

Propagation of scrapie in peripheral nerves after footpad infection in normal and neurotoxin exposed hamsters

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Abstract – As is known from various animal models, the spread of agents causing transmissible spongiform encephalopathies (TSE) after peripheral infection affects peripheral nerves before reaching the central nervous system (CNS) and leading to a fatal end of the disease. The lack of therapeutic approaches for TSE is partially due to the limited amount of information available on the involvement of host biological compartments and processes in the propagation of the infectious agent. The *in vivo* model presented here can provide information on the spread of the scrapie agent via the peripheral nerves of hamsters under normal and altered axonal conditions. Syrian hamsters were unilaterally footpad (f.p.) infected with scrapie. The results of the spatiotemporal ultrasensitive immunoblot-detection of scrapie-associated prion protein (PrP^{Sc}) in serial nerve segments of both distal sciatic nerves could be interpreted as a centripetal and subsequent centrifugal neural spread of PrP^{Sc} for this route of infection. In order to determine whether this propagation is dependent on main components in the axonal cytoskeleton (e.g. neurofilaments, also relevant for the component 'a' of slow axonal transport mechanisms), hamsters were treated -in an additional experiment- with the neurotoxin β,β' -iminodipropionitrile (IDPN) around the beginning of the scrapie infection. A comparison of the Western blot signals of PrP^{Sc} in the ipsilateral and in the subsequently affected contralateral sciatic nerve segments with the results revealed from IDPN-untreated animals at pre-clinical and clinical stages of the TSE disease, indicated similar amounts of PrP^{Sc}. Furthermore, the mean survival time was unchanged in both groups. This *in vivo* model, therefore, suggests that the propagation of PrP^{Sc} along peripheral nerves is not dependent on an intact neurofilament component of the axonal cytoskeleton. Additionally, the model indicates that the spread of PrP^{Sc} is not mediated by the slow component 'a' of the axonal transport mechanism.

scrapie / PrP^{Sc} / nerve / footpad / IDPN

1. INTRODUCTION

Transmissible spongiform encephalopathies (TSE), such as the Creutzfeldt-Jakob Disease (CJD) in humans, bovine spongiform encephalopathy (BSE) in cattle or scrapie in sheep are fatal diseases

of the central nervous system (CNS) in mammals. The etiological classification differentiates between genetic, acquired or sporadic forms.

The key molecular pathological event, characteristic to all forms of TSE, seems to have been elucidated: The normal host cellular prion protein (PrP^C) converts into a disease-specific form [11], that is resistant

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to proteinase K digestion [42]. The glycoprotein PrP^C is strongly expressed by cells of the nervous and lymphoreticular systems. During biogenesis, PrP^C migrates to the outer plasma membrane where it is anchored by glycosyl-phosphatidylinositol [47]. In vivo studies showed that PrP^C is transported in nerves by axonal anterograde and retrograde transport mechanisms [8,36,45]. However, the normal function of PrP^C is still unclear.

The abnormal scrapie specific prion protein (PrP^{Sc}) is a biochemical marker for TSE and is linked with infectivity. This fact coupled with the failure to successfully transmit the disease in PrP^C knock-out mice models [11], the in vitro generation of infectious PrP [13,32] and the futile search for specific prion-associated nucleic acids are all strong indicators that PrP^{Sc}, according to the protein-only hypothesis [42], is the causative TSE agent. Post-mortem histological examinations of brain tissue of clinically affected individuals revealed astrocytosis and vacuolar degeneration besides the detection of antibody-positive granular deposits of PrP^{Sc}.

The natural route of infection for acquired forms of TSE is the peripheral uptake of the infectious agent. Known sources of iatrogenic CJD in humans are transmission via infected grafts (cornea or meninx), via neurosurgical devices or transmission by the administration of human pituitary hormones [10]. There is some evidence that TSE can be transmitted by blood transfusion from infected donors [33, 40]. A large number of in vivo experiments suggest that the most important epidemiological modus for scrapie, BSE or variant-CJD (vCJD) transmission is the oral route [2, 39]. The endemic appearance of Kuru in Papua New Guinea is due to endocannibalistic rites [1]. Nevertheless, in vivo experiments showed successful transmission of TSE-disease not only by oral, intracerebral, intravenous or intraperitoneal application of infectivity but also by

subcutaneous injection, through defect mucosa [5, 12] or, even very effectively, by scarification of the intact skin [48].

The sequential detection of abnormal prion protein in lymphoreticular tissue and along neural projections to the CNS after peripheral infection in various animal models led to the conclusion that these tissues are involved in the spread of the TSE agent to the CNS [3, 7, 17, 28, 35, 44, 46]. The sciatic nerve is a useful structure for monitoring the centripetal neural spread of the scrapie agent [29] and rapid disease onset was observed after intrasciatic injections of strains of transmissible mink encephalopathy (TME) in rodents [4]. Kimberlin [29] debated that the velocity of the spread of the scrapie agent to the CNS is comparable to the velocity of slow axonal transport. In all eukaryotic cells, including neurons, intracellular transport of membranous and nonmembranous cargoes (e.g. vesicles, proteins, mRNA) is of vital importance. Axonal transport proceeds in a fast or slow manner in anterograde or retrograde direction (from the cell body to the axon tip and vice versa). With respect to the rate and nature of moving structures, the slow axonal transport can be distinguished in two components. The component 'a' of slow axonal transport moves material bidirectionally at an overall rate of around 1 mm per day. Amongst moving material are the main proteins of the axonal cytoskeleton, neurofilament proteins and tubulin, whereby cytoskeletal polymers act as carrier structures [9]. It is still unclear whether the development of TSE interferes with the axonal transport of neurofilaments [20].

In order to answer the question whether axonal transport mechanisms are involved in the spread of the infectious agent along axons, previous studies were carried out in four-repeat tau over-expressing mice and in dynein mutant mice models [25, 31]. The results suggested that the fast host retrograde axonal transport mechanism did

not serve as a “ferry” for the centripetal propagation of PrP^{Sc}.

In this study Syrian hamsters were unilaterally footpad (f.p.) infected with 263K scrapie, approximating a natural route of infection, in order to characterize the normal neural pathway of the disease for this route. In a further experiment, animals were treated with iminodipropionitrile (IDPN), known to disorganize neurofilaments and axonal transport mechanisms [14–16, 19, 21, 23, 30, 37, 38]. We analyzed Western blot signals of protease resistant PrP from the sciatic nerve segments of f.p. infected animals in non- and IDPN-exposed groups at the preclinical and clinical stages of scrapie. Furthermore, we compared the time required to reach the terminal stage of disease, to clarify whether the assumed centripetal spread of the infectious agent to the CNS and its subsequent centrifugal travel to the periphery are dependent on the intact neurofilament component of the axonal cytoskeleton.

2. MATERIALS AND METHODS

2.1. Animal inoculations

The animal experiments complied with German legal provisions.

Adult male and female Syrian hamsters were sedated with isofluran and f.p. infected with 20 μ L of a 2% (wt/vol) terminally ill 263K-hamster brain homogenate or mock-infected with normal hamster brain homogenate in the sole of the right foot by injection using a 27 gauge cannula. The animals were regularly monitored for clinical signs of scrapie ranging from an increased sensitivity to touch and sound to muscle tremor, kiphosis, ataxia and rapid weight loss.

2.2. Tissue collection for spatiotemporal PrP^{Sc} analysis in sciatic nerves after f.p. infection

The hamsters were sacrificed by CO₂ asphyxiation at different time points

following infection or when terminally ill. Samples of both distal sciatic nerves were divided into quarters weighing 3.6 ± 1.4 mg (mean \pm SD) for Western blot analyses. Specimens were TBS-washed and frozen at -80°C immediately after preparation until further processing. To avoid cross-contamination, the instruments used for the preparation of samples were carefully cleaned after removal and processing of each specimen.

Western blot results were reported for samples at the following time points: 2 days post infection (dpi, $n = 2$), 42 dpi ($n = 5$), 60 dpi ($n = 2$), 70 dpi ($n = 6$), 80 dpi ($n = 6$), 90 dpi ($n = 7$), 100 dpi ($n = 4$) and when terminally ill with severe symptoms at 100 dpi ($n = 6$) and 119 dpi ($n = 2$), and for samples of mock-infected hamsters manifesting no clinical signs which were sacrificed at 42 dpi and at > 100 dpi.

Other animals were transcardially perfused with periodate-lysine-paraformaldehyde (PLP [34]); nerve and brain samples were removed and post-fixed for 4 h in PLP or the removed tissue was directly immersion fixed for a maximum of 48 h with neutral buffered 3.7% formalin. Tissue samples for immunohistochemistry (IHC) were taken 42 dpi, 70 dpi, 80 dpi, 90 dpi, 100 dpi (a total of $n = 4$ preparations for every time point) and when terminally ill at 104 dpi and 110 dpi ($n = 2$, respectively). After incubation in phosphate-buffered saline, tissue samples were transferred to 70% ethanol, processed in a tissue processor and subsequently embedded in paraffin wax.

2.3. Chronic IDPN intoxication and spatiotemporal tissue collection for PrP^{Sc} analysis

A tolerable treatment regime for IDPN intoxication was determined in preliminary tests. Hamsters were f.p. infected and treated intraperitoneally (i.p.) five times

with IDPN (20 mg/100 g). The first injection was administered 10 days before and the final injection 28 days after infection. Before administration, IDPN was diluted in saline and the pH was adjusted with hydrochloric acid to 7.4. Nerve tissue of a mock-infected, a scrapie-infected and a scrapie-infected IDPN-intoxicated hamster at 42 dpi (PLP fixed as described above) were the controls for immunohistological analysis to confirm the effect of the intoxication.

At 45 dpi peripheral blood of 6 untreated and 7 IDPN-treated animals was collected by puncturing the retrobulbar plexus. White blood cells were quantitatively and qualitatively compared by counting using a hemocytometer and by Giemsa staining of a blood smear.

Analogous to untreated hamsters, the IDPN-treated animals were sacrificed at different time points following infection or when terminally ill. Samples of both distal sciatic nerves were divided into quarters weighing 3.4 ± 1.3 mg (mean \pm SD) for Western blot analyses. Specimens were TBS-washed and frozen at -80 °C immediately after preparation until further processing. Western blot results were reported for samples at the following time points: 42 dpi ($n = 4$), 60 dpi ($n = 4$), 70 dpi ($n = 4$), 80 dpi ($n = 4$), 90 dpi ($n = 4$), 100 dpi ($n = 2$) and when terminally ill with severe symptoms at 109 and 126 dpi ($n = 2$). Mock-infected and IDPN-treated hamsters without clinical signs were sacrificed 42 dpi and at > 100 dpi. For histological investigations, nerve and brain samples were removed 42 dpi ($n = 4$), 60 dpi ($n = 4$), 70 dpi ($n = 4$), 80 dpi ($n = 4$), 90 dpi, 100 dpi ($n = 3$) and when terminally ill at 112 dpi ($n = 1$), fixed in PLP or formalin and processed as described above.

The incubation times until the development of terminal scrapie disease were determined in separate groups with IDPN-treated ($n = 7$) or nontreated animals ($n = 4$).

2.4. Western blot

For positive controls, nerve samples of uninfected animals were spiked with 10 μ L of 0.1% 263K-hamster brain homogenate (corresponding to 10^{-5} g of scrapie brain homogenate) prepared by ultrasonification of 1 g brain tissue of a terminally ill donor hamster in 10 mL Tris-buffer, pH 7.4. All nerve samples were incubated at 37 °C for 60 min with 500 μ L 0.25% collagenase in TBS buffer containing 2 mM CaCl_2 . After halting the reaction by adding 10 μ L 250 mM EDTA, tissue-extraction of PrP^{Sc} in the form of the protease resistant 27–30 kD fragment (PrP²⁷⁻³⁰) was initiated in line with a previously published protocol [6], including the use of proteinase K (PK) to destroy normal PrP^C. The final pellets were resuspended with 20 μ L of $2 \times$ sample loading buffer and heated to 100 °C for 10 min prior to Western blotting using the monoclonal anti-PrP antibody (mAb) 3F4 [27] and an alkaline phosphatase-conjugated secondary goat-anti-mouse antibody. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blot analyses were performed as described elsewhere [6, 49]. Antibody binding was visualized using CDP-Star (Tropix) as a chemoluminescent substrate for alkaline phosphatase and a Kodak X-OMAT AR film, exposed for 30–60 min.

2.5. Immunohistochemistry

For the detection of granular PrP^{Sc}, 2 μ m thick paraffin slides were pretreated with guanidiniumthiocyanate after deparaffinization and rehydration and heating in citrate buffer in a microwave. After blocking with 0.2% (wt/vol) casein (Tropix), the slides were first incubated with mAb 3F4, diluted 1:250 in blocking solution for 90 min at room temperature followed by incubation for 60 min at room temperature with an alkaline phosphatase-conjugated

goat-anti-mouse antibody (DakoCytomation), diluted 1:500 in blocking solution. Immunostaining was performed using new fuchsin as substrate for alkaline phosphatase. On selected sections, 3F4 binding was visualized using a biotin-conjugated goat-anti-mouse antibody, the avidin biotin-complex (Vectastain) technique and with diaminobenzidine (DAB) as a chromogen. DAB-stained sections were pre-treated with amino acid after deparaffinization. Subsequently, the slides were counterstained with hemalaun.

To verify the effects of IDPN, sections of sciatic nerve tissues were stained using, as the first antibody, a marker for neurofilaments (mAb 2F11, DakoCytomation), diluted 1:100 in phosphate-buffered saline and a fluorescent (Oregon green) labelled secondary antibody, diluted 1:100. 2F11 reacts with the phosphorylated form of a 70 kD component of the three neurofilament major polypeptide units. An uninfected/untreated animal and an uninfected/IDPN-treated animal as well as a 263K infected/IDPN-treated animal were compared at 42 dpi.

3. RESULTS

3.1. Spatiotemporal PrP^{Sc} analysis in sciatic nerves after f.p. infection

After unilateral infection in the right foot pad of hamsters with 263K brain homogenate, the scrapie specific clinical phase started with the symptom of muscle tremor around 78 dpi.

To determine the distribution of PrP^{Sc} in nerve tissue at different time points, quarters of both distal sciatic nerves (femoral level) were collected and Western blots of the nerve segments were performed after extraction and PK digestion in order to detect only protease resistant PrP^{Sc} in the form of PrP27-30. Figure 1 shows the typical results for the Western blot detection of PrP27-30 at different time points in serial segments of ipsilateral (lanes 2-5 in

A-G) and contralateral sciatic nerves (lanes 2-5 in H-N) of one animal, respectively. Nerve segments and spiked nerve segments of uninfected animals served as negative and positive controls for the PrP27-30 extraction procedure. Additionally, controls for blotting efficiency (lane 9 in A-N) were carried out to align the signal intensities. No noticeable signals for PrP27-30 in segments of both nerves were detectable 2 dpi (data not shown) and 42 dpi (Fig. 1). The f.p. infection led to the first clear detection of PrP27-30 in the ipsilateral sciatic nerves at 60 dpi, earlier than in contralateral nerves (around 80 dpi). The time frame from finding the first weak signals up to finding strong signals in the nerves was very short (about 10–20 days). Western blot results indicate an increasing level of PrP^{Sc} in the sciatic nerves at the respective site with time, whereby signal intensities of segments of one nerve did not differ substantially.

Immunohistological examinations of the brain revealed the tendency for the region of the contralateral red nucleus (RN), correlating with the ipsilateral sciatic nerve, to show stronger granular PrP-immunoreactive signals than the red nucleus of the other side (Fig. 2).

Granular PrP-positive deposits could be detected very rarely in the sciatic nerve. Figure 3 shows a cross section through the sciatic nerve with central axons, peripherally surrounded by myelin sheaths. The light microscopical investigations indicated that the deposits were located at the axonal periphery, in a close position to the myelin sheath.

3.2. Effects of IDPN-treatment, spatiotemporal PrP^{Sc} analysis in sciatic nerves after f.p. infection in neurotoxin-exposed animals and survival after infection

The IDPN-dose and treatment regime were well tolerated in all animals. One

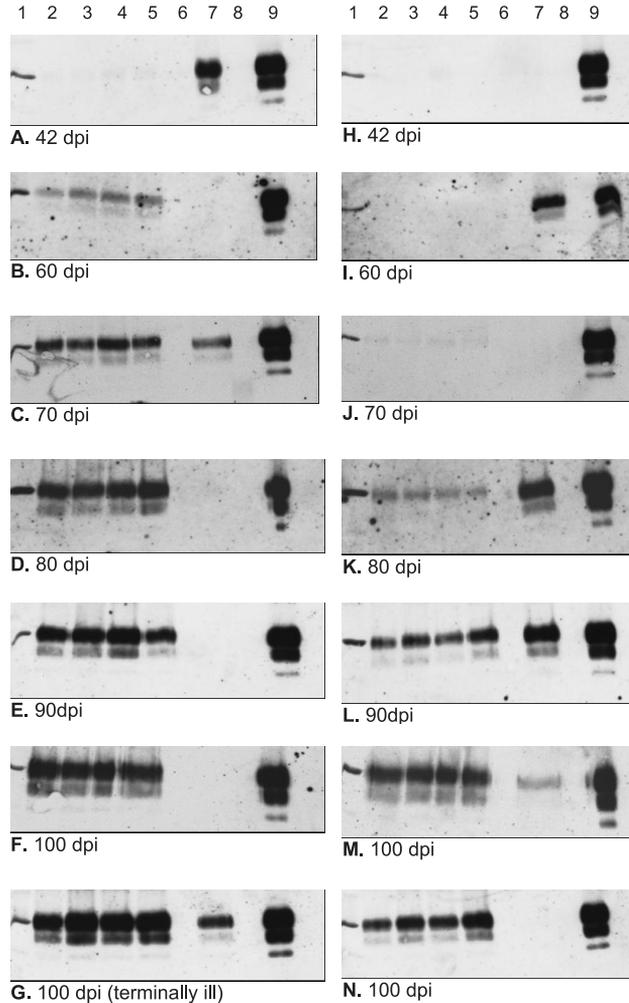


Figure 1. Detection of PrP²⁷⁻³⁰. Representative Western blots of ipsilateral (A-G) and contralateral (H-N) sciatic nerve tissue of 263K f.p. infected hamsters for indicated time points after infection. Lane 1: marker; lanes 2-5: sciatic nerve quarters from proximal to distal of the same individual at the indicated time point (samples correspond to the following amounts of tissue (mg): A2, 2.4; A3, 2.1; A4, 2.9; A5, 4.7; B2, 3.3; B3, 3.2; B4, 3.3; B5, 5.2; C2, 2.4; C3, 2.3; C4, 2.2; C5, 8.7; D2, 3.0; D3, 2.6; D4, 3.1; D5, 6.1; E2, 4.1; E3, 3.9; E4, 4.7; E5, 5.5; F2, 4.5; F3, 2.2; F4, 4.0; F5, 5.5; G2, 2.9; G3, 2.6; G4, 3.9; G5, 3.0; H2, 3.1; H3, 3.0; H4, 2.2; H5, 5.5; I2, 5.5; I3, 2.8; I4, 4.2; I5, 4.8; J2, 3.0; J3, 2.6; J4, 2.4; J5, 2.8; K2, 3.0; K3, 2.6; K4, 3.0; K5, 3.9; L2, 3.1; L3, 3.5; L4, 3.6; L5, 10.5; M2, 2.8; M3, 1.9; M4, 2.2; M5, 4.8; N2, 3.4; N3, 3.8; N4, 2.6; N5, 4.0); lane 7 (A, C, G, I, K, L, M): spiked processing controls, 10^{-5} g 263K brain homogenate (samples correspond to the following amounts of normal sciatic nerve tissue (mg): A7, 7.5; C7, 2.3; G7, 2.6; I7, 6.4; K7, 2.5; L7, 1.5; M7, 2.7); lane 7 (B, D, E, F, H, J, N): uninfected nerves as controls for PK digestion (samples correspond to the following amounts of normal sciatic nerve tissue (mg): B7, 2.6; D7, 2.8; E7, 1.8; F7, 2.6; H7, 2.9; J7, 2.2; N7, 2.5); lane 9: blot controls, 10^{-6} g 263K brain homogenate. Lanes 6 and 8 were loaded with LPP.

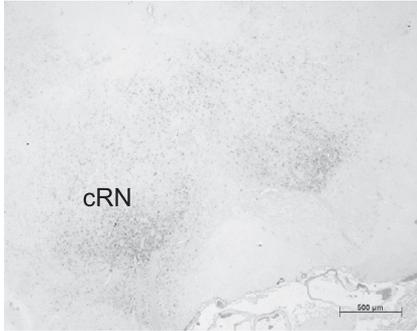


Figure 2. Representative micrograph for immunohistological staining of a 6 µm brain section of a f.p. infected hamster 70 dpi using mAb 3F4 and DAB as chromogen, without counterstaining. Brown deposits for PrP^{Sc} accumulate early in both red nuclei. There are slightly stronger deposits in the contralateral red nucleus (cRN). (A color version of this figure is available at www.edpsciences.org/vetres.)

observed side effect was the occurrence of temporary salivation 30 min after i.p. injection. One week after the last IDPN-application (45 dpi), peripheral blood of separated animals was collected for monitoring general inflammatory side effects. The number and morphology of white blood cells were comparable in IDPN-treated and untreated animals (data not shown).

In further selected animals, tissue samples for immunohistological examinations were taken two weeks after the last IDPN-application to verify the neural effects of the neurotoxin. Immunolabeling of neurofilaments using the mAb 2F11 showed that peripheral nerve tissue of IDPN-exposed hamsters had a stronger immunoreactivity than nerve tissue of untreated animals (Fig. 4).

After scrapie f.p. infection, the disease-specific clinical phase started in the IDPN-treated animals, similar to the non-IDPN-treated animals, with symptoms of muscle tremor around 80 dpi.

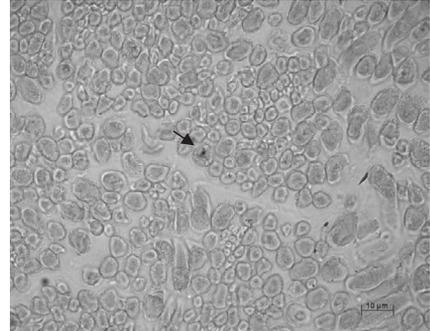


Figure 3. Representative micrograph for immunohistological staining of a 1 µm ipsilateral sciatic nerve section of a f.p. infected animal (PLP-fixed tissue) at 80 dpi using mAb 3F4 and new fuchsin as chromogen. Red deposit (arrow) for PrP^{Sc}. (A color version of this figure is available at www.edpsciences.org/vetres.)

Figure 5 indicates no difference in survival after scrapie infection in non-treated and IDPN-treated animals (106 ± 5 dpi and 105 ± 4 dpi, respectively, mean \pm SD).

Temporal Western blot signal intensities for PrP²⁷⁻³⁰ in serial nerve segments at all time points indicated comparable amounts of PrP^{Sc} in analogous sciatic nerve segments in IDPN- and non-IDPN-treated animals for both ipsilateral or contralateral nerves (Fig. 6, compare with Fig. 1).

4. DISCUSSION

We chose an *in vivo* model mimicking injuries as a possible natural exposure to the TSE agent [48]. However, peripheral uptake via ingestion is the most likely route of exposure for several TSE diseases. Experimental oral infection of hamsters with 263K scrapie required high doses and resulted in relatively long incubation periods until the terminal stage of the disease was reached [43, 49]. In contrast, footpad infection with lower doses showed a relatively short mean incubation time with small variance, characterizing this route as

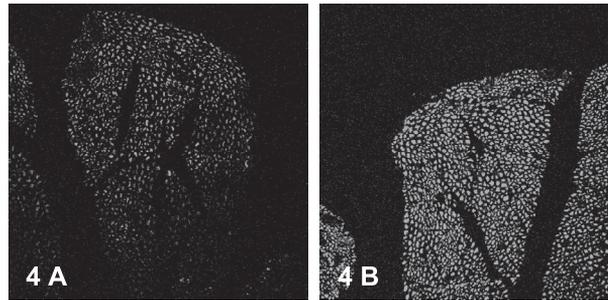


Figure 4. Cross sections of the distal sciatic nerve of an untreated animal (A) and an IDPN-treated animal (B). Immunostaining with a neurofilament antibody showed strong immunoreactivity in the IDPN-treated animal (confocal laser scanning microscopy, magnification $\times 400$.) (A color version of this figure is available at www.edpsciences.org/vetres.)

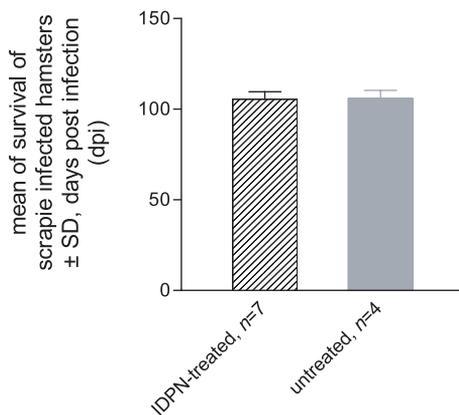


Figure 5. Mean survival time (dpi) after footpad scrapie infection with 20 μ L 2% 263K brain homogenate in neurotoxin exposed and non-exposed hamsters

very effective. Therefore, skin lesions or injuries may be a relevant natural site of entry for the TSE agent.

Western blot analyses of protease resistant prion protein in both distal sciatic nerves and the immunohistochemical detection of PrP^{Sc} in the corresponding brain areas after unilateral footpad infection with the scrapie agent (Figs. 1 and 2) pointed to the postulated centripetal propagation to the CNS and to the subsequent centrifugal spread of PrP^{Sc} along nerval projections:

Cell bodies of the sciatic nerves are localized in the spinal cord. Corresponding areas in the brain of the sciatic nerve are the red nuclei. As most prominent projections, nerve fibers arising in the cells of RN cross ventral to the nuclei and course within the lateral funiculi of the spinal cord (Tractus rubrospinalis).

Spread along nerval projections was also suggested by Kimberlin [29] and Bartz [4] for the intrasciatic route of experimental scrapie- or TME-infection. Moreover, Kimberlin [29] assumed that the intrasciatic route bypasses extraneural agent replication. Nevertheless, for the footpad route, extraneural loci of agent replication cannot be ruled out since early and continuous accumulation of PrP^{Sc} could also be detected in the regional lymphoid system (data not shown). However, up to now no method has been available to differentiate between the exogenously administered and endogenously formed pathological prion protein.

Our immunohistological investigations of the distal sciatic nerve concerning the localisation of PrP^{Sc} rarely showed granular PrP-positive deposits, located apparently between the myelin sheath and axon. However, it was not possible to exactly determine the axonal or extraaxonal location of these depositions by light

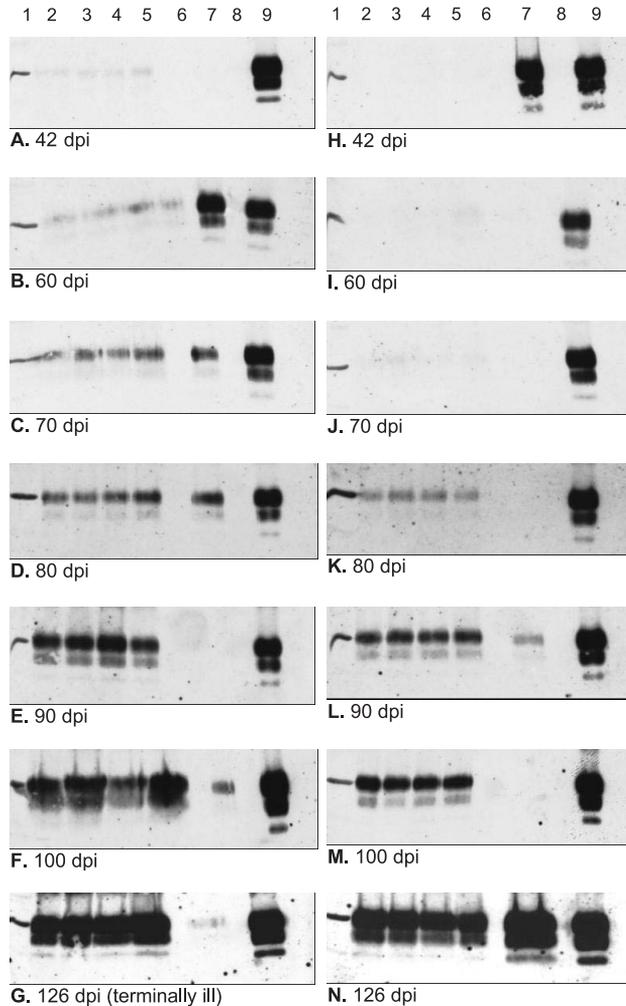


Figure 6. Detection of PrP27-30. Representative Western blots of ipsilateral (A-G) and contralateral (H-N) sciatic nerve tissue of 263K f.p. infected and IDPN-treated hamsters for indicated time points after infection. Lane 1: marker; lanes 2-5: sciatic nerve quarters from proximal to distal of the same individual at the indicated time point (samples correspond to the following amounts of tissue (mg): A2, 2.6; A3, 2.5; A4, 2.9; A5, 4.6; B2, 1.2; B3, 1.0; B4, 1.5; B5, 1.9; C2, 2.5; C3, 2.1; C4, 2.0; C5, 3.7; D2, 2.9; D3, 1.9; D4, 2.9; D5, 3.3; E2, 6.2; E3, 4.3; E4, 4.5; E5, 5.5; F2, 3.9; F3, 3.3; F4, 3.5; F5, 5.6; G2, 2.6; G3, 2.1; G4, 1.9; G5, 2.8; H2, 5.4; H3, 2.8; H4, 4.4; H5, 4.0; I2, 1.8; I3, 1.7; I4, 1.4; I5, 1.6; J2, 2.1; J3, 2.2; J4, 2.2; J5, 4.6; K2, 3.4; K3, 3.3; K4, 3.4; K5, 4.4; L2, 4.9; L3, 4.1; L4, 3.4; L5, 5.0; M2, 4.7; M3, 3.1; M4, 3.3; M5, 4.0; N2, 3.1; N3, 1.7; N4, 2.6; N5, 4.8); lane 7 (B, C, D, F, H, L, N): spiked processing controls, 10^{-5} g 263K brain homogenate (samples correspond to the following amounts of normal sciatic nerve tissue (mg): B7, 2.5; C7, 2.7; D7, 3.5; F7, 2.5; H7, 2.4; L7, 1.9; N7, 2.7); lane 7 (A, E, G, I, J, K, M): uninfected nerves as controls for PK digestion (samples correspond to the following amounts of normal sciatic nerve tissue (mg): A7, 3.6; E7, 1.6; G7, 2.2; I7, 3.0; J7, 2.3; K7, 2.7; M7, 2.1); lane 9: blot controls, 10^{-6} g 263K brain homogenate.

microscope. Groschup et al. [24] suggested adaxonal location of PrP^{Sc} deposits in peripheral nerves for the i.p. route of infection.

Western blot signals for PrP27-30 from the sciatic nerve segments did not indicate any clear direction of PrP^{Sc}-propagation: the blot signal intensities from all segments of a unilateral nerve in the same individual at one time point were almost equal (Figs. 1 and 6). This may be due to the limited resolution of the detection method and/or to the comparative observations along a relatively short neural distance (quarters of a length of 2–3 cm nerve). Another possible explanation may be that the infectious agent rapidly passes neural tissue at earlier points in time of the incubation period, something that is also assumed for vagal transit [35].

Another objective of this study was to identify or narrow down potential mechanisms for the neural centripetal/centrifugal propagation of the TSE agent. In order to determine whether this propagation is dependent on an intact axonal cytoskeleton we studied the spread of scrapie in IDPN intoxicated animals. The effects of IDPN as a toxin causing changes in cytoskeletal architecture and in axonal transport of proteins are well characterized by various *in vivo* models including a broad range of species (e.g. rats, cats, chickens, guinea pigs). IDPN treatment induces structural abnormalities in axonal segments [14, 15], neurofilaments displace toward the axonal periphery while microtubuli displace toward the axonal center [23, 37]. Giant axonal swellings appear, containing maloriented neurofilaments [16]. In motor end plates, the immunoreactivity of neurofilaments is decreased [18]. The neuronal cytoskeleton in IDPN-treated animals can be characterized as “disorganized”. Several data indicate that axonal transport mechanisms are impaired: predominantly the slow anterograde ones but also the retrograde and fast ones [19, 21, 30, 38]. Com-

pared to the doses described in the literature for experiments with various species, the administered dose in the present study ranged in the lower level. Chronic low dose intoxication with IDPN in our test animals induced an increased immunoreactivity of neurofilaments in sections of the distal sciatic nerve, confirming an effect on the axonal cytoskeleton. Regardless of possible changes in the phosphorylation status of the neurofilament proteins leading to a possibly higher affinity to the antibody labelling of the neurofilaments, we would interpret this observation as indicating an enrichment of neurofilaments in distal axons, analogous to results in IDPN-intoxicated cats [22]. Moreover, based on visual subjective judgement, the axonal diameter seemed to be slightly increased (Fig. 4). The occurrence of salivation shortly after i.p. application in hamsters could be the effect of the neurotoxin on neurotransmitters involved in the control of salivary gland secretion. The quality and quantity of the animals’ white blood cells suggested that there was no evidence of general inflammation caused by IDPN-treatment regime. Inflammations were shown to have an influence on the detection of PrP^{Sc} or on scrapie pathogenesis [26].

A comparison of the incubation times in neurotoxin exposed and unexposed animals (Fig. 5) led to the assumption that the neuroinvasive progression of disease is independent of the neurofilament component of the axonal cytoskeleton. Moreover, the comparison of the results of spatiotemporal PrP^{Sc} detection in sciatic nerves without or with IDPN treatment (Figs. 1 and 6) did not indicate any influence of the axonal neurofilament component on the propagation of the pathological prion protein in peripheral nerves.

One conceivable mode of transportation along peripheral nerves for the abnormal prion protein is the host’s axonal transport machinery. Although the molecular basis is not completely understood, members

of the *Herpesviridae* family are examples of agents using this transport system [50] as well as neurotoxins (i.e. tetanus toxin, [41]). IDPN intoxication mainly affects the slow axonal transport mechanism. Regarding the relevance of neurofilaments for slow component 'a' [9], the IDPN model has the potential to specify the influence of host slow axonal transport mechanism 'a' on propagation of PrP^{Sc}. Although the degree of impairment of the axonal flow achieved by IDPN-intoxication was not monitored in this study, it is strongly indicated that axonal propagation of PrP^{Sc} does not correlate with the slow axonal transport system component 'a'.

Finally, we only focused on the neuronal pathway and its structural-functional modification in one TSE in vivo model. Alternative pathways of spreading should be examined, too, including simultaneous observations of other tissues. Further investigations are necessary to elucidate the impact of neuronal vs. nonneuronal pathways for the footpad model.

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