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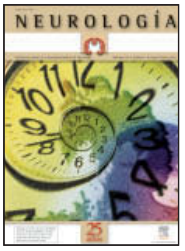

ORIGINAL ARTICLE

Cellular prion protein in the central nervous system of mammals. Anatomoclinical associations

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KEYWORDS
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Abstract
Introduction: The scrapie prion protein (PrPsc) requieres the celular prion protein (PrPc) for its propagation and replication. In this work we studied the expression and localization of the PrPc in the central nervous system (SNC) of the rat, mouse, cat, cow and human, using immunohistochemistry and Western blot techniques to understand more about prinopathies and Alzheimer's disease (EA).
Material and methods: For the immunohistochemistry study we used human, cat, rat and cow samples to analyse frontal, temporal and occipital cortex, as well as the hippocampus and the thalamus. For the Western blot analysis we used mouse, cat, cow and human brain samples.
Results: We observed a decrease in the amount of PrPc in the central nervous system (CNS) in a rostrocaudal shift in the species mentioned above. We observed inhibitory cells in the cat cortex. The Western blot analysis showed a similar pattern of expression in the different species studied with a preponderance of the diglycosylated band, in relation to the other bands observed in the analysis.
Discussion: These data suggest that in prionopathies PrPsc could be transmitted and could be replicated in and from the areas with most expression of PrPc. Similarly, a higher amount of this protein (PrPc) in some brain areas could explain some histopathological aspects of Alzheimer's disease (AD).
Conclusions: Our findings support the hypothesis of a retrograde transport of PrPsc in the CNS. PrPc could be related to the pathophysiology of AD.

PALABRAS CLAVE
Proteína priónica celular;

La proteína priónica celular en el sistema nervioso central de mamíferos. Correlatos anatomoclínicos

Resumen
Introducción: La proteína priónica celular patógena (PrPsc) necesita de la presencia de la fisiológica (PrPc) para su propagación y replicación. Se estudia comparativamente la ex-

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presión y localización de PrPc en el sistema nervioso central (SNC) de rata, ratón, gato, vaca y humano, mediante técnicas inmunohistoquímicas y de *Western blot,* con el objetivo de un mejor conocimiento de las prionopatías y de la enfermedad de Alzheimer (EA).
Material y métodos: Se emplearon encéfalos humanos y de gato, rata y vaca, para estudios por técnicas inmunohistoquímicas; se analizaron las cortezas frontal, temporal y occipital, así como hipocampo y tálamo. Se utilizaron técnicas de *Western blot* para encéfalos de ratón, gato, vaca y humano.
Resultados: Existe una disminución rostrocaudal de la cuantía de PrPc en el SNC de dichas especies. PrPc se sitúa en la membrana y en el citoplasma de las neuronas. Se observan neuronas inhibitorias en el córtex del gato. El patrón general del *Western blot* es análogo en las especies estudiadas, con predominio de la banda diglucosilada sobre las bandas monoglucosilada y no glucosilada.
Discusión: Los datos indican que en las prionopatías, PrPsc puede transmitirse y replicarse de forma retrógrada en y a partir de las zonas más PrP positivas. La mayor cuantía de PrPc en algunas zonas del encéfalo humano podría estar en relación con los hallazgos anatomopatológicos de la EA.
Conclusiones: Los datos apoyan un transporte retrógrado de la PrPsc en el SNC. La PrPc debe de tener relación con la fisiopatología de la EA.

Introduction

Cellular prion protein (PrPc) is a membrane glycoprotein that forms part of various organs in most animal species studied. It is especially abundant in the central nervous system (CNS)[1].

There is a different isoform of this protein: the scrapie prion protein (PrPsc or prion, anagram of *PROteinaceous INfectious particle*). Prions are the causative agents of transmissible spongiform encephalopathies (TSEs), for whose establishment and subsequent evolution, the presence of PrPc along with PrPsc is required. In fact, it has been observed that knockout mice for PrP did not develop the disease when inoculated with PrPsc[2]. Both PrP isoforms are encoded by the same gene and present the same amino acid sequence, although with differences in their secondary structure. PrPc can be digested by proteinase K, while PrPsc is only partially digested.

PrPsc appears only in prionopathies, and reaches brain concentrations 10-20 times higher than PrPc. There are sometimes accumulations of PrPsc in the form of plaques in TSEs.

The most common form of TSE in humans is Creutzfeldt-Jakob disease (CJD). Severe or fatal familial insomnia (FFI) shows similarities to CJD. However, what is characteristic of FFI are the initial pathological changes of the midline of the dorsomedial thalamic nucleus (DM) and of the anteroventral nucleus of the complex of the anterior thalamic nuclei (AN)[3-5]. Interestingly, the mentioned thalamic areas have a different connectivity with respect to other DM and AN portions, as well as with respect to neighbouring thalamic nuclei to DM and AN, in both cats and rats[6,7]. After a time, in FFI cases with longer duration, other structures become affected, including the deep layers of the cerebral cortex[3,8]. The most affected cortical zones are the frontal and cingulate cortices, and the least, the occipital[8,9].

We have proposed the existence of a retrograde propagation of PrPsc from the DM and AN to various CNS areas in FFI[6,10]. Similarly, in other prionopathies, the propagation must be retrograde (for example, in bovine spongiform encephalopathy[11]). Moreover, in the rat CNS we have observed the co-localisation of PrPc with several neurotransmitters[7], which suggests a multiplicity of pathogenic mechanisms in both FFI and in TSEs in general.

There is also another special circumstance: we have observed a specific PrPc behaviour in brains with Alzheimer's disease (AD)[12].

This study aims to determine whether PrPc, both in prionopathy and in AD, must have a special role in relation to the pathophysiology of both groups of clinical entities.

Material and methods

We used human brains as well as those from the cat, rat, mouse and cow.

The brains of cat, rat, cow and human were studied by immunohistochemistry techniques, with a special focus on the analysis of the frontal, temporal and occipital cortices, the hippocampus and the thalamus.

The tissue was fixed by immersion, to obtain a correct antigen protection. It was included in paraffin and cut into 4Ìm sections. The best results were from polyclonal antibodies, Anti-Prion Protein 91511 (Assay Designs Inc.) and ARP-01-8634 (American Research Products).

We also carried out Western blot techniques with fresh tissue, not fixed, which was dissected into areas of interest for this purpose; samples were quickly frozen with dry ice and stored at -80°C until the quantification of the total protein content in each of the study areas. Subsequently, proteins were separated on 12% acrylamide gels and transferred to nitrocellulose membrane, with an incubation in which the antibody for PrP mAb 6H4 (Prionics) was used. Once the right conditions were obtained, we proceeded to