厚生労働科学研究費補助金（難治性疾患克服研究事業）
平成２３年度　分担研究報告書

セルロース誘導体に関連する低分子化合物に関する研究

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研究要旨
セルロース関連グルコース誘導体や三糖誘導体を 33 種類化学合成した。次いで、プリオン脳内感染マウスを用いた実験を行ったところ、6 種類の低分子化合物に弱いながらもプリオン病発症遅延効果を認めた。活性を改善する必要が残されているものの、これら化合物の発見はプリオン病予防薬開発の可能性を一歩前進させたと言える。

A．研究目的
これまでに研究代表者の堂浦らにより、セルロース誘導体（ＣＥ）に異常型プリオン蛋白の産生を阻害する抗プリオン活性があることが見いだされている。しかしながら、不均一反応で調製される高分子ＣＥは化学構造的に不均一であるため、ＣＥのメチル基の置換状態はグルコース内の３つの水酸基での置換状態の違い、分子鎖に沿ったメチル基の置換位置の偏り、分子鎖間でのメチル基置換度の違いが生じ、化学構造-活性相関を詳細に検討することは不可能である。
そこで、本研究では化学構造の明確なセルロース誘導体を有機合成し、その化学構造が抗プリオン活性に与える影響を詳細に検討することとした。本年度はＣＥ関連低分子誘導体、特にグルコース誘導体の化学合成を行った。最終的に、抗プリオン活性を与えるセルロース誘導体の部分構造を明らかにし、より抗プリオン活性の高いセルロース系化合物を開発することを目的とした。

B．研究方法
グルコース、メチル alpha-D-グルコシド、セロビオースを原料とし、位置特異的な保護基の導入、メチル基の導入、グリコシル化、保護基の除去などの工程を経て、33 種類のＣＥ系化合物を化学合成した。次いで、プリオン感染細胞 N167 を用いてそれら化合物の異常型プリオン蛋白産生抑制効果を検討した。
さらに、プリオン脳内感染マウスを用い、感染後、脳室内に化合物を 14 日間投与し平均余命により効果評価を行った。
（倫理面への配慮）
動物実験は施設の動物実験委員会の審査

を受け、施設管理者の許可を得て動物実験指針を遵守して行った。

C．研究結果

本年度合成した化合物、ＣＥ関連グルコース誘導体25種類、三糖誘導体8種類についてプリオン感染細胞を用いて抗プリオン活性を検討した結果、ＣＥ関連グルコース誘導体および三糖誘導体には効果が認められなかった。
一方、プリオン脳内感染マウスを用いた実験を行ったところ、6 種類の低分子化合物に弱いながらもプリオン病発症遅延効果を認めた。

D．考察

プリオン病発症遅延効果が認められた 6 種類のＣＥ関連グルコース誘導体には、1 置換、2 置換、3 置換、4 置換誘導体が含まれていた。また活性の認められなかった化合物３種類には 1 置換、2 置換誘導体が含まれていた。この結果は高分子ＣＥでこれまでに得られている結果と矛盾しなかった。

E．結論

化学構造の明確な位置特異的置換、あるいは位置特異的メチル化グルコース誘導体、三糖誘導体33種類を化学合成した。プリオン脳内感染マウスを用いた in vivo 実験の結果、6 種類の低分子化合物に弱いながらもプリオン病発症遅延効果を認めた。これら化合物の発見はプリオン病予防薬開発の可能性を一歩前進させたと言える。今後、全ての置換パターンを有するＣＥ関連グルコース誘導体をプリオン脳内感染マウス実験に供することにより、明確な構造活性相関が得られることが期待される。

F．健康危機情報

なし

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

発明の名称：セロオリゴ糖誘導体およびその製造方法

出願人：京都大学

発明者：上高原 浩、中坪 文明、Dieter Klemm

出願番号（出願年月日）：特願 2006-548688, PCT/JP2005/0121127 (2005 年 6 月 30 日)

公開番号(公開年月日): WO2006/067883, (2006 年 6 月 29 日)

特許番号（登録年月日）：第 4749339 号 (2011 年 5 月 27 日)

２．実用新案登録

なし

３．その他

なし

厚生労働科学研究費補助金（難治性疾患克服研究事業）
平成２３年度　分担研究報告書

セルロース誘導体の最適化に関する研究

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研究要旨
効果の高いＣＥ修飾体の化学構造や物性と共通点を持つグルコシド化合物の中で、プリオン感染細胞で抗プリオン活性を発揮する化合物について、新たに７種類の誘導体を化学合成し、プリオン感染細胞で検討したが、新たに有効なものは見つからなかった。

Ａ．研究目的
経口投与でも抗プリオン活性が高いセルロース誘導体（ＣＥ）に関連した低分子化合物を開発するため、昨年度は、効果の高いＣＥ修飾体の化学構造や物性の特徴を抽出した。その化学構造や物性と共通性を持ち、低分子化合物で抗プリオン活性を有するものとして 4-methoxyphenyl-2-amino-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside を発見している。そこで、このグリコシド化合物について構造を最適化するため構造活性相関解析を行った。

Ｂ．研究方法
4-methoxyphenyl-2-amino-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside のベンジル基の位置特性について調べるため、新たに７種類の誘導体を化学合成した。それらをプリオン感染細胞に投与して、抗プリオン活性を評価した。
（倫理面の配慮）
該当しない。

Ｃ．研究結果
4-methoxyphenyl-2-amino-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside とはベンジル基の位置が異なる７種類の化合物（4-methoxyphenyl-6-*O*-benzyl-β-D-glucopyranoside、4-methoxyphenyl-2-*O*-benzyl-β-D-glucopyranoside、4-methoxyphenyl-2,6-di-*O*-benzyl-β-D-glucopyranoside、2,3,6-tri-*O*-benzyl-D-glucose、2,6-di-*O*-benzyl-D-glucose、3,6-di-*O*-benzyl-D-glucose、

6-*O*-benzyl-D-glucose）を化学合成し、抗プリオン活性をプリオン感染細胞で調べたところ、いずれの化合物も、細胞障害が観察されない濃度では抗プリオン活性は観察されなかった。

D．考察

効果が高いＣＥの特徴としては、親水性であると同時に、側鎖や還元末端が分解を受けにくい構造であった。その化学構造や物性と共通点がある4-methoxyphenyl-2-amino-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosideは、プリオン感染細胞で抗プリオン活性を発揮する。この化合物に関連する誘導体として今回合成した７種類のグルコシド化合物の解析では、新たに有効なものは確認されなかった。さらに側鎖構造を展開して、構造活性相関解析を進めるとともに、プリオン感染細胞での抗プリオン活性が必ずしもプリオン感染動物での効果と相関するわけではないので、新たに化学合成した７種類のグルコシド化合物についてもプリオン感染動物での効果を調べる必要があると考えている。

E．結論

効果の高いＣＥ修飾体の化学構造や物性と共通点を持つグルコシド化合物の構造展開を行ったが、すでに見つけているグルコシド化合物以外には抗プリオン活性を発揮する化合物は見つからなかった。

F．健康危険情報

なし

G．研究発表

10、11、12ページに記載

厚生労働科学研究費補助金（難治性疾患克服研究事業）
平成２３年度　分担研究報告書

既製品の薬効評価に関する研究

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研究要旨

昨年度の成果を踏まえ、GM-CSF の効果を他のプリオン病動物モデルで検討した。また、これまでの解析から、ＣＥの作用機序に糖代謝関連因子の関与も考えられたため、糖代謝に影響する因子として SGLT 阻害剤や SGLT 選択的透過糖誘導体、さらには PPARγアゴニストやレプチンをプリオン感染マウスで検討した。GM-CSF の検討では、ＣＥと同様に Tg7 マウスでは効果は見られたが、Tga20 マウスでは効果は乏しいという結果が得られた。一方、糖代謝関連では、検討した中では SGLT 選択的透過糖誘導体が最も効果があり、SGLT 阻害剤との併用では効果が減弱した。

Ａ．研究目的

マウスを用いたセルロース誘導体の作用機序解明研究において、投与後に長期間にわたる泡沫状貪食細胞の出現を踏まえ、網羅的解析を行ったところ、G-CSF/GM-CSF/M-CSF などのサイトカインが長期間にわたり誘導されていることを発見した。この発見をもとに、プリオン感染マウスに感染末期より GM-CSF を投与したところ、臨床症状の改善及び生命予後の改善が観察された。G-CSF/GM-CSF は既製薬品であり、早期にリスク保有者や患者への応用が可能であることより、本年度は他のプリオン病感染動物モデルにおいても効果の有無を検討した。また、これまでの解析から、ＣＥの作用機序に糖代謝関連因子の関与も考えられたため、糖代謝に影響する因子として SGLT 阻害剤や SGLT 選択的透過糖誘導体、さらには PPARγアゴニストやレプチンをプリオン感染マウスで検討した。

Ｂ．研究方法

GM-CSFの効果を、22Lプリオン株に脳内感染させたTga20マウスで検討した。具体的には感染後期の未発症期より発症末期までマウス組換え型GM-CSFを一日一回(週5日間）、一回に500 ngを皮下に投与を実施した。一方、糖代謝に影響する因子としてSGLT阻害剤であるphloridinや、SGLT選択的透過糖誘導体であるmethyl-α-D-glucoside(αMG)やmethyl-4-fluoro-4-deoxy-D-glucopyranoside(Me-4FDG)や3-O-methyl-glucose(3OMG)やmethyl-β-D-glucoside(βMG)、さらにはPPARγアゴニストであるpioglitazone、その対照としてPPARαアゴニストである

benzafibrate、またマウス組換え型レプチンを早発系プリオン病発症動物である263Kプリオン株脳内感染Tg7マウスで検討した。SGLT関係は脳内感染後3日目より浸透圧ポンプを用い120 μg/日の量を２週間にわたり脳室内に投与した。PPARのアゴニストやレプチンは既報告に基づき薬効量を脳内感染後3日目より浸透圧ポンプを用いて２週間にわたり皮下に投与した。

(倫理面の配慮)

動物実験は施設の動物実験委員会の審査を受け、施設管理者の許可を受け動物実験指針を遵守して行った。

C．研究結果

GM-CSF の効果を 22L プリオン株感染 Tga20 マウスで検討したところ、Tg7 マウスと異なり、潜伏期間を延長する効果に乏しかった。さらに、投与開始時期や投与量を変えて再検討したが、効果の程度は変わらなかった。一方、糖代謝に影響する因子の中では、SGLT 選択的透過糖誘導体に効果が見られた。Me-4-FDG＞αMG＞βMG の順に効果が見られ、3OMG では効果がなかった。また、αMG に phloridin を同時に併用すると、αMG の効果が低下した。PPARγアゴニストやレプチンや PPARαアゴニストには、効果は観察されなかった。

D．考察

昨年度の発見を踏まえ、既製薬品が応用できないかどうかを検討した。GM-CSF の効果はＣＥ効果と共通した振る舞いを呈し、マウス系統による違いが見られた。したがって、ＣＥ効果の一部は GM-CSF に依っていると考えられる。一方、SGLT 選択的透過糖誘導体に効果が見られ、その効果は SGLT 阻害剤により抑えられた。糖代謝に関わる PPARγアゴニストやレプチンには効果がなかったことより、糖代謝の中でも SGLT で取り込まれた糖アナログが何らかの働きを発揮することにより治療効果に結びついているものと推察される。糖代謝に関わる DPP4 阻害剤の効果ついては、既製薬の aloglipitin を用いたプリオン感染マウスでの検討を実施していたが、本年度内に結論を得られなかった。また、同様にサイトカインである CXCL5 についても、本年度にプリオン感染マウスでの結論を得られず、継続して検討している。

引き続き、サイトカイン製剤や糖代謝関連薬を中心とした既製薬の有効性評価を継続し、CE の効果を代用できる既製薬を早期に患者に応用できるよう努める。

E．結論

GM-CSF は、ＣＥと共通した振る舞いを呈し、その効果はマウス系統により違いが見られた。一方、糖代謝関連では、SGLT 選択的透過糖誘導体の効果があらためて確認されたが、PPARγアゴニストやレプチンは効果がなかった。

F．健康危険情報

なし

G．研究発表

14、15 ページに記載

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

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
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Original Article

Protease-resistant PrP and PrP oligomers in the brain in human prion diseases after intraventricular pentosan polysulfate infusion

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Intraventricular infusion of pentosan polysulfate (PPS) as a treatment for various human prion diseases has been applied in Japan. To evaluate the influence of PPS treatment we performed pathological examination and biochemical analyses of PrP molecules in autopsied brains treated with PPS (one case of sporadic Creutzfeldt-Jakob disease (sCJD, case 1), two cases of dura mater graft-associated CJD (dCJD, cases 2 and 4), and one case of Gerstmann-Sträussler-Scheinker disease (GSS, case 3). Six cases of sCJD without PPS treatment were examined for comparison. Protease-resistant PrP (PrP^{res}) in the frontal lobe was evaluated by Western blotting after proteinase K digestion. Further, the degree of polymerization of PrP molecules was examined by the size-exclusion gel chromatography assay. PPS infusions were started 3–10 months after disease onset, but the treatment did not achieve any clinical improvements. Postmortem examinations of the treated cases revealed symmetrical brain lesions, including neuronal loss, spongiform change and gliosis. Noteworthy was GFAP in the cortical astrocytes reduced in all treated cases despite astrogliosis. Immunohistochemistry for PrP revealed abnormal synaptic deposits in all treated cases and further plaque-type PrP deposition in case 3 of GSS and case 4 of dCJD. Western blotting showed relatively low ratios of PrP^{res} in case 2 of dCJD and case 3 of GSS, while in the treated sCJD (case 1), the ratio of PrP^{res} was comparable with untreated cases. The indices of oligomeric PrP were reduced in one sCJD (case 1) and one dCJD (case 2). Although intraventricular PPS infusion might modify the accumulation of PrP oligomers in the brains of patients with prion diseases, the therapeutic effects are still uncertain.

Key words: Creutzfeldt-Jakob disease, oligomer, pentosan polysulfate, prion protein, size-exclusion gel chromatography.

INTRODUCTION

Prion diseases, also known as transmissible spongiform encephalopathies (TSEs), are fatal neurodegenerative disorders that include Creutzfeldt-Jakob disease (CJD) and Gerstmann-Sträussler-Scheinker disease (GSS) in humans, and scrapie and bovine spongiform encephalopathy in animals. In these diseases, histopathological changes in the brain are characterized by spongiform change, reactive changes of astrocytes and variable loss of neurons.[1] In addition, deposition of a protease-resistant isoform of prion protein (PrP^{res}) is detected in the brain. This PrP^{res} contains high β-sheet content and is composed of polymerized PrP molecules post-translationally converted from normal, cellular PrP (PrP^{c}) of 254-amino acid 32–35 kDa glycolipid-anchored, plasma membrane protein that is widely expressed in the CNS.[2]

There is currently no effective remedy for human prion diseases, but several therapeutic compounds including quinacrine and pentosan polysulfate (PPS) have been tested for patients with prion diseases on experimental trial bases. Quinacrine is reportedly effective in inhibiting PrP^{res} formation in prion-infected cells.[3,4] However, subsequent studies showed no apparent beneficial effects of quinacrine in either experimental animals or humans.[5–7] By comparison, PPS has been shown to prevent the propagation of

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PrP^{res} in prion-infected cells.[8] Additionally, in experimental animals PPS has been administered directly into the CNS via an intra-hemiventricular canula, resulting in significant prolongation of the incubation periods accompanied with the laterality of neuropathological changes.[9] PPS inhibits PrP^{res} formation by interfering with the interaction of PrP^{c} and PrP^{res} with endogenous glycosaminoglycan or proteoglycan.[10] In addition, PPS stimulates endocytosis of PrP^{c}, reducing the amount of PrP^{c} present on the cell surface.[11] Experimental trials of intraventricular PPS infusion in the patients with prion diseases have been performed on observational bases, and thus it has been difficult to prove its efficacy.[12,13] In fact, Tsuboi *et al.* reported that PPS treatment showed no apparent improvements of clinical features in Japanese patients with prion disease.[14] In comparison, Bone *et al.* reported that mean survival of seven patients with PPS treatment was longer than reported values for the natural history of prion diseases in the United Kingdom, although possible reasons for this finding remain unclear.[15]

The main pathogenic component in prion diseases has been suggested to be PrP^{res} composed of PrP polymers. Recently, PrP oligomers, equivalent to 14–28 PrP molecules were reported as the most infectious units.[16,17] In addition, Kristiansen *et al.* reported that disease-associated PrP oligomers inhibit the 26S proteasome and cause neurotoxicity.[18] We have previously developed a gel-filtration chromatography method using spin columns to examine PrP oligomers, and revealed that increased PrP oligomers correlated with the degree of histopathological changes such as spongiform change and gliosis.[19] In other neurodegenerative diseases, including Alzheimer's disease, dementia with Lewy bodies and Parkinson's disease, soluble oligomers of amyloidogenic proteins were proposed to be the principal neurotoxic agents.[20,21]

In this report, to clarify the influence of PPS treatment on brains affected with piron diseases, we performed pathological examination and biochemical analyses of PrP, including its degree of polymerization, in four cases of prion diseases treated with PPS.

MATERIALS AND METHODS

We investigated the degree of polymerization of PrP molecules in four prion diseases cases that received PPS treatment, denoted PPS(+): one case of sporadic CJD (sCJD), two cases of dura mater graft-associated CJD (dCJD), and one case of GSS. We also examined six cases of sCJD without PPS treatment, denoted PPS(–), for comparison. The profiles of the patients are summarized in Table 1. At autopsy the brains were weighed and fixed with 10% buffered formalin. Six micrometer-thick sections of paraffin-embedded tissue from the CNS were stained with HE and the KB staining method. Immunohistochemistry was performed with primary antibodies against anti-prion antibody (mouse monoclonal 3F4, 1:400; Signet, Dedham, MA, USA) and GFAP (rabbit polyclonal, 1:1000; Dako, Glostrup, Denmark). The sections were then treated with appropriate biotinylated secondary antibodies and the reaction products were detected using the avidin-biotinylated peroxidase complex method (ABC; Vector Laboratories, Burlingame, CA, USA) coupled with a diaminobenzidine (Dojindo, Kumamoto, Japan) reaction.

Brain homogenate preparation

Human brains were collected at autopsy from four prion disease cases that had received PPS treatment and six cases of sCJD that had not received PPS treatment. Samples of frontal cortex were frozen fresh and stored at –80°C until used. The brain samples were homogenized to a final concentration of 10% in lysis buffer with sodium dodecyl sulfate (SDS) (100 mM Tris-HCl, 100 mM NaCl, 10 mM EDTA, 1% SDS, pH 7.6) for the size-exclusion gel chromatography assay. Most PrP^{c} could be solubilized as monomers in lysis buffer with SDS.[19] Samples were homogenized

Table 1 Summary of patient profiles

Case	Diagnosis	Genotype/PrP^{res} type	Age at death	Sex	Duration of illness (months)	Duration of PPS treatment (months)	Brain weight (g)
1	sCJD	129MM/type 1	74	F	23	20	660
2	dCJD	129MM/type 1	67	M	12	9	950
3	GSS	P102L/8 kDa	70	F	20	14	1055
4	dCJD	129MM/type 1	55	M	14	4	1460
5	sCJD	129MM/type 1	71	M	10	–	562
6	sCJD	129MM/type 1	61	M	30	–	745
7	sCJD	129MM/type 1	69	M	15	–	940
8	sCJD	129MM/type 1	73	F	4	–	1100
9	sCJD	129MM/type 1	68	F	2	–	1260
10	sCJD	NA/type 1	66	M	2.5	–	1435

dCJD, dura CJD; F, female; GSS, Gerstmann-Sträussler-Scheinker disease; M, male; MM, methionine homozygote at prion protein gene codon 129; NA, not available; PPS, pentosan polysulfate; PrP^{res}, proteinase resistant isoform of prion protein; sCJD, sporadic CJD.

at 5000 rpm for 30 s in a bead disrupter homogenizing system (MicroSmash MS-100; Tomy Seiko Co., Ltd, Tokyo, Japan). Homogenates were then clarified by centrifugation at 250 *g* for 5 min and the supernatant was stored at –80°C.

Detection of PrP^{res}

Conventional procedure for the detection of PrP^{res} was conducted as follows: 1% brain homogenate was prepared in extraction buffer (100 mM Tris-HCl, 100 mmol NaCl, 10 mmol EDTA, 0.5% Nonidet P-40, 0.5% sodium deoxycholate, pH 7.6) and incubated with 50 μg/mL proteinase K (PK) at 37°C for 1 h. Protease activity was then abolished by the addition of 1 mmol Pefabloc SC (Roche, Indianapolis, IN, USA). Undigested PrP^{res} fragments were separated by SDS-PAGE in 12% NuPAGE Bis-Tris gels (Invitrogen, Carlsbad, CA, USA) and transferred onto polyvinylidene difluoride membranes (Immobilon-P; Millipore, Billerica, MA, USA). PrP was detected using anti-PrP antibody (mouse monoclonal 3F4, 1:10 000) as the primary antibody and peroxidase-conjugated anti-mouse IgG as the secondary antibody (AP192P, 1:20 000; Chemicon, Temecula, CA, USA). The immunoreaction was visualized using the ECL plus Western Blotting Detection System (GE Healthcare; Chalfont St. Giles, Buckinghamshire, UK).

Size-exclusion gel chromatography assay

We performed the size-exclusion gel chromatography assay using the spin-column kit CHROMA SPIN-200 (Clontech, San Francisco, CA, USA) that clearly separated oligomeric PrP from monomeric PrP.[19,22] The samples were first centrifuged at 120 *g* for 2 min, and the first fraction was collected in the collection tube. Another 40 μL of lysis buffer was added, and the samples were centrifuged at 120 *g* for 2 min to collect the size-exclusion fractions sequentially. In these operations, we used a centrifuge with a swing-bucket rotor (A-4–62; Eppendorf, Hamburg, Germany). Fractionated PrP was detected without PK treatment by SDS-PAGE and Western blot analysis, as described above.

RESULTS

Case reports and brain pathology

Details of PPS treatment and clinical findings from the patients were described in a previous paper.[14] In all four PPS-treated cases, the PPS infusion catheter was inserted into the right lateral ventricle, and the PPS dose was gradually escalated to the target dosage of 120 μg/kg/day. PPS treatment showed no apparent improvement of clinical features in all the cases. Clinicopathological findings from each case that had received PPS treatment are described below.

Case 1

A 72-year-old woman showed truncal ataxia and progressive gait disturbance. She had no family history of prion or neurological disease. Diffusion weighted imaging (DWI) demonstrated diffuse bilateral high-intensity signals in the cerebral cortex and striatum. Periodic synchronous discharge was seen in electroencephalography 2 months after disease onset. She was diagnosed with sCJD. Myoclonus and startle reaction were observed 3 months after the onset. The PPS infusion was started 3 months after the onset; however, no improvement in clinical features was observed. She developed akinetic mutism 6 months after the onset. Tonic seizures in extremities were also seen. The patient died of pneumonia and autopsy was performed 14.5 h after death. The brain weighed 660 g and showed severe atrophy with bilateral subdural hematoma and fluid collection. Microscopy demonstrated severe neuronal loss, rarefaction of neuropil, and gliosis across the cerebral cortices (Fig. 1A). Although astrocytosis was noted in HE staining, GFAP expression was weak in the cerebral cortices, except in subpial astrocytes (Fig. 2A). These pathological changes were also seen in the basal ganglia and thalamus. Synaptic PrP deposition was detected in the cerebral cortices, basal ganglia and thalamus (Fig. 1B). There was no laterality of spongiform change, neuronal loss, gliosis or PrP deposition.

Case 2

A 66-year-old man showed dysarthria 25 years after dura mater graft implantation because of cerebral hemorrhage. He manifested right hand clumsiness and progressive gait disturbance. Myoclonus was also seen in the right extremities. He had no family history of prion disease or neurological disease. DWI showed abnormal high-intensity signals in bilateral temporal cortices. In addition, brain biopsy was performed and revealed a type 1 PrP^{res} accumulation. The patient was diagnosed with dCJD. The electroencephalogram showed no periodic synchronous discharges. He developed akinetic mutism 2 months after disease onset. PPS infusion was started 3 months after the onset, but no improvement of clinical features was observed. The patient died of pneumonia and autopsy was performed 9 h after death. The brain weighed 950 g and showed severe atrophy with bilateral subdural fluid collection. Spongiform change, severe neuronal loss and gliosis were seen in the cerebral cortices (Fig. 1C). Astrocytosis was found in all layers of the cerebral cortex; however GFAP expression was weak (Fig. 2B). Synaptic PrP

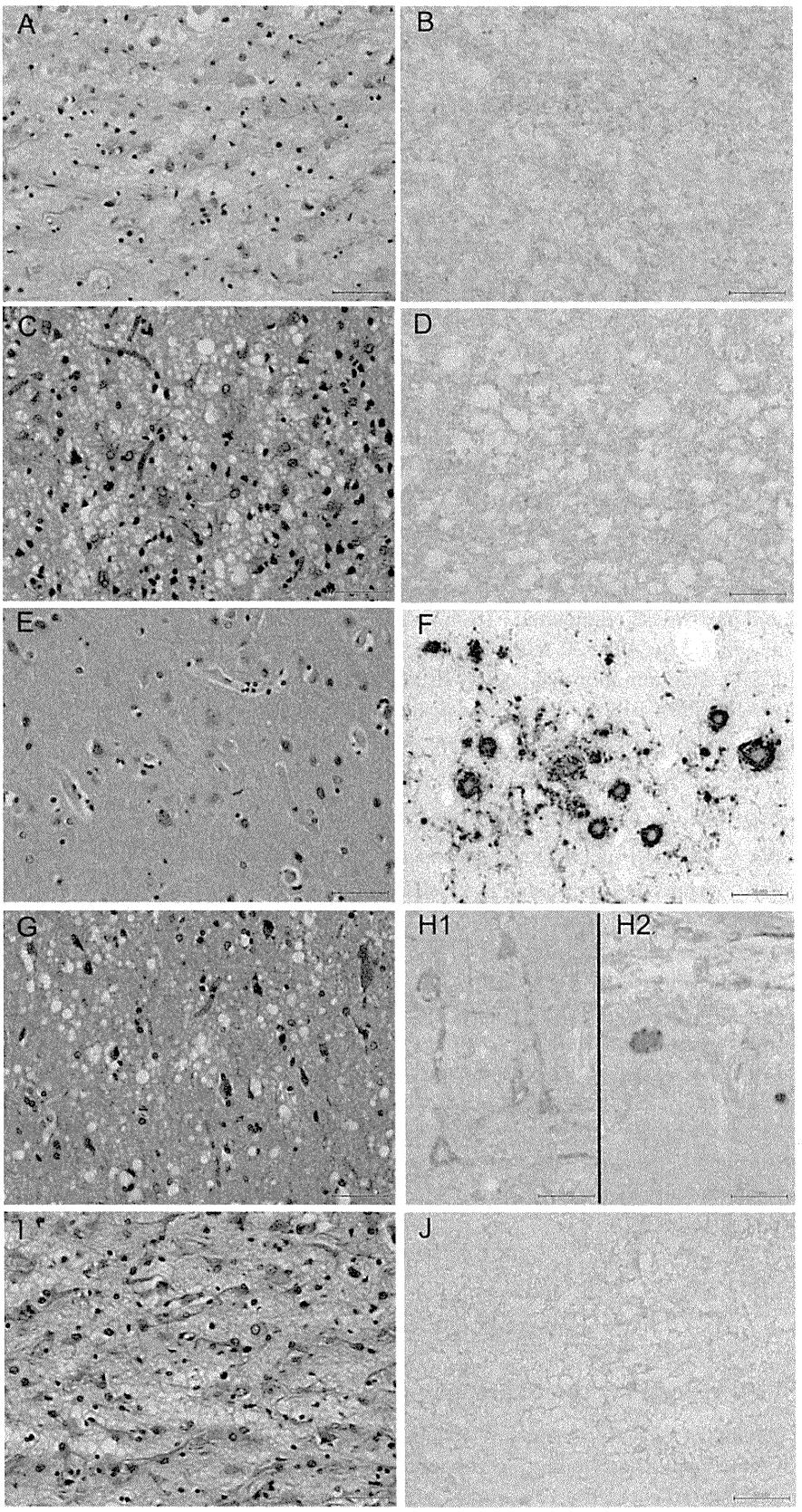

Fig. 1 Light micrographs of frontal cortices in prion diseases. A, C, E, G, I: HE stain. B, D, F, H1, H2, J: immunohistochemistry for PrP. Case 1 shows severe neuronal loss and significant rarefaction of neuropil (A) and synaptic PrP deposition (B). Case 2 shows typical spongiform change (C) and synaptic PrP deposition (D). Case 3 shows several amyloid plaques, neuronal loss and gliosis; however spongiform change is very mild (E). Both plaque-type deposition and synaptic deposition of PrP are detected (F). Case 4 shows neuronal atrophy and spongiform change (G). Synaptic deposition of PrP is mainly seen around pyramidal neurons of the deep cortical layer (H1) and plaque-type depositions are mainly found in the subpial layer (H2). Case 5 without pentosan polysulfate (PPS) treatment shows severe neuronal loss and remarkable rarefaction (I) and synaptic PrP deposition (J).

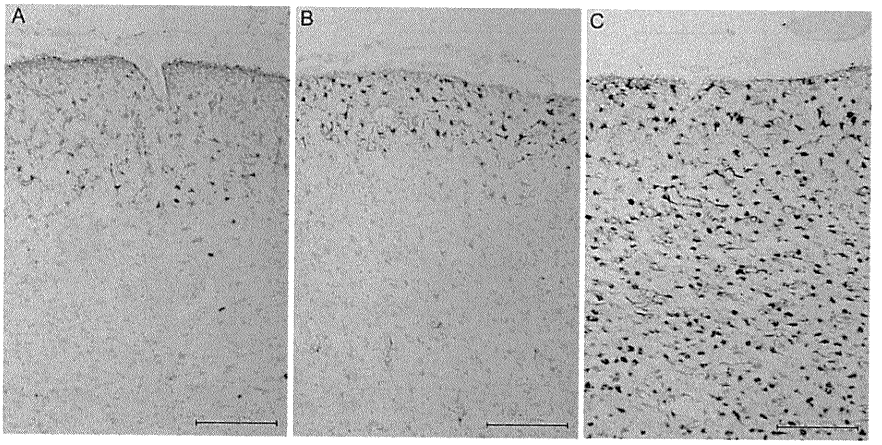

Fig. 2 Immunohistochemistry for GFAP of the frontal cortices. In case 1 (A) and case 2 (B) with pentosan polysulfate (PPS) treatment, subpial astrocytes show strong immunoreactivity for GFAP, but most cortical astrocytes show negative or weak immunoreactivity for GFAP despite their reactive morphology. In comparison, cortical astrocytes in all layers show strong GFAP immunoreactivity in case 5, which did not receive PPS treatment (C).

deposition was also detected in the cerebral cortices (Fig. 1D). No plaque-type deposition of PrP was noted. There was no laterality of spongiform change, neuronal loss, gliosis or PrP deposition.

Case 3

A 68-year-old woman showed progressive gait disturbance and dysarthria. Upper limb ataxia was also observed 5 months after disease onset. DWI showed no apparent intensity changes. The electroencephalogram showed no periodic synchronous discharges. She had a family history of prion disease. Analysis of the PrP gene revealed a P102 L mutation. She was diagnosed with GSS and the PPS infusion was started 6 months after the onset. No clinical improvement was observed. The patient died of pneumonia and autopsy was performed 27 h after death. The brain weighed 1055 g and showed moderate atrophy with bilateral subdural fluid collection. Spongiform change and neuronal loss were mild (Fig. 1E). Although astrocytosis was found in all layers of the cerebral cortices, GFAP immunoreactivity was seen mainly in the superficial cortical layers. Numerous plaque-type PrP depositions were noted in all layers of the cerebral cortices (Fig. 1F), the basal ganglia, thalamus and cerebellar granular layer. Synaptic PrP deposition was also seen in the molecular layer of the cerebral cortices, basal ganglia and thalamus. No laterality of pathological change was seen.

Case 4

A 55-year-old man showed dizziness 19 years after dura mater graft implantation. He developed dysarthria, memory deficits and character changes 8 months after disease onset. Myoclonus and startle reaction were also observed 10 months after the onset. The electroencephalogram showed no periodic synchronous discharges. DWI demonstrated high-intensity signals in the right caudate nucleus and the right thalamus. He had no family history of prion disease or neurological disease. Brain biopsy was performed and showed a type 1 PrP^{res} accumulation. The patient was diagnosed with dCJD. Although PPS infusion was started 10 months after the onset, no clinical improvement was observed. The patient died of pneumonia and autopsy was performed 13 h after death. The brain weighed 1460 g. Right subdural hematoma was noted, but brain atrophy was not apparent. Spongiform change, severe neuronal loss and gliosis were apparent in the precentral gyrus, entorhinal cortex, anterior cingulate gyrus, thalamus and putamen (Fig. 1G). Even in regions where astrocytosis was found in all layers, GFAP immunoreactivity was seen exclusively in the superficial layer of the cerebral cortices. Both synaptic deposition of PrP (Fig. 1H1) and plaque-type deposition of PrP (Figs 1,2) were noted in the cerebral cortices, basal ganglia and hippocampus. There was no laterality of spongiform change, neuronal loss, gliosis or PrP deposition.

In PPS(−) cases, HE staining revealed that the levels of neuronal loss, spongiosis and gliosis advanced in accordance with the loss of brain weight. Various levels of synaptic PrP deposition were noted in each case. In case 5, which showed severe brain atrophy, neuronal loss and rarefaction of neuropils were evident (Fig. 1I). Both astrocytosis and marked GFAP immunoreactivity were noted in all layers of the cerebral cortices (Fig. 2C). Synaptic PrP deposition was also detected in cerebral cortices (Fig. 1J).

The ratio of PrP^{res}/total PrP

Western blot analysis detected PrP^{res} and total PrP. Cases 1 and 2 showed a type 1 pattern (Fig. 3A,B). Case 3 showed PrP^{res} fragments with molecular masses of around 8 kDa (Fig. 3C). Case 4 showed PrP^{res} fragments with intermediate size between types 1 and 2 (Fig. 3D). We calculated the ratio of PrP^{res}/total PrP based on the signal intensities of the immunoblots. In PPS(−) cases, the ratio of PrP^{res}/total PrP was already increased in case 9 with mild brain