INTRACEREBROVENTRICULAR INFUSION OF PENTOSAN POLYSULPHATE IN HUMAN VARIANT CREUTZFELDT-JAKOB DISEASE

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Abstract

Objective: Creutzfeldt-Jakob disease (CJD) is a fatal neurodegenerative disease associated with the accumulation of an abnormal isoform of the prion protein (PrP^{sc}) in the brain. Variant CJD (vCJD) is a form of CJD believed to be caused by the bovine spongiform encephalopathy (BSE) agent. Unlike the sporadic form, vCJD mostly affects young adults and adolescents. At present there is no specific or effective treatment available for any form of CJD.

Pentosan polysulphate (PPS), a large polyglycoside molecule with weak heparin-like activity, has been shown to act against prion infection in a rodent scrapie model. PPS seems to prevent the production of further PrP^{sc} and to remove existing PrP^{sc} when given by systemic injection. These properties of PPS prompted the intracerebro-ventricular PPS administration in one patient with vCJD.

Case report: A 19 years old man with advanced vCJD and persistent neurological deficits was treated with PPS. The drug was infused directly into the cerebral ventricles via a permanently implanted ventricular catheter connected to a subcutaneous reservoir. A dose escalation regime was used, with the PPS dose increasing from 1 µg/kg/day (total daily dose 66 µg) to a final dose of 11 µg/kg/d (660 µg/d). There were no serious adverse effects due to PPS infusion in the CSF space at any dose level. The drug did not have any measurable systemic anticoagulation activity.

A subcutaneous programmable pump was subsequently implanted and PPS was administered in the ventricular CSF on a continuous basis as a long-term infusion. The total daily dose of 660 µg PPS was delivered in a volume of 330 µl/d. Long-term continuous infusion of PPS for 6 months did not cause any side effects. Follow-up CT

scans demonstrated no visible changes in the brain, such as haemorrhage or periventricular lucencies. No hydrocephalic enlargement of the ventricles was noted.

Conclusion: Intracerebroventricular infusion of PPS at 11 µg/kg/d appears safe for continuous long-term CSF application in humans. Ongoing clinical/neurological and laboratory follow-ups are underway for assessment of the efficacy of PPS administration.

Key words: brain, intraventricular, pentosan polysulphate, variant CJD

Introduction

Creutzfeldt-Jakob disease (CJD) is a fatal neuro-degenerative disorder associated with the accumulation of an abnormal isoform (PrP^{sc}) of the normal prion protein (PrP^{c}) in the brain. Variant CJD (vCJD), unlike the sporadic form of CJD, mostly effects young adults and adolescents and is now considered to be caused by the bovine spongiform encephalopathy (BSE) agent.[1-3] Currently there is no specific and/or effective treatment for any form of CJD in man.

Pentosan polysulphate (PPS) is a large polysulphonated polyglycoside molecule (molecular weight 5-8 kDa) with a weak heparin-like activity. Some experimental strategies using PPS have shown promise in cell culture and in various animal models.[4-6] It acts as a prophylactic agent against prion infection in a rodent scrapie model [7], prevents the production of further PrP^{sc} in neuroblastoma cell culture models rendering them sterile for prions [8], and permits the removal of existing prion infection outside the nervous system when given by systemic injection in scrapie-infected mice [9]. The biologic activity of systemic (intramuscular or subcutaneous) PPS given systemically in man is known [10], but given systemically it is considered ineffective as a therapeutic agent after the prion infection has invaded the brain. This is probably because PPS is a large hydrophilic molecule which poorly crosses the blood brain barrier. A recent animal study reported the infusion of PPS directly into the ventricles of mice, rats and dogs via an intraventricular catheter.[11] PPS administered at between 110-230 μg/kg/d significantly prolonged the survival of mice intracranially infected with scrapie even when given late in the disease incubation period. Symptoms were not seen to progress in the mice until

several weeks after the drug infusion had stopped. Higher doses of PPS (345-460 μg/kg/day) caused no side effects in mice or rats, but when given to dogs were associated with seizures and/or death in some dogs which was in some cases consequent upon intracranial haemorrhage.[11]

Considering the favourable side effect profile of low dose systemic PPS and the available preclinical data from cell culture and animal experiments, we then proceeded to administer PPS intracerebroventricularly in a young man with advanced vCJD.

We report here the results of the preliminary stage of PPS treatment – a study designed to establish the safety of long-term intraventricular PPS at a dose which, based on projections from the animal studies [11] was thought likely to have a significant biological effect in humans.

Case presentation

Initial clinical and neurological findings

The patient is a 19 year old man who presented at the age of 17 with behavioural disturbance and mild ataxia. His symptoms rapidly progressed with increasing ataxia, dementia, pyramidal signs, and myoclonus. The clinical picture combined with abnormal MR findings in the FLAIR sequence (pulvinar sign) and positive tonsil biopsy allowed a diagnosis of probable vCJD according to internationally accepted criteria.[12] The patient had advanced neurological disability prior to PPS administration. Also he was repeatedly

treated for aspiration pneumonia due to severely disfunctioning swallow reflex necessitating frequent suctioning of saliva. Also he was fed through a percutaneous gastrostomy tube (PEG).

The baseline neurological assessment was carried out at the time of inpatient admission for intracerebroventricular catheter implantation. Eyes were intermittently open spontaneously. There was some fluctuation in the level of responsiveness but with no clear sleep/wake cycle. The patient could not consistently fix on an examiner or family member. He was unable to follow single stage commands. He had intact brainstem reflexes, with preserved vestibulo-ocular reflexes and slow but consistent direct and consensual pupilary reflexes. Pout and grasp reflexes were also elicitable. There was grimacing and flexion to painful stimuli in all four limbs. There were incomprehensible sounds when disturbed but no verbalisation attempts. There was an increase in the frequency of myoclonus in response to increased background noise, or to sudden loud sounds. The patient was consistently more settled in the presence of his family.

Consent

The patient was not legally competent. He had expressed an advance wish (when competent), for all active measures to be taken to treat him. To confirm the ethical and legal rightfulness for a proposed experimental application of PPS with therapeutic intentions, the High Courts in London (Family Division) and Belfast (North Ireland High Court) were asked to hear the case. The decisions of both High Courts were that intraventricular treatment with PPS was in the patient's best interests. An independent local research ethics committee (LREC) also voted that it was in the patient's best interest to receive intraventricular PPS.

Study Design

This was a pilot single-case prospective dose-escalation study of PPS given as a chronic intraventricular infusion. A permanently implanted right frontal intraventricular catheter connected to a subcutaneous Ommaya reservoir was used. An external syringe pump delivering the drug was attached to the Ommaya reservoir. After an uneventful completion of the dose escalation part, a programmable drug infusion pump (Synchromed EL 18 ml with side port, Medtronic Inc., Minneapolis, MN) was implanted subcutaneously in the abdominal area and was connected to the intraventricular catheter. Long-term intraventricular infusion of PPS was continued.

We used PPS at approximately 10% of the lowest effective dose in mice (110 µg/kg/d).[11] On that basis the final target dose was 11 µg/kg/d. We set up a dose-escalation study starting at 1 µg/kg/d and escalating on a daily basis until the target dose of 11 µg/kg/d was achieved. Sterile PPS in a 100 mg/ml stock solution (Pentosan polysulphate SP54, Bene Arzneimittel GmbH, Munich, Germany) was diluted to the respective desired concentration in 5 ml of 0.9% sterile NaC1. The total daily infusion volume of 5 ml was infused within 20 hours. CT scans were performed to detect intracranial haemorrhage. Systemic clotting parameters were measured on a daily basis. Samples of urine, blood and CSF were taken daily.

Treatment Course

A standard right frontal ventricular catheter was placed via a right frontal burr hole into the right lateral ventricle and connected to a subcutaneous (Ommaya) reservoir. Following uneventful recovery from surgery, a CT scan was performed as planned after

three days, prior to administration of the first dose of PPS. This revealed the catheter to be in a satisfactory position. There was an area of low attenuation and most like post-procedural local bleeding in the line of the catheter tract (**Figure 1 A**). The first dose of PPS (60 μg) was given as a single bolus injection. There were no adverse clinical events, however a CT scan the next day showed an increased amount of blood surrounding the catheter (**Figure 1 B**). Treatment was suspended for some days to allow this haematoma to resolve. Unfortunately during this period, a swab of an erythematous area around the pre-existent gastrostomy site revealed the presence of methicillin resistant *Staphylococcus aureus* (MRSA). In view of the potentially disastrous consequences of an MRSA ventriculitis, all further treatment was postponed for two weeks to allow MRSA to be successfully eliminated using doxycycline.

Following recommencement of treatment by intraventricular infusion, the PPS dose was successfully escalated for five days with no side effects detected clinically or radiologically. However, on the sixth day, initial aspiration of the reservoir revealed uniformly blood stained fluid. CT immediately prior to the procedure had shown no evidence of blood. The blood stained CSF cleared following further aspiration, becoming only slightly blood tinged. Analysis revealed 800 RBC/μl in the final sample. CT scan on the following day showed a small amount of blood in the occipital horn of the right ventricle (**Figure 1 C**) and analysis showed a falling RBC count in the CSF. This was felt to represent traumatic puncture of the reservoir rather than an intraventricular bleed and treatment was recommenced. The dose was further escalated on a daily basis up to the target dose of 660 μg without further complications. Systemic clotting parameters remained normal over the whole period.

No adverse events were noted clinically. Specifically, the patient remained apyrexic and there were no epileptic seizures or convulsions. Neurological evaluation at the end of the dose escalation study was unchanged compared with baseline.

The implantation of the programmable pump was carried out under general anaesthesia. The pump was placed in a subcutaneous pocket formed in the upper right abdomen, and an extension catheter was passed subcutaneously to the right frontal area where it was connected to the existing ventricular catheter.

Two weeks after pump implantation, low-volume PPS infusion (660 μg in 330 μl/d) was commenced. No clinical side effects were noted during the following 6 subsequent months of continuous PPS infusion. Cranial CT scans failed to show any visible intracranial haemorrhage or hydrocephalus (**Figure 1 C**).

Neurologically, the patient remained objectively unchanged, although subtle clinical signs appeared which may signal ongoing improvement of the neurological deficits. Such were the reduced frequency of myoclonus, the regained ability to fix his eyes on persons, to obey simple commands, and to verbalise single words in response to stimuli. Also of significance is the fact that the patient showed much improved swallowing and suctioning of the oral cavity became unnecessary. Also he has had no aspiration pneumonia during the course of PPS administration and has gained weight while on the same nutritional regime.

Discussion

In this pilot study we infused escalating doses of PPS in the cerebroventricular system of a 19 years old male patient with advanced vCJD. All doses up to 660 µg/d were safe and well tolerated, and no serious permanent adverse effects attributable to the drug itself or to the infusion procedure were observed. Early in the course of the treatment, some blood was seen on CT scans, however there was no clinical correlation to the neuroradiological observations. Long-term CSF infusion of PPS (660 µg/d) for 6 months via an implanted pump was also safe and resulted in no further side effects. No pathological intracranial changes were noted on follow-up CT scans.

Rationale for the use of intraventricular PPS

PPS may be currently, in the opinion of some authors, the best candidate drug for vCJD treatment.[6,8,11] Experimental studies have demonstrated that PrP^{sc} infected cell cultures were rendered sterile by the presence in the culture medium of exceptionally low quantities of PPS.[8] When given systemically, PPS has been also shown to act as a prophylactic agent against prion infection in rodents. It seems to cause the removal of PrP^{sc} infection from a mouse even when given by subcutaneous injection during a short period after PrP^{sc} has been inoculated.[7]

The mode of action of PPS in scrapie infected rodents suggested that it might be expected to act similarly in human vCJD.[11] Obviously dose-dependent complications might be different in humans compared to the murine model. Preliminary biosafety studies of intraventricular PPS in large animals (dogs) without scrapie infection have identified a

maximum tolerated dose (MTD) for the drug in the normal dog brain, with the main serious adverse effects being epileptic seizures and intracerebral haemorrhage.[11]

Adverse effects and complications after PPS infusion

Since PPS has a weak heparin-like effect, intraventricular infusion of PPS carries at least a theoretical risk of causing intracerebral haemorrhage. In the presented case, there were no clinical adverse effects due to intracerebral haemorrhage. CT confirmed, however, the occurrence of blood around the distal part of the catheter after the first dose of PPS. It is possible that this modest bleeding present at catheter introduction was facilitated by PPS, although spontaneous bleeding along the catheter track without clinical significance is not uncommon on CT scans performed early after placement of a ventricular catheter. The second episode of haemorrhage occurred on day six of the restarted PPS treatment schedule, and had similarities to a traumatic lumbar puncture. Blood appeared as soon as the skin/reservoir was punctured and with aspiration of blood and then CSF, clear CSF was finally obtained. The CT scan on the following day showed a little blood in the occipital horn exactly as one would have expected from a small amount of blood tracking back down the catheter.

In future studies we would recommend commencing PPS infusion at least one week after catheter placement to allow for complete cessation of any traumatic bleeding around the catheter, and for the development of a gliotic reaction along the catheter track.

We would like to emphasise that there was no evidence of any direct adverse effect of the intraventricular infusion of PPS, such as subependymal, intracerebral, or subarachnoid

haemorrhage. In addition, there was no local or generalised brain oedema visible on serial CT scans. Also the systemic coagulation was not influenced by any of the infused doses of PPS, as demonstrated by the screening of blood coagulation parameters. In terms of the neurological and general condition, PPS infusion was also safe and did not provoke any epileptic activity or additional neurological deficits above the baseline level. Also there were no haemorrhagic complications elsewhere in the body.

PPS dosing and application modalities

There are no previously published data on the safest and most effective dosage of intraventricularly infused PPS in human patients with prion diseases. A recent preclinical study of intraventricular PPS investigated a small rodent scrapie infection model and also healthy dogs.[11] The PPS dose used in scrapie-infected mice was adapted for human use by a 10-fold reduction based on body surface area and weight differences. In addition, the final dose of 11 µg/kg/d was reached after stepwise escalation starting with 1 µg/kg/d. However, because PPS is not administered systemically but rather into a single compartment, the CSF, it could be hypothesised that even the maximum dose in our case, 11 µg/kg/d, is considerably lower than the maximum tolerated dose (MTD) in humans. A possible therapeutic dose-effect relationship for intracerebroventricular PPS in humans with prion disease remains unknown, and therefore dose escalation would only be limited by side effects. In our case, there were no dose limiting side effects up to 11 µg/kg/d. A further dose escalation is being considered.

Despite the fact that the current protocol for PPS infusion seems to be safe and well tolerated, the effectiveness of the treatment still needs to be addressed. Measuring

therapeutic effects in a disease such as vCJD is very difficult, at least in part because so many of the outcome parameters are variable, for example time from diagnosis to death and speed of onset of neurological and/or associated functional impairment. Possibly motor or sensory evoked potentials or physiologic tests measuring the function of the autonomous nervous system would be suitable as treatment endpoints, although no standardised data exist in that respect. The pharmacokinetics of PPS distribution within the brain and outside the CNS also needs investigation and, ideally, quantification.

In summary, the first results obtained with long-term intracerebroventricular infusion of PPS in a young man with advanced vCJD are encouraging and certainly justify further clinical and basic research to establish the efficacy of PPS in larger cohorts of patients with prion disease.

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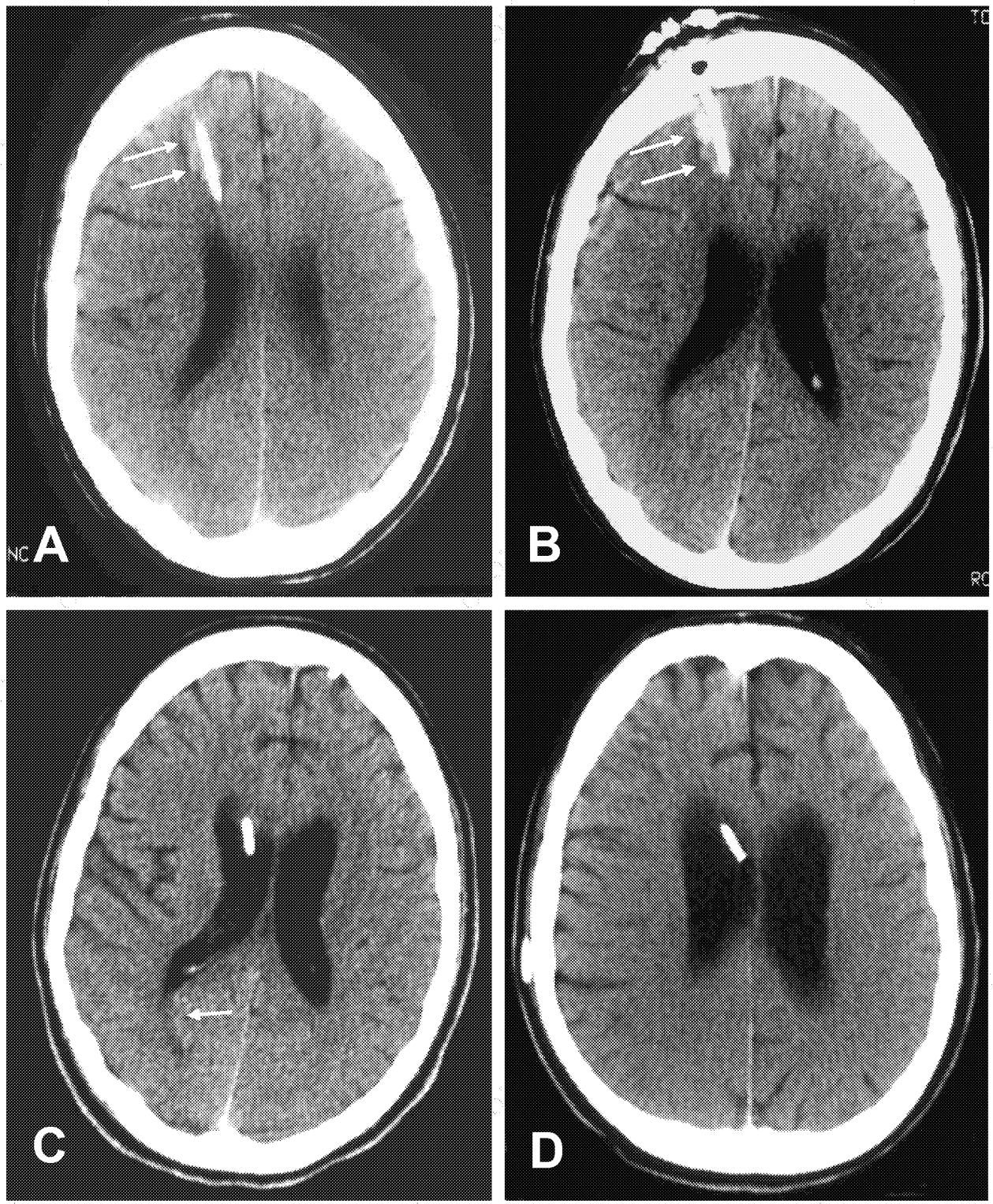
Figure 1: **A**: Non-enhanced CT scan 3 days after intraventricular catheter placement. Note the presence of blood surrounding the catheter (arrows) **B**: CT scan after the first dose of PPS. Note slightly increased size of haematoma around catheter (arrows). **C**: CT scan on day 6 after recommencement of PPS infusion. Note the small amount of blood in the posterior horn of the lateral ventricle (arrow). **D**: CT scan 2 months after the implantation of the programmable infusion pump. The anatomy of the brain and ventricles appears normal and there is no intracranial blood visible.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