

DEVELOPMENT OF A CONFORMATION DEPENDENT IMMUNOASSAY FOR THE DETECTION OF THE ABNORMAL FORM OF THE PRION PROTEIN IN BLOOD

Researchers

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Aim

Variant Creutzfeldt-Jakob disease is a fatal neurodegenerative disease resulting from human exposure to the bovine spongiform encephalopathy agent. Although clinical cases of variant Creutzfeldt-Jakob disease are in decline in the UK, asymptomatic individuals incubating the diseases can inadvertently transmit it to others by donating blood for transfusion. In order to prevent this happening the blood transfusion services urgently require methods to remove the infectious agent from blood, or means of testing blood donations for infectivity. This project's aim was to investigate whether a sensitive blood test could be developed using a technique called conformation dependent immunoassay.

Project Outline/Methodology

Conformation dependent immunoassay is a method which can distinguish between the normal prion protein that is found in everyone's blood, and the abnormal prion protein, which is associated with infectivity. Although conformation dependent immunoassay is a very sensitive test, it is not in itself sufficiently sensitive to detect the very small quantities of the abnormal prion protein thought to be present in the blood of those suffering from the disease, still less those incubating it. We have therefore sought to develop a combined test in which small quantities of abnormal prion protein are amplified, using a method called protein misfolding cyclic amplification, to levels that can be easily detected by conformation dependent immunoassay. Our work has focussed on the practical issues of combining these two techniques to produce a highly sensitive test for abnormal prion protein that might be applicable to blood testing.

Key Results

We have shown that minute amounts of the abnormal prion protein from Creutzfeldt-Jakob disease brain can be amplified by protein misfolding cyclic amplification to levels that can be readily detected by conformation dependent immunoassay. Extracts of normal blood cells called platelets, that are often surplus to the requirements of the

transfusion services were found to provide a suitable and convenient medium to support this amplification. Additional steps to overcome inhibition of amplification by a hitherto unrecognised factor found in human plasma, and measures to make the assay compatible with different genetic groups needed to be introduced into the assay. In its current form the assay can reach the sensitivity required to detect the amounts of abnormal prion protein thought to be present in the blood of individuals with variant Creutzfeldt-Jakob disease.

Conclusions

Protein misfolding cyclic amplification coupled with conformation dependent immunoassay provides a highly sensitive assay for the detection of the abnormal form of the prion protein found in Creutzfeldt-Jakob disease.

What does this study add to the field?

The biochemical sensitivity and specificity of this assay suggests that it is a strong candidate confirmatory screening test for variant Creutzfeldt-Jakob disease infectivity in blood and tissues.

Implications for Practice or Policy

Should a commercial Creutzfeldt-Jakob disease blood screening test become available it will be important to implement a confirmatory assay at the same time to minimise the impact of potential false positive test results on UK blood donors and the UK blood supply.

Where to next?

This combination of protein misfolding cyclic amplification and conformation dependent immunoassay is being further evaluated as a potential confirmatory variant Creutzfeldt-Jakob disease blood screening assay using the procedure recommended by the National Institute for Biological Standards and Control.

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