Use of fluorescence correlation spectroscopy to detect single prion particles (surface-FIDA)

Area of research interest: Foodborne pathogens
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Background

The pathological isoform of the prion protein (PrP^{Sc}) can be considered to be an early biomarker for transmissible spongiform encephalopathy (TSE) infection such as scrapie in sheep or BSE in cattle. PrP^{Sc} is composed of a proteinase K (PK) resistant ($resPrP^{Sc}$) and PK-sensitive portion. The non-pathogenic, cellular isoform, PrPC, is also sensitive to PK-digestion. Most TSE-diagnostic approaches today are based on the detection of the $resPrP^{Sc}$-portion only, however this research will investigate the aggregated PrP^{Sc} structure as a specific marker, not depending upon PK resistance

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Research Approach

A new method has been developed in which PrP^{Sc}-particles, the PK-sensitive as well as the PK-resistant portion, are bound to a surface via capture antibodies. The PrP^{Sc}-particles are labelled with two different antibodies carrying different fluorescent labels. The labelled PrP^{Sc}-particles are evaluated using Fluorescence Intensity Distribution Analysis (FIDA) which captures fluorescence from the two different antibodies. To enhance sensitivity a prion protein amplification (PPA) method will be used which in combination with surface-FIDA should result in an ultra sensitive detection method for TSE-associated particles. This could aid the detection of low levels of PrP^{Sc} - in the early state of disease and in non-central nervous system tissues or body fluids. The research aims to optimise surface-FIDA in respect to sensitivity and specificity of detecting single PrP^{Sc}-particles. Body fluids such as blood will be used to investigate the application of surface-FIDA to living animals. Brain tissue and CSF from preclinical animals will also be used to investigate the very early state of disease.

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Results

The preparation of PrP^{Sc}-aggregates had to be adapted to the analysis of blood samples. As major progress a step to digest the lipoprotein in blood was introduced, otherwise these would

cover up the epitopes for antibodies on the PrP^{Sc}-aggregates and make the PrPSc aggregates inaccessible to antibody binding. The assay was able to detect PrP^{Sc}-particles in the plasma fraction of blood from Scrapie sheep.

It had previously been shown that Scrapie infectivity could be transmitted by blood but this research showed for the first time the molecular PrP^{Sc}--particles in blood. A series of samples obtained from VLA (Weybridge) were tested. Over 60% of the samples from infected sheep were identified correctly; a number which could potentially be improved later. Samples from a pathogenesis study on BSE cattle at the Friedrich-Löffler Institut in Germany were also analysed. As a major difference compared to sheep no PrP^{Sc}-particles could be detected in the blood of BSE-cattle. In brain samples of BSE-cattle, however, PrP^{Sc}-aggregates could be detected in some animals several months before the cattle showed symptoms.

Finally the researchers succeeded in amplifying the amount of PrP aggregates by using the PrP^{Sc}-aggregates present in blood to convert further PrP-molecules added to the sample, which has the potential of improving the sensitivity of the test in the future.

The goal was to deliver a live animal test for Scrapie and BSE to the agency. A blood test for Scrapie in sheep could be achieved, for BSE-cattle it was not possible but for particular applications on single animals a live test based on cerebrospinal fluid (CSF) is possible.

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Published Papers

1. Panza, G., Luers, L., Stohr, J., Weiss, J., Riesner, D., Willbold, D. & Birkmann E. (2010) Molecular interactions between prions as seeds and recombinant prion proteins as substrates resemble the biological interspecies barrier in vitro. Published online *PLoS One* 5: e14283

Research report

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