に評価する必要がある。

本申請では慢性期完全脊損患者に術前・術後に積極的リハビリテーションを導入したうえで嗅粘膜移植を行い、より効率的な下肢機能回復を目指すことを目的とする。

B. 研究方法

本研究では機能保存的リハビリテーション・脊髄神経再生・脳神経機能の変化の観点から、下記 6 つの工程を設ける。

①術前に廃用下肢筋のリハビリテーションにより、筋肉由来神経栄養因子の産生と下位運動神経の維持を図る。②自家嗅粘膜移植による脊髄神経軸索の再生。③術後のバイオフィードバックを用いた随意的筋放電の誘発。　④長下肢装具装着による積極的歩行訓練。さらに、これら機能回復のプロセスの客観的指標として、下肢運動指標に加え、新たに ⑤DTI(Diffusion Tensor Imaging)で損傷脊髄移植部位の組織的再生を可視化する。⑥脳 fMRI で脳神経活動の再構築を解明する。

（倫理面への配慮）

本研究は、【ヘルシンキ宣言】【臨床研究に関する倫理指針】ならびに本臨床研究実施計画書および同意説明文書を尊守して実施している。

① 同意説明と同意所得

研究責任医師等は治療に先立ち、未来医療臨床研究審査・評価委員会の承認を得た同意説明文書を用いて文書による同意を得る。同意取得のため研究責任医師等は、治療への参加に関し、被験者に強制するなど不当な影響を及ぼすことのないよう留意する。

本臨床研究への参加は被験者本人の自由意思による同意を、同意書に署名または記名・捺印し、日付を自ら記入することにより取得する。同意取得後、同意書の写し及び同意説明文書を同意者本人に交付する。

② 同意の撤回

一旦書面による同意を行った被験者であっても、嗅粘膜移植術実施前であればいつでも撤回できる。

③ 臨床研究内容の開示

同意説明を行った患者、または被験者に本臨床研究実施計画書の開示を要求されれば、それに応じるものとする。

④ 同意書および同意説明文書の改訂

研究責任医師等は、研究に継続して参加するか否かについて被験者の意思に影響を与える可能性のある情報や、被験者の同意に関連しうる新たな情報を入手した場合には、当該情報を直ちに口頭で被験者に伝える。また、情報提供した旨を診療録に記録し、被験者が研究に継続して参加するか否かを確認する。被験者が未成年の場合は、同時に法定代理人に対してもこれを行う。

C. 研究結果

嗅粘膜移植においては脊髄損傷後、骨損傷に対する治療やリハビリテーションを行ったにもかかわらず、12か月後に完全対麻痺を呈する胸髄損傷患者を対象とした。採取可能な嗅粘膜の量が限られているため、損傷部位の長さは3cm 以下である。術前2ヶ月にわたりリハビリテーションを行い、リハビリによってはやはり下肢運動機能が改善しないことを確認するとともに、術後リハビリが可能な下肢関節の拘縮などがないかを評価した。術後早期から連日リハビリテーションを行うと、4例中3例において6カ月後より運動機能の改善がみられ、4名いずれの患者においても体幹支持性が向上し、日常生活上何らかの運動機能改善が自覚された。ASIA Scoringのうち、運動スコアは、１名では改善が認められなかったが、他の3例では24週以後50から52-57に改善した。下肢筋収縮による筋電図の発現を認め、さらにうち2例で経頭蓋磁気刺激によるmotor evoked potential(MEP)の下肢からの導出に成功し、慢性期の完全脊髄損傷において、電気生理学的に神経軸索の再建を証明し得た。感覚および膀胱直腸障害においては変化を認めなかった。

慢性期脊髄完全損傷に対する嗅粘膜移植術は、現在まで8例実施済みである。うち5例において下肢の随意性運動の発現を認め、うち3例でMEPの検出に成功した。うち1例では、杖歩行ながら自力歩行500m以上が可能となった。

DTIでは術前に比し、術後リハビリ後損傷部位に向けて多くの神経線維が伸長し、一部その連続性が伺えるものも存在した。

FMRIによる脳機能解析では、一部減少していた下肢運動野の拡大がうかがわれた。

D. 考察

慢性期の完全脊髄損傷患者に、本移植法とリハビリテーションによって、下肢運動機能の回復と皮質脊髄路の再建を電気生理学的に証明し得たことは、不可能とされてきたこれまでの医学常識を覆すものであり、意義深いと考えられる。しかし、その効果にはばらつきがあり、また自力歩行を再獲得し得たのは 1 例のみであり、効果を増強させる新たな方策が望まれる。

移植後のリハビリテーションは、完全両下肢運動麻痺慢性期患者の歩行という、これまでにないリハビリテーションを実施しなくてはならなかった。中枢神経の神経ネットワークの再構築のため、長期間にわたるハードなものとなった。HAL を導入し、検出される生体信号が徐々に下位に延びてくるのにあわせてプログラムを変更することで、科学的リハビリが可能となった。またトレッドミルと免荷装置を併用することで、安全且つ省力的なリハビリが可能となった。さらに初期の段階から患者に歩行を体感させることが可能となり、これは長く単調になりがちなリハビリテーションに対する患者のモチベーションの維持に、大きく貢献したものと思われた。

DTI, fMRI の解析については、症例数が少なく十分な解析となっていない。症例数をさらに増やし、検討を加える必要が有る。

E. 結論

慢性期完全脊髄損傷患者に対し、嗅粘膜移植と積極的リハビリテーションを行い、一定の機能回復を導き、かつ下肢筋電図の導出に初めて成功した。このことは、損傷後数年以上を経た慢性期脊髄損傷患者の機能再建とQOLの向上に新たな道を拓くものである。

F. 健康危険情報

全ての症例において、これまで当研究と関連があると判断される感染症、悪性新生物の発生を認めていない。有害事象として一過性の嗅覚低下や、頭痛および脊損領域の痛みが出現した症例もある。

G. 研究発表

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H. 知的財産権の出願・登録状況

１．特許取得

なし

２．実用新案登録

なし

３．報道実績

見出し：脊髄損傷の再生医療
掲載紙：日本経済新聞　夕刊１頁
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見出し：On His Own Two Feet
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平成25年2月13日（水）

別紙４

研究成果の刊行に関する一覧表

[雑誌]

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Scientific Research Publishing

Early Diagnosis of Spontaneous Spinal Epidural Hematoma with Echo-Planar Gradient-Echo T2*-Weighted MR Imaging

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Abstract

Spontaneous spinal epidural hematoma (SSEH) is a rare idiopathic condition that leads to the acute onset of neurological deficits, which can have catastrophic consequences if not recognized early. It is important to make an early precise diagnosis. Spinal epidural hematoma has been increasingly recognized since the advent of magnetic resonance imaging (MRI). However, T1- and T2-weighted gradient-echo sequences are relatively less sensitive to the magnetic susceptibility effects of hemorrhage. Echo-planar gradient-echo T2*-weighted MR imaging (T2* MRI) is sensitive to these magnetic susceptibility effects and is commonly used for the detection of hemorrhage. We reported that the case of a 76-year-old man who presented with tetra paresis had an early diagnosis of spontaneous spinal epidural hematoma early diagnosed by T2* MRI.

Keywords

Spinal Epidural Hematoma, T2* MRI, Diagnosis, Hemorrhage

1. Introduction

Spontaneous spinal epidural hematoma (SSEH) is a rare condition that requires early diagnosis and treatment to ensure complete recovery of function. This condition has been increasingly recognized because patients presenting with rapid progressive neurological deficits of spinal cord origin undergo early evaluation with MRI. The diagnosis of SSEH, however, remains debated in the presentation of a differential diagnosis with spinal cord

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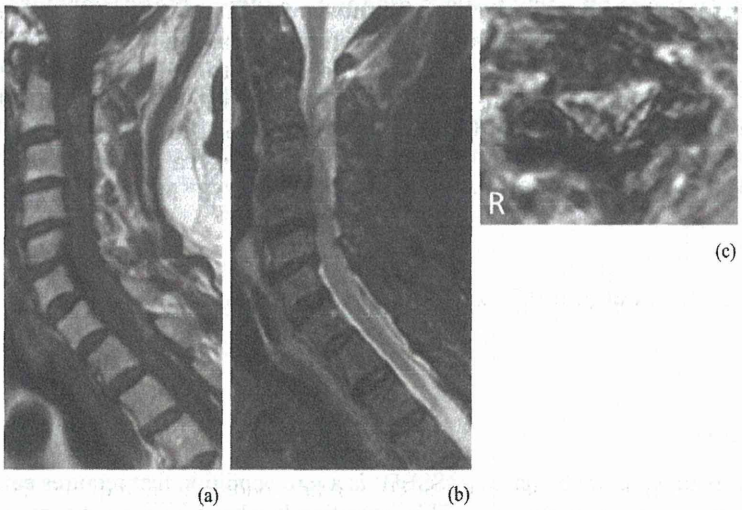
involvement. Early diagnosis of SSEH is important to aid the therapeutic strategy decision. We report a case of spontaneous cervical SSEH diagnosed with T2* MRI in a patient with natural recovery without a surgical intervention.

2. Case Report

A 76-year-old male presented with acute onset progressive tetra paresis for 24 h. His symptoms started as severe neck pain associated with the radiation of pain along his upper limb and difficulty in neck movements. Within a few hours, he recognized progressive tetra paresis and difficulty in walking. The next day, the symptoms progressed to tetraparesis with an inability to walk and hesitancy of micturition. He was transported to our emergency unit. His medical history was unremarkable: no trauma, smoking, drinking, or drug use. He was neither on anticoagulation nor antiplatelet medication at the time of presentation. Upon neurological examination, the power in extremities was 2/5 (medical research council grading) with urinary retention. There was bilateral hypotonia with absent reflexes and the Babinski sign was negative. Sensory examination showed absent vibration sensation below C5 level and an impairment of joint position sensation in both feet.

An urgent MRI of the cervical spine revealed a dorsal extradural lesion at the C2-Th3 level which was iso/hyper intense to the cord on T1-weighted images (T1WI) (Figure 1(a)) and iso/hyper intense with a band of hypo intensity on T2-weighted images (Figure 1(b)). A T2* MRI was taken under the suspicion of hemorrhage. It disclosed hypo intensity spots within the hyper intensity mass and a clear hypo intense rim (Figure 1(c)). The lesion showed blooming displacing the spinal cord anteromedially. His initial laboratory workup failed to show any significant abnormalities and the coagulation profile was normal. Given the acute presentation and the MRI findings, in particular the T2* MRI, a diagnosis of spontaneous cervico-thoracic epidural hematoma causing cord compression was suspected.

After emergency hospitalization, he showed gradual natural recovery. He could stand and walk 3 days after hospitalization. His urination had normalized 2 days after hospitalization. An MRI taken after 5 days, which revealed that the lesion was thinner, a hyper intensity on T1WI, and mixed intensity on T2WI and T2* images (Figures 2(a)-(e)). MRI Imaging Studies MRI was performed with a 1.5 T superconducting unit (Magnetorom Vision, Siemens) with a standard head coil to obtain axial fast spin-echo T2-weighted images (repetition time [TR]/echo time [TE]/excitations, 3600/96/2, slice thickness = 5 mm, gap = 1 mm). The imaging matrix and field of view were 224 Å~ 256 and 23 cm, respectively. Axial single-shot echo-planar gradient-echo T2-weighted imaging (TE/excitations 25/1, flip angle 90˚) was also obtained with a slice thickness and gap of 5 mm and 1 mm, respectively. The imaging matrix and field of view were 128 Å~ 128 and 23 cm, respectively.



(a) (b) (c)

Figure 1. (a) A dorsal extradural lesion at the C2-Th3 level, which was iso/hyperintense to the cord on T1-weighted images (b) iso/hyperintense with a band of hypointensity on T2-weighted images (c) hypointensity spots within a hyperintensity mass with a clear hypointense rim on T2* MRI.

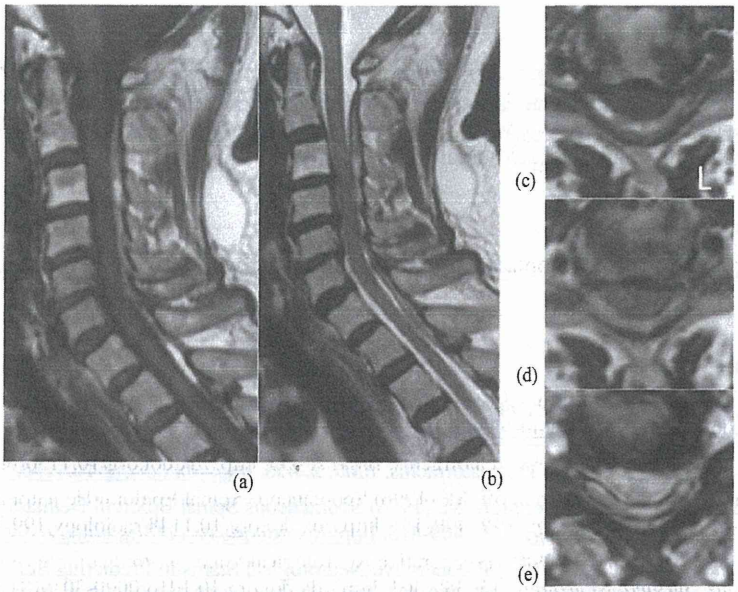


Figure 2. (a) (c) The lesion was becoming thin and revealed hyperintensity on T1WI and mixed intensity on T2WI (b) (d), T2* MRI (e).

3. Discussion

Spinal epidural hematoma was first described by Jackson in 1869 [1] and first treated surgically by Bain in 1897 [2]. The incidence of SSEH is estimated at $0.1.100000^{-1}\cdot year^{-1}$ [3]. Spinal epidural hematomas occur most frequently in the elderly but can occur at any age [4]. They are classified into two groups: nonspontaneous and spontaneous. Nonspontaneous epidural spinal hematomas may result from spinal taps, spinal anesthesia, trauma, pregnancy, bleeding diathesis, anticoagulant therapy, spinal hemangiomas, vascular malformations, hypertension, and neoplasms. Spinal epidural hematoma is capable of producing severe and irreversible neurologic deficits, and acute surgical intervension may be needed. The early precise diagnosis is crucial. Current literature supports both venous and arterialorigins as the source of spontaneous epidural hematomas [5]. The most widely accepted hypothesis for the source of bleeding is venous, because spinal epidural veins have no valves and are thus unprotected from changes in abdominal or thoracic pressure [5]. Increasing intrathoracic and intra-abdominal pressure leads to brief increases in intravenous pressure in valveless and thin-walled epidural veins, subsequently leading to their rupture. This accounts for cases that are reported occurring with activities such as straining, bending, coitus, coughing, and sneezing [6].

The investigation of choice is MRI. Following the inclusion of MRI into standard medical practice, the mean incidence of SSEH cases reported in the literature has increased further [7]. In the first 24 h, an epidural hematoma is isotense to the cord on T1WI and is usually hyperintense, although it may be heterogeneous on T2WI. By 48 h, the hematoma appears hyperintense on both T1WI and T2WI [3] [8]. The radiological differential diagnosis includes epidural abscess and spinal epidural lymphoma.

SSEH is acute onset and surgical management is recommended in the case of progressive symptoms, which can have catastrophic consequences if they are not recognized early in presentation. MRI is useful to verify diagnosis; however, T1WI and T2WI are relatively less sensitive to the magnetic susceptibility effects of hemorrhage. T2* MRI is sensitive to these magnetic susceptibility effects and is commonly used for the detection of hemorrhage [9]-[11]. T2* MRI requires a very short time for complete acquisition and is also sensitive to the effects of the local static magnetic field in homogeneities induced by the presence of hemosiderin [12]. Furthermore, T2* MRI can detect hyperacute hemorrhage because susceptibility is increased by the paramagnetic effect of deoxyhemoglobin, which is the earliest observable hemoglobin breakdown product on MRI [13]. Thus, T2* MRI is thought to be highly sensitive for detecting hemoglobin degradation products. In the case presented here, we could make a precise diagnosis of SSEH early on with T2* MRI and following up with it helped our decision on clinical course. T2* MRI might be recommended for evaluation of SSEH.

4. Conclusion

Spinal epidural hematoma is capable of producing severe and irreversible neurologic deficits, and acute surgical intervention may be needed. The early precise diagnosis is crucial. We reported a case of spinal epidural hematoma diagnosed with T2* MRI early on and it was followed without a surgery. T2* MRI was useful for the early precise diagnosis of spinal epidural hematoma.

5. Consent

Written informed consent was obtained from the patient for publication of this case report and any accompanying images.

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Transplantation of Olfactory Mucosa as a Scaffold for Axonal Regeneration Following Spinal Cord Contusion in Rats

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ABSTRACT

Object: The inability of the spinal cord to regenerate after SCI is due to the extremely limited regenerative capacity of most central nervous system (CNS) axons, along with the hostile environment of the adult CNS, which does not support axonal growth. It seems that for successful axonal regeneration to take place, a supportive local environment is required after the injury. We have previously reported that transplantation of the olfactory mucosa is effective in restoring functional recovery in rats following spinal cord transaction. In this study, we examined histological features of olfactory mucosa grafts in rats subjected to a spinal cord contusion protocol. Respiratory mucosa was utilized as a control, as we have previously found that respiratory mucosa does not support neuronal generation. **Methods:** The rats spinal cords were crash-injured by dropping a 10-g metal rod from a height of 7.5 cm, and a couple of weeks later, the injury sites were exposed, and both olfactory and respiratory mucosae were inserted into the posterior sulcuses of the spinal cord. The each number of olfactory and mucosa transplanted rats were five. The Basso, Beattie, and Bresnahan (BBB) score was observed. Immunohistochemical study for neurofilament was performed. **Results:** Olfactory mucosa transplanted rats following spinal cord injury can support at least partial hind limb motor recovery compared with respiratory mucosa transplanted rats and we identified numerous axons surrounding the transplanted olfactory mucosa cells, and penetrating the olfactory mucosa at the transplant site. **Conclusion:** Olfactory mucosa might be a suitable scaffold for axonal regeneration.

Keywords: Olfactory Mucosa; Spinal Cord Injury; Transplantation; Scaffold

1. Introduction

Traumatic spinal cord injury (SCI) is relatively common, and can result in severe damage leading to partial or complete loss of motor and sensory function caudal to the level of injury. This occurs as a result of severing of descending and ascending fiber tracts. One of the most devastating permanent complications following SCI is paraplegia, management of which has been a constant challenge in clinical medicine. Facilitating restoration of tract structure, and with it recovery of function, after SCI is of great interest to neuroscientists. The inability of the spinal cord to regenerate after SCI is due to the extremely limited regenerative capacity of most central nervous system (CNS) axons, along with the hostile environment of the adult CNS, which does not support axonal growth. After an SCI, astroglial scarring occurs within lesioned areas [1]. It has been shown that axonal regeneration is in fact initiated in the injured spinal cord but that it is blocked by glialscar formation [2]. It seems that for successful axonal regeneration to take place, a supportive local environment is required from an early stage after the injury. Recently [3], a team reported partial success in bridging the ends of the spinal cord after a complete resection using grafts of smooth muscle, peripheral nerve [4], fetal brain cells [5], semi-fluid collagen material [6], and embryonic spinal cord segments in the neonatal rat. These experiments suggest that regeneration of spinal nerve fibers across a spinal cord defect could be possible, under favorable conditions. To date, there have been very few studies regarding events that occur in the early stages of autografts transplantation.

We have previously reported that grafts of the olfactory mucosa are effective in restoring functional recovery in rats following spinal cord transaction, with histological evidence of neuronal regeneration [7-9]. In the present study, we examined histological features of olfactory mucosa grafts in rats subjected to a spinal cord contusion protocol. Respiratory mucosa was utilized as a control, as we have previously found that respiratory mucosa does not support neuronal generation.

2. Materials and Methods

Spinal cord injury model Male Sprague-Dawley rats, weighing 250 - 300 g, were anesthetized using a pentobarbiturate sodium/atropine mixture (5/5 mg/kg, intraperitoneally). Rectal temperature was maintained at 37°C ± 0.5°C using a heating pad. A laminectomy was performed at the thoracic (Th) 8 - 9 vertebrae using a microsurgery bone rongeur to expose the spinal cord without touching it. The spinal cord, covered by the dura mater, was crush-injured by dropping a 10-g metal rod from a height of 7.5 cm using a New York University (NYU) impactor. Although crush injury is commonly simulated by dropping a weight from a height of 2.5 - 5.0 cm 4 mild or moderate injuries tend to produce high rates of spontaneous locomotor recovery in controls. We dropped a rod from a height of 7.5 cm to cause severe crush injuries. Dissection and preparation of olfactory and respiratory mucosa Rats were deeply anesthetized using sodium pentobarbital (100 mg/kg) and sacrificed by decapitation. The nasal septum was freed by removing the lower jaw, upper teeth, and nasal turbinates. Both olfactory and respiratory mucasae were identified on the septum. The olfactory mucosa is located in the dorsocaudal portion and is easily identifiable by the yellowish appearance of its surface. The respiratory mucosa is located ventrorostral to the olfactory mucosa and identified by the grayish color of its surface. Each mucosa was carefully dissected to exclude the border region between the mucosae in order to avoid cross-contamination between the 2 types. Transplantation of olfactory and respiratory mucosa A couple of weeks after injury, the injury site was exposed, and the posterior sulcuses of the spinal cord were opened. Both olfactory and respiratory mucosae were divided into approximately 0.5 - 1.0-mm sections.

Next, 2 - 3 sections of the olfactory and respiratory mucosae were gently inserted into the sulcuses respectively. The each number of olfactory and mucosa transplanted rats were five. The wound was sealed by suturing the muscle and the skin overlying the exposed spine. Behavioral assessment The Basso, Beattie, and Bresnahan (BBB) score is an operationally defined 21-point scale. It is designed to assess the degree of hind limb locomotor recovery following impact injury to the thoracic spinal cord in rats [10]. In the present study, the BBB score in each animal was determined by 2 independent observers, who were blinded to the purpose and other protocols of this study. The scores were averaged and compared between the 2 groups using the Student's t test (unpaired). Statistical significance was set at $p < 0.05$. Preparation of tissue for histology and immunohistochemistry for immunohistochemical examination, 3 rats from each of the transplantation groups were sacrificed 8 weeks after the transplantation. Rats were deeply anesthetized by an intraperitoneal injection of sodium pentobarbital (100 mg/kg), and perfused intracardially with 50 ml PBS, followed by 200 ml of a fixative containing 2% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4. Specimens were processed using a standard procedure for embedding in OCT compound, and cut horizontally into 7-μm-thick frozen sections with a cryostat (CM1510S; Leica). Frozen sections were mounted on coated glass slides.

For histological examination, horizontal sections were stained with hematoxylin and eosin (HE), and used for observing blood vessels, and measuring the volume of cavities in the spinal cord. For immunohistochemistry, sections were washed 3 times with PBS, and blocked with a 0.1% bovine serum albumin solution containing 0.1% Tween 20 in PBS for 30 min. Sections were then incubated overnight in a solution containing primary antibodies as follows: anti-p75NGFR (Chemicon, Cat. No. MAB365; 1:500 in 0.1 M PBS pH 7.4) for olfactory ensheathing cells, anti-glial fibrillary acidic protein (GFAP) monoclonal antibody (1:300; Sigma) for astro- cytes, and anti-neurofilament 200 kD rabbit polyclonal antibody (1:100; Chemicon) for axons. After washing, the sections were incubated overnight with secondary antibodies as follows: FITC- or Cy-3-labeled anti-mouse IgG antibody (1:1000; Amersham Biosciences) for astrocytes, or Cy-3-labeled anti-rabbit IgG antibody (1:1000; Amersham Biosciences) for axons. Sections were then mounted and examined by a fluorescence microscope (Axio Imager MI; Carl Zeiss).

All experimental procedures were approved by the Animal Ethics Committees of the Osaka University Medical School.

3. Results

The averaged BBB scores of the olfactory mucosa transplanted rats ($n = 5$) were 3.13 ± 1.12, 5.25 ± 1.21, 6.88 ± 1.34, and 10.83 ± 1.23, measured 1, 2, 4, and 8 weeks after transplantation, respectively. The averaged BBB scores of the respiratory mucosa transplanted rats ($n = 5$), measured over the same time frame, were 2.2 ± 0.84, 2.8 ± 1.15, 3.5 ± 1.02, and 4.0 ± 0.71. These data indicate that the recovery of hind limb movement in the olfactory

mucosa transplanted rats improved significantly in comparison to the control, respiratory mucosa transplanted rats ($p < 0.05$) (**Figure 1**). In the histological assessment, expression of neurofilament was observed strongly at the injury site in the olfactory mucosa transplanted rats (**Figures 2** and **3**). The numerous fibers that were strongly stained with neurofilament were surrounding the GFP-positive cells and penetrating the transplanted olfactory mucosa (**Figure 3**). In contrast, there were no apparent neurofilament stained fibers at the marginal spinal cord of the respiratory mucosa transplanted rats (**Figure 4**).

4. Discussion

Injuries to the central nervous system (CNS) in humans are usually associated with a low degree of neurological recovery and, in the majority of cases, life-long debilitation. This lack of recovery, however, is not due to any intrinsic inability of CNS axons to regenerate; rather, the environment of the CNS is strongly inhibitory to axonal regeneration. Following SCI, astroglial scars form within lesioned areas of the spinal cord [1]. Although the majority of known inhibitors of neurite outgrowth are myelin membrane proteins, equally potent inhibitors have also been identified in astroglial scars, for example, chondroitin sulfate proteoglycans and semaphoring 3A.

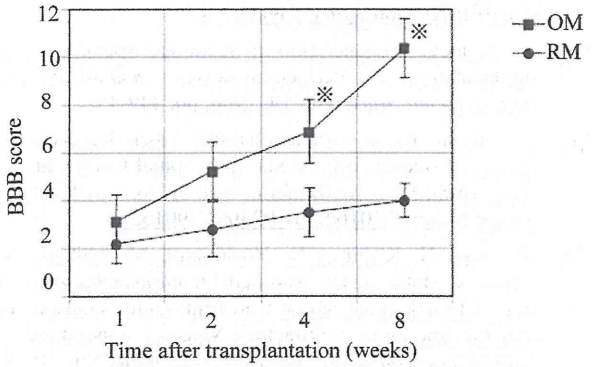

Figure 1. A significantly greater degree of functional recovery as measured by hindlimb usage was observed in the olfactory mucosa transplanted rats (OM) compared with the respiratory mucosa transplanted rats (RM) 4 weeks after the transplantation (※). BBB: Basso, Beattie, and Bresnahan locomotor rating scale.

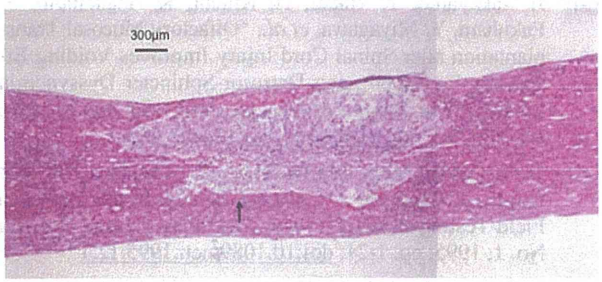

Figure 2. Histology (HE). A transplanted mucosa (indicated by an arrow) is recognizable in the contused spinal cord.

Figure 3. Immunohistological study. Numerous fibers (arrow), strongly stained with neurofilament, are seen penetrating the transplanted olfactory mucosa (a); The fibers surround the GFP-positive cells (b).

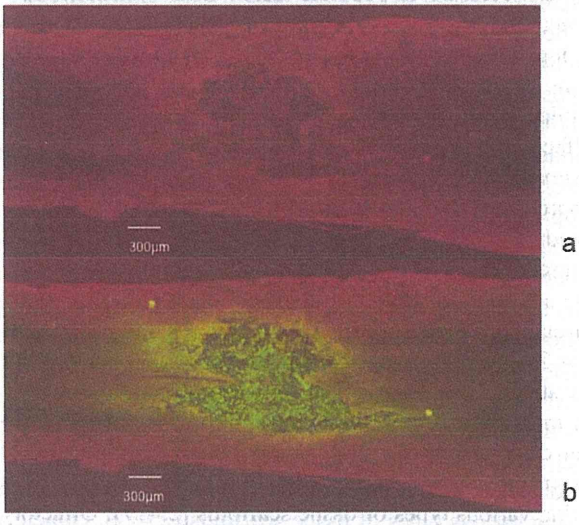

Figure 4. Immunohistological study. No apparent fibers stained with neurofilament are found in the respiratory mucosa (GFP-positive) transplanted spinal cord.

Manipulating the local environment in order to provide a favorable scaffold, supportive of axonal regeneration, is one of the more promising strategies for treatment of SCI. Spinal cord reconstruction using implantation of cells from various sources has been gaining attention in recent years [11,12]. Neuronal stem cells have the potential to differentiate into both neuronal and glial cells, and are therefore prime candidates for cell replacement therapy following CNS injury. Neuronal stem cells constitutively

secrete significant quantities of several neurotrophic factors that act to support host axonal regeneration after SCI [13]. Partial restoration of function after contusion of the spinal cord has been accomplished by injecting neural/glial precursors (NSCs), differentiated in vitro from mouse embryonic stem cells, into the lesion 9 days after injury [14]. However, implantation of NSCs alone did not produce any significant restorative effect because the majority of the NSCs grafted into the spinal cord differentiated with an astrocytic phenotype [13,15]. Although astrocytes can secrete neurotrophic factors and limit the extent of the inflammatory reaction, extensive astroglial scarring within the lesioned area blocks axon growth.

However, one of the major disadvantages associated with implantation or injection of cells alone is the limited proportion of viable cells surviving in the injury site after the procedure, as cells tend to migrate away from the injury site [16]. To achieve significant functional reconstruction of the spinal cord after spinal cord injuries, it is either necessary to populate lesion sites with tissue-specific, regeneration-competent cells that replace or rescue dying cells, or to activate endogenous neural progenitor cells that do likewise [17]. In this study, numerous neurofilaments were observed strongly in the transplanted olfactory mucosa. Unlike respiratory mucosa, it permits axonal regeneration after SCI and therefore may be an appropriate scaffold on which to reconstruct axons. Indeed, the olfactory mucosa is an excellent autologous source of adult neuronal precursor cells. The neurons and the sustentacular cells there renew themselves constantly throughout life by proliferation of basal global stem cells [18-20]. Furthermore, the mucosa contains olfactory ensheathing cells, which have previously been the subject of much attention for their potential in the repair of spinal cord injuries [21-24]. Recent studies of spinal cord axon regeneration have reported good long-term results using various types of tissue scaffolds [25-27]. Olfactory tissue would allow autologous transplantation, is easily accessible, and can be obtained by a simple biopsy that is performed through the external nares [28]. These considerations, combined with the results of the present study, make nasal mucosa an attractive potential scaffold for axonal regeneration.

5. Conclusions

As we have previously reported, olfactory mucosa transplantation following spinal cord injury can support at least partial hind limb motor recovery. In this study, we identified numerous axons surrounding the transplanted cells, and penetrating the mucosa at the transplant site without marginal spinal white matter. Olfactory mucosa might therefore be a more suitable scaffold for axonal regeneration than white matter, which contains inhibiting factors for axonal regeneration in the spinal cord.

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Involuntary muscle spasm expressed as motor evoked potential after olfactory mucosa autograft in patients with chronic spinal cord injury and complete paraplegia

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ABSTRACT

Object: The efficacy of olfactory mucosa autograft (OMA) for chronic spinal cord injury has been reported. New activity in response to voluntary effort has been documented by electromyography (EMG), but the emergence of motor evoked potential (MEP) reflecting electrophysiological conductivity in the central nervous system, including the corticospinal pathway, after OMA, and the best indications for OMA, have not been clarified. Here, we report the emergence of MEPs after OMA and offer recommendations for appropriate indications based on the presence of involuntary muscle spasm (IMS). We used analysis of MEP to examine the efficacy of OMA for patients with complete paraplegia due to chronic spinal cord injury. To clarify the indications for OMA, we investigated the association of IMS and efficacy of OMA. Methods: Four patients, 3 men and 1 woman, were enrolled. The mean age of the cases was 30.3 ± 9.5 years (range, 19 to 40 years). All 4 cases were American Spinal Injury Association (ASISA) grade A. The mean duration from injury to OMA was 95.8 ± 68.2 months (range, 17 to 300 months). Samples of olfactory mucosa were removed, cut into smaller pieces, and grafted into the sites of spinal cord lesions after laminectomy. Postoperative subcutaneous fluid collection, postoperative meningitis, postoperative nosebleed, postoperative infection in the nasal cavity, impaired olfaction, neoplastic tissue overgrowth at the autograft site, new sensory disturbance, and involuntary muscle spasm were investigated as safety issues. Improvements in ASIA grade, variations in ASIA scores, EMG, SSEP, and improved urological function were evaluated as efficacy indicators. Results: There were no serious adverse events in this series. In 2 of the 4 cases, an improvement in motor function below the level of injury was recognized. In one, the motor score was 50 until 16 weeks after surgery, and it increased to 52 from 20 weeks after surgery. In the other, the motor score was 50 until 20 weeks after surgery, and it increased to 52 at 24 weeks after surgery with a further increase to 54 at 48 weeks after surgery. The emergence of MEP was recognized in the latter case at 96 weeks after surgery. The other 2 cases had no improvement in ASIA motor score. Both of these cases who showed improvements in the ASIA motor scores exhibited relative IMS compared with those who had no ASIA motor score recovery. Conclusions: We recognized the emergence of MEPs in a case with complete paraplegia due to chronic spinal cord injury after OMA. IMS might be a candidate of indication of OMA.

Keywords: Olfactory Mucosa Autograft; Spinal Cord Injury; Transplantation; Voluntary Movement; Motor Evoked Potential

1. INTRODUCTION

The olfactory mucosa is an excellent autologous source of adult neuronal precursor cells. The neurons and sustentacular cells of the olfactory mucosa constantly renew themselves throughout life by proliferation of basal global stem cells [1-3]. Furthermore, the mucosa contains olfactory ensheathing cells, which have previously received much attention for their potential application in

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the repair of spinal cord injuries (SCIs) [4-7]. Recent studies of spinal cord axon regeneration have reported good long-term results using various types of tissue scaffolds [8-10]. Olfactory tissue, which allows autologous transplantation, is easily accessible, and can be obtained by a simple biopsy performed through the external nares [11].

We have previously reported that grafts of the olfactory mucosa are effective in restoring functional recovery in rats following spinal cord transection, with histological evidence of neuronal regeneration [12-14]. Lima *et al.* performed a clinical trial of olfactory mucosal autograft (OMA) in humans with chronic traumatic SCI and reported restoration of voluntary electromyography (EMG) responses in 15 of 20 cases (75%) and mean American Spinal Injury Association (ASIA) motor score improvement of 4.95 ± 7.1 over a mean follow-up period of 27.7 months. The range of improvement was various, and some cases demonstrated no response to OMA. Therefore, evaluating the possible factors that could predict the efficacy of OMA will be useful. Inclusion criteria for the study by Lima *et al.* included age, extent of the lesion, and time from the injury, but the authors did not assess the neuronal condition of the severed caudal spinal cord [15]. Furthermore, they did not assess the emergence of motor evoked potential (MEP), which reflects electrophysiological conductivity in the central nervous system, including the corticospinal pathway [16,17], after OMA.

The emergence of involuntary muscle spasm (IMS) after SCI is an indirect measure of the recovery of motor neurons and general motor function [18]. It may indicate plasticity of the spinal cord and the potential for successful regenerative interventions in patients with chronic SCI.

Our pilot study was conducted to examine the emergence of MEP and IMS in OMA patients with chronic SCI and complete paraplegia.

2. MATERIAL & METHODS

2.1. Patient Selection and Inclusion Criteria

This phase I/II nonrandomized, non-controlled, prospective study was approved by the Ethical Committee of the Osaka University Medical School in Osaka, Japan. All procedures were performed after obtaining written informed consent, which included permission to culture and analyze a biopsy from the tissue to be grafted. Patients were fully aware of the experimental nature of the treatment, the uncertain outcomes, and possible side effects including pain, spasticity, autonomic dysreflexia, worsening of motor or sensory function, infection, and unforeseen adverse events.

Patients who had sustained SCI more than 6 months previously with chronic paraplegia (**Table 1**) were included. Our rationale for selecting chronic SCI patients (more than 6 months from injury) was to circumvent the spontaneous recovery bias [19]. The other inclusion criteria of this study were generally consistent with those of Lima *et al.* [15] and comprised ASIA Ggrade A or B; age ≥ 7 and ≤ 40 years; presence of a spinal cord lesion ≤ 3 cm; absence of significant nasal and paranasal sinus pathology; and absence of additional serious medical problems including respiratory disturbance, brain disease, or psychological disturbance.

Four patients were enrolled in the study, 3 males and 1 female. Demographic data, clinical findings, and imaging/radiological characteristics of the patients are presented in **Table 1**. The mean age of the patients was 30.3 ± 9.5 years (range, 19 to 40 years). Injuries were due to traffic accidents in 2 patients, fall in 1 patient, and hemorrhage of unknown origin in 1 patient. The mean maximum lesion size on the vertical axis as measured on both the T1- and T2-weighted MRI was 2.25 ± 0.57 cm (range, 1.55 to 2.94 cm). All 4 patients were ASISA grade A. The mean time from injury to OMA was 95. 8 ± 68.2 months (range, 17 to 300 months).

2.2. Transplantation Protocol and Surgical Procedure

Our procedure essentially followed that reported by Lima *et al.* [15,20]. Samples of olfactory mucosa were removed, cut into smaller pieces, and grafted into the spinal cord lesion site after laminectomy. Microbiological examinations of the nasal cavities were performed routinely before surgery and during the operation just prior to transplantation.

Table 1. Summary of demographic and clinical characteristics of 4 patients with olfactory mucosa autografts (OMA).

Case No. (years)	Age at OMA	Sex	Months Post-SCI	SCI Level	Length of Lesion	AIS Grade
1	40	Male	300	T4-5	2.2	A
2	19	Female	30	T7-9	2.3	A
3	26	Male	17	T12	1.55	A
4	36	Male	36	T7-8	2.94	A

Abbreviations: SCI, spinal cord injury; T, thoracic; AIS, ASIA Impairment Scale.

