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Short Communication

Molecular profiling and comparison of field transmissible spongiform encephalopathy cases diagnosed in Catalunya

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Abstract

Molecular profiling of the proteinase K resistant prion protein (PrP^{res}) is a technique that has been applied to the characterisation of transmissible spongiform encephalopathy (TSE) strains. An interesting example of the application of this technique is the ability to differentiate, at the experimental level, between bovine spongiform encephalopathy (BSE) and scrapie infection in sheep, and to distinguish between classical and atypical BSE and scrapie cases. Twenty-six BSE cases and two scrapie cases from an active TSE surveillance program and diagnosed at the PRIOCAT, TSE Reference Laboratory (Centre de Recerca en Sanitat Animal, Universitat Autònoma de Barcelona, Catalunya, Spain) were examined by Western blotting. Molecular profiling was achieved by comparing the glycosylation profile, deglycosylated PrP molecular weight and 6H4/P4 monoclonal antibody binding ratio. The results obtained during the characterisation of these field cases indicated an absence of atypical BSE cases in Catalunya.

Keywords: Molecular profile; BSE; Scrapie; Prion protein

Until recently, techniques based on mice bioassay (Fraser and Dickinson, 1968) have been the only tools available for transmissible spongiform encephalopathy (TSE) strain differentiation. However, molecular profiling of the proteinase K resistant prion protein (PrP^{res}) is a technique, which has been lately applied to the characterisation of strains and has been used to differentiate between bovine spongiform encephalopathy (BSE) and scrapie infection in sheep (Stack et al., 2002; Thuring et al., 2004) or atypical BSE cases as described in France (Biacabe et al., 2004) and Italy (Casalone et al., 2004). Mice lesion profiling studies are, however, still required to confirm a diagnosis of a specific strain infection in a field case, and involve inoculating mice strains with a certain TSE isolate. Parameters such as the incubation period and the quantification of vacuoles in several brain sites yield a profile that is characteristic for each prion strain (usually several passages from mouse to mouse are required to stabilise one strain's profile) (Fraser and Dickinson, 1968).

Here we present molecular profiling studies of TSE cases diagnosed in the active surveillance program in the PRIOCAT TSE Reference laboratory in Catalunya, Spain. The work was undertaken with a view to ruling out the existence of atypical TSE cases in the region. To this end, molecular profiling, including glycosylation pattern, deglycosylated PrP^{res} molecular weight and monoclonal antibody (mAb) 6H4/P4 immunolabelling was carried out on 26 BSE cases and the only two scrapie cases diagnosed in Catalunya. Although there have been 45 BSE cases diagnosed in the region, we selected 26 samples (57%) based both on post mortem delay and the amount of sample available. Samples which were autopsied beyond 48 h were discarded (these were usually samples from fallen stock,

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and heavily autolysed). All other animals were humanely killed in slaughterhouses.

In both scrapie cases, the genotype for the Prnp gene (encoding for the host prion protein) was homozygous for the ARQ gene. Sheep carrying this genotype are susceptible to the scrapie agent (Atkinson, 2001).

Proteinase K (PK) digestion and Western blotting (WB) were performed following the manufacturers' instructions (Prionics-Check WB Kit). For antibody binding comparison, the original antibody supplied (6H4 mAb 1:5000) was substituted for the P4 mAb (1:2500, kindly provided by B. Öesch). PrP^{res} deglycosylation was performed using PNGase F following the manufacturers' instructions (New England Biolabs). Glycosyl digestion was carried out by incubating proteinase K-treated denatured homogenates with 0.5 PGNase F U/μL for 1 h at 37 °C. A Bio-Rad Multi Analyst TM V1.1 was used to determine glycoform proportions and the molecular weight of the deglycosylated PrP^{res} blots by densitometry of the immunolabelled Western blot membranes. For each sample an average of eight runs were evaluated (ranging from a minimum of 3 to a maximum of 19). Origin 7.0 software was used for data analysis.

All BSE and scrapie samples presented the characteristic three banded pattern corresponding to diglycosylated (top band), monoglycoslylated (middle band) and unglycosylated (bottom band) forms of the prion protein. The 26 BSE cases included in this study presented a similar glycosylation profile, which differed from that of the two studied scrapie cases (Fig. 1).

In cases of both BSE and scrapie, the diglycosylated form was the most abundant glycoform, although in the BSE cases this was almost 12% more abundant than in the scrapie cases (Table 1). This difference reflected the finding that a major proportion of the monoglycosylated

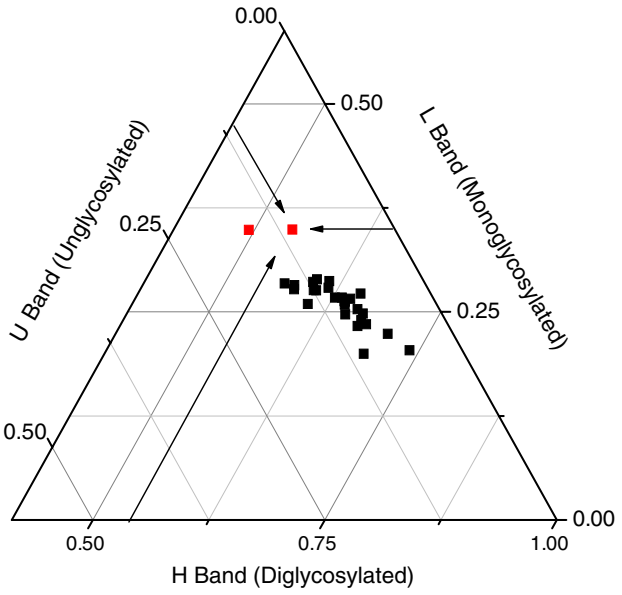

Fig. 1. Triangular plot for the individual glycoform proportions of BSE (black) and scrapie (grey) cases. Note the different location of the scrapie cases from the BSE ones.

Table 1
PrP^{res} molecular weight (kDa) and proportion (%) of the PrP^{res} glycoforms of BSE and scrapie cases

PrP^{res}TSE	MW (kDa)	H (%)	L (%)	U (%)
Scrapie	22.0 ± 0.1	51.7 ± 3.3	34.1 ± 0.0	10.4 ± 2.3
BSE	20.0 ± 0.7	63.5 ± 4.1	25.9 ± 2.6	10.4 ± 2.3

Scrapie cases showed a higher proportion of the L glycoform and a lower proportion of the H glycoform when compared to BSE cases. The U band proportion is indistinguishable from both PrP^{res} forms. Glycoforms are represented as: (U) unglycosylated, (L) monoglycosylated and (H) diglycosylated forms. When compared within the same gel, a consistent 2 kDa difference was observed between scrapie and BSE unglycosylated moiety. Mean values ± standard deviations are shown.

form was found in the scrapie cases as the proportion of both unglycosylated bands was similar. The glycoform pattern found in our BSE and scrapie cases fell within the range of data published by other authors (Baron et al., 2004, 1999; Gretzschel et al., 2005; Nonno et al., 2003; Stack et al., 2002). Glycosylation pattern analysis allowed us not only to discriminate between BSE and scrapie cases but also to confirm the absence in these samples of atypical BSE (Biacabe et al., 2004) or scrapie (Benestad et al., 2003).

In the regular blots used to determine the glycoform ratios, a difference in the molecular weight of the unglycosylated band between BSE and scrapie cases was identified. To analyse this difference better, PrP deglycosylation was performed before Western blotting (Fig. 2). Deglycosylation of the resistant prion protein increased the unglycosylated band signal. The measurement of the molecular weight of the deglycosylated band showed a 2 kDa higher mean value for the scrapie prion protein (22.0 ± 0.1 kDa) when compared to the BSE prion protein (20.0 ± 0.7 kDa) (Fig. 2). Differences in the thickness of the bands due to different PrP^{res} load were minimized by diluting these samples to achieve a similar PrP^{res} concentration. In some cases, a double band could be seen (see, for example, Fig. 2, B18), increasing the molecular weight variation among the samples. This phenomenon was deemed to have occurred as a consequence of the different proteinase K N-terminal truncation sites of the BSE PrP^{res} glycosylated and unglycosylated glycoforms (Nonno et al., 2003). Accordingly, the resulting band after deglycosylation would be composed of fragments with slight differences in molecular weight depending on the parent glycoform.

In previous studies, similar differences in MW between scrapie and BSE cases have been found (Gretzschel et al., 2005; Nonno et al., 2003; Stack et al., 2002). The slightly higher MW of the scrapie cases PrP^{res} is explained by a conformational underexposure of the PK cleavage regions associated to the scrapie agent (Thuring et al., 2004) (Fig. 3).

P4 mAb immunolabelling has been used to distinguish between scrapie and BSE PrP^{res} by Western blotting (Stack et al., 2002; Thuring et al., 2004; Gretzschel et al., 2005), since this antibody immunolabels PrP^{res} from scrapie but not from BSE due to the absence of the mAb-P4 epitope in the PK digested PrP^{res} from BSE (Fig. 3). MAb 6H4 is