

- Grade 3 toxicity for neurological symptoms or for symptoms in other organ systems lasting longer than 5 days and attributed to PPS

Patients considered for PPS administration had to have a probable diagnosis of one of the above PrD in accordance to WHO criteria. Normal haematological, renal, and liver function was also a requirement. Because of the surgical procedure for implantation of the ventricular catheter and subcutaneous pump and infusion system, ongoing treatment with anticoagulants such as warfarin, heparin, clopidogrel, or aspirin was not allowed. Also the presence of any active infection or any viral syndrome within two weeks prior to treatment was an exclusion criterion.

Patients undergoing surgery had standard ventricular catheters placed in the anterior horn of the right lateral ventricle or in a few cases in both frontal horns, unless clinical reasons dictated another point of access to the ventricular system. In the first case with PPS administration, the catheter was connected initially to an external pump for trial administration of PPS, and later attached to a subcutaneously programmable pump (Synchromed EL, 18 ml reservoir, with side port, Medtronic Inc.) (Figure 4) permanently implanted in the abdominal wall. Later cases had simultaneous implantation of the catheter system and the infusion pump in the same surgical session. After a period of time after the surgical procedure (3-14 days) in which the pump was not active and scar tissue formation was expected to occur, PPS infusion commenced at a low dose level. The decision to proceed to the next higher dose level was based on the absence of clinical side effects and on normal findings on non-enhanced CT scans (e.g. exclusion of hydrocephalus or intracranial blood).

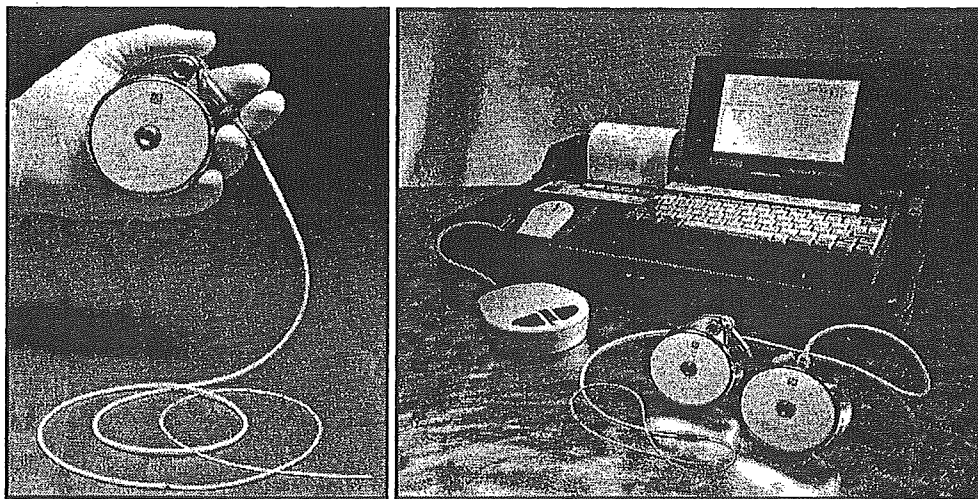


Figure 4. The implantable externally programmable pump (Synchromed EL with side port, Medtronic Inc., Minneapolis, MN) is shown with attached catheter (A). (B) shows a portable pump programming unit (telemetry unit) with printer.

There are no previously published data on a safe or potentially effective dose of cerebroventricularly infused PPS in human patients with PrD. Based on preclinical animal work, a dose escalation schedule was set up starting at 1 $\mu\text{g}/\text{kg}/\text{d}$ and escalating on a daily basis until a target dose of 11 $\mu\text{g}/\text{kg}/\text{d}$ was reached. This represented a 10-fold dose reduction, based on body surface area and weight differences, from the lowest effective PPS dose used in scrapie-infected mice in a preclinical study of intraventricular PPS [80]. The maximum daily dose of cerebroventricular PPS administered to the first 6 patients on a long term basis was 11 $\mu\text{g}/\text{kg}/\text{day}$. All further cases have received a maximum dose of 110 $\mu\text{g}/\text{kg}/\text{d}$ in 10-20 $\mu\text{g}/\text{kg}/\text{d}$ escalation steps, but long term follow up with this dose is still limited (Table 1).

The clinical source of PPS is *Pentosanpolysulfat SP54* in sterile 1 ml vials, supplied by the pharmaceutical company Bene Arzneimittel GmbH (Germany). Each vial contains 100 mg of Sodium-PPS (100 mg/ml) with 1% sodium-4-oxopentanoate as a stabiliser. For filling of the pump reservoir, PPS SP54 100 mg/ml is diluted with 0.9% NaCl to a final concentration of 1-10 mg/ml. The pump is then programmed to deliver the total daily dose in a continuous simple infusion mode (constant volume and infusion rate over time).

There are no standardised or widely accepted criteria for assessment of treatment efficacy in PrD. Surrogate criteria for efficacy were thus adopted and included overall survival, speed of disease progression before PPS infusion compared with disease progression after start of PPS, neuro-radiological imaging, and changes in the general and neurological condition of the patients.

The first patient to receive PPS infusion was a young man suffering from vCJD [98]. He presented initially with subjective signs of behavioural disturbance, followed a few months later by progressive ataxia, pyramidal signs and myoclonus, which led to the clinical diagnosis of possible vCJD. The clinical picture combined with abnormal MR findings in the FLAIR sequence (pulvinar sign) and positive tonsil biopsy allowed the diagnosis of probable vCJD 8 months after the occurrence of initial clinical symptoms. At the time of first administration of PPS, the patient had symptoms of advanced vCJD, such as ataxia, dementia, dysphagia, dysphasia, myoclonus, and was confined to bed and unable to care for himself. He was fed via percutaneous gastrostomy. The initial PPS dose of 1 $\mu\text{g}/\text{kg}/\text{d}$ was escalated without drug-related complications to the target dose of 11 $\mu\text{g}/\text{kg}/\text{d}$. Continuous infusion of PPS for 23 months has not caused any drug-related side effects. Cerebroventricular PPS at the above dose did not have any measurable systemic anticoagulant activity in serum, as confirmed by unchanged INR (international normalised ratio) before and during PPS infu-

sion. Follow-up CT scans demonstrated no intracerebral haemorrhage, and there were no seizures. Subdural fluid collections first over the right hemisphere and subsequently over the left hemisphere necessitated surgical (burr hole) evacuation of fluid. Repeated surgical revisions of the fluid collections were necessary (Figure 5).

This first patient is currently alive and in a stable condition. Although there were no major improvements in the neurological and general condition, there were a few notable changes. The patient is now able to fix his eyes on persons, to obey simple one stage commands, and to make verbalization attempts in response to stimuli. The sleep/wake cycle and the reflex swallow are restored and the myoclonus is reduced. The patient has gained 5 kg of weight compared to pre-PPS baseline, while on the same nutritional regime. Regular follow-up CT scans have shown progressive brain atrophy during PPS administration, which could not be correlated to any worsening of the clinical condition (Figure 5).

Since January 2003, a total of 13 patients with PrD have undergone surgery and continuous cerebroventricular administration of PPS. Anonymised clinical and follow-up data are presented in Table 1. The most important clinical finding is the safety of PPS administration to the cerebral ventricles. The maximum tolerated dose of PPS has not been reached. There were no cases with side effects attributable to PPS, even in patients receiving 110 $\mu\text{g}/\text{kg}/\text{d}$ of PPS.

Focal seizures have been observed in one patient on 11 $\mu\text{g}/\text{kg}/\text{d}$, and generalised tonic-clonic seizures in one patient with 110 $\mu\text{g}/\text{kg}/\text{d}$. It remains to be clarified if these seizures were a side effect of PPS or of surgery, since in both cases they occurred months after start of PPS and during infusion with a stable dose of PPS.

It is currently unclear if a higher dose of PPS has a stronger effect, and if dose escalation should be continued above 110 $\mu\text{g}/\text{kg}/\text{d}$. In most cases it seems that PPS administration results in a temporary halt of disease progression, but this conclusion is not based on hard evidence or objective measurements. PPS administration seems not able to reverse the clinical course of advanced disease and to achieve functional recovery of established neurological deficits.

Furthermore, surgery in the brain affected by PrD may result in a higher rate of surgical complications than usually encountered in comparable non-PrD cases. Brain atrophy may progress while PPS is administered, and there is no apparent correlation between degree of atrophy and clinical status of the patients. Therefore, in accordance with results from the pre-clinical animal studies [80], cerebroventricular infusion of PPS should be commenced as early as possible after disease diagnosis and, if possible, before the occurrence of fixed neurological deficits.

In conclusion, despite the encouraging preliminary results in PrD patients receiving long term cerebroventricular PPS, further clinical, neuroradiological and laboratory investigations in the context of a prospective clinical study will be essential for the evaluation of genuine clinical benefits of PPS administration.

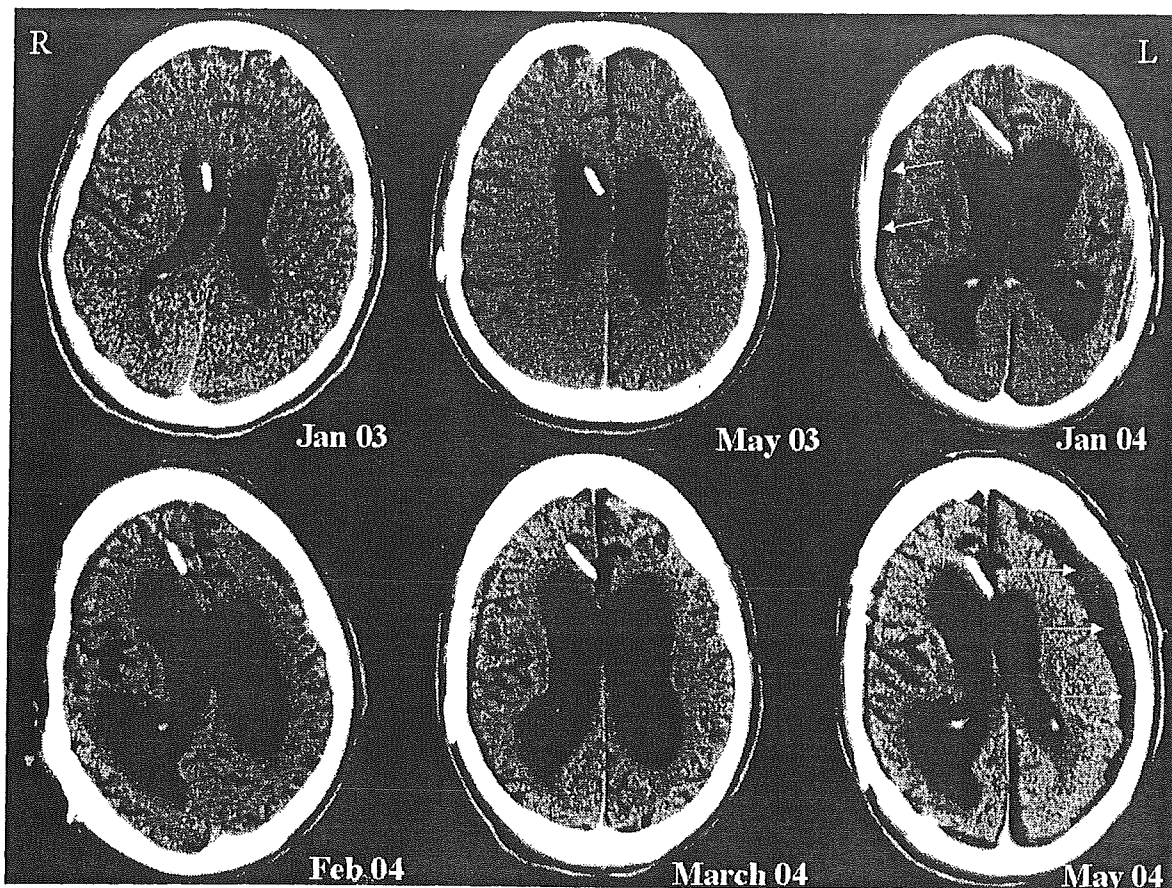


Figure 5. Serial CT scans of patient #1 demonstrating sequential occurrence of right parietal subdural fluid collection (upper right, arrows) and left parietal subdural collection (lower right, arrows). Note the progression of brain atrophy over time.

Table 1. Summary of clinical data of all current patients with PPS administration.

Patient number	Sex	Age at Dx ^a (years)	Diagnosis and clinical course after start of PPS	Survival (months after Tx ^a)	Maximum PPS dose ($\mu\text{g}/\text{kg}/\text{d}$)
1	M	17	vCJD. Stable disease, swallowing and myoclonus improved, brain stem function improved. PPS started at very advanced stage of disease.	23	11
2	M	19	sCJD. Initially neurological improvement, later slow progression. Weight gain 10 kg. Reduction of myoclonus. Partial seizures occurring a few months after start of PPS, currently on phenytoin.	10	11
3	F	12	vCJD. Stable disease, wheelchair bound. Currently speech deficit, stable weight, swallowing remained intact.	13	11
4	M	15	vCJD. Stable after PPS, but disease progressed rapidly before PPS started.	9	11
5	F	34	GSS Stable disease, but surgical complications (brain haemorrhage) giving rise to neurological deficits.	10	11
6	F	32	GSS Stable disease. Initially only very mild neurological symptoms present.	3	11
7	M	37	Iatrogenic CJD (GH ^b administration) Cerebellar syndrome, initially stable condition. Rapid deterioration despite PPS.	6	110
8	F	27	Iatrogenic CJD (GH ^b administration) Stable disease, but rapid progression before start of PPS. Alive but in state of limited awareness.	9	110
9	F	39	vCJD. Presented with psychiatric syndrome. Continuous neurological deterioration while on PPS. Generalized seizures occurring 2 months after start of PPS. Died of disease progression.	4 ^c	110
10	M	44	GSS Continued neurological deterioration. Increase in mental symptoms and disorientation while on PPS.	4	110
11	M	34	Iatrogenic CJD (GH ^b administration) Stable disease.	1	110
12	F	39	GSS Mild neurological deficits at start of PPS.	- ^d	110
13	F	66	sCJD	- ^d	110

^a - Dx/Tx - Diagnosis/Therapy.

^b - Growth hormone.

^c - Patient deceased.

^d - Follow-up period < 1 month.

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プリオン病の治療

—経口キナクリン療法とペントサン硫酸の脳室内持続投与法の現状—

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プリオン病は伝染性海綿状脳症と考えられ、発症後その進行は早く、平均約2ヵ月で無動無言になることが多い。これまでに有効な治療法は全くなし、診断後は対症療法しかない。しかし、英国における変異型CJD、およびほぼ同時期に本邦で発生が多かった硬膜移植後の医原性CJDの発生後、即戦力となる治療の開発が求められた。キナクリン(抗マalaria薬)やペントサンポリサルフェートは実験的に抗プリオン効果が認められ、臨床応用が期待された。これまでの本邦および海外における最新の治療研究を概説する。

はじめに

プリオン病は伝染性海綿状脳症として知られ、いったん発症すると、進行性でかつ致死性の疾患である。臨床病型は孤発性、遺伝性(家族性)、感染性の3つに分類され、感染性には伝染が疑われるものと医原性のものが含まれる¹⁾。プリオン病のあらゆる病型において共通してプリオン蛋白の代謝異常が認められ、プロテアーゼ抵抗性の異常型プリオン蛋白(PrP^{sc})の蓄積がみられる。PrP^{sc}の異常な蓄積は、主に中枢神経に起こる。ヒトのプリオン病はクロイツフェルト・ヤコブ病(CJD)、Gerstmann - Sträussler - Scheinker (GSS)症候群、クールー病(kuru)、致死性家族性不眠症(FFI)などの病型を呈する。このうち80%は孤発性のCJDであり、一般にその進行は早く、発症後は平均約2ヵ月で無動無言になる。これまでに有効な治療法は全くな

く、いったん診断されたら後は対症療法しか考えられなかった。しかし、この疾患に対する考えが一変した理由は、英国で発生した変異型CJDであり、ほぼ同時期に本邦で発生が確認された硬膜移植後の医原性CJDであった。どちらも古くから認められた疾患ではなく、人為的な行為の結果作られた疾患(man-made disease)であり、若年者で多発したことから、即戦力となる治療が求められたのである。

1 プリオン蛋白とは

正常のプリオン蛋白(PrP^c)はすべての動物において存在し、主に中枢神経系で、少量はリンパ球組織で発現する。プリオン遺伝子がコードする蛋白質であり、転写後にゴルジ体で糖鎖修飾を受け、細胞膜に移動する。培養細胞では細胞膜上での半減期は3~6時間であり、その後分解されるとされて

Key words

■プリオン病
■クロイツフェルト・ヤコブ病(CJD)
■キナクリン
■ペントサンポリサルフェート

いる²⁾。正常 PrP^cは 253 アミノ酸蛋白で、分子量は 35~36 kDa で、その生理的作用は不明であるが、銅結合蛋白質としての機能や酸化ストレスに関与している可能性が考えられている。

一方、異常プリオン蛋白 (PrP^{sc}) はすべてのプリオン病の病理で認められ、ヒトのみならず動物のプリオン病、たとえばスクレイピーにおいても、脳にその蓄積が認められる。正常の PrP^c に転写後に何らかの構造変化を引き起こされ、 β シート構造に富む PrP^{sc} へと変化し、病的蓄積を生じる³⁾と考えられている。

脳における PrP^{sc} の病的蓄積が神経細胞死、反応性のグリオシス、ミクログリアの増加、そして海綿状変化を引き起こす。これらの脳病理はプリオン病の診断に必須で、アミロイド斑が認められることもある。PrP^{sc} は感染性を有し、種を超えて伝播が可能であり、新たなプリオン病を生じる可能性を有する。しかし、PrP^c が発現していない宿主にはプリオン病は感染しない。

2 プリオン病の現状

孤発性の CJD は 1921 年に初めて報告されたが、患者のほとんどは 40~80 歳までの間に発病し、発生率は年間 100 万人に一人とされている。本邦では年間に 100 名近くの患者が新たに診断されており、その平均年齢は 65.6 歳である⁴⁾。典型的な症状としては、進行性の痴呆とミオクローヌス、

小脳性運動失調や視覚異常などの臨床症状のほか、脳波では周期性同期性放電が認められ、診断に有用とされている。また、脳脊髄液中の 14-3-3 蛋白、neuron-specific enolase (NSE) やタウ蛋白が、CJD における神経細胞死の生物学的マーカーとして有用であることが報告されている⁵⁻⁸⁾。また、プリオン蛋白遺伝子 (PRNP) 内コドン 129 のメチオニンとバリンの遺伝子多型は、臨床表現型に影響を与えることがわかっている⁹⁾。

医原性プリオン病は、本邦で 100 例を超えて報告⁴⁾されており、ヒト硬膜の移植後に数年から十数年経った後に発症している。まれに角膜移植からの感染が疑われる報告もある。遺伝性プリオン病の発生は全体の約 10% を占めている。遺伝性プリオン病は、いずれも常染色体優性遺伝で PRNP 内に点変異あるいは挿入変異を有する。

プリオン病の臨床症状は、必ずしも均一ではなく多彩である。先ほど述べた進行性痴呆、ミオクローヌス、小脳性運動失調、視覚異常などは頻度が高く、他にうつ、無気力、不安神経症などの精神症状が強い場合や、ジストニア、舞踏様運動や錐体路症状、感覚症状(感覚過敏など)が出現する。

変異型 CJD は 1996 年に報告され、ウシ海綿状脳症 (BSE) との関連が確実視されている¹⁰⁾。孤発型 CJD との違いは若年発症(平均 29 歳)で進行は遅い。脳病理も異なり、海綿状病理の周囲に florid plaques が認められる。英国での発生がほとんどであるが、最近本邦でも 1 例目が報告された¹¹⁾。

どの型の CJD も発症年齢、初発症状に違いはあるものの進行性であり、ほぼ数ヵ月から長くても数年で無動無言となり死に至る。診断のみ確立されて、その後はただ対症療法のみであった CJD 治療は、近年一変している。表 1 はこれまでの実験室的研究、すなわち培養細胞や動物実験での研究に使われた主な薬物である。この中のいくつかは、若干なりとも予防的効果のあることが確認されている¹²⁾¹³⁾。しかしながら動物の研究において、症状が出現した時にはすでに脳内には PrP^{sc} の沈着が多く認められることから、理想の予防的治療は症状発症前、すなわち PrP^{sc} の蓄積がまだ脳機能障害を起こす以前に開始されなければ理論上有効ではない。しかしながらこの発症前診断は現在困難であり、やはり発症のできるだけ早期に治療を開始する以外に方法はない。しかし今後、どのような治療法の開発においても、早期あるいは発症前診断というものは必要となる。

治療法の探求 —キナクリンの報告とペンタサン硫酸 脳室内投与の取り組み—

これまでに実験室的に有効性の確立された薬剤が臨床的に使用された報告は数少ない(表 1)が、この中でこれまでの治療研究の概略と、福岡大学で行われたキナクリン治療の結果、ペンタサンポリサルフェート脳室内投与の海外と本邦の取り組み状況などを述べる。

抗マラリア薬であるキナクリン、キニーネや抗精神病薬であるクロルプロ

表1 プリオン病治療研究に使われた化合物

抗ウイルス剤	抗寄生虫剤	免疫賦活剤	ホルモン
Amantadine	Quinacrine*	Bacterial polysaccharide	Adrenalin
Adenine arabinoside	Chloroquin*	BCG	Estradiol
Cytosine arabinoside	Glycobiarsol	Phytohemagglutinin	Insulin
Isopinosine	Metronidazole	Vaccinia virus	Prednisone
Methisazone	Niclosamide	OpG-deoxyoligonucleotide*	Propylthiouracil
Phosphonoacetic acid	Suramin		Testosterone
Rifampicin		免疫抑制剤	
Sodium butyrate	ポリアニオン	Antilymphocytic serum	その他
Sodium thiocyanate	Carrageenan*	Arachis oil	Chlorpromazine
Thiamphenicol	Chondroitin sulfate	Indomethacin	Colchicine
Virazole	Dextran sulfate*		DEAE-dextran
	Heparan sulfate	抗癌剤	DTA-R Ig
抗細菌剤	HPA-23*	Actinomycin D	Cysteine protease inhibitors
Dapsone	Pentosan polysulfate*	Cyclophosphamide	Neutral dextran
Rifampicin	Polyvinyl sulfate	Methotrexate	Quabain
Sulfamethoxazole	Silicotungstate	Streptozotocin	Silica
Tetracycline			Tetrapyrroles
Trimethoprim	インクレーブウイルス		Trypan blue
Thiamphenicol	およびその誘導体		Vitamin C
	Newcastle disease virus		
抗真菌剤	PolyI-PolyC		
Amphotericin B	Sendai virus		
Griseofulvin	Statofen		
Mepartricin	Tilorone HCl		
MS-8209*			
Sinetungin			

*: 実験的に何らかの有効性を報告されているもの。

(文献 12 より引用)

マジンは、培養細胞における実験系で PrP^cから PrP^{sc}への構造変化を防止する作用がある。また、キナクリンは神経芽腫細胞における異常型プリオン蛋白の蓄積を阻害する¹⁴⁾¹⁵⁾。キナクリンはマラリアに対する治療薬として60年以上の歴史があるために、CJDに対する治療的研究が、米国および英国にて始まり、現在進行形である(文献 13, PRION -1 study: <http://www.ctu.mrc.ac.uk/studies/cjd.asp>)。

本邦でもプリオン病に対して、抗マ

ラリア薬であるキナクリン、キニーネの効果および副作用の検討を行った¹⁶⁾¹⁷⁾。キナクリンを孤発性CJD 22例、医原性(硬膜移植後発症)CJD 5例、および遺伝性プリオン病4例の計31症例に、300 mg/dayを経口または経管投与を行った。両者とも副作用出現がない限り、原則として12週間連日投与とした。

その結果、キナクリン治療を行った12例(39%)に臨床症状の部分的改善を認めた(表2, 3)。効果の平均持続

期間は3.2±3.5週(1~12週)で、効果の内容は覚醒度の改善、自発語の増加、固視反応の改善などであった。しかし、この効果は一過性で、キナクリン投与が継続されていたにもかかわらず消退した。孤発性CJD症例においては、治療開始時の覚醒、意識レベルが高い症例に治療効果がみられた。すなわち、自発語や聴覚・視覚刺激に反応を認めた10例中の8例(80%)に部分的改善を認めた。一方で、症状が進行した無動無言状態で治療を開始した

表2 孤発性CJDにおけるキナクリン治療効果

効果	性別 男/女	年齢 (歳)	発症から投与 までの期間 (月)	開始時の認知機能 レベル 刺激に反応あり	開始時の認知機能 レベル 無動性無言	効果持続 期間 (週)
あり(N=9)	3/6	63.2±8.6	9.1±11.1	0	1	3.2±3.5
なし(N=12)	4/8	64.6±10.1	7.3±6.3	2	10	0
p値	ns	ns	ns	<0.001		—

ns：有意差なし。

表3 キナクリン治療の効果とその内容

性/年齢	診断	投与後の認知機能その他の変化	効果の持続 期間(週)
F/67	医原性CJD	閉眼時間の延長、発語の増加。	4
M/76	孤発性CJD	自発閉眼時間の延長、Eye-to-Eyeコンタクトが可能。	1
M/63	孤発性CJD	投与2週後に痛み刺激に患者への視覚注視が出現。	1
F/37	医原性CJD	感情失禁、外部からの刺激に突ったり泣いたり表情の表出、光・音・呼びかけに直視あり、自発運動の出現、PSDの消失、背景脳波の出現。	4
F/72	孤発性CJD	痛覚刺激にて上肢の動きが出現。	4
F/64	孤発性CJD	投与1週後で覚醒状態の改善がみられた。	1
F/62	孤発性CJD	投与1～2週後で発語が少しみられ、意志疎通性が上昇した。自動運動の増加。	1
F/59	遺伝性GSS	投与10日～3週後に意味のある発語が増加した。投与10日後で坐位保持可となったがすぐに不可となった。	2
M/46	孤発性CJD	投与4～5日後には反射性ミオクローヌス減少、「うー」と唸り声が聞かれるようになった。以後持続。投与2～5週後に音の方向を注視する反応あり。	3
F/68	孤発性CJD	指示動作や挨拶が可能。	12
F/59	孤発性CJD	追視、笑顔がみられた。四肢の自発運動は増加した。不随意運動も粗大な運動に変わったが、その後、もとのミオクローヌスに戻った。	4
F/59	孤発性CJD	投与1週後に呼びかけに反応、ミオクローヌスの減弱。家族の話しかけに笑顔。このような変化は2週間後にはもとに戻る。	2

12例では、1例(8%)にしか確かな変化は認められなかった。一方、医原性CJD症例では5例中2例(40%)に覚醒度の改善を認めた。遺伝性プリオン病症例では4例のうち長期経過の1例(GSS¹⁰²)において効果を認めたが、他の3例(いずれもCJD²⁰⁰)では無効であった。

治療中止を余儀なくされた副作用として、肝機能障害を16例(52%)に、溶血性貧血、発熱、誤嚥、偽膜性大腸炎、および皮疹をそれぞれ1例に認めた。副作用症状はキナクリン投与中止後に全例で改善した。

プリオン病に対するキナクリン治療は、短期間ではあるが、臨床的に部分的改善を示すことを初めて明らかにした。一方で、半分以上の症例に肝機能障害が出現し、中止を余儀なくされた。これらの点から治療プロトコルの再検討が必要と考えられる。

ペントサンポリサルフェート(PPS)は動物感染実験において、末梢から感染する前に投与すれば発症を遅らせる効果があること、また、神経芽腫細胞におけるPrP^{sc}の蓄積を阻害する¹⁸⁾¹⁹⁾ことなどがわかっていた。この効果は、PPSがプリオン蛋白の線維形成を阻害するか、あるいは細胞膜上のプリオン蛋白を減少させるためと推察されている²⁰⁾²¹⁾。PPSは血液脳関門を通らないために、脳室内に直接投与する必要があった。Doh-uraら²²⁾は脳内感染させたマウスに対して、4週間の脳室内薬物投与を感染後10日目および35日目に開始した。使用薬物はPPSの他キナクリン、アンホ

テリシンBなどであった。

このうちアンホテリシンBは感染後10日目の投与で30%、35日目の投与で12%の発症遅延効果を認めた。PPSはアンホテリシンBよりさらに強力に発症を抑制した(10日目投与で173%、35日目投与で93%)。この時、最も有効な投与量は230 $\mu\text{g}/\text{kg}/\text{day}$ であった。投与開始が早いほど発症抑制効果は強力であった。脳の免疫組織学的検討やウェスタンブロットでも脳へのPrP^{sc}蓄積は著明に抑制されており、また、230 $\mu\text{g}/\text{kg}/\text{day}$ の投与量では明らかな副作用は認めず、イヌにおける実験でも230 $\mu\text{g}/\text{kg}/\text{day}$ までの投与量ではいかなる副作用も示さなかった。

PPSは経口投与や静脈内投与では血液脳関門を通過せず、脳室内に直接投与する必要がある。PPSの脳室内持続投与法は、プリオン感染マウスにおける実験で発症を遅延させる効果が証明された。臨床効果のみならず、マウスの脳病理において、異常プリオン蛋白の蓄積が投与側で抑制されていることが認められた。ヒトプリオン病に対する臨床試験は、2003年英国で変異型CJDに対して同治療の1例目が行われて以降、全世界で現在までに15例のプリオン病患者に同治療が行われ、患者での安全性と有効性が評価されているところである(文献23, personal communication)。

現在、福岡大学で作成したプロトコルは、腹部皮下体内埋め込み型微量注入器具の留置および脳室内カテーテルの留置手術を行い、術後8日目より

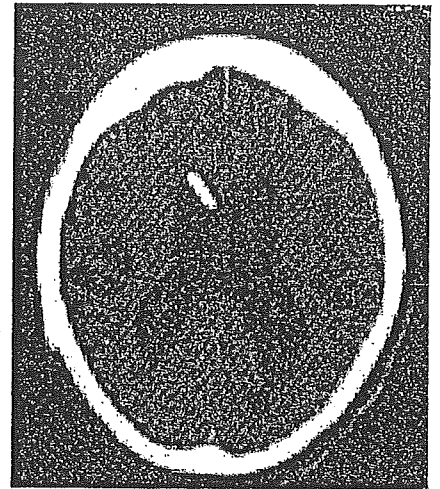


図1 脳室内カテーテルの留置後のCT

PPS投与を低濃度で開始する。その後、漸増し維持量に到達させる。至適維持量に関してはまだ検討中である。その後、4週間ごとに腹部皮下の微量注入器具中の薬液を新しい薬液に交換充填する。本邦では1例目のPPSの脳室内持続投与は孤発性CJDの66歳女性例に行われた²⁴⁾。治療開始は発症から9ヵ月後であった。PPSはプロトコルどおりに腹部に埋め込んだ持続注入ポンプから右側脳室に注入された。手術後1週間はポンプに生食を満たし、頭部CTで出血がないことを確認した後(図1)にPPSを低濃度(1 $\mu\text{g}/\text{kg}/\text{day}$, 50 $\mu\text{g}/\text{day}$)より始め、徐々に濃度を上げて60 $\mu\text{g}/\text{kg}/\text{day}$ (3.0 mg/day)とした。現在、同濃度にて維持療法中である。治療開始7ヵ月現在、明らかな臨床効果を示していない。一方で、血算、生化学、凝固検査、頭部CTで副作用は認められない。現在のところ、PPS脳室内持続投与

法は抗プリオン作用を持つ, 最も期待できる方法と考えられるが, 今後は経過の注意深い観察と, 安全域を考えた治療濃度設定を検討する必要がある。

おわりに

プリオン病の基礎研究から臨床治験に至るまでを概説したが, 治療法の開発に関して, 今ようやく始まったばかりである。今後の発展のためには, この疾患の病態の把握と, 薬物の機序に関してのさらなる研究, 知見や, 診断学の向上による早期の発見, 治療評価法の確立, そして今回の PPS 治療にみられるように, 脳外科医, 麻酔医や手術室のスタッフの協力など, 集学的な取り組みが必要である。

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6-*O*-Sulfo sialylparagloboside and sialyl Lewis X neo-glycolipids containing lactamized neuraminic acid: Synthesis and antigenic reactivity against G159 monoclonal antibody[†]

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Synthesis and antigenic reactivity of 6-*O*-sulfo sialylparagloboside (SPG) and sialyl Lewis X (sLe^x) neo-glycolipids containing lactamized neuraminic acid are described. The suitably protected GlcNAc- β (1→3)-Gal- β (1→4)-GlcOSE derivative was glycosylated with NeuTFAc- α (2→3)-Gal imidate to give NeuTFAc- α (2→3)-Gal β (1→4)-GlcNAc- β (1→3)-Gal- β (1→4)-GlcOSE pentasaccharide. The partial *N,O*-deacylation in the NeuTFAc- α (2→3)-Gal part afforded *N*-deacetylated SPG derivative which was converted to the desired oligosaccharide containing lactamized neuraminic acid. Similar treatment of the sLe^x hexasaccharide derivative, NeuTFAc- α (2→3)-Gal- β (1→4) [Fuc- α (1→3)]-GlcNAc- β (1→3)-Gal- β (1→4)-GlcOSE, gave the key hexasaccharide intermediate containing lactamized neuraminic acid. These suitably protected SPG and sLe^x oligosaccharides were converted stepwise into the desired neo-glycolipids (GSC-551 and GSC-552) by the coupling with 2-(tetradecyl)hexadecanol, 6-*O*-sulfation at C-6 of the GlcNAc residue, and complete deprotection.

Both lactamized-sialyl 6-*O*-sulfo SPG (GSC-551) and sLe^x (GSC-552) neo-glycolipids were clearly recognized with G159 monoclonal antibody showing that both the lactamized neuraminic acid and the 6-*O*-sulfate at C-6 of GlcNAc would be involved in the G159-defined determinant. However, the Fuc residue and the lipophilic (ceramide) part may not be critical for this recognition.

Published in 2005.

Keywords: Selectin, glycolipid, sialic acid, sialyl Lewis X, sialylparagloboside, carbohydrate, antibody, sulfation

Introduction

Selectins (L-, E- and P-selectin) are a family of carbohydrate-binding cell adhesion molecules which play important roles in homing of lymphocytes, recruitment of leukocytes to sites of inflammation, thrombosis, cancer metastasis, etc. [2,3]. It has been demonstrated that sialyl 6-*O*-sulfo Lewis X (I, Figure 1) is an endogenous L-selectin ligand on the human high endothelial venule (HEV) [4–8]. Recently, de-*N*-acetyl sialyl 6-*O*-sulfo Lewis X [9,10] was found to be a superior ligand for L-selectin,

which may be inactivated (down regulation) by conversion into the cyclic structure detected with G159 monoclonal antibody (G159 mAb) [11].

In the previous study, we have shown that the structure defined by G159 mAb may be lactamized sialyl 6-*O*-sulfo Lewis X (III) suggesting the existence of a new immunity adjustment mechanism regulated by the structural change of sialic acid [4,11] (Figure 1).

Since this discovery, we have systematically synthesized a series of gangliosides [12] and neo-glycolipids [1] containing lactamized neuraminic acid for mapping the G159 mAb recognition sites in more detail. As described in a preceding paper [1], the lactamized-sialyl 6-*O*-sulfo Lewis X B₃₀ neo-glycolipid (GSC-534) (Figure 2) was not recognized with G159 mAb to suggest that the lactose part and/or ceramide in lactamized-sialyl 6-*O*-sulfo Lewis X ganglioside (GSC-535) may be critically important for the recognition by G159 mAb.

[†]Synthetic studies on sialoglycoconjugates, Part 138. For part 136, see Ref [1], and for part 137, see Ref [19].

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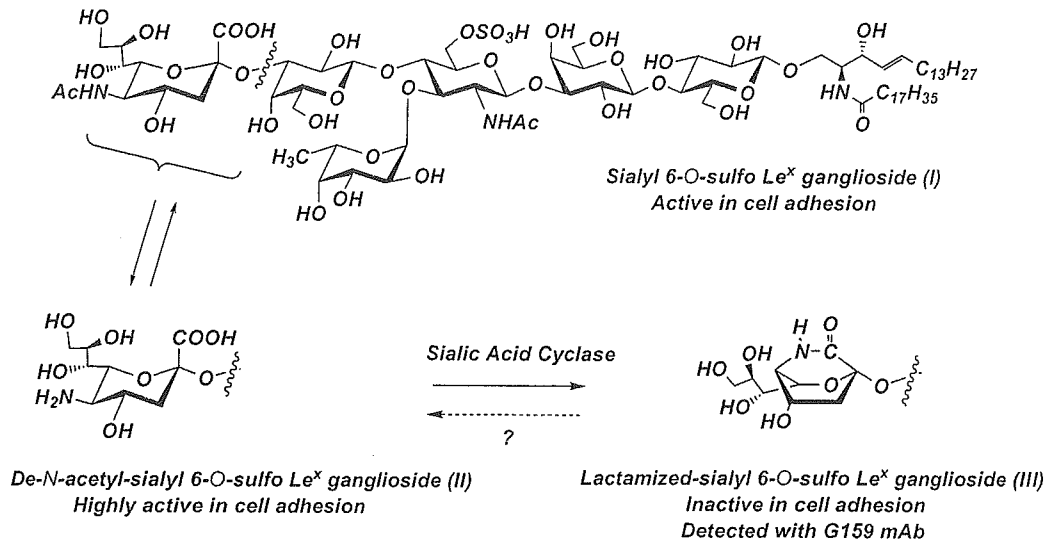


Figure 1. Proposed ligand processing pathway and binding reactivity for human L-selectin.

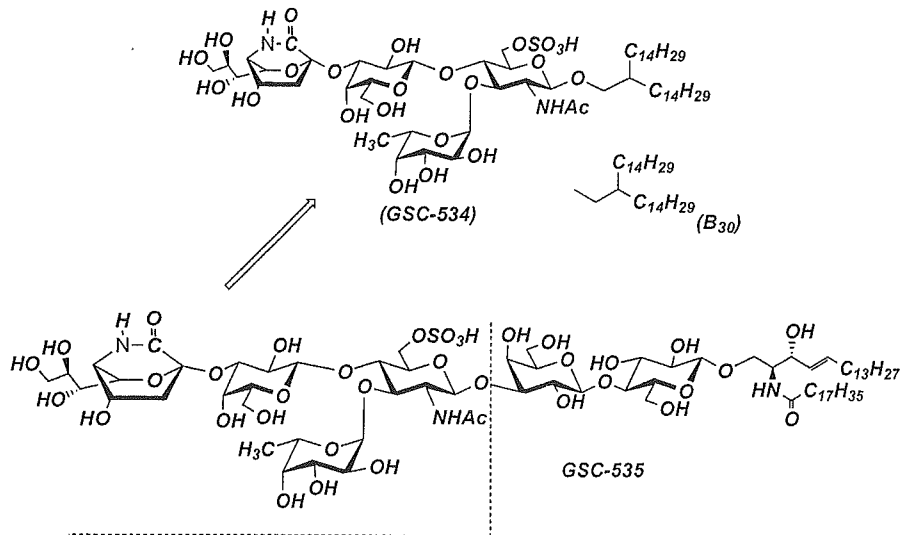


Figure 2. Lactamized sialyl 6-O-sulfo LewisX ganglioside (GSC-535) and the sLe^x tetrasaccha neo-glycolipid (GSC-534).

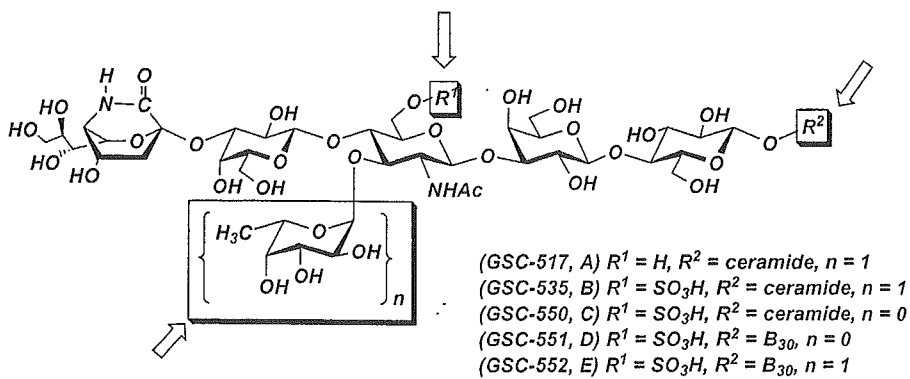
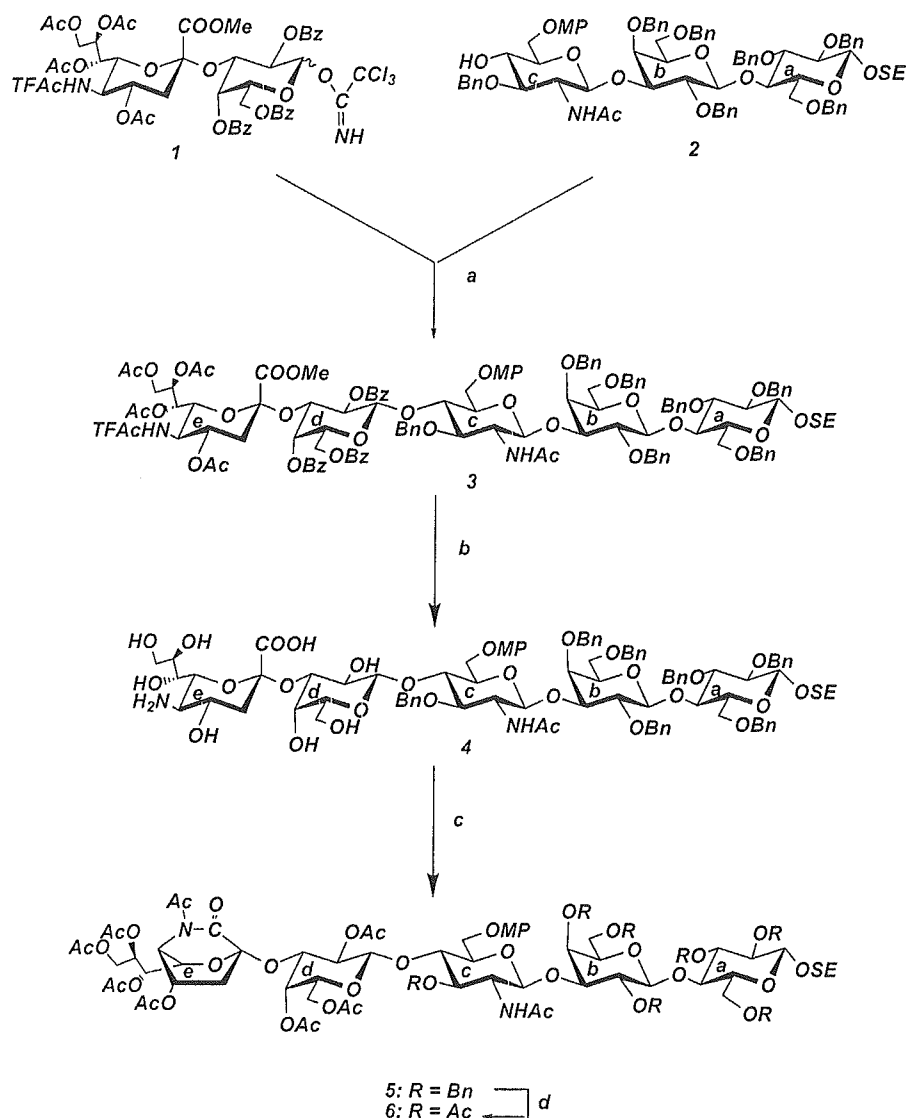


Figure 3. Three modification sites of Lactamized sialyl 6-O-sulfo Lewis X ganglioside to clarify the recognition specificity of G159 mAb.



Scheme 1. (a) TMSOTf/CH₂Cl₂, MS4Å, 3°C, 76%; (b) NaOMe, MeOH, then H₂O, 45°C, 80%; (c) 1, WSC-HCl, HOBT, DMF, 70°C, 2, Ac₂O, Pyr., 46% (two steps); (d) 1, H₂, Pd(OH)₂, EtOH, 2, Ac₂O Pyr., 93% (two steps).

We here report the synthesis of novel sialylparagloboside (**D**, GSC-551) and sialyl Le^X (**E**, GSC-552) neo-glycolipids containing both lactamized neuraminic acid and lactose (Figure 3), and examine the structural requirements for recognition with G159 mAb by comparing the immunostaining reactivity of the related gangliosides (**A**, GSC-517; **B**, GSC-535; **C**, GSC-550) [12].

Results and discussion

Coupling of **1** [13] and suitably protected trisaccharide **2** [14] in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf) and powdered molecular sieves 4 Å (AW 300) gave the expected pentasaccharide **3** in 76% yield (Scheme 1).

The partial *N,O*-deacetylation of **3** with sodium methoxide in methanol for 72 h at 45°C, and subsequent saponification of the methyl ester group afforded **4** in 80% yield. Lactamization

of the neuraminic acid residue in **4** was achieved by treatment with WSC-HCl and HOBT in DMF at 70°C, followed by acetylation, to give **5** (46% in 2 steps). In the ¹H NMR spectrum of **5**, the values of vicinal couplings (*J*_{3,4}) changed dramatically (Table 1) to indicate clearly that typical ^{5,2}*B* boat conformation was formed by lactamization of the neuraminic acid part of **4** (²C₅ chair conformation). The lactamization reaction was greatly improved by using *O*-benzotriazol-1-yl-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HBTU) and HOBT in DMF at 65°C as described for the sialyl Le^X derivative **13** (Scheme 3). Hydrogenolytic removal of the benzyl (Bn) groups in **5** and the following acetylation gave **6** (93% in 2 steps). Compound **6** was then transformed into the corresponding trichloroacetimidate **7** by selective removal of the 2-(trimethylsilyl)ethyl (SE) group with trifluoroacetic acid (TFA) and subsequent imidate formation (Scheme 2). Glycosylation of