

## Short Communication

Department of Comparative Anatomy and Pathological Anatomy, Veterinary Faculty, University of Córdoba, Córdoba, Spain

### Purkinje Cell Apoptosis in Arabian Horses with Cerebellar Abiotrophy

A. BLANCO<sup>1</sup>, R. MOYANO<sup>2</sup>, J. VIVO<sup>1</sup>, R. FLORES-ACUÑA<sup>1</sup>, A. MOLINA<sup>2</sup>, C. BLANCO<sup>1</sup> and J. G. MONTERDE<sup>1,3</sup>

Addresses of authors: Departments of <sup>1</sup>Comparative Anatomy and Pathological Anatomy; <sup>2</sup>Pharmacology and Toxicology, Veterinary Faculty, University of Córdoba, 14071 Córdoba, Spain; <sup>3</sup>Corresponding author: Tel.: +34 957218675; fax: +34 957218847; E-mail: jg.monterde@uco.es

With 2 figures

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#### Summary

Purkinje cerebellar cells were studied in three Arabian horses aged between 6 and 8 months with clinical disorders in their movements, tremors and ataxia; the occurrence of apoptosis in this cell population was investigated by the (terminal deoxynucleotidyl transferase biotin-dUTP nick-end labelling (TUNEL) method. Both optical and electron microscopical images showed a scant number of Purkinje cells, most of them with morphological features of apoptosis such as condensation of the nucleus and cytoplasm as well as segregation and fragmentation of the nucleus into apoptotic bodies. The TUNEL technique revealed a substantial number (65%) of positive immunoreactive Purkinje cells.

cell death of cerebellar Purkinje cells, have demonstrated that the Purkinje cells undergo apoptosis via the activation of caspase 3 and, subsequently, a fragmentation of their DNA. The apoptotic cells show a series of structural changes: blebbing of the plasma membrane; condensation of the cytoplasm and nuclei; and cellular fragmentation into apoptotic bodies, but the most common biochemical property of apoptosis is the endonucleolytic cleavage of chromatin, which can be detected by the terminal deoxynucleotidyl transferase biotin-dUTP nick-end labelling (TUNEL) method.

This work describes the presence of positive reactions to the TUNEL apoptosis detection method as well as ultrastructural signs of apoptosis in the Purkinje cells of three cases of cerebellar abiotrophy in Arabian horses.

#### Introduction

The cerebellum is the part of the brain concerned with the motor function, balance and coordination of movements. Cerebellar abiotrophy is characterized by a premature degeneration of Purkinje cells, whose progressive death causes clinical signs associated with poor coordination and lack of balance; an autosomal recessive pattern of inheritance is suspected, although any definitive evidence is lacking at this time (de Lahunta, 1990).

This neurodegenerative condition has been reported in Arabian horses and affects foals between the time of birth and 6 months of age. The occurrence of cerebellar abiotrophy in horses is, moreover, not well-known, because breeders are usually reluctant to disclose that their breeding stock has produced foals with a neurological disease. Clinical signs include head tremors and ataxia and although some improvement phases are possible, the condition is considered as untreatable. Cerebellar abiotrophy can be difficult to diagnose, until it is at an advanced stage, the loss of Purkinje cells in the cerebellar cortex being the most consistent sign of it in a histopathological diagnosis (DeBowes et al., 1987).

Although the neuronal degeneration and Purkinje cell death found in cerebellar abiotrophy have been attributed to an unidentified metabolic defect, different from the programmed cell death, some signs indicate that the apoptosis mechanism could be implicated in the neuronal loss that characterizes this disease (Sandy et al., 2002). Recently, Kyuhou et al. (2006), using Purkinje cell degeneration mice, a classic model for hereditary cerebellar degeneration characterized by the neural

#### Materials and Methods

We studied the cerebella of three Arabian horses aged between 6 and 8 months. Although the animals were from the same breeding herd, the parentage of all the three affected horses was unknown because multiple stallions were used in a random pasture-mating programme. These animals displayed clinical disorders in their movements, tremors and ataxia. After carrying out the necropsy, the randomly selected samples of the cerebellar cortex were fixed in 10% buffered formalin for 24 h at 4°C; then, they were immediately dehydrated in graded series of ethanol, immersed in xylol and embedded in paraffin wax by using an automatic processor. Sections (4 µm thick) were stained with haematoxylin and eosin and with the TUNEL for the morphological study. The TUNEL (In situ Cell Death Detection, POD; Roche Molecular Biochemicals, Barcelona, Spain) technique was applied following the steps recommended in the manufacturer's instructions.

For the ultrastructural study, small randomly selected samples of each cerebellar cortex were primarily fixed in a 2% glutaldehyde solution in 0.1 M phosphate buffer (pH 7.4) overnight at 4°C and then refixed in 1% osmium tetroxide in 0.1 M phosphate buffer (pH 7.4) for 30 min. After dehydration in graded ethanol series and embedding in Araldite, semithin and ultrathin sections were cut on an LKB ultramicrotome, Stockholm, Sweden. Semithin sections were stained with toluidine blue, whereas ultrathin sections were double-stained with uranyl acetate and lead citrate. Ultrathin sections were

viewed and photographed in a Philips CM10 transmission electron microscope (Philips, Mahwah, NJ, US).

## Results

Haematoxylin and eosin staining revealed a marked loss of Purkinje cells. Besides neurons that appeared normal, many of the remaining Purkinje cells displayed many morphological changes ranging from shrinking, chromatolysis, to nuclear and cytoplasmic condensation (Fig. 1a).

The TUNEL technique revealed a substantial number of immunoreactive positive Purkinje cells (65%) which also showed other morphological features of apoptosis such as condensation of the nucleus and cytoplasm and margination of condensed chromatin in the nuclear rim as well as segregation and fragmentation of the nucleus into apoptotic bodies (Fig. 1b).

Electron microscopical analysis (Fig. 2) showed neurons with a clearly apoptotic ultrastructural morphology. The apoptotic changes varied from moderate condensation of both nucleus and cytoplasm, and nuclear membrane hyperchromatosis, to a pronounced reduction and reshaping of both the nucleus and cytoplasm. Considerable condensations of chro-

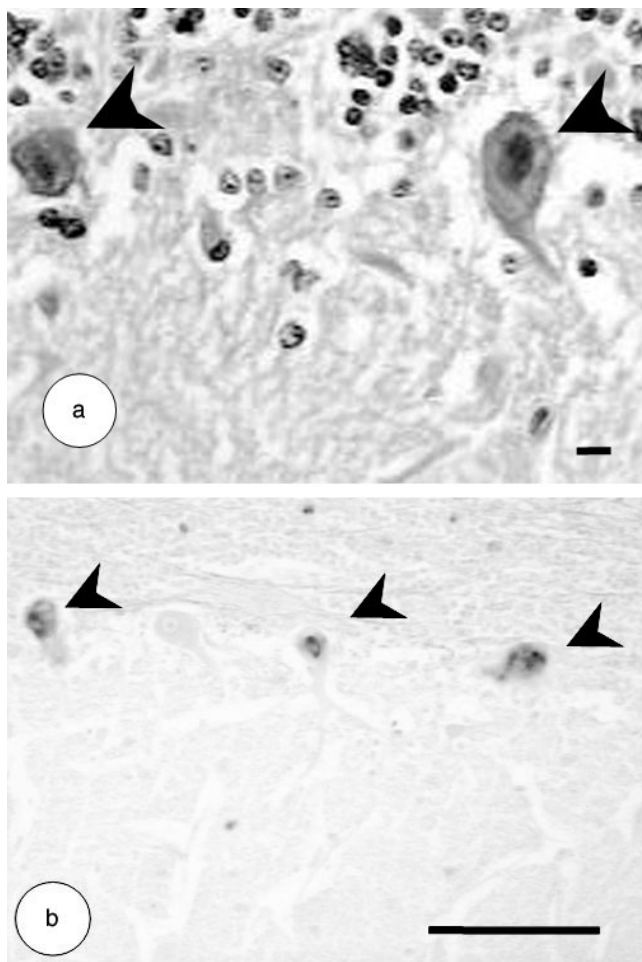


Fig. 1. Purkinje cells stained with haematoxylin and eosin (a) and terminal deoxynucleotidyl transferase biotin-dUTP nick-end labelling (b) showing signs of apoptotic degeneration (arrowheads): shrinkage and chromatin condensation in (a); positive immunoreactivity in (b); scale bars: (a) 10  $\mu$ m; (b) 100  $\mu$ m.

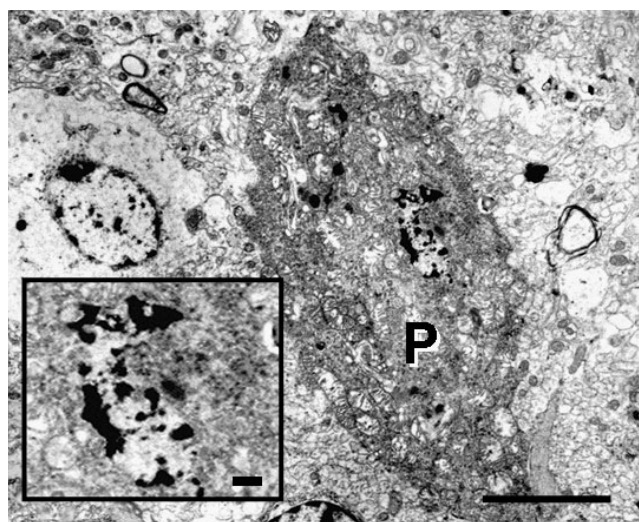


Fig. 2. Electron microscopic appearance of a Purkinje cells (P) with a considerable condensation of both nucleus and cytoplasm (scale bars, 10  $\mu$ m). In the insert, detail of the chromatin fragmentation (scale bars, 1  $\mu$ m).

matin masses and the presence of apoptotic bodies were also seen.

## Discussion

Although the primary degeneration of Purkinje cells has been attributed to excitotoxic degeneration, which is a pathological phenomenon mediated by an excessive stimulation of glutamate receptors, the mechanisms by which it leads to neuronal death are not fully understood (de Lahunta, 1990; Sandy et al., 2002). While the role of apoptosis during the development of central nervous system is unquestioned, its contribution to neurodegeneration is controversial (Graeber and Moran, 2002). During excitotoxicity, both apoptotic DNA fragmentation and morphologic evidence of necrosis have been detected. Our observations clearly indicate that a significant percentage of the scant number of cerebellar Purkinje cells, detected in three cases of cerebellar abiotrophy in horses, presented morphological features of apoptosis, which was further corroborated by the detection of an extensive fragmentation of the chromatin identified by the TUNEL method. These results point to the apoptosis mechanism as being clearly implicated in the loss of Purkinje cells that characterize cerebellar abiotrophy in horses.

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