APOPTOTIC NEURONS IN A CAT WITH CEREBELLAR ABIOTROPHY

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ABSTRACT

Cerebellar abiotrophy is defined as a premature spontaneous progressive loss of neurons. In an 11-months-old cat with cerebellar abiotrophy, clinical symptoms developed progressively after castration. Histologically a loss of Purkinje cells, granular layer cells and demyelination of white matter were observed. Apoptotic neurons were found using the TUNEL technique. In this case the cerebellar abiotrophy was due to apoptosis of neuronal populations. An episode of anoxia/hypoxia of the nervous system during anesthesia may have played a role in the induction of cerebellar apoptosis.

SAMENVATTING

Cerebellaire abiotrophy wordt gedefinieerd als een prematuur spontaan progressief verlies van neuronen. Bij een kat van 11 maanden oud met cerebellaire abiotrofie, ontwikkelden de klinische symptomen zich progressief na castratie. Histologisch werd een verlies van Purkinje cellen, cellen uit de granulaire laag en demyelinisatie van de witte stof opgemerkt. Apoptotische neuronen werden vastgesteld met de TUNEL-techniek. Bij dit geval was de cerebellaire abiotrophy te wijten aan apoptose van neuronenpopulaties.

Een periode van anoxie/hypoxie van het zenuwstelsel tijdens anesthesie zou een rol kunen gespeeld hebben bij de inductie van cerebellaire apoptose.

Keywords: Cat - Cerebellar abiotrophy - Purkinje cells - TUNEL

INTRODUCTION

In Gower's concept of abiotrophy, it is postulated that in susceptible individuals certain groups of cells are programmed to degenerate prematurely (Gower, 1902). In principle, abiotrophy results in atrophy of the affected area and is of unknown cause. Some authors, however, have restricted the term abiotrophy to organ atrophies of genetic origin (de Lahunta, 1990). Along the same lines, abiotrophy has been defined as a degenerative process which is the consequence of a metabolic disorder (Summers *et al.,* 1995). Abiotrophy may affect any organ, but it is most commonly described in the retina and in the cerebellum.

Cerebellar abiotrophy has been described in many different animal species, including dogs, cattle, sheep, horses and pigs (Thomas and Robertson, 1989; Sustronck *et al.,* 1990; Riber *et al.,* 1991; Tatalick *et al.,* 1993; Mitchell *et al.,* 1993; Gruys *et al.,* 1994; Kemp *et al.,* 1995; Summers *et al.,* 1995; Wallace *et al.,* 1996; Milne and Schock, 1998). More recently, the disorder also has been described in cats (Inada *et al.,* 1996; Shamir *et al.,* 1999).

The neurological symptoms usually start several weeks to months after birth. The condition can usually be traced to a hereditary origin (Summers *et al.,* 1995; Inada *et al.,* 1996).

This manuscript describes a case of cerebellar abiotrophy after castration. A possible connection between the abiotrophy and the castration is postulated. To our knowledge, this is the first reported case in domestic animals where cerebellar abiotrophy could be associated with apoptosis of cerebellar neurons.

Apoptosis is a form of regulated cell death characterized by specific morphological changes. These include cell shrinkage, membrane blebbing, chromatin condensation and cell fragmentation into small apoptotic bodies. At the molecular level, the activation of an endogenous endonuclease results in the fragmentation of DNA into oligosomal length fragments which can be immunhistologically stained with the TUNEL technique (Pihlgren *et al.*, 1996).

MATERIALS AND METHODS

Case History:

A tomcat (*Felis domesticus*) was clinically normal and without any history of disease up to the age of 7 months.

At that time it was castrated under xylazin and ketamin anaesthesia. Moderate to severe loss of blood was observed during the operation and the day after.

Three months after castration the cat was presented with neurological signs: tremor, exaggerated fear reactions and incoordination (especially of the hind limbs). No nystagmus or paresis was observed. According to the owner, the symptoms developed gradually after the castration and grew progressively worse. Treatment with 1ml Vit B1–B12® (Vetoquinol – Aartselaar, Belgium) intramuscularly and 1 mg/kg body weight Moderin® (methylprednisolone – Upjohn – Puurs, Belgium) orally (alternate day) had no success.

After one month, the condition of the cat had deteriorated. The animal defecated and urinated in lateral decubitus and could very easily be pushed over. The gait was slow and careful and the animal fell over easily. The tremor had also increased. Due to the progressive character of the symptoms the animal was euthanized.

At autopsy, samples for histological examination were taken of the cerebrum, the cerebellum, the brainstem and the spinal cord. Control samples of the cerebellum of another cat of the same age were included. These samples were processed according to standard methods: fixation in a 4% phosphate-buffered formaldehyde solution, paraffin-embedded and sectioned at 5μm. Sections were stained with hematoxillin and eosin (HE). To examine the myelination, a Luxol fast blue / periodic acid shiff / toluidine blue staining (LFB/PAS/Tol) was made.

To demonstrate apoptotic cells in the cerebellum, the TUNEL technique (TdT-mediated dUTP NickEnd Labeling) was used according to Gavrieli *et al.* (1992) in combination with a sensitive detection method based on peroxidase activated biotine-tyramide, TSA (Tyramide Signal Amplification – Du Pont NEN, Boston). This technique uses terminal deoxynucleotidyl transferase (TdT) and allows the labeling of double-stranded DNA breaks (free 3'-OH DNA ends), which can be immunohistochemically visualized (Pihlgren *et al.*, 1996).

RESULTS

Autopsy

At autopsy, overfilling of the urinary bladder and accumulation of large amounts of feces in the rectum were the most important findings. No other noteworthy lesions were observed. The different parts of the central nervous system appeared normal in size and shape.

Histology

In the cerebrum no obvious abnormalities were found. The cerebellum showed a severe loss of Purkinje cells. The remaining Purkinje cells had a swollen aspect, with granular cytoplasm and excentric nucleus. The granular layer was reduced in thickness with a diminished cell population. In the granular layer and the layer of Purkinje cells, many eosinophilic remnants of cells were visible. Histiocytic cells were also observed in these layers. The molecular layer showed no abnormalities. Using the LFB-PAS-Tol staining, a mild to moderate diffuse demyelination of the cerebellum was noted, which was not found in the normal cat.

In the brainstem, a mild non-suppurative perivasculitis was found.

TUNEL technique

Many Purkinje cells, cells of the granular and molecular layers stained positive (= apoptotic cells) in the affected cat (Fig. 1), whereas the cerebellum of the control cat stained negative (Fig. 2).

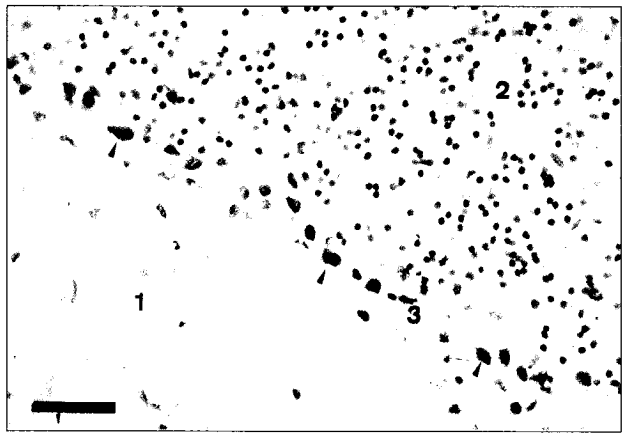

Fig. 1: Microphotograph of the cerebellum with positive staining (apoptotic) cells (arrowheads) in the affected cat (including Purkinje cells).
Molecular layer (1) – Granular layer (2) – Layer of Purkinje cells (3).
Bar = 50 µm

Fig. 2: Microphotograph of the cerebellum with absence of positive staining cells in the control cat.
Molecular layer (1) – Granular layer (2) – Layer of Purkinje cells (3).
Bar = 50 µm

DISCUSSION

Clinically progressive deteriorating tremor, ataxia, spasticity, loss of balance and wide-based posture are typical of cerebellar abiotrophies. Sometimes the symptoms are worse on the hind quarters than on the forehand. The symptoms in this cat were similar.

A reduced size of the cerebellum (<10% of the total brain weight) is observed at autopsy only in advanced cases. This feature was not observed in the present case. Microscopically, abiotrophy of the cerebellum is characterized by normal areas gradually merging into areas with neuronal depletion. The Purkinje cells are usually first affected and are strongly reduced in number. The remaining Purkinje cells have a dark and eosinophilic aspect or are swollen and chromatolytic with an excentric nucleus. Generally, the depletion of the Purkinje cells is followed by a loss of cells in the granular layer (Braund, 1994; Summers *et al.*, 1995). In advanced cases there is also a shrinkage of the molecular layer. Especially where Purkinje cells are lost, astrogliosis can be observed. Wallerian degeneration (demyelination) can be detected in the white matter of the folia of the cerebellum as a consequence of Purkinje cell degeneration. All of these degenerative changes were observed in the present case. Thus this patient was diagnosed as a case of cerebellar abiotrophy.

In the traditional concept of abiotrophy as put forward by Gower (1902), it is postulated that certain groups of cells are programmed to degenerate prematurely. This theory has lost ground over time because diseases previously believed to be degenerative, have been shown to result from specific causes (Powers and Horoupian, 1996). It is likely that metabolic or genetic causes for most of these diseases will be elucidated in the future (Powers and Horoupian, 1996).

In an abiotrophic process, the organ is affected after it has initially fully developed. Cerebellar abiotrophy patients are neurologically normal at birth and gradually develop cerebellar symptoms that progressively deteriorate (Summers *et al.*, 1995). In contrast, viral agents (e.g. feline panleukopenia) affect the development of the cerebellum at a specific period of the gestation. These viral infections produce cerebellar abiotrophy at birth. In cases of cerebellar abiotrophy of genetic origin, an autosomal recessive gene is assumed (Summers *et al.*, 1995; Inada *et al.*, 1996). The onset of the clinical expression of the genetic disorder differs from species to species, varying from several weeks to several months after birth.

A loss of cells that does not induce an inflammatory response is by definition an indication of apoptosis. To our knowledge this is the first case in a domestic animal where abiotrophy was shown to be associated with apoptosis of neurons

Apoptosis of cerebellar neurons can be induced by ischemia (Hara *et al.*, 1995), prions (Giese *et al.*, 1995), low potassium (D'Mello *et al.*, 1993), hypoxia/asphyxia (Kalda *et al.*, 1998; Li *et al.*, 1998; Dell'Anna *et al.*, 1997), carbon monoxide poisoning (Piantadosi *et al.*, 1997), lead poisoning (Oberto *et al.*, 1996), methylmercury poisoning (Kunimoto, 1994) or episodes of severe hyperthermia, anoxia or hypoglycemia (Chrisman *et al.*, 1983). In these cases it is more likely that the clinical signs will appear shortly after the 'insult'. The affected cat developed symptoms shortly after castration, suggesting that the cause of the cerebellar degeneration is to be found during or after the castration.

Studies performed in mice have confirmed that the pathogenesis of abiotrophy is associated with apoptosis of Purkinje cells (Gillardon *et al.*, 1995). A great number of apoptotic cells, including Purkinje cells, were found in the cerebellum of this cat using the TUNEL technique. Watson *et al.* (1998) reported apoptosis due to a lack of survival signals from other neurons. Secondary apoptosis of the cells of the granular layer could be the result of such a mechanism because Purkinje cells are usually first affected (Braund, 1994; Summers *et al.*, 1995).

Anesthesia for castration is routinely done with xylazin and ketamin. It is possible that a respiratory depression or lowering of blood pressure could have resulted in an episode of anoxia/hypoxia. Cerebellar apoptosis can indeed be induced by anoxia/hypoxia (Chrisman *et al.*, 1983; Dell'Anna *et al.*, 1997; Kalda *et al.*, 1998; Li *et al.*, 1998). Post-anesthetic cerebral necrosis, as described in adult horses (Summers *et al.*, 1995), gives rise to various clinical symptoms and histological lesions.

In conclusion, cerebellar abiotrophy in this cat was associated with neuronal apoptosis in the cerebellum. The apoptosis could have been induced by an episode of anoxia/hypoxia during the anesthesia for castration. It cannot be excluded however that an inherited heightened susceptibility predisposed this patient to develop the cerebellar abiotrophy, thus explaining why this cat developed the lesion whilst many others going through similar episodes of vascular and hypoxemic stress recover uneventfully.

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