6. ABSTRACT

Scrapie is a neurodegenerative disease that is characterized by the accumulation of the misfolded prion protein, PrP^{Sc}, which carries the infectivity. The function of the normal prion protein is still unclear; however, it has been shown to bind copper and may play a role in copper homeostasis, which may also be involved in the neurotoxicity of the disease. To date, the majority of studies on prion protein infection have been performed on terminally ill animals so the molecular events underlying the disease, especially at early stages, are still poorly understood. Thus, the main goal of the thesis was to examine the chemical composition of scrapie infected nervous tissue at pre-clinical time points in order to investigate prion-induced molecular differences. To address the compositional changes associated with scrapie infection, a time-course study was used. 263K scrapie-infected (N=24) and mock-infected (control, N=15) hamsters sacrificed at five time points: 70 days post infection (dpi), 100 dpi, 130 dpi, first clinical signs (~145 dpi), and terminally diseased (~180 dpi) were studied. Protein composition, structure, and distribution were examined in the dorsal root ganglia (DRG) with 3F4 immunostaining and synchrotron Fourier Transform InfraRed Microspectroscopy (FTIRM). Trace metal content and distribution was determined from serial sections of the same DRG using synchrotron x-ray fluorescence (SXRF) microprobe. Results showed that protein-related changes occur at the pre-clinical stages of scrapie, before the transformation of PrP^{C} to PrP^{Sc}. The scrapie-infected animals exhibited a significant increase in protein expression, yet the β-sheet protein content was significantly lower than controls. Regions of elevated β-sheet content were observed only at the plasmalemma and in surrounding satellite cells. Then, over the course of the disease, the β-sheet content increased significantly, leading to higher numbers of terminally diseased animals. Comparison with the same tissue, subsequently immunostained for PrP, proved that this increase in ß-sheet is at least partly related to increasing amounts of PrP^{Sc}. Thus, the spectral findings are likely to be specific for prion diseases such as scrapie. At the terminal stage of the disease, the relative protein expression declined significantly, likely due to the induced neuronal death. Moreover, there was a strong inverse correlation between the distribution of α-helical and β-sheet protein content (R^2 = -0.636), where cells exhibiting very high β-sheet content had a very low α-helical content – a correlation that was much lower in earlier stages of the disease. Based on these findings, we suggest that the preclinical

stages of scrapie are characterized by an overexpression of proteins low in β-sheet content. As the disease progresses, PrP^{C} is converted to PrP^{Sc}, along with the conversion or replacement of other α-helical-rich proteins by β-sheet rich proteins. The dramatic changes in protein content and structure at pre-clinical time points emphasizes the need for identifying protein alterations involved in early pathogenesis, which are important for understanding the disease and may provide a mechanism for early TSE diagnosis and treatment.

Furthermore, 70 dpi infected brains examined with the aid of synchrotron light showed significant alterations in the spectral fingerprint region, as calculated by the ratio of the symmetric and asymmetric P=O stretching in $>PO_2^-$, indicating changes in nucleic acids, carbohydrates or phospholipids at very early stages of the disease. Furthermore, some of the investigated animals exhibited a lower content of β-sheet and slightly more total protein, as was already seen in pre-clinical DRG, suggesting that molecular alterations in central and peripheral nervous system during scrapie pathogenesis are similar time dependent events.

SXRF microprobe results from trace metal imaging showed that copper, zinc and phosphorus are highest intracellular, while iron and calcium are abundant in the ECM. It was also shown that physiological concentrations of phosphorus are about a magnitude higher than that of calcium which in turn is about twice that of iron. Copper and zinc concentrations are about 2-3 times less than that of iron but about twice as much as manganese, the least concentrated of the studied elements. With scrapie infection, increase in copper, zinc, iron, calcium and manganese levels in scrapie infected animals was detected at the terminal stage of the disease, while potassium and phosphorus levels decreased. Furthermore, calcium levels were altered at pre-clinical time points, indicating to play an important role in scrapie pathogenesis at early time points.

In order to investigate the specificity of these compositional changes to 263K scrapie infection, a similar set of experiments was performed on DRG infected with the ME7 scrapie strain and also hamster brain infected by Reovirus T3C9, another neurological disease. Specifically, alterations in the brains of reovirus infected animals were compared to those of terminally scrapie infected hamsters. Results showed that reovirus induces alterations in the same spectral region as scrapie. However, the appearance of a shoulder in original spectra at ~1050 cm^{-1}, indicative of alterations in complex sugar ring vibrations of carbohydrates was not observed in the virus group

and therefore possibly only occurs in scrapie. Finally, we compared compositional changes induced in DRG of hamsters by the two different scrapie strains 263K and ME7. Spectra from 263K and ME7 infected animals differed remarkably in several spectral regions. Firstly, both strains exhibited significantly more β-sheet compared to control, while ME7 showed even more than 263K. The result of the Western Blot analysis, however, did not indicate more PrP^{Sc} in the ganglia of ME7 infected animals. Therefore, the detected increase in β-sheet in ME7 infected animals might partly be caused by other proteins besides PrP^{Sc}, probably to a greater extent than was seen for 263K. Due to the integrative nature of information provided by FTIRM, the amide I band of tissue spectra represents the sum of all proteins in the investigated area, which might also explain why no remarkable differences between the strains in the amide I band were detected. Both 263K and ME7 exhibited significant differences in the lipid region (3000 – 2800 cm^{-1}) compared to the control, ME7, again, greater than 263K, indicating changes in e.g. the neurons membrane system. Finally, ME7 and 263K exhibited oppositional changes in the peak intensities around 1238 cm^{-1} and ~1084 cm^{-1}. Thus, ME7 and 263K induce different alterations in nucleic acid phosphodiester groups; sugarsm, phosphorylated lipids, proteins and other molecules. It seems therefore possible that the determined spectral patterns could even be specific on a strain level.