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Tubulovesicular structures are present in brains of hamsters infected with the Echigo-1 strain of Creutzfeldt-Jakob disease agent

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Tubulovesicular structures (particles; TVS) are virion-like particles 25–30 nm in diameter found by thin-section electron microscopy in brains of all prion diseases including scrapie, Creutzfeldt-Jakob disease (CJD), fatal familial insomnia (FFI) and Gerstmann-Sträussler-Scheineker disease (GSS), as well as in cell cultures infected with TSE agents. TVS are regarded as a disease-specific ultrastructural marker for TSEs and, by those not completely satisfied with the prion hypothesis, they are even considered to be a possible candidate for the infectious TSE agent itself. A caveat regarding that interpretation stemmed from previous failures to find TVS by electron microscopic studies of tissues from animals infected with the Echigo-1 strain of CJD agent. We now report detecting TVS in brains of hamsters infected with that strain of CJD agent, albeit with a very low frequency.

Key words: prion diseases, tubulovesicular structures, Echigo-1, Creutzfeldt-Jakob disease

INTRODUCTION

The Echigo-1 strain of Creutzfeldt-Jakob disease (CJD) agent is one of two described panencephalopathic strains of CJD agent. Echigo-1 was first isolated by Mori and colleagues (1989) and then passaged several times in hamsters in our laboratory. A detailed description of the immunohistochemical and ultrastructural characteristics of this model was published recently (Liberski et al. 2004, Sikorska et al. 2004).

Tubulovesicular structures (particles) are virion-like particles 25–30 nm in diameter found by thin-section electron microscopy in brain tissues from humans and animals with all transmissible spongiform encephalopathies (TSEs) including scrapie (David-Ferreira et al. 1968), CJD (Liberski et al. 1992), fatal familial insomnia (FFI) and Gerstmann-Sträussler-Scheineker disease (GSS) (Liberski and Budka 1994, Liberski et al. 2005), as well as in cell cultures infected with TSE agents (Manuelidis et al. 2007). The tubulovesicular structures (TVS) are regarded as a disease-specific ultrastructural marker for TSEs (Liberski and Jeffrey 2004, Liberski and Brown 2007, Manuelidis 2007, Manuelidis et al. 2007) and somehow involved in the TSEs pathogenesis. A caveat regarding that interpretation stemmed from previous failures to find TVS by electron microscopic (EM) studies of tissues from animals infected with the Echigo-1 strain of CJD agent (Liberski and Mori 1997). We now report detecting TVS in brains of hamsters infected with that strain of CJD agent, albeit with a very low frequency.

METHODS

Prion strain, animals, incubation period of illness

Outbred 6-week-old golden Syrian hamsters (Medical University of Lodz, Department of Oncology, Lodz, Poland) were inoculated intracerebrally with 0.05 ml of a 10% (w/v) centrifugation-clarified hamster brain suspension containing the Echigo-1 strain of CJD agent (Liberski and Mori 1997).

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Control animals were sham inoculated intracerebrally with the same volume of saline. We used five hamsters for each experiment; the control group consisted of 3 hamsters. In the first passage in our laboratory (7th serial passage after initial isolation), the incubation period of illness in hamsters following intracerebral inoculation with a 10% clarified suspension of Echigo-1-infected brain was approximately six months. The experiment was repeated twice with the similar results.

Neuropathology and immunohistochemistry

For neuropathology, H & E staining and GFAP immunohistochemistry with DAKO antibodies were used. Anti PrP antibodies – 3F4 (DAKO) or 6H8 (Prionics, Zurich, Switzerland) were used

Electron microscopy

Hamsters in the terminal stage of CJD and control sham-inoculated hamsters at the same interval after inoculation were anaesthetized deeply with ketamine. Hamsters were perfused by intracardiac injection with saline followed by 150 ml of 1.25% glutaraldehyde and 1% paraformaldehyde prepared in cacodylate buffer (pH 7.4) and then by 50 ml of 5% glutaraldehyde and 4% paraformaldehyde. Perfused animal carcasses were held at 4°C for at least 2 hours, after which brains were removed and several 1-mm^3 samples were dissected under a binocular microscope from parietal cortex, corpus callosum, CA2 region of the hippocampus, thalamus, cerebellum and the brain stem. Those samples were postfixed in 1% osmium tetroxide for 1–2 hours, dehydrated through a series of graded ethanols and propylene oxide, and then embedded in Embed 812 resin (Electron Microscopy Sciences, Ft. Washington, PA). Semi-thin sections were stained with toluidine blue, blocks trimmed, and ultrathin sections stained with lead citrate and uranyl acetate. Specimens were examined using a JEM 100 C transmission electron microscope.

RESULTS

Immunohistochemistry

Several distinct patterns of PrP^d deposits were observed: fine granular „synaptic"/diffuse type of accumulation which, especially in the cerebral cortex, appeared laminar; plaque-like structures and robust perineuronal deposits (Fig. 1).

After prolonged search and examination of tens of serial sections, we identified two neuronal processes containing typical TVS (Figs 2 and 3). As reported previously, TVS (diameter approximately 27 nm) were smaller than synaptic vesicles and larger than microtubules; the TVS had high electron density, and they were somewhat disorganized in appearance, never forming the paracrystalline arrays sometimes seen in previous studies with other TSEs. They were readily discriminated from other vesicular structures – i.e. multivesicular bodies (Figs 4 and 5), autophagic vacuoles (Fig. 5) and glycogen granules (Fig. 6).

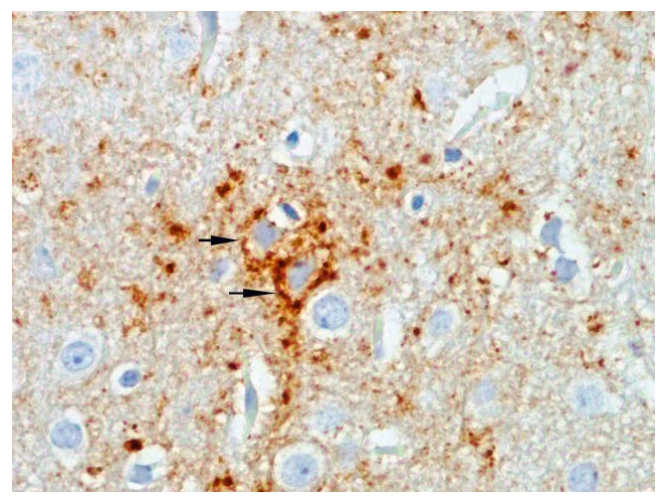

Fig. 1. Immunohistochemical staining for PrP reveals perineuronal accumulations (arrows). Original magnification, ×1 000.

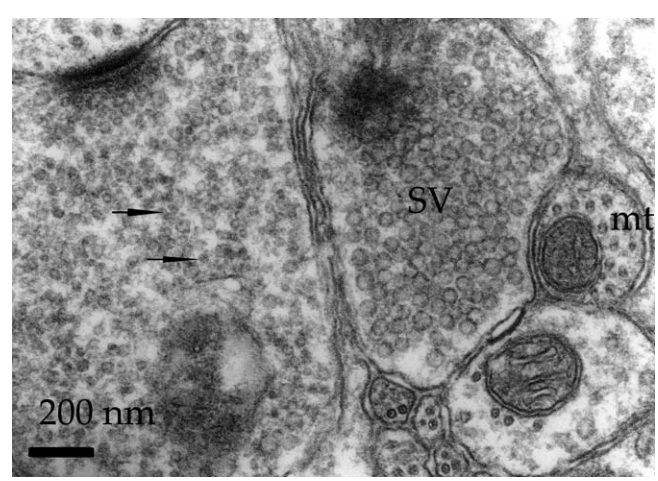

Fig. 2. Tubulovesicular structures (arrows) in comparison to synaptic vesicles (SV) and microtubules (mt)

DISCUSSION

We have now been able to demonstrate TVS in every TSE model available to us for study (Liberski and Brown 2007). The small number of processes containing TVS in brains of hamsters infected with the Echigo-1 agent is strikingly different from other models of TSE in hamsters: brains of hamsters infected with both the 263K and the 22C-H strains of scrapie agent had many processes containing TVS; however in both those scrapie models the titers of infectivity were extremely high, while very little infectivity is present in brains of hamsters infected with the Echigo-1 CJD agent.

The significance of TVS remains unknown, and arguments for and against the hypothesis that they represent the TSE agent are summarized in a companion review. It is sufficient to note here that the size of TVS clearly fits not only the cut-off size for infectivity estimated by early results of ultrafiltration studies (Gibbs et al. 1965), but also the results of studies using field flow sedimentation following nuclease digestion of infected material (Sklaviadis et al. 1992) that showed maximal infectivity titers in a 120-S peak containing marker spheres 25–30 nm in diameter (Manuelidis 2007) and, by EM, dense-cored particles of the same size, while more than 75% of the prion protein spiked into the starting materi-

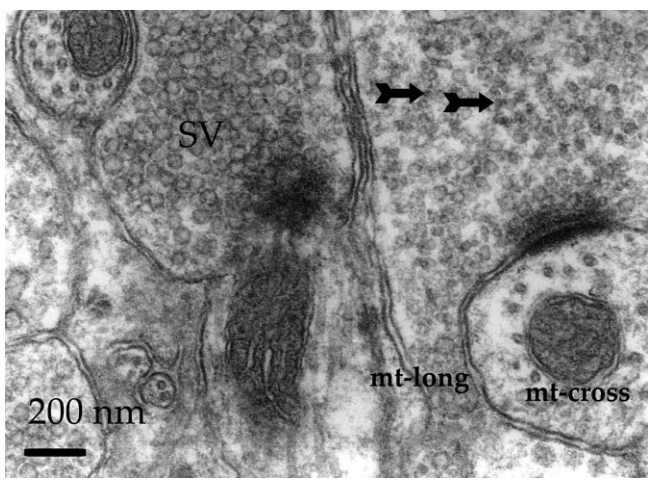

Fig. 3. Tubulovesicular structures (arrows) in comparison to synaptic vesicles (SV) and microtubules in either longitudinal (mt-long) or cross-section (mt-cross) microtubules

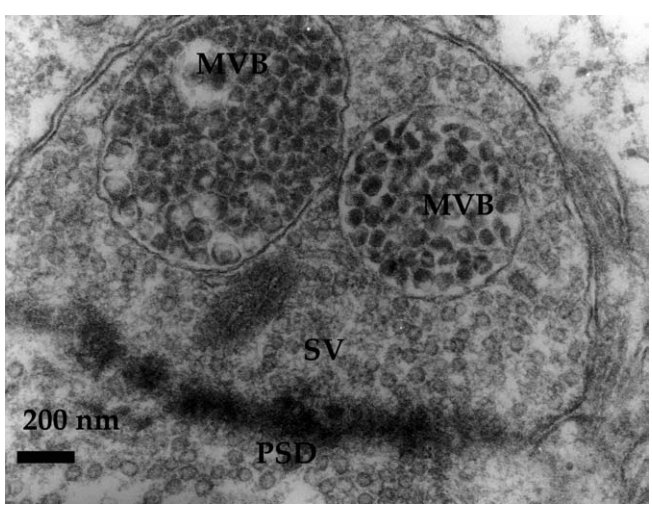

Fig. 4. Enlarged synaptic terminal containing two large multivesicular bodies (MVB), and synaptic vesicles (SV). Presynaptic density (PSD) is also visible

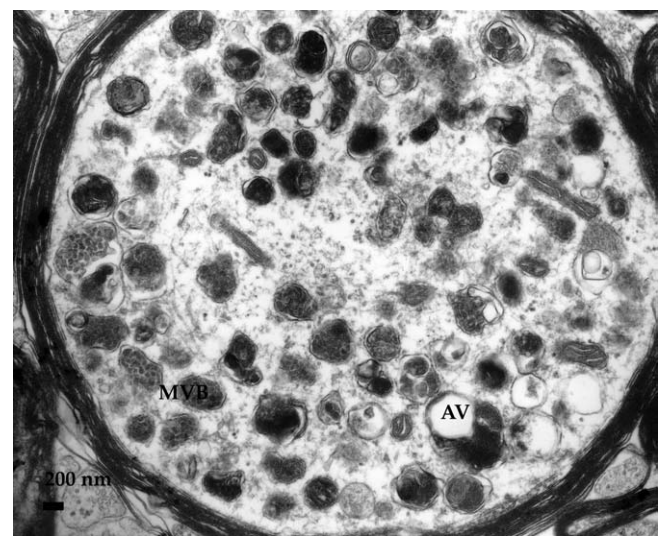

Fig. 5. A large myelinated axon containing numerous multivesicular bodies (MVB) and autophagic vacuoles (AV)

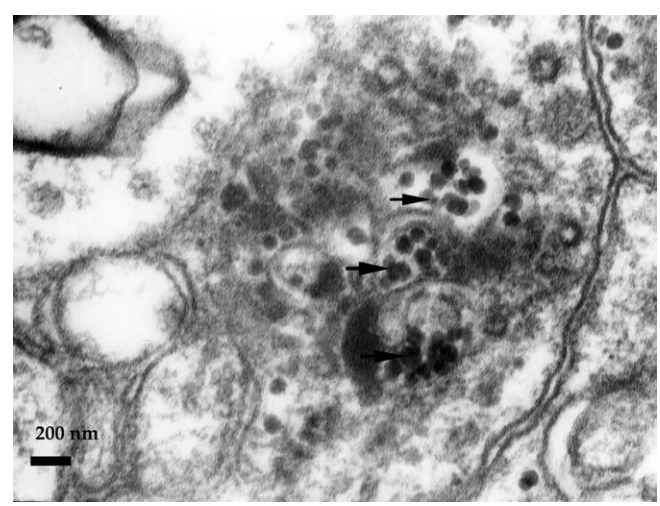

Fig. 6. Numerous glycogen granules (arrows) in unidentified cellular process

al was found in fractions containing only minimal amounts of infectivity. The size of TVS also corresponds well to the probable size of the smallest infectious unit of scrapie agent calculated in a recent molecular study (Silveira et al. 2005). We suggest that such a consistent association of infectivity with structures having the same size as TVS is unlikely to be coincidental.

CONCLUSION

In conclusion, there is growing evidence that virion-like particles 25–30 nm in diameter are consistently found in all TSEs, both *in vivo* and *in vitro*, which strongly suggests that they are likely to play an essential role in TSE pathogenesis.

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