厚生労働科学研究費補助金（難治性疾患克服研究事業）
プリン病２次感染に対する現実的滅菌法の開発研究
平成１９年度　分担研究報告書

SDS処理によるステンレス刃の電顕的検討

分担研究者：　秋田定伯　　長崎大学医学部・歯学部附属病院・助教

研究要旨

プリオン病感染後の二次対策として、鋭利は先端の手術器具の３％ドデシル硫酸ナトリウム水溶液煮沸消毒後の形態変化を最大５０回まで検討した。手術で頻用されるメス刃、１８，２２，２３ゲージのカヌラ針を用いて、電顕での形態変化を観察したところ、経時的に堆積物、付着物の増加を認め、針では内腔の狭いものでより顕著であった。刃、針先の摩耗は認めず、３％ドデシル硫酸ナトリウム水溶液煮沸消毒の手術器具への直接的な機械的変化はないと推察された。今後、より定量的な解析を必要とするが、手術器具の３％ドデシル硫酸ナトリウム水溶液煮沸消毒は鋭利なものであっても有用であると思われた。

A．研究目的

プリオン病感染後の二次対策として、特に感染者手術時に使用された場合でも再利用する可能性の高い持針器、メスホールダに直接接する、メス刃、針の３％ドデシル硫酸ナトリウム水溶液煮沸消毒後の形態変化について電子顕微鏡を用いて観察検討する。

B．研究方法

３％ドデシル硫酸ナトリウム水溶液でメス刃、１８ゲージ、２２ゲージ、２３ゲージ　カヌラを煮沸消毒する。検討したカヌラ、刃は各々①NN-1838R　刃の形状 R・B 針の長さ 1 1/2　(1.20 ×38mm)、テルモ社製、②NN-2238R　刃の形状 R・B 針の長さ 1 1/2(0.70 × 38mm) 、テルモ社製、③No.11 メス、Feather 社、２５Gy gannma irradiated、④JMS　注射針　２３G　R.B　(0.6 × 25mm)であった。

煮沸時間が５分間、直ぐに水洗・乾燥させる。その回数を１０，２０，３０、４０、５０回とする。対照１として、蒸留水で５分間煮沸消毒、各々テイッシュペーパー上で５分乾燥したものを用意し、対照２としてサンドペーパーで５０回スクラッチ下ものを準備した。

電顕試料ホルダーにセットアップのためメスの刃、及び注射針は適切な長さに切断する。

検鏡位置の損傷を回避するため切断位置の両端をクランプ固定し切断する。

また、実体顕微鏡で事前に表面を確認し、切断位置の確認、切断する衝撃による表面付着物の剥離を無くすため慎重に切断する。

検鏡には走査電子顕微鏡（FIELD EMISSION SCANNING ELECTORON

MICROSSOPE)
型式：JSM-6700F/IV（JEOL,日本電子）：高電圧 15kV、エミッション電流 10μA　使用した。

（倫理面への配慮）
本研究はプリオン病感染後の手術器具を３％ドデシル硫酸ナトリウムを用いて、煮沸創毒後の主に形態変化を検討しており、研究対象は手術時に使用する鋭利な刃物などであり、倫理的問題はないと思われる。

C．研究結果
３％ドデシル硫酸ナトリウム煮沸により、経時的な付着物、堆積物が注射針、刃に付着した。内腔の狭い方が、付着物が多い。刃の先端には付着物はあるものの、摩耗物はなかった。針の先端が摩耗した場合堆積物の付着は多量となった。

D．考察
３％ドデシル硫酸ナトリウム煮沸により、メス刃、１８，２２，２３ゲージ針の形状は付着物、堆積物の沈着を認めたものの、刃および針先の摩耗は認めなかった。また、機械的な刺激で先端を摩耗した場合（対照２）堆積物の付着はより多く認められた。また、３％ドデシル硫酸ナトリウム煮沸消毒後の堆積物の付着を防ぐ方法が検討されるべきである。

E．結論
今後、定量的解析を必要とするものの、５０回までの３％ドデシル硫酸ナトリウム煮沸消毒単独では、メス刃、針など鋭利な手術器具の形態変化は電顕上問題ないと推測された。

F．健康危機情報
特記事項なし

G．研究発表
１．論文発表

１．Akita S, Akino K, Yakabe A, Imaizumi T, Tanaka K, Anraku K, Yano H, Hirano A. Combined surgical excision and radiation therapy for keloid treatment. J Craniofac Surg, 18: 1164-1169, 2007

２．攝田麻里、安倍久仁子、林徳真吉、木下直江、今泉敏史、野中大樹、皆川知広、秋田定伯、上谷雅孝、平野明喜
多毛を伴った fibrous hamartoma of infancy の一例
臨床皮膚科　61: 263-266, 2007

３．諸藤陽一、松尾孝之、竹下朝規、林健太郎、黒川季代子、秋田定伯、永田　泉
開頭術と顔面神経側頭枝損傷に対する神経意匠術を一期的に行った症例
Neurosurgical Emergency 12: 100-106, 2007

４．Imaizumi T, Akita S, Akino K, Hirano A
Acceleration of sensory neural regeneration and would healing with human mesenchymal stem cells in immunodeficient rats. Stem Cells, 25: 2956-2963, 2007

5．Nakashima M, Yano H, Akita S, Tokunaga K, Anraku K, Tanaka K, Hirano A.Traumatic unilateral temporomandibular joint dislocation overlooked for more than two decades. J Craniofac Surg, 18: 1466-1470, 2007

6．秋田定伯
臨床各領域の動向 2006-2007
「形成外科」
治療　89: 107-110, 2007

7．秋田定伯
ＦＧＦ製剤の治療効果について
PEPARS 16: 54-63, 2007

8．秋田定伯
FGF による皮膚損傷修復作用
細胞　39: 352-358, 2007

9．Akita S, Akino K, Hirano A, Ohtsuru A, Yamashita S. Reconstruction for local radiation injuries and proposed regeneration therapy for acute systemic radiation injuries.
Radiation Risk and Perspectives edited by Shibata Y, Namba H, Suzuki K and Tomonaga M. Elsevier, International Congress Series 1299, Amsterdam, 2007, Pp. 196-202, total 298 pages.

10. 秋田定伯、秋野公造、今泉敏史、田中克己、安楽邦明、矢野浩規、平野明喜
広範囲熱傷患者における血中白血病抑制因子の上昇
日本熱傷学会会誌、印刷中、2007 年

11. Akita S, Akino K, Tanaka K, Anraku K, Hirnao A. A basic fibroblast growth factor improves lower extremity wound healing with a porcine-derived skin substitute. J Trauma, in press

12. Akino K, Akita S, Yakabe A, Mineda T, Hayashi T, Hirano A. Human mesenchymal stem cells may involve in keloid pathogenesis. Int J Dermatol., in press.

２．学会発表

1. 秋田定伯、平野明喜
線維芽細胞増殖因子―２は下腿創傷治癒の質を改善する
第 50 回日本形成外科学会　（口演）
（東京）、4 月、2007 年

2. Akita S, Akino K, Hirano A.
Fibroblast growth factor-2 improves the quality of lower extremity wound healing.
2007 Wound Healing Society Annual Meeting, Tampa, April-May, 2007.

3. Yakabe A, Akita S, Fujioka M, Hirano A.
Easy and objective skin color analysis in plastic surgery
2007 Wound Healing Society

Annual Meeting, Tampa, April-May, 2007.

4. 秋田定伯
ケロイドの国際分類比較
第2回瘢痕・ケロイド治療研究会 【パネルディスカッション】《ケロイドの分類》、2007年 8月、東京

5. Akita S
Novel therapeutic approach using reconstructive surgery, cytokine and human bone-marrow derived mesenchymal stem cells. Scientific-Practice Conference "Cell technologies and regenerative medicine", Saint Petersburg State Medical Institution "Pokrovskaya Municipal Hospital", Invited Lecture, September 24, St. Petersburg, 2007

6. Akita S
Stem cell therapy and Plastic surgical management for local radiation injury. 2nd International Seminar-"Radiation Medicine in Research and Practive", Wuerzburg, Germany, Invited Symposium, October 4-5, 2007

7. 秋田定伯
座談会 Part II～何故、私はこの研究をしているのか～
第16回日本形成外科学会（神戸）、10月、（企画、発表、座長）、2007年

8. 秋田定伯、秋野公造、平野明喜
創傷治癒促進から瘢痕改善へ連動するヒト間葉系幹細胞
第16回日本形成外科学会（神戸）、10月、2007年

9. 秋田定伯、平野明喜
5HT2A 受容体拮抗薬を血管化結合織膜弁に用いた下肢虚血モデルでの血流回復と創傷治癒促進
第16回日本形成外科学会（神戸）、10月、2007年

10. 秋田定伯、矢加部 文、秋野公造、平野明喜
皮膚色差解析にいる術前後、カラーマッチの検討
16回日本形成外科学会（神戸）、10月、2007年

11. 秋田定伯
創傷治癒促進から瘢痕改善へ関連する間葉系幹細胞
第37回 日本創傷治癒学会 主題演題、12月、2007年

H. 知的財産権の出願・登録状況
（予定も含む。）
1.特許取得
無し
2.実用新案登録
無し
3..その他
無し

研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

(2007年4月1日ー2008年3月31日迄)

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
Akita S, Akino K, Hirano A, Ohtsuru A, Yamashita S	Reconstruction for local radiation injuries and proposed regeneration therapy for acute systemic radiation injuries.	Shibata Y, Namba H, Suzuki K and Tomonaga M.	Radiation Risk and Perspectives, Internatioal Congress Series 1299	Elsevier	Amsterdam	2007	196-202, total 298 pages.

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
Shiga Y, Satoh K, Kitamoto T, Kanno S, Nakashima I, Sato S, Fujihara K, Takata H, Nobukuni K, Kuroda S, Takano H, Umeda Y, Konno H, Nagasato K, Satoh A, Matsuda Y, Hidaka M, Takahashi H, Sano Y, Kim K, Konishi T, Doh-Ura K, Sato T, Sasaki K, Nakamura Y, Yamada M, Mizusawa H, Itoyama Y	Two different clinical phenotypes of Creutzfeldt-Jakob disease with a M232R substitution	J Neurol.		(in press)	2007

Noguchi-Shinohara M, Hamaguchi T, Kitamoto T, Sato T, Nakamura Y, Mizusawa H, Yamada M	Clinical features and diagnosis of dura mater graft associated Creutzfeldt-Jakob disease	Neurology	69	360-367	2007
Shin RW, Ogino K, Shimabuku A, Taki T, Nakashima H, Ishihara T, Kitamoto T	Amyloid precursor protein cytoplasmic doma in with phospho- Th668 accumulates in Alzheimer's disease and its transgenic models: a role to mediate interaction of Abeta and tau	Acta Neuropathol	113	627-636	2007
Hamaguchi T, Noguchi-Shinohara M, Nakamura Y, Sato T, Kitamoto T, Mizusawa H, Yamada M	Ophthalmic surgery in prion diseases	Emerg Infect Dis	13	162-164	2007
Kobayashi A, Asano M, Mohri S, Kitamoto T	Cross-sequence transmission of sporadic Creutzfeldt-Jakob disease creates a new prion strain	J Biol Chem	282	30022-30028	2007
Iwasaki Y, Mimuro M, Yoshida M, Hashizume Y, Ito M, Kitamoto T, Wakayama Y, Sobue G	Enhanced Aquaporin-4 immunoreactivity in sporadic Creutzfeldt-Jakob disease	Neuropathology.	27	314-323	2007
Yoshioka M., Murayama T., Miwa T., Miura K., Takata M., Yokoyama T., Nishizawa K. and Mohri S	Assessment of Prion Inactivation by a Combined Use of 4 *Bacillus*-derived Protease and SDS	Biochemistry & Molecular Biology	Oct;71(10)	2565-8	2007
Murayama Y., Yoshioka M., Okada H., Takata M., Yokoyama T. and Mohri S	Urinary Excretion and Blood Level of Prions in Scrapie Infected Hamsters	Journal of General Virology.	Vol.88	2890-2898	2007
Suyama K., Yoshioka M., Akagawa M., Murayama Y., Horii H., Takata M., Yokoyama T. and Mohri S.	Assessment of Prion Inactivation by Fenton Reaction Using Protein Misfolding Cyclic Amplification and Bioassay	Bioscience, Biotechnology, and Biochemistry	vol.71(8)	2069-2071	2007

Suyama K., Yoshioka M., Akagawa M., Murayama Y., Horii H., Takata M., Yokoyama T. and Mohri S	Prion inactivation by the Maillard reaction	Biochem Biophys Res Commun	vol.356	245-248	2007
Akita S, Akino K, Yakabe A, Imaizumi T, Tanaka K, Anraku K, Yano H, Hirano A	Combined surgical excision and radiation therapy for keloid treatment	J Craniofac Surg	18	1164-1169	2007
Imaizumi T, Akita S, Akino K, Hirano A	Acceleration of sensory neural regeneration and would healing with human mesenchymal stem cells in immunodeficient rats	Stem Cells	25	2956-2963	2007
Nakashima M, Yano H, Akita S, Tokunaga K, Anraku K, Tanaka K, Hirano A	Traumatic unilateral temporomandibular joint dislocation overlooked for more than two decades	J Craniofac Surg,	18	1466-1470	2007
秋田定伯	臨床各領域の動向2006-2007	「形成外科」	治療 89	107-110	2007
秋田定伯	ＦＧＦ製剤の治療効果について	PEPARS	16	54-63	2007
秋田定伯	FGFによる皮膚損傷修復作用	細胞	39	352-358	2007
秋田定伯、秋野公造、今泉敏史、田中克己、安楽邦明、矢野浩規、平野明喜	広範囲熱傷患者における血中白血病抑制因子の上昇	日本熱傷学会会誌		印刷中	2007
Akita S, Akino K, Tanaka K, Anraku K, Hirnao A	A basic fibroblast growth factor improves lower extremity wound healing with a porcine-derived skin substitute	J Trauma		in press	
Akino K, Akita S, Yakabe A, Mineda T, Hayashi T, Hirano A	Human mesenchymal stem cells may involve in keloid pathogenesis	Int J Dermatol		in press.	

研究成果の刊行物・別刷

International Congress Series 1299 (2007) 196–202

ELSEVIER

www.ics-elsevier.com

Reconstruction for local radiation injuries and proposed regeneration therapy for acute systemic radiation injuries

Sadanori Akita[a,*], Kozo Akino[b], Akiyoshi Hirano[a], Akira Ohtsuru[c], Shunichi Yamashita[c,d,e,f]

[a] *Department of Plastic and Reconstructive Surgery, Nagasaki University, Graduate School of Biomedical Sciences, 1-7-1 Sakamoto, Nagasaki 8528501, Japan*
[b] *Department of Anatomy and Neurobiology, Nagasaki University, Graduate School of Biomedical Sciences, Japan*
[c] *Takashi Nagai Memorial Int. Hibakusha Medical Center, Nagasaki University Hospital, Japan*
[d] *Department of Molecular Medicine, Atomic Bomb Disease Institute, Nagasaki University, Japan*
[e] *Department of International Health and Radiation Research, Nagasaki University Nagasaki, Japan*
[f] *Department of Public Health and Environment, World Health Organization, Geneva, Switzerland*

Abstract. Skin and soft tissue radiation injuries are life-threatening as well as of esthetic concern in local tissues. As for local radiation injuries, clinically developed procedures such as local flaps and free vascularized flaps result in better and less-invasive reconstructive outcomes for victims of therapeutic radiation of malignant tumors, prolonged fluoroscopic procedures for cardiovascular diseases or possibly direct contact with radioactive waste, while systemic radiation injuries should find other solutions. In systemic radiation injuries, there is a good chance to save lives when the initial treatment is efficient, although donors of the skin grafting after debridement of the radiation-damaged tissue removal are unrealistically autogenous. Thus, exceeding certain extent and degree, systemic radiation injury-induced burns were not the medical objectives. Recent understanding and application of use in adult stem cells, however, create a new insight for severe systemic radiation injuries. Among the adult stem cells, the human mesenchymal stem cells are able to be freeze-dried, easily thawed and able to be cultured in vitro. A basic fibroblast growth factor is already of clinical use and efficient for the human mesenchymal stem cell growth both in vitro and in vivo. An artificial dermis is useful for temporal coverage of the raw surface of severe bedsores. The combined use of these successfully regenerates both dermis and epidermis after 20 Gy whole body irradiation in a

* Corresponding author. Tel.: +81 95 849 7327; fax: +81 95 849 7330.
E-mail address: akitas@hf.rim.or.jp (S. Akita).

0531-5131/
doi:10.1016/j.ics.2006.09.017

nude rat model. Therefore, even severe radiation injuries, local tissue management and therapy are well-handled and systemic radiation injury may be hopeful with understanding and the specialized practice of regenerative medicine. © 2007 Published by Elsevier B.V.

Keywords: Local and systemic radiation injury; Human mesenchymal stem cell; Basic fibroblast growth factor; Artificial dermis; Regenerative medicine

1. Introduction

There are increasing concerns about both systemic and local radiation injuries, possibly caused by nuclear power plant accidents, therapeutic irradiation for malignant tumors or prolonged fluoroscopic procedures for cardiovascular diseases. These should be treated properly for the sake of life-saving and improved wound healing [1–4].

A plastic surgeon is one of the surgical sub-specialists, who deals with the devastating soft and hard tissue wounds and is always concerned about the quality of the healed wounds. Recent innovations lead to better reconstructive surgical options for difficult wound-healing and more realistic regenerative medicine may be applicable for systemic radiation injures, which may be impossible for rescue with conventional medical and surgical modalities. Plastic surgery procedures such as skin grating, local flap and free vascularized flap are carefully selected on a case-by-case basis for local radiation injuries. Although the therapeutic effect of local irradiation can control malignant tumors, it also alters the remaining normal structure of the surrounding tissues in both a permanent and progressive manner. This includes a loss of healing, skin atrophy, fibrosis, altered micro-circulation and potential necrosis. Also interventional procedures under fluoroscopy are increasing because more radical procedures are adopted as more precise and accurate diagnosis is required for intervention. The characteristics of the sustained fluoroscopic tissue injury are unrecognized and under-treated.

For systemic acute radiation injuries, immediate and precise diagnosis of the extent and the degree of the affected tissues should be elucidated. Re-surfacing the injured skin and soft tissues are always the great concern and if autogenous skin is not sufficient enough for coverage, other modalities such as allogeneic skin grafting or the more advanced therapies with adult stem cells may necessitate acute systemic radiation injuries of the skin and soft tissues. We, therefore, demonstrate the efficacy of the plastic surgical modalities in local radiation injuries and investigate the possibility of the use of human adult stem cells (human mesenchymal stem cells) against the severe radiation injury models. Here the roles of plastic surgery in emergency radiation thus far are reviewed and the futuristic possibility of integration of regenerative medicine in this field is also discussed.

2. Materials and methods

2.1. Patients and methods for local radiation injuries

From January 1990 to April 2004, 9 (8 females and 1 male) patients who demonstrated radiation injuries such as telangiectasia, xerosis, epidermal atrophy karatoses, and fibrosis

as well as deep ulcers in the costal ribs and sternum, were surgically treated and included in this investigation.

2.2. Materials and methods for systemic radiation injuries

2.2.1. Human mesenchymal stem cells

Human mesenchymal stem cells from a single human bone-marrow donor were isolated by density gradient centrifugation and strictly sorted as positive for markers such as CD105, CD166, CD29 and CD44, and negative for cell surface markers such as CD14, CD34 and CD45. Human mesenchymal stem cells (hMSCs) were purchased from BioWhittaker, Inc. (Cat # PT-2501, Walkersville, MD, USA) and the cryopreserved cells were thawed immediately according to the manufacturer's instructions. Two different donor-derived hMSCs, whose gender were female and whose racial backgrounds were Caucasian (Lot # 1F0658 and 1F1061), were used in the experiments. The cells were cultured in "basic medium" of Dulbecco's modified Eagle Medium (DMEM) containing low glucose supplemented with 10% fetal bovine serum (FBS, heat-inactivated, cat # 16000-044, GIBCO, Invitrogen™, Life Technologies, Japan K.K.), 200 mM L-glutamine, and penicillin (100 U/mL) and streptomycin (100 μg/mL) at 37 °C in 95% humidified air and 5% CO_2. The medium was exchanged every 3 days until the cells were confluent, then the cells were passaged up to 4 times. The growth characteristics during the four passages in FBS were indistinguishable. The cells were washed using 10 mL of phosphate-buffered saline (PBS) and then liberated by exposure to 0.25% trypsin/1mM EDTA (GIBCO, cat # 25200-056) for 3 min at 37 °C, followed by tapping of the dishes and the addition of 5 mL of culture medium. The cells were centrifuged at 400×*g*, and then re-suspended in basic medium for the following in vitro examinations. The other cells were stored at −70 °C until use in a solution containing 5% human serum albumin (IS Japan, Co., Ltd., Saitama, Japan, Cat # 9988) and 10% dimethylsulfoxide (DMSO, Sigma-Aldrich Japan K.K., Tokyo, Japan, Cat # 41641) according to the manufacturer's instruction. The hMSCs were cell counted by a Beckman Coulter® cell and particle counter (Beckman Coulter KK., Tokyo, Japan). After cell counting, the pellets were dissolved in total amount of 100 μL of culture medium 30 min before in vivo use [5].

2.2.2. Animals and whole body irradiation by X-ray generator

Animals aged 10 weeks and weighing 200–300 g, were used. Animals were obtained from CLEA JAPAN (Tokyo, Japan), housed in the laboratory animal centre for biomedical research, Nagasaki University School of Medicine (Nagasaki, Japan), and the protocol of the animal experiment was approved by the Institutional Animal Care and Use Committee of Nagasaki University, no. 0204080111. They were handled according to the guidelines established for animal care at the centre. Each rat had free access to both sterile water and standard rodent soft chow *ad libitum* [6].

20 Gy whole body irradiation to 10 nude rats (F344/NJCl-rnu), which have deleted T-cell function and thus acute immune rejection to human derived cells is minimized, were performed at the Atomic Bomb Disease Institute, Nagasaki University, by an X-ray radiation generator (EXS-300-5, Toshiba, 200 kV, 15 mA, 0.405 Gy/min). Animals were divided into two groups of five each, control group and hMSCs treated group; surgical procedures were performed immediately after irradiation.

2.3. The basic fibroblast growth factor, Fiblast®

Genetically recombinant human bFGF (Fiblast®, Trafermin) was purchased from Kaken Pharmaceutical Co. (Tokyo, Japan). The freeze-dried samples were dissolved in PBS at a concentration of 1 mg/mL and dissolved in culture medium 30 min before in vivo use.

2.3.1. The bilayer dermal substitute, Pelnac®

The bilayer dermal substitute, Pelnac®, was kindly provided by Gunze (Kyoto, Japan). This dermal substitute has two layers: the inner layer is made from pig tendon-derived atelocollagen, which is freeze dried at −135 °C, and the outer layer is made from a silicone polymer of 150 μm thickness [7]. The Pelnac® was soaked in DMEM containing bFGF with or without hMSCs.

2.3.2. Surgical procedures

In an aseptic surgical room and with sterile surgical instruments, skin tissue of 3×3 cm^2, including the panniculus carnosus, was excised from F344/NJCl-rnu nude rats, and subsequently from the artificial dermal substitute impregnated with 10 μg bFGF with or without the hMSCs and, after anaesthetizing with a 40-mg kg^{-1} intra-peritoneal injection of pentobarbital sodium, U.S.P. (Nembutal; Abbot Laboratories, North Chicago, IL, U.S.A.). Five microliters of DMEM containing hMSCs and bFGF were added to the inner layer of the same-sized (3×3 cm^2) Pelnac® for 30 s at room temperature. The wound was covered with the impregnated artificial dermis and sutured using 5-0 nylon. Each animal was then housed in its own cage to avoid damaging the dermis.

The control group (medium and bFGF) received 5 μL of DMEM. The hMSC treatment group received 5 μL of DMEM. The experimental treatment group received 5 μL of DMEM containing 5×10^6 hMSCs and 10 μg of bFGF at 30 min before transplantation for each wound defect (Fig. 1).

2.3.3. Histology

After obtaining the fixed tissues, 5-μm thickness slides were stained with hematoxylin and eosin. The staining samples were tested from each tissue.

3. Results

3.1. Treatment for local radiation injuries

All surgeries were uneventfully performed. The mean postoperative follow-up was 9 years and 3 months (1 to 14 years). The average age was 65 years (51 to 75 years). Eight female patients received radiation therapy after mastectomy ranging from 35 Gy to 50 Gy (mean 46.1 Gy) administered in fractions. One male patient underwent percutaneous transluminal coronary angioplasty (PTCA) under fluoroscopy, which was unexpectedly prolonged. The time to reconstructive surgery averaged 19 years and 8 months (4 to 30 years). Some patients had more than one operation, and the average number was 1.8 (1 to 4 surgeries). Rectus abdominis myocutaneous flaps were used for 4 cases and one of which had a free vascularized flap. Latissimus dorsi myocutaneous flaps were used for

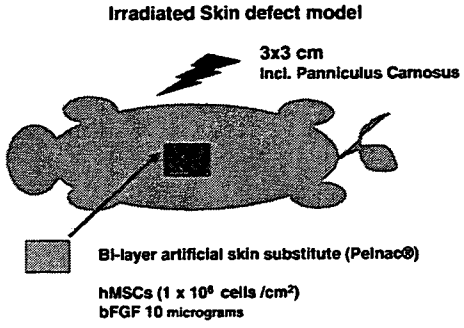

Fig. 1. Schematic drawing of the human mesenchymal stem cell transplantation to a nude rat.

4 cases. One of the latissimus dorsi myocutaneous cases was reconstructed with an osteo-musculo-cutaneous flap. Other reconstruction modalities include the trapezius muscle flap, abdominal wall flap, deltopectoral flap, and free vascularized groin flap. One case required three major flaps (rectus abdominis myocutaneous flap and trapezius muscle flap, latissimus dorsi flap, and free vascularized groin flap). Five patients suffered from heart diseases such as angina, aortic regurgitation and heart failure. Two cases experienced PTCAs, one case suffered from an old myocardial infarction, another case suffered from angina pectoris and developed heart failure, and one other case demonstrated of hypertension as well as aortic regurgitation.

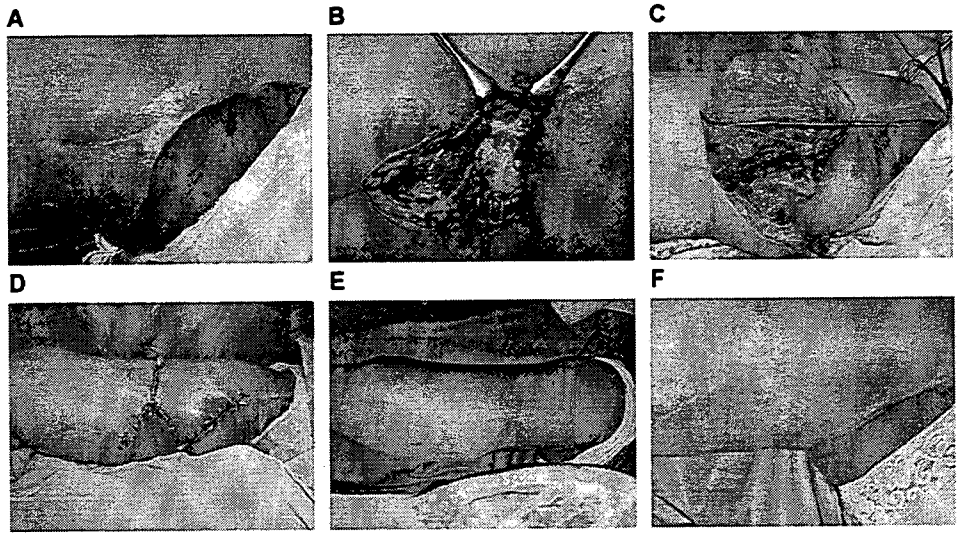

Fig. 2. A 72-year-old female, post-35 Gy irradiation in her right inner thigh. A: Prolonged fistulae in the scar; B: Intra-reconstructive operative view; C: Fistulae continued to her inguinal ligament; D: Immediately after local flap reconstruction; E: 2 years after reconstruction; F: Close-up view. No recurrence.

3.1.1. Case report

A 72-year-old female underwent right thigh radical liposarcoma resection by an orthopedic surgeon and subsequent 35 Gy therapeutic sequential radiations. Fistulae developed in 2 months after radiation therapy and odor exudates limited her quality of life. The swollen tissues and consolidated scar tissues also evoked tender pain during walking. She was referred to us 2 years after final radiation therapy. The scar tissue resection and reconstruction were planned under general anesthesia. The fibrosis and fistulae continued up to her inguinal ligaments. The local fasciocutaneous flap with Z-plasty resulted in complete wound healing in 2 years after reconstructive surgery (Fig. 2).

3.2. Experimental investigation for acute systemic radiation injuries

All the animals were healthy after 20 Gy whole body irradiation and subsequent surgery. At day 7, the wound seemed most dramatically healed in the experimental group; the regenerated tissues including raw surfaces were harvested for histology. There were less desquamations of the epidermis adjacent to the wound as well as progressed healed regenerated tissues in experimental group (Fig. 3).

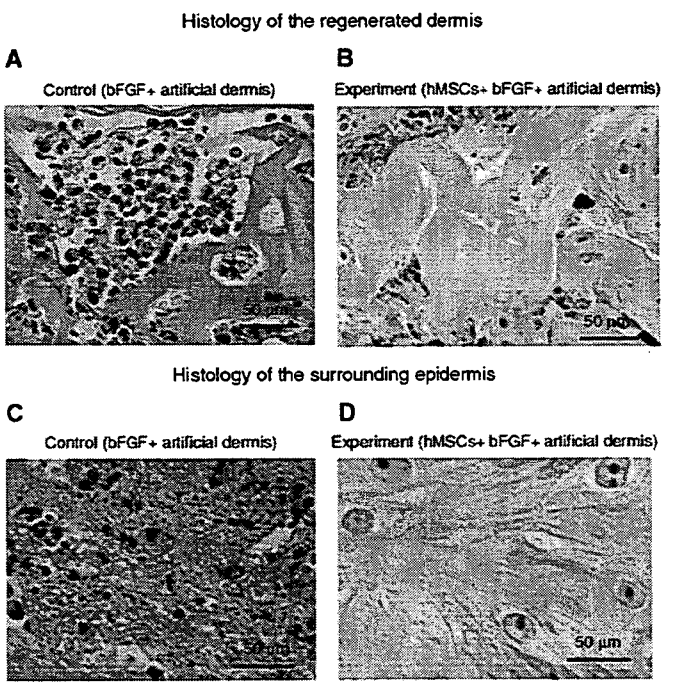

Fig. 3. Histology of the regenerated tissues (×200). A: The dermal component in control. More inflammatory cells invaded. B: The regenerated dermis in the experiment. More organized arrays of the dermal fibers. C: The surrounding epidermis of control. More necrotic tissues and disarrays of the keratinocytes. D: The surrounding epidermis and partial regenerated epidermis of the experiment. Well-developed keratinocytes.

4. Discussion

Plastic surgery often involves reconstructive procedures in various cases of burns, friction trauma and post-malignant tumor resection. The local reconstruction modalities can be selected from simple skin grafting, local flap and free vascularized flap. As the radiation exposed tissues sometimes prolong wound healing problems due to lack of sufficient blood supply, irreversible scarring and damage to microcirculation of the blood and lymphatic vessels. Our series for reconstruction of local radiation injuries demonstrated remarkable benefits in less invasive, reduced donor-site morbidities, and less frequency of surgery.

For systemic acute radiation injury, prepared medical supplies should be considered first. In this context, the human mesenchymal stem cells (hMSCs) used in this experiment are the realistic cell source. The hMSCs were normally freeze-dried and immediately thawed upon necessity of use. The characteristics of this cell type are highly proliferative, cytokine-inducible and able to differentiate into skin and soft tissue components [6]. In a 20 Gy whole body irradiation, there were progressed dermal components in the experimental group with more matrix deposition and less polymultinuclear cell invasions, which reflect the inflammatory reactions. The surrounding epidermis was also superior in the quality of the regeneration as there are less necrotic tissues and keratinocyte morphology seems more ordered. The combined use of the hMSCs, bFGF and artificial dermis (Pelnac®) brings about all readily available resources for systemic acute radiation injury. Our approaches for both local and systemic radiation injuries are clinically feasible and highly recommended for emergency situations, which are normal for devastating radiation injuries.

We propose our therapeutic options for both local and systemic radiation injuries, especially for skin and soft tissue injuries.

References

[1] S. Akita, K. Akino, T. Imaizumi, A. Hirano, A basic fibroblast growth factor improved the quality of skin grafting in burn patients, Burns 31 (2005) 855–858.

[2] S. Akita, H. Nakagawa, K. Tanaka, A. Hirano, Analysis of pediatric burns in Nagasaki University from 1983 to 2002, Burns 31 (2005) 1041–1044.

[3] S. Akita, K. Akino, S.G. Ren, S. Melmed, T. Imaizumi, A. Hirano, Elevated circulating leukemia inhibitory factor levels in burn patients: marker of mortality, J. Burn Care Res. (formerly J. Burn Care. Rehabil.) 27 (2006) 221–225.

[4] S. Akita, K. Akino, T. Imaizumi, et al., Polyurethane dressing is beneficial for split-thickness skin-graft donor wound healing, Burns 32 (2006) 447–451.

[5] K. Akino, T. Mineta, M. Fukui, T. Fujii, S. Akita, Bone morphogenetic protein-2 regulates proliferation of human mesenchymal stem cells, Wound Repair Regen. 11 (2003) 354–360.

[6] H. Nakagawa, S. Akita, M. Fukui, T. Fujii, K. Akino, Human mesenchymal stem cells successfully improve skin-substitute wound healing, Br. J. Dermatol. 153 (2005) 29–36.

[7] S. Akita, K. Tanaka, A. Hirano, Lower extremity reconstruction after necrotizing fasciitis and necrotic skin lesions using a porcine-derived skin substitute, J. Plast. Reconstr. Aesthet. Surg. (formerly Br. J. Plast. Surg.) 59 (2006) 759–763.

J Neurol (2007) 254:1509–1517
DOI 10.1007/s00415-007-0540-9

ORIGINAL COMMUNICATION

Yusei Shiga
Katsuya Satoh
Tetsuyuki Kitamoto
Sigenori Kanno
Ichiro Nakashima
Shigeru Sato
Kazuo Fujihara
Hiroshi Takata
Keigo Nobukuni
Shigetoshi Kuroda
Hiroki Takano
Yoshitaka Umeda
Hidehiko Konno
Kunihiko Nagasato
Akira Satoh
Yoshito Matsuda
Mitsuru Hidaka
Hirokatsu Takahashi
Yasuteru Sano
Kang Kim
Takashi Konishi
Katsumi Doh-ura
Takeshi Sato
Kensuke Sasaki
Yoshikazu Nakamura
Masahito Yamada
Hidehiro Mizusawa
Yasuto Itoyama

Two different clinical phenotypes of Creutzfeldt-Jakob disease with a M232R substitution

Received: 28 June 2006
Received in revised form: 8 January 2007
Accepted: 6 February 2007
Published online: 2 November 2007

Y. Shiga, MD, PhD* (✉) · S. Kanno, MD · I. Nakashima, MD, PhD · K. Fujihara, MD, PhD · Y. Itoyama, MD, PhD
Dept. of Neurology
Tohoku University Graduate School of Medicine
1-1 Seiryo-machi, Aoba-ku
Sendai 980-8574, Japan
Tel.: +81-22/717-7189
Fax: +81-22/717-7192
E-Mail:
yshiga@em.neurol.med.tohoku.ac.jp

K. Satoh, MD, PhD
The First Department of Internal Medicine
Graduate School of Medicine
Nagasaki University, Japan

T. Kitamoto, MD, PhD*
Division of CJD Science and Technology
Graduate School of Medicine
Tohoku University, Japan

S. Sato, MD, PhD
Dept. of Neurology
Kohnan Hospital, Japan

H. Takata, MD, PhD · K. Nobukuni, MD, PhD
Dept. of Neurology
Minami Okayama National Hospital, Japan

S. Kuroda, MD, PhD*
Dept. of Neuropsychiatry
Graduate School of Medicine, Dentistry and Pharmaceutical Sciences
Okayama University, Japan

H. Takano, MD, PhD · Y. Umeda, MD, PhD
Dept. of Neurology
Brain Research Institute
Niigata University, Japan

H. Konno, MD, PhD
Dept. of Neurology
Nishitaga National Hospital, Japan

K. Nagasato, MD, PhD
Dept. of Neurology
Isahaya General Hospital, Japan

A. Satoh, MD, PhD
Dept. of Neurology
Nagasaki Kita Hospital, Japan

Y. Matsuda, MD, PhD
Dept. of Neuropsychiatry
Graduate School of Medicine
Yamaguchi University, Japan

M. Hidaka, MD, PhD
Yokohama Miyazaki Hospital of Neurosurgery, Japan

H. Takahashi, MD, PhD
Dept. of Neurology
Matsudo Municipal Hospital, Japan

Y. Sano, MD, PhD
Dept. of Neurology
Graduate School of Medicine
Yamaguchi University, Japan

K. Kim, MD, PhD
Dept. of Neurology
Shizuoka General Hospital, Japan

T. Konishi, MD, PhD
Dept. of Neurology
Shizuoka National Medical Center, Japan

K. Doh-ura, MD, PhD
Division of Prion Protein Biology
Graduate School of Medicine
Tohoku University, Japan

T. Sato, MD, PhD*
National Center for Neurology and Psychiatry
Kohnodai Hospital, Japan

K. Sasaki, MD, PhD
Dept. of Neuropathology
Neurological Institute
Graduate School of Medical Sciences
Kyushu University, Japan

Y. Nakamura, MD, PhD*
Dept. of Public Health
Jichi Medical School, Japan

M. Yamada, MD, PhD*
Depts. of Neurology and Neurobiology of Aging
Graduate School of Medical Science
Kanazawa University, Japan

H. Mizusawa, MD, PhD*
Dept. of Neurology and Neurological Science
Graduate School
Tokyo Medical and Dental University, Japan

* The Creutzfeldt-Jakob Disease Surveillance Committee, Japan.

JON 2540

Abstract *Objective* To describe the clinical features of Creutzfeldt-Jakob disease with a substitution of arginine for methionine (M232R substitution) at codon 232 (CJD232) of the prion protein gene (PRNP). *Patients and methods* We evaluated the clinical and laboratory features of 20 CJD232 patients: age of onset, initial symptoms, duration until becoming akinetic and mute, duration until occurrence of periodic sharp and wave complexes on EEG (PSWC), MRI findings, and the presence of CSF 14-3-3 protein. Immunohistochemically, prion protein (PrP) deposition was studied. *Results* None of the patients had a family history of CJD. We recognized two clinical phenotypes: a rapidly progressive type (rapid-type) and a slowly progressive type (slow-type). Out of 20 patients, 15 became akinetic and mute, demonstrated myoclonus, and showed PSWC within a mean duration of 3.1, 2.4, and 2.8 months, respectively (rapid-type). Five showed slowly progressive clinical courses (slow-type). Five became akinetic and mute and four demonstrated myoclonus within a mean duration of 20.6 and 15.3 months, respectively, which were significantly longer than those in the rapid-type. Only one demonstrated PSWC 13 months after the onset. Diffuse synaptic-type deposition was demonstrated in four rapid-type patients, and perivacuolar and diffuse synaptic-type deposition in two, and diffuse synaptic-type deposition in one slow-type patient. Three of 50 suspected but non-CJD patients had the M232R substitution. *Conclusions* Patients with CJD232 had no family history like patients with sCJD, and showed two different clinical phenotypes in spite of having the same PRNP genotype. More studies are needed to determine whether M232R substitution causes the disease and influences the disease progression.

Key words Creutzfeldt-Jakob disease · M232R · clinical phenotype · uncommon variant · diffusion-weighted MRI

Introduction

Human prion diseases are divided into three types: sporadic, genetic, and infectious prion disease. Genetic prion disease, which is defined as prion disease with causative abnormalities of the prion protein gene (PRNP), accounts for approximately 10 to 15% of all prion disease cases, and includes genetic Creutzfeldt-Jakob disease (gCJD), Gerstmann-Sträussler-Scheinker disease (GSS), and fatal familial insomnia (FFI) [1]. In general, the clinical features of gCJD are more various compared with those of sporadic CJD (sCJD) and are regulated by the genotype [2]. Therefore, gCJD, even if its clinical features are quite different from those of sCJD, especially those of the most often encountered type of sCJD with methionine homozygosity at codon 129 of PRNP and type 1 protease-resistant prion protein (MM1) [3], can be diagnosed by examining the genotype. To clarify the clinical features of CJD, which associates with a substitution in PRNP, will provide an important clue that can lead to genetic examination.

To date, more than 30 causative mutations have been recognized and individual PRNP mutations show variable geographical distribution and frequency. The cardinal characteristic of gCJD is that more than half of the patients lack family history.

CJD patients associated with a substitution of arginine for methionine at codon 232 (M232R substitution) in PRNP with no relevant family history have been reported in Japan [4–10]. Previously, the clinical features of CJD with the M232R substitution (CJD232) were thought to be similar to those of typical sCJD with MM1 [3], which accounts for the vast majority of sCJD in terms of clinical features, including EEG findings [5, 6, 9]. However, cases of CJD232 that showed a longer clinical course and lacked the characteristic periodic sharp and wave complexes (PSWC) have been reported [7, 8]. We have experienced eight cases of CJD232. Five of them showed a rapid clinical course and typical CJD features, while the others showed very slow progression and atypical features. We studied the clinical features of 20 CJD232 patients, including our original patients, and found that there were two different major clinical phenotypes with the same genotype, including polymorphisms at codons 129 and 219 of PRNP; one progressed rapidly, and the other progressed slowly. Better understanding of the clinical features of CJD232 would contribute to the diagnosis of CJD232, especially in patients with atypical clinical features.

Patients and methods

Twenty-four patients with CJD232 were included in this study: eight were our original cases, seven were obtained by reviewing the literature [5–10] and nine were found by reviewing the clinical records of CJD patients reported to the Creutzfeldt-Jakob disease Surveillance Committee, Japan. We excluded two patients because they had double point mutations at codon 180 and at codon 232 [10] and one patient because her polymorphism at codons 129 and 219 of PRNP was uncertain [5]. Therefore, 21 patients were enrolled in this study. The nine who were proven at autopsy are indicated by asterisks in Fig. 1.

We first evaluated the duration from onset until the patients manifested akinetic mutism. As shown in Fig. 1, 15 became akinetic and mute within six months, while five did not become so until 15 months after the onset. These CJD232 patients appeared to be comprised of two different groups: one was a rapidly progressive type (rapid-type) and the other was a slowly progressive type (slow-type). We evaluated the age of onset, initial symptoms, duration from onset to the appearance of myoclonus, duration from onset to akinetic mutism, du-

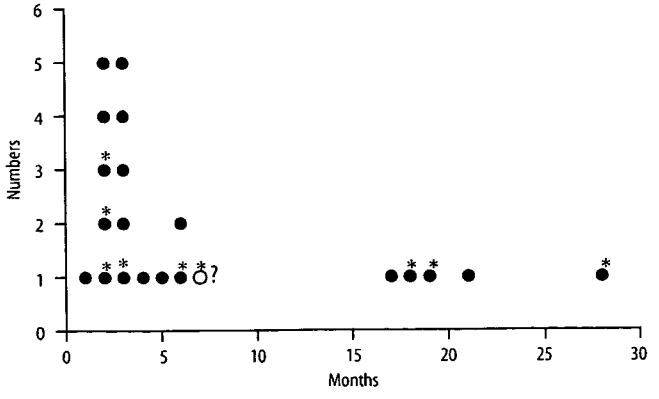

Fig. 1 The duration from the onset to akinetic mutism. The X-axis shows the duration (months) and the Y-axis shows the accumulative number of patients. Black circles indicate patients who became akinetic and mute; the white circle indicates a patient who had not become akinetic and mute. The white circle with a question mark indicates a 50-year-old-male patient who suddenly died seven months after the onset because of a myocardial incident. Since he had not become akinetic and mute, and was able to converse with simple words, we excluded him from further analyses. Asterisks indicate autopsy proven patients. We recognize two different groups concerning the duration from the onset to akinetic mutism: a rapidly progressive type and a slowly progressive type

ration from onset to occurrence of PSWC, results of MRI, and the presence of 14-3-3 protein in the CSF of the two types. The patient marked by a question mark in Fig. 1 was excluded from the evaluation. We were unable to determine which group this 50-year-old man belonged to because he had not become akinetic and mute and was still able to converse with simple words seven months after the onset when he suddenly died due to a myocardial incident [8]. Thus, the clinical data of 20 patients were finally used for this study.

In one of the rapid-type patients and in three of the slow-type patients including a previously reported 64-year-old woman [7], immunohistochemical staining of PrP using monoclonal antibody 3F4 (Prionics, Schlieren, Switzerland) was performed. Including the previously reported pathological findings of three patients belonging to the rapid-type [6], immunohistochemical staining of PrP in both groups were studied. In each group, the molecular type of the abnormal isoform of prion protein (PrP^{Sc}) was studied.

The Mann-Whitney U test was used for statistical comparison of the age of onset and the duration until the appearance of myoclonus and akinetic mutism from the onset between the rapid-type and the slow-type. The Grubbs-Smirnov critical test was used for statistical analysis of the duration until the appearance of PSWC from the onset between the rapid-type and the slow-type. Fisher's exact probability test was used for comparison of the male to female ratio, and the rates of myoclonus, akinetic mutism, and PSWC between the two types. It was also used for comparison of the positive rate of 14-3-3 immunoassay and MRI between the two types.

Results

Reviewing the clinical records of the enrolled patients, we found that no patients of either group had a family history of prion disease or dementia.

Fifteen patients, eight men and seven women, with a mean onset age of 65.4 ± 5.2 (Mean ± SD) years could be categorized as the rapid-type. Of those, seven with an initial symptom of progressive dementia or memory disturbance, two with visual symptoms, two with cerebellar ataxia, two with involuntary movement, and two with other symptoms. All except for one uncertain patient demonstrated myoclonus 2.4 ± 1.8 months after the onset. All became akinetic and mute within a mean duration of 3.1 ± 1.5 months, and demonstrated PSWC (Fig. 2A and B) within a mean duration of 2.8 ± 1.8 months. CJD-related high intensity lesions [11] were detected in eight of the nine patients examined by MRI. Similar to sCJD, three patterns existed: in one, high intensity lesions appeared mainly in the striatum (Fig. 3A); in another, they appeared in the striatum and the cortical ribbon equally (Fig. 3B); and in yet another, they appeared mainly in the cortical ribbon (Fig. 3C). The 14-3-3 protein assay was positive in all eight patients examined. All 15 patients showed MM129, 14 showed glutamic acid homozygosity at codon 219 (GG219) and one showed glutaminic acid/lysine heterozygosity at codon 219 in the PRNP analysis. These clinical features closely resembled typical sCJD with MM1 [3]. Immunohistochemical staining of PrP in four patients (one original patient and three previously reported patients [6]) revealed a diffuse synaptic-type deposit (Fig. 4A). The molecular type of PrP^{Sc} in one patient was type 1.

Five patients, two men and three women, with a mean onset age of 59.0 ± 12.8 years could be categorized as the slow-type. Three had an initial symptom of progressive dementia or memory disturbance, one showed psychiatric symptoms, and one had dressing apraxia. Four of five patients demonstrated myoclonus 15.3 ± 12.3 months after the onset, and the remaining one did not demonstrate myoclonus during the 13-month observation period. All became akinetic and mute within a mean duration of 20.6 ± 4.4 months. Only one demonstrated PSWC within the observation period of 23.8 ± 13.7 months (Fig. 2C and 2D). CJD-related high-intensity lesions were detected in four of the five patients examined by MRI [11]. One showed high-intensity lesions in the cortical ribbon (Fig. 3D and 3E), while in the others such lesions appeared in both the striatum and cortical ribbon (Fig. 3F). The medial thalami showed high-intensity lesions in all three patients examined by DWI (white arrows in Fig. 3D and E, and black arrows in Fig. 3F). The 14-3-3 protein assay was positive in all four patients examined. In the PRNP analysis, all five patients showed MM129 and GG219. Immunohistochemical staining in two patients revealed predominantly perivacuolar-type PrP deposits in the cerebral cortex (Fig. 4B), but also partly the diffuse synaptic-type deposits. In one patient, only the diffuse synaptic-type deposits were revealed. The molecular type of PrP^{Sc} in one patient who had predominantly perivacuolar-type PrP deposits was type 1 + 2.

Between the two groups, there were no differences in the age at onset, male to female ratio, or positive rate of

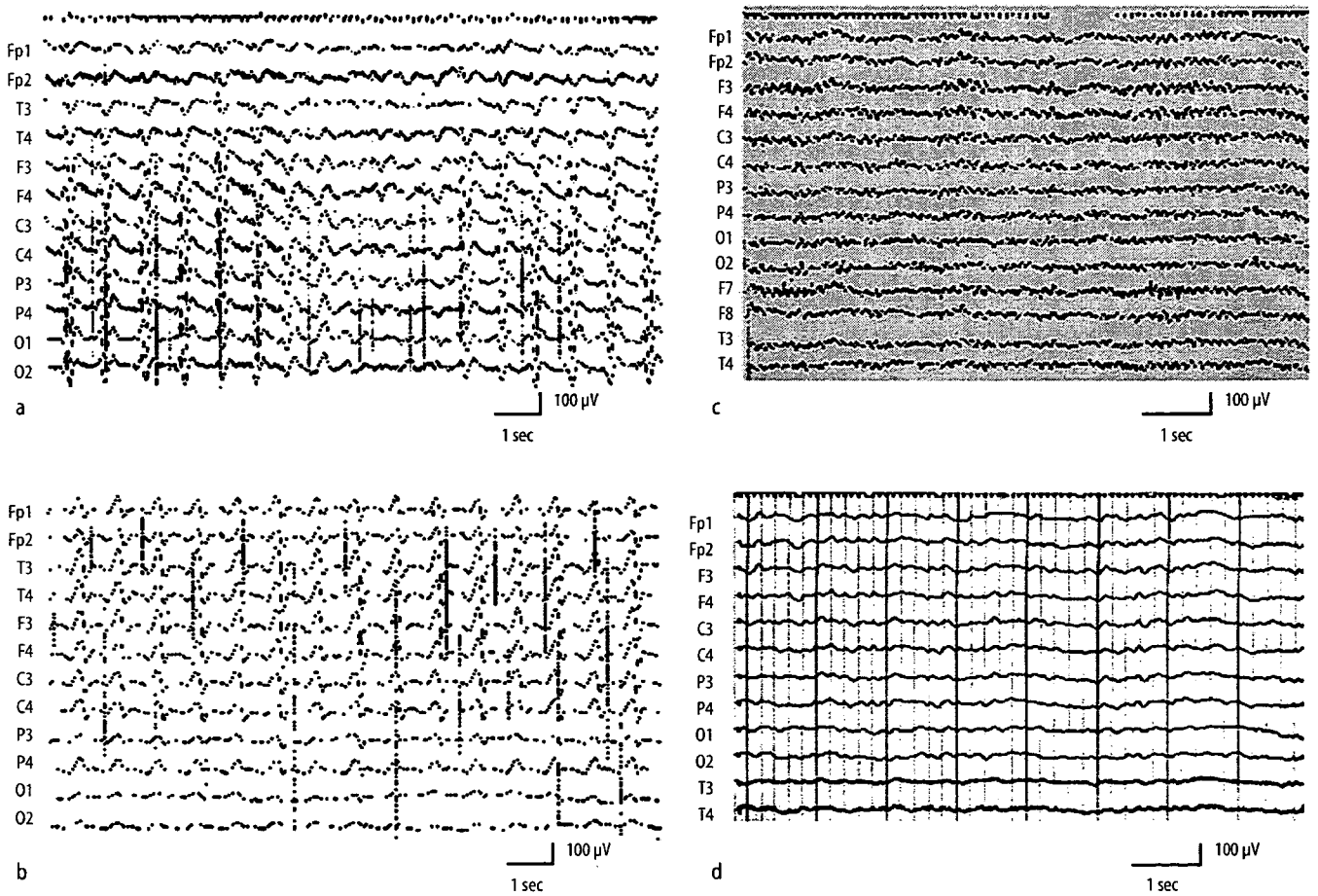

Fig. 2 EEG of representative patients of the rapid-type group and the slow-type group. **A** and **B** were recorded from the same 55-year-old woman in the rapid-type group. **C** and **D** were recorded from the same 69-year-old woman in the slow-type group. **A** EEG obtained two and half months after onset demonstrated high amplitude periodic sharp and wave complexes (PSWC) at a frequency of 1.5 Hz characteristic of CJD. **B** EEG obtained five months after the onset demonstrated PSWC at a frequency of 1 Hz. The amplitude was lower than that of Fig. 1A, and the background activities were flattened. EEG rapidly deteriorated. **C** EEG obtained four months after the onset. The background activities were 8 Hz mixed with no apparent slow activities. PSWC was not demonstrated. **D** EEG obtained twelve months after the onset. The background activities were 5 Hz mixed with δ activities. However, PSWC was not yet demonstrated

14-3-3 protein immunoassay. Similar to sCJD, there were three patterns of high-intensity lesions shown by MRI in the rapid-type. We were unable to distinguish the rapid-type of CJD232 from sCJD based on the clinical features including MRI findings. Patients with the slow-type did not have fewer lesions than patients with the rapid-type at diagnosis. High-intensity lesions in the medial thalamus depicted by DWI were a common finding of the slow-type (Fig. 3A–F). There was no difference in the rate of myoclonus between the two groups, but the duration until the appearance from the onset was longer in the slow-type compared with the rapid-type ($p < 0.005$). All patients became akinetic and mute in both types, but the duration until becoming akinetic and mute from the onset in the slow-type was longer than that in the rapid-type ($p < 0.001$). Concerning PSWC, all patients in the rapid-type demonstrated PSWC 2.8 ± 1.8 months after the onset. However, in the observation period of 21.6 ± 12.8 months, only one patient with the slow-type demonstrated PSWC 13 months after onset, which was later compared with that of the rapid-type ($p < 0.01$). The rate of PSWC in the slow-type was lower than that in the rapid-type ($p < 0.01$). Since there were no differences in the polymorphisms of codons 129 and 219 between the two groups, such polymorphisms would not be determinants of the disease subtype. Based on the differences in the clinical and laboratory findings (Table 1), we considered that these two types represented completely different phenotypes of exactly the same genotype.

By reviewing the investigative reports collected by the Creutzfeldt-Jakob Disease Surveillance Committee, Japan, as of February 2006, PRNP information was available from 511 patients: 317 were acknowledged as sporadic CJD, 41 as infectious CJD, 103 as genetic prion disease that included 28 CJD with V180I (CJD180), 27 GSS with P102L, 23 CJD with E200K, and 13 CJD232, and 50 as non-CJD. Three of the 50 non-CJD patients who had