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Intraventricular pentosan polysulphate in human prion diseases: an observational study in the UK

I. Bone[a], L. Belton[b], A. S. Walker[b] and J. Darbyshire[b]

[a]*Department of Medical and Cardiovascular Studies, Western Infirmary, Glasgow, UK and* [b]*MRC Clinical Trials Unit, London, UK*

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Background, purpose and methods: This observational study assessed the effect of continuous intraventricular infusion of pentosan polysulphate (PPS) in seven patients at different clinical centres in the UK. *Results:* Complications of intraventricular catheterization were frequent. PPS was well-tolerated over a wide dose range (11–110 μg/kg/day) during the 6-month study. Four patients were assessed for the entire study period: one remained stable, two showed minimal deterioration and one progressed significantly. *Conclusion:* Mean survival of all patients was longer than reported values for natural history of specific prion disorders. Possible reasons for these findings are explored.

Introduction

The rare neurodegenerative human prion diseases include sporadic, inherited and iatrogenic forms of Creutzfeldt–Jakob disease (CJD), variant CJD and Gerstmann–Sträussler–Scheinker (GSS) disease. There are no proven disease-modifying therapeutic options, although various strategies have been proposed by Collinge and others [1–3]. Polyanions, for example, delay progression of animal transmissible spongiform encephalopathies [4–7].

Pentosan polysulphate (PPS; syn SP54; Tavron; Elmiron) is a sulphated, semi-synthetic, polysaccharide polyanion with a low molecular weight [8] and a heparin-like structure. It does not penetrate the blood–brain barrier after oral or parenteral administration [9]. Experiments with direct PPS brain infusions in animals [10] have led to speculation about a role in human prion diseases [11–13], but there are difficulties in extending such findings to prion diseases in people.

Direct intraventricular infusion of PPS in a vCJD patient was described by Todd *et al*. [14] using doses extrapolated from animal studies [10]. It was difficult to assess efficacy and, although the infusion did not lead to specific toxicities, it was suggested that CJD patients may be particularly susceptible to risks associated with intraventricular catheterization. In two case series, Rainov *et al*. [15,16] found no toxicities associated with intraventricular PPS over a wide dose range and suggested that it was associated with increased survival in vCJD. More recently, a patient with vCJD survived for 51 months after diagnosis with 31 months of PPS infusion, with no side-effects reported [17].

Correspondence: Prof. Ian Bone, Department of Medical and Cardiovascular Studies, Western Infirmary, Glasgow G11 6NT, UK (tel.: 44 1436 671472 e-mail: gcl136@clinmed.gla.ac.uk).

Most studies on PPS have been single case reports or case series, which may not have been personally observed, and anonymization may mean that some are reported more than once. Problems encountered in comparing survival in small numbers of highly selected patients and in estimating disease onset are similar to the assessment of quinacrine in the Prion-1 trial [18].

In the UK, patients who are receiving PPS are geographically scattered, have different types of human prion disease and are managed and assessed in different ways. Standardization of clinical observations and collection of existing data are essential and underpin the rationale for this study.

Methods

Patients with human prion disease receiving PPS infusions were identified from records of the National Prion Clinic and the National CJD Surveillance Unit. Information on a patient from a separate study [14] was included to provide context for discussions about survival. The initial monitoring protocol, developed by an independent consultant neurologist, was approved by caregivers and treating clinicians. Consent was obtained from caregivers and/or patients. The Eastern Multicentre Research Ethic Committee approved the study. The full protocol is available online [19].

Records from clinicians, the registries and multidisciplinary meetings were examined by the neurologist for information on disease progression, catheter insertions, pumps, drug dosages and drug safety using standard case record forms. Side-effects were graded using the National Cancer Institute's common toxicity criteria. Clinical assessments were conducted in the patients' homes by the neurologist over 6 months, including structured interviews of caregivers to assess disease status, general health, concomitant drug treatment,

hospital visits, level of care and use of aids/supports. Side-effects, drug interactions and episodes of infection were documented. Routine neurological assessments were performed, using standard operational definitions of conscious state. All data were anonymized.

Results

Table 1 summarizes the characteristics of the participants.

Surgical procedures and complications

Surgery was performed in five neurosurgical units using standard ventricular catheters, pumps and reservoirs. Pumps were implanted in subclavicular or epigastric pouches. Catheters were placed uni- or biventricular (using stereotactic neuronavigation in two patients). Catheter-siting problems occurred in four patients and early complications in two of these four – symptomatic subcortical ischaemic stroke (Fig. 1) and postoperative pyrexia. A further patient had an asymptomatic right caudate haematoma detected on postoperative imaging. CT scans were performed around insertion in all patients. Time from surgery to PPS infusion was 5–28 days. Pumps were refilled regularly by different clinical disciplines (two neurosurgery, three neurology and two anaesthetics). One was re-sited due to erosion through the skin, and

Figure 1 Postoperative CT of symptomatic subcortical (lacunar) infarction with haemorrhagic transformation in patient with inherited CJD receiving continuous intraventricular infusions of pentosan polysulphate (patient 3).

Table 1 Characteristics of seven patients with prion disorders receiving continuous intraventricular infusions of pentosan polysulphate (PPS)

Patient number	1	2	3	4	5	6	7
Year of birth	1965	1985	1961	1964	1989	1976	1963
Gender	Female	Female	Female	Female	Male	Female	Female
Type of disease	Iatrogenic/ hGH	Variant CJD	Inherited/ GSS	Inherited/GSS	Variant CJD	Iatrogenic/ hGH	Variant CJD
Genotype/mutation	N/A	129:M/M	P102L	P102L	129:M/M	129:M/M	129 M/M
Disease onset	December 2003	June 2003	June 2001	February 2001	November 2002	April 2003	February 2003
PPS initiation	July 2004	October 2003	November 2003	September 2004	December 2003	March 2004	February 2004
Maintenance dose (mg/kg/day)	110	11	11	11	32	110	110
Date recruited	September 2005	October 2005	October 2005	October 2005	October 2005	October 2005	January 2006
Mobility at recruitment	Bed-bound	Chair-bound	Bed-bound	Mobile	Chair-bound	Bed-bound	–
Home visits	September 2005	October 2005	October 2005	October 2005	October 2005	October 2005	–
	November 2005	December 2005	–	January 2006	January 2006	–	–
	March 2006	February 2006	–	April 2006	January 2006	–	–
Date of death	August 2006	–	October 2005	–	–	September 2005	June 2004
Survival (to August 2007) (months)	32	50	52	64[a]	57	29	16
Months from disease onset to PPS (months)	7	4	29	43	13	11	12[c]
Duration of PPS treatment[b] (months)	25	46	21	21[a]	44	18	4[c]

GSS, Gerstmann–Sträussler–Scheinker; hGH, human growth hormone.

[a]To June 2006.

[b]To August 2007 or death.

[c]PPS discontinued 4 weeks before death.

one had late complications (disconnection). CT checks of catheter position after insertions were performed variably, if at all.

Doses of PPS

Doses were extrapolated from animal studies [10] and escalated over 15–40 days to 11–110 μg/kg/day (weight-adjusted 0.55–7.5 mg/day) (Table 1). One patient was undertreated at two separate hospitals for 4–6 weeks due to dose miscalculation. There was no commercially available assay for drug stability.

Safety of PPS treatment

Potential risks of PPS treatment include seizures and intracranial haemorrhage. Patients were monitored retrospectively from records and assessed prospectively for skin bruising, other haemorrhagic complications, seizures or significant worsening of myoclonus. None occurred in seven patients over 148 patient months. Two patients died from pneumonia (despite aggressive treatment with antibiotics). One other patient had tonic–clonic seizures, associated with raised inflammatory markers and sepsis, which did not recur when infusion resumed. No specific toxicity was observed.

Clinical data

The frequency and reasons for emergency admissions were similar before and after PPS treatment (none were related to treatment or catheter/pump failure). Acute interventions at home were infrequent. Four patients required antibiotics for chest/urinary infections. None required fluid replacement or assisted ventilation. One underwent regular oropharyngeal suction. Standard tube-feeding procedures (percutaneous endoscopic gastrostomy or radiologically inserted gastrostomy) were required by five patients. Previous putative anti-prion treatments included quinacrine, flupertine, thalidomide, memantine hydrochloride. Oral PPS was taken in two patients, one of whom also took minocycline.

Clinical assessment

One patient (7) died before the first visit. Two (3, 6) died after the first visit (respiratory complications). All planned neurological assessments were conducted in one patient. All three visits (but not all neurological assessments) were completed in four patients; one died after the third visit, two are known to be alive in August 2007 (as is the patient from Todd's study [14]) and the third was not contacted after the end of the study but was known to be alive in June 2006 (Table 1).

Pre-PPS symptoms were minimal to moderate in two patients and severe in five. Eleven specific activities (milestones) were defined to monitor functional ability, communication, sensory ability and emotionality. These were charted for each patient (example given in Fig. 2) and for each activity (Fig. 3; see [19] for full details). Functional ability deteriorated before and following PPS initiation and during prospective assessment, with an estimated median of 16 (range 6–30) milestones passed since PPS initiation to last known alive in the entire group of seven patients, the specific activities with milestones and numbers passed within each activity varying substantially across patients. The total number of milestones passed in all patients following PPS initiation was 30, 17, 19, 11, 9, 6 and 16, respectively. In the four patients assessed over three visits during prospective follow up a total of nine milestones were passed (0, 0, 8 and 1, respectively), the level of disability in two of these, during assessments, leaving little scope for further worsening.

Neurological assessment included some or all of Glasgow Coma Scale, Modified Rankin Scale, Barthel's Index for Activities of Daily Living, Disability Rating Scale, Level of Cognitive Function Scale, Mini-Mental

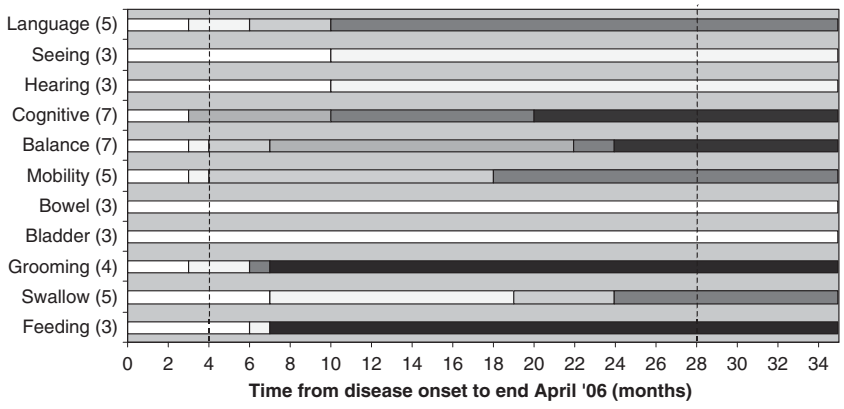

Figure 2 Milestones (levels 1–5) of disease progression in patient with variant CJD (patient 2) receiving continuous intraventricular infusions of pentosan polysulphate (PPS). Dotted lines indicate initiation of PPS 4 months after onset and time of first assessment at 28 months.

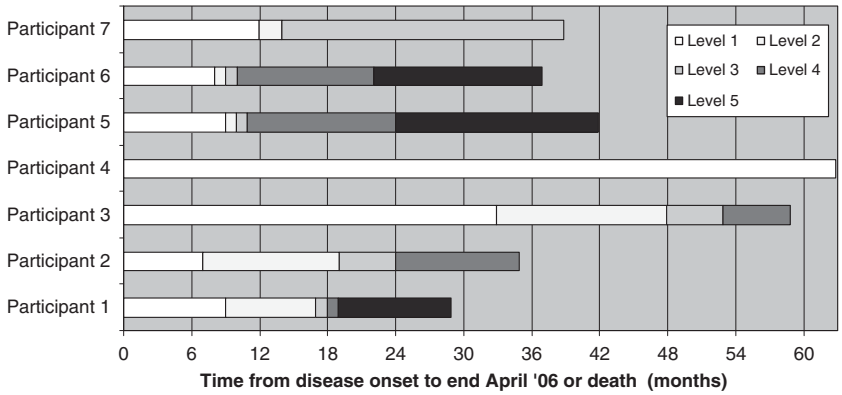

Figure 3 Milestones for swallowing disability (levels 1–5) in seven patients with various prion disorders receiving continuous intraventricular infusions of pentosan polysulphate.

State Examination, Alzheimer's Cognitive Assessment Scale, Clinician's Dementia Rating, Global Impression of Change and Brief Psychiatric Rating Scale.

Whilst repeated standardized clinical examinations showed some extrapyramidal signs and involuntary movements resolving, these were only replaced by others. Four patients met criteria for minimal consciousness state (none for permanent vegetative state). Progressive changes were subtle in three of four patients visited on three occasions, with no sustained worsening of arousal, conscious level or brainstem function during this time. Patient 4 remained cognitively stable, but had temporary declines in neurological function coinciding with catheter disconnection between second and third visits.

Full details of the assessments can be found online [19].

Disease duration and survival

Survival of the seven patients from estimated disease onset to death or last known alive (see 'clinical assessment' section) is documented in Table 1. Table 2 compares data for seven patients plus the extra case [14] with those of natural history patients. Individual survival of all patients in all disease groups (16–75 months) was at or beyond the reported means [20] and medians [M. Pocchiari, personal communication; 21]. All but one vCJD patient also survived beyond the ranges of survival determined by both Pocchiari (personal communication) and Stewart *et al*. [21].

Table 2 Survival data to August 2007 for patients with prion disorders receiving continuous intraventricular infusion of pentosan polysulphate (PPS) and untreated patients in two natural history studies [20,21]

	Survival (months) in PPS-treated patients	Survival (months) in untreated (natural history) patients Pocchiari *et al*. [personal communication; 20]	Stewart *et al*. [21]
Iatrogenic CJD (hGH)			
Number in study	2	85	162
Actual value/range	29, 32	4–45	2–30
Median		13	9
Inherited GSS			
Number in study	2	24	21
Actual value/range	52, 64	3–133	2–84
Median		39	48
Variant CJD			
Number in study	4[a]	86	159
Actual value/range	16, >50, >57, >75[a]	6–39	6–39
Median		13	14

Note: Where patients last seen alive, survival time denoted by '>'. GSS, Gerstmann–Sträussler–Scheinker; hGH, human growth hormone.

[a]Including survival data (75 months) from additional patient from Todd *et al*. [14].

Other investigations

Patients underwent various tests, including coagulation, electrolyte and cerebrospinal fluid protein 14-3-3 levels, differential cell counts, erythrocyte sedimentation rate, urinalysis, chest X-rays, analysis of gait, heart rate variability and detailed neuropsychometry. All had initial CT scans, and some had further CT/MRI monitoring (between 1 and 18 scans per patient). Sequential scans (Fig. 4) were carried out in only three patients.

Discussion

PPS was administered by the intraventricular route and complications with the catheterization procedure occurred in four of seven patients, as previously observed [14,15]. Some catheters were positioned biventricular and some univentricular, though substances

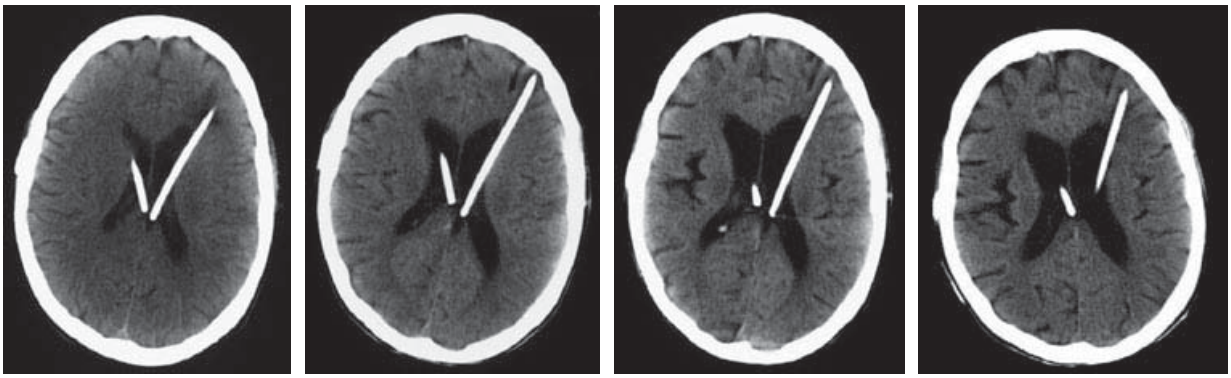

Figure 4 Sequential follow-up CT scans over 3 months in patient with variant CJD (patient 2) receiving continuous intraventricular infusions of pentosan polysulphate.

injected into just one ventricle may distribute evenly throughout the CNS [22,23]. The extent of PPS distribution will be critical, given the diffuse pathology of the different prion disorders [24,25].

No safe or effective dosing regimen has been established for PPS in humans. Doses varied 10-fold in this study. The dose of 11 μg/kg/day, derived from animal studies [10] and calculated from total body area [14], may be an underestimate for infusion into a single compartment (M. Rawlins, personal communication). Doses up to 110 μg/kg/day were tolerated, with no toxicity, increase in seizures or haemorrhagic complications. The low patient numbers made conclusions of safety unreliable – only overt toxicity could be detected.

Standardized follow-up imaging, which might have allowed crude assessment of brain atrophy [26], was not performed regularly in these patients (not at all in one) [26]. Assessment was further confounded by movement artefacts. Sequential scans were rarely taken, but did reveal continuing atrophy throughout therapy (Fig. 4). Frisoni *et al*. [27] emphasize the need for prospective imaging to assess brain atrophy, preferably at a single site and with movement artefacts minimized.

Of the four patients assessed prospectively for the entire 6-month study, one remained stable, two showed minimal deterioration and one deteriorated significantly. PPS efficacy was difficult to quantify because of the short time frame, low patient numbers, variable patient/disease characteristics and differences in management and assessment. It was not possible to recruit a control group with matching baseline features as planned in the initial protocol.

S*urvival*, as measured by the interval between disease onset and death, can be a robust end-point for measuring efficacy. However, it is very difficult to establish retrospectively when the first symptoms emerged. Increased public awareness of these diseases and differences in the ability to identify/recall details of early non-specific symptoms may mean that diagnosis is being made earlier in the disease course. Comparisons with previous data may, therefore, be subject to 'lead-time' bias. Even if the precise point of onset can be determined, disease survival is probably to be influenced by age and gender, genotype, disease-modifying treatments, level of care, treatment complications, infections, immobility and nutritional status. A collaborative study [20] used data from 10 national registries of prion disorders, including untreated (natural history) cases, and revealed differences in survival in vCJD, iatrogenic (human growth hormone) and inherited (GSS) forms of the disease (Table 2). Additional survival data were provided by Stewart *et al*. [21] in a systematic review that included studies showing variability in disease progression, levels of care, life-ending decisions and life-prolonging interventions. Table 2 compares the survival of the seven patients with published figures [M. Pocchiari, personal communication; 21].

There are many possible reasons why survival of PPS-treated patients might appear longer compared with such untreated patients: including chance alone and biases such as lead-time bias from attentive carers diagnosing onset early; selection bias from included patients having prolonged survival whilst awaiting PPS or bias from increased use of active interventions for complications in more actively managed PPS patients amongst others. Self-selection related to longer survival is less probably in this study because there were variable delays from disease onset to treatment (4–19 months in vCJD cases; 7–11 months in iatrogenic cases and 29–43 months in GSS cases).

Survival of all seven patients exceeded mean survival of natural history patients [20] but were within reported ranges for iatrogenic and inherited cases [21]. Three out of four vCJD patients (including the additional patient

from [14]) survived longer than the reported median and range for both natural history studies [M. Pocchiari, personal communication; 21]. By definition, for every new patient surviving longer than the median survival time, one will not – thus the probability of surviving longer than the median survival time is 0.5. If there were no effect of PPS on survival, the probability of two patients with iatrogenic or inherited prion disease both exceeding the median survival time of previous untreated patients just by chance is 0.25 (0.5^2). The probability of four vCJD patients exceeding the median survival time by chance alone is 0.0625 (0.5^4). Compared with natural history data [M. Pocchiari, personal communication; 21], the patients with inherited GSS had survival times of 52 and 64 months (last seen alive), which lie within reported range [21] for untreated patients and thus do not differ significantly. The two patients with iatrogenic disease died 29 and 32 months after diagnosis, both similar to the maximum reported by Stewart *et al*. [21]. Rank-sum testing suggests the probability of two treated patients surviving beyond the maximum of 111 untreated patients (if there were no difference between treated and untreated patients and also none of the other potential biases described above) is 0.02, which indicates there may be a very weak suggestion/possibility of some effect given the fact that survival times were similar to the maximum, and the strong possibility of lead-time bias in this group of patients. Of the four vCJD patients, in whom untreated comparison patients are more contemporaneous, one survived for 16 months (beyond the median of 13 months in (M. Pocchiari, personal communication) and 14 months in [21]) and three are still alive in August 2007 (all exceeding the mean, median and range of untreated patients in both natural history studies). The probability of this occurring (if there were no difference between the treated and natural history patients and no other potential biases) is 0.01, indicating some suggestion/possibility of an effect.

These findings must, of course, be interpreted cautiously because of the difficulty in determining disease onset from non-specific and subtle symptoms and the very small numbers. An accurate date of onset and a longer period of prospective assessment would yield more data for evaluating efficacy. However, if other explanations can be discounted, then prolonged survival in these patients may suggest a treatment effect of PPS.

Conclusions

This small case series found that widely varying doses of PPS delivered intraventricularly were safe, resulting in patient survival similar to or greater than in previously reported historical controls, in most cases. However, complications from the surgical procedures were common. Small observational studies with this much variability cannot quantify efficacy and offer only limited data on safety. They can, however, be valuable if procedural and follow-up protocols are strictly controlled. Recent draft guidelines on clinical trials in small populations by the Committee for Medicinal Products for Human Use recognize the importance of observational studies and case reports when data are collected in a controlled manner. They recommend use of a surrogate marker when recruitment of sufficient patients is difficult or will take a very long time. This commonly occurs in studies of the rare human prion diseases, yet there are no obvious surrogate markers. More experimental work in animal models is clearly needed, both on efficacy and on the distribution and binding of PPS following intraventricular administration. If results are encouraging, a controlled trial in human prion disease should be considered, having determined optimum doses. Until then, all patients with prion diseases considering PPS therapy should be informed of existing evidence and, if opting for treatment, managed and monitored in a standardized manner.

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References

1. Collinge J. Molecular neurology of prion disease. *Journal of Neurology, Neurosurgery and Psychiatry* 2005; **76:** 906–919.
2. Caughey B, Caughey W, Kocisko D, *et al*. Prions and transmissible spongiform encephalopathy (TSE) chemotherapeutics. A common mechanism for anti-TSE compounds. *Accounts of Chemical Research* 2006; **39:** 646–653.
3. Head MW, Farquhar CF, Mabbott NA, Fraser JR. The transmissible spongiform encephalopathies: pathogenic mechanisms and strategies for therapeutic intervention. *Expert Opinion on Therapeutic Targets* 2001; **5:** 569–585.
4. Kimberlin RH, Farquhar CF, Walker CA, Dickinson AG. Suppression of scrapie infection in mice by inorganic and organic polyanions. In: Tateishi J ed. *Proceedings of Workshop on Slow Transmissible Diseases*. Tokyo: Japanese Ministry of Health and Welfare, 1984: 119–128.
5. Ehlers B, Diringer H. Dextran sulphate 500 delays and prevents mouse scrapie by impairment of agent replication

in spleen. *Journal of General Virology* 1984; **65:** 1325–1330.
6. Farquhar CF, Dickinson AG. Prolongation of scrapie incubation period by an injection of dextran sulphate 500 within the month before or after infection. *Journal of General Virology* 1986; **67:** 463–473.
7. Kimberlin RH, Walker CA. Suppression of scrapie infection in mice by heteropolyanion 23, dextran sulphate and some other polyanions. *Antimicrobial Agents and Chemotherapy* 1986; **30:** 409–413.
8. Janssen-Ortho Inc. *Product Monograph: Elmiron (Pentosan Polysulfate Sodium)*. Janssen-Ortho Inc., Toronto: Canada, January 2006. Available at: http://www.janssen-ortho.com
9. MacGregor IV, Dawes J, Paton L, Pepper DS, Prowse CV, Smith M. Metabolism of sodium pentosan polysulphate in man. Catabolism of iodinated derivatives. *Thrombosis and Haemostasis* 1984; **51:** 321–325.
10. Doh-ura K, Ishikawa K, Murakami-Kubo I, *et al*. Treatment of transmissible spongiform encephalopathy by intraventricular drug infusion in animal models. *Journal of Virology* 2004; **78:** 4999–5006.
11. Farquhar C, Dickinson A, Bruce M. Prophylactic potential of pentosan polysulphate in transmissible spongiform encephalopathies. *Lancet* 1999; **353:** 117.
12. Dealler S, Rainov N. Pentosan polysulphate as a prophylactic and therapeutic agent against prion disease. *IDrugs* 2003; **6:** 470–478.
13. Dealler S. Post-exposure prophylaxis after accidental prion inoculation. *Lancet* 1998; **351:** 600.
14. Todd N, Morrow J, Doh-ura K, *et al*. Cerebroventricular infusion of pentosan polysulphate in human variant Creutzfeldt–Jacob disease. *Journal of Infection* 2005; **50:** 394–396.
15. Rainov N, Whittle I, Doh-ura K. Treatment options in patients with prion disease. The role of long term cerebroventricular infusion of pentosan polysulphate. In: Kitamoto T ed. *Prions (Food and Drug Safety)*. Tokyo: Springer-Verlag, 2005.
16. Rainov NG, Tsuboi Y, Krolak-Salmon P, Vighetto A, Doh-ura K. Experimental treatment for human transmissible spongiform encephalopathies: is there a role for pentosan polysulphate? *Expert Opinion Biological Therapy* 2007; **7:** 1–14.
17. Parry A, Baker I, Stacey R, Wimalaratna S. Long-term survival in a patient with vCJD treated with intraventricular pentosan polysulphate. *Journal of Neurology, Neurosurgery and Psychiatry* 2007; **78:** 733–734.
18. MRC Clinical Trials Unit. Prion-1 Trial: Quinacrine for human prion disease. A partially randomised patient preference trial to evaluate the activity and safety of quinacrine in human prion disease. Available at: http://www.ctu.mrc.ac.uk/studies/cjd.asp (accessed 28/07/2007).
19. Medical Research Council. Intraventricular pentosan polysulphate in human prion disease – a study of experience in the United Kingdom (issued 1 June 2006). Available at: http://www.mrc.ac.uk/Utilities/Documentrecord/index.htm?d = MRC003453 (accessed 28/07/2007).
20. Pocchiari M, Puopolo M, Croes AE, *et al*. Predictors of survival in sporadic Creutzfeldt–Jacob disease and other human transmissible spongiform encephalopathies. *Brain* 2004; **127:** 2348–2359.
21. Stewart L, Rydzewska L, Keogh G, Knight RSG. A systematic review of therapeutic interventions in human prion disease. *Neurology* (in press).
22. Abbott NJ. Evidence for bulk flow of the brain interstitial fluid: significance for physiology and pathology. *Neurochemistry International* 2004; **45:** 545–552.
23. Fenstermacher J, Ghersi-Egea J, Finnegen W, *et al*. The rapid flow of cerebrospinal fluid from ventricles to cisterns via the subarachnoid velae in the normal rat. *Acta Neurochirurgica Supplement* 1997; **70:** 285–287.
24. Ironside JW, Head MW. Neuropathology and molecular biology of variant Creutzfeldt–Jacob disease. *Current Topics in Microbiology and Immunology* 2004; **284:** 133–159.
25. DeArmond SJ, Ironside JW, Bouzamondo-Berstein E, Peretz D, Fraser JR. Neuropathology of prion diseases. In: Prusiner SB, ed. *Prion Biology and Diseases*, 2nd edn. New York: Cold Spring Harbor, 2004: 777–856.
26. Yerby MS, Sundsten JW, Larson EB, Wu SA, Sumi SM. A new method of measuring brain atrophy: the effect of aging in its application for diagnosing dementia. *Neurology* 1985; **35:** 1316–1320.
27. Frisoni GB, Scheltens P, Galluzzi S, *et al*. Neuroimaging tools to rate regional atrophy, subcortical cerebrovascular disease, and regional cerebral blood flow and metabolism: consensus paper of the EADC. *Journal of Neurology, Neurosurgery and Psychiatry* 2003; **74:** 1371–1381.

