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Calbindin positive Purkinje cells in the pathology of human cerebellum occurring at the time of its development

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Abstract

Development of cerebellum continues over an extremely long period of time extending from the early embryonic phase until the first postnatal years. During an extended time of maturation the cerebellum is vulnerable to harmful agents. A group of cytoplasmic proteins that may protect cells against injury are the calcium binding proteins, among others calbindin. The distribution of this protein is not well known in cerebellar pathology, thus in the present study the localisation and appearance of calbindin expressing Purkinje cells in different pathological conditions occurring at the time of cerebellar development was examined. The investigations were carried out on human maturing cerebellar cortex (age range 30 gestational weeks – 2 years) of cases with paraneoplastic cerebellar degeneration and cerebellar neuronal migration disturbances. The Purkinje cells located in cerebellar heterotopias and dysgenesias were morphologically well developed and strongly immunostained with calbindin antibody. In paraneoplastic cerebellar degeneration the progressive decrease of calbindin content and disintegration of Purkinje cells were observed. Our results show that intrauterine harmful agents that disturb migration of the cerebellar neurons do not affect the content of calbindin in misoriented neurons and that this protein may play a role in development of Purkinje cells located in heterotopias and cerebellar dysgenesias. The progressive decrease of calbindin content in the Purkinje cells undergoing degeneration and death during paraneoplastic changes in the cerebellum also supports the hypothesis that this protein is very important component of intracellular homeostasis in cerebellar neurons.

Key words: *CalbindinD28k, cerebellum, Purkinje cells, development, human, pathology.*

Introduction

Development of cerebellum continues over an extremely long period of time extending from the early embryonic phase until the first postnatal years [1]. Agents that interrupt normal growth may have different effects depending on the stage of development. During an early development focal disruption of neuronal migration leads to localised cortical dysplasia and heterotopias [7,11]. In general, cerebellar heterotopias exist in the white matter of the hemispheres and consist of nests of large cells resembling Purkinje cells, which may have an admixture of granular neurons. When an agent acts after the Purkinje cells have matured but granular cells are still dividing and migrating diffuse lesions affecting the entire granular layer may appear [8].

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After birth cerebellar injury usually consists of diffuse loss of Purkinje cells with less conspicuous damage of granular cells. The cerebellar white matter show evidence of secondary degeneration and the deep cerebellar nuclei are only rarely involved. It has been estimated that cerebellar degeneration is most often associated with neoplastic diseases [4].

A group of cytoplasmic proteins that may protect cells against injury are the calcium binding proteins. This group includes such proteins as calretinin, calbindin D-28K and parvalbumin [14]. All of them are characterised by the presence of a variable number of helix-loop-helix motives, which bind calcium ions with high affinity and are considered to be cytosolic calcium buffers that may modify the spatiotemporal aspects of calcium transients in cells [12]. These proteins are particularly enriched in specific cerebellar neurons but distribution each of them in these neurons is different [3]. The distribution of all above mentioned proteins that may protect or defend human cerebellum against different harmful agents is not well known in cerebellar pathology, thus in the present study the localisation and appearance of calbindin expressing cells in different developmental stages and pathological conditions occurring at the time of cerebellar maturation were examined.

Material and Methods

The investigations were carried out on 33 human brains obtained following autopsy. The cerebellar cortex of cases with paraneoplastic cerebellar degeneration, cerebellar neuronal migration disturbances and uninjured controls from the age of 30 weeks of gestation to 2 years were examined.

Nine brains of children who died in the course of neoplastic diseases, at age 2 months-2 years have been studied. The brain was involved neither by tumour nor cerebral and meningeal metastases. The neoplasms had various localisations. Autopsy examination showed 5 neuroblastomas, hepatoblastoma, adenocarcinoma of submandibular gland, and 2 cases of histiocytosis. Seven of them were not treated; others were treated by polytherapy (chemotherapy and radiotherapy). Antimitotic drugs were used in classical compositions.

Ten brains with abnormal clusters of neurons arrested in the cerebellar white matter and dysgenesias were chosen to the study. The age of cases was 30, 32, 34, 36 gestational weeks, term newborns, 2, 3, 6 months and one year infants.

Fourteen brains of age matched controls were used in the study. Ten cases aged 30, 32, 34, 36, 38 gestational weeks and term were found among newborns delivered after normal pregnancies. All of them died due to acute perinatal pathology and were not put on controlled respiration. Four infants aged 2 months, 6 months, one year and two years died after short, severe illnesses or sudden death. Death was caused by accidental intoxication, aspiration, car accidents. These children were not treated at all or only a few hours by controlled respiration. After autopsy all brains were fixed in formalin, embedded in paraffin, and stained with haematoxylin eosin and cresyl violet to identify morphology. These brains were diagnosed as nonpathological.

Neuropathological examination was performed on representative slides from the cerebellar hemispheres and vermis at several levels.

Sections of cerebellum were incubated in primary antibody generated against calbindin D-28k (CB) (Sigma) at dilution of 1 : 200. As the secondary antibody anti-rabbit IgG was used. For visualization of the reaction sites the sections were treated with peroxidase (brown stain) or alkaline phosphatase (red stain). Some sections were counterstained by toluidin blue.

Results

In the normal cerebellar cortex of newborns all Purkinje cells displayed intense calbindin immunoreactivity (Fig. 1).Their cell bodies were strongly stained and their processes were also positive. Few cells scattered in the external granule layer were immunoreactive. The intensity of staining increased with the development of Purkinje cells soma. The dendritic tree arborisation in the molecular layer was well visible. In the older cases dendrites of Purkinje cells exhibited high calbindin immunoreactivity imparting an intense coloration to the molecular layer where they arborize.

The Purkinje cells located in cerebellar heterotopias and dysgenesias were morphologically well developed and strongly immunostained with CB antibody (Fig. 2). The disorderly scattered cell bodies with well visible dendritic arborisation were calbindin immunopositive. The intensity of calbindin immunoreactivity corresponded to age matched controls.

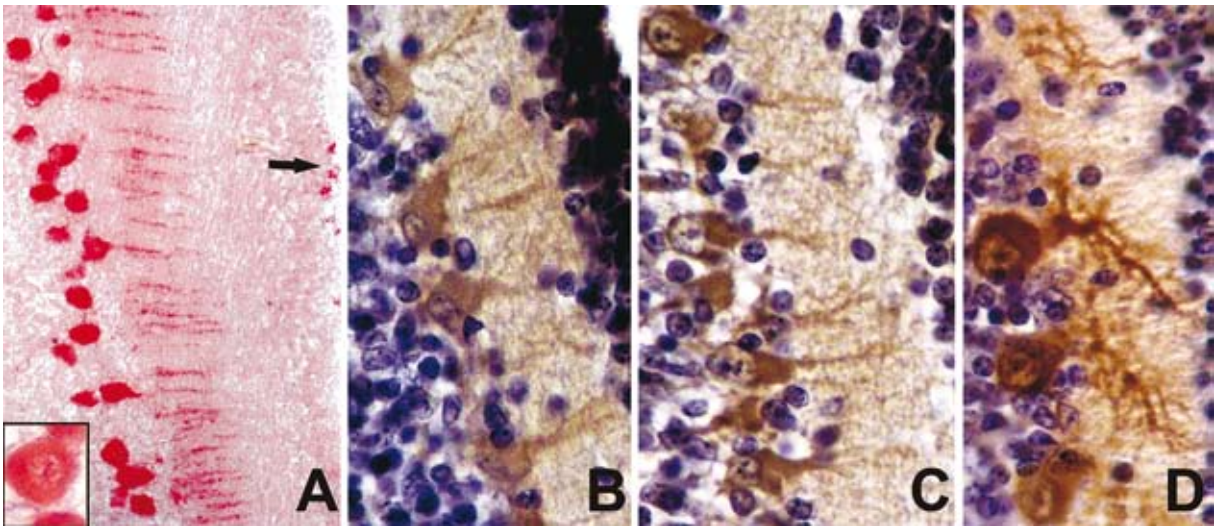

Fig. 1A-D. Calbindin immunoreactivity in the Purkinje cells of normal cerebral cortex. A. Premature newborn 30 weeks of gestation. Arrow: immunoreactive cells in the external granular layer. Insert: Purkinje cell soma. Magn. ×20, insert ×40; B. Premature newborn 32 gestational weeks; C. Newborn 36 gestational weeks; D. Full term newborn; Magn. B,C,D ×40.

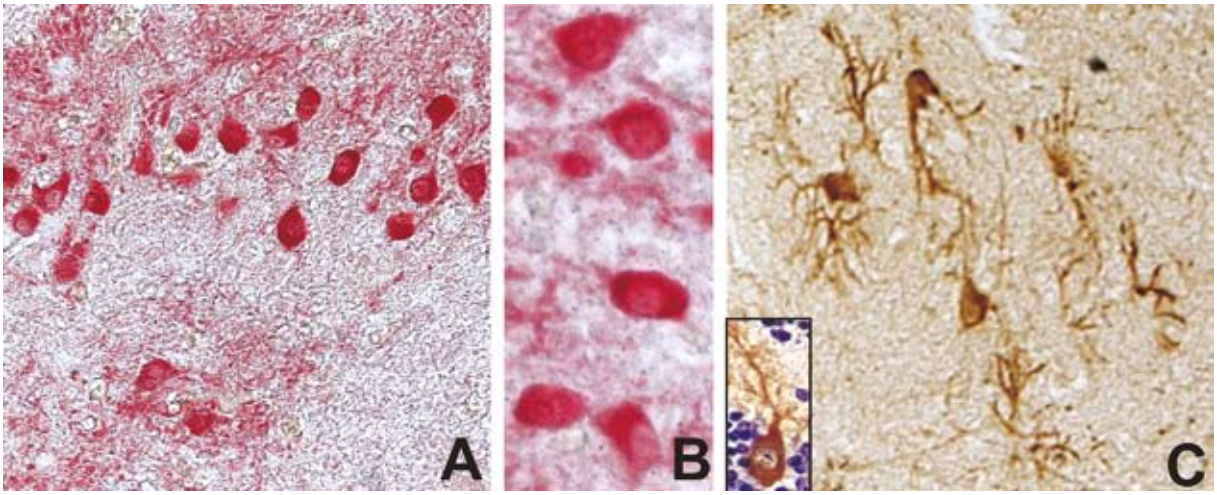

Fig. 2A-C. Calbindin immunoreactivity in the heterotopic cells in the cerebellar white matter. A. CalbindinD28K positive cells scattered in the white matter. Full term newborn. Magn. 20×; B. Well stained calbindin positive soma of the heterotopic cells, the same case. Magn. 40×; C. Group of heterotopic cells with well visible arborisation. Two months old infant. Magn. 20×, insert: cortical Purkinje cell, age matched control, Magn. ×20.

In paraneoplastic cerebellar degeneration a diffuse loss of Purkinje cells was observed (Fig. 3). In the cells undergoing degeneration the progressive decrease of calbindin was found. Among the Purkinje neurons some steps of calbindin soma disintegration were determined. Beside normal appearing neurons the cells with fade, dissolute cytoplasm emerged. The intensity of calbindin immunoreactivity decreased. The neurons changed their shape and formed cell globose spheroids. Segmentally unshapely formed cell shadows were seen. The progressive decrease of calbindin immunoreactivity beside cells soma was observed in the dendrites. The dendrites became shortened and tortuous. Dendritic tree disin-

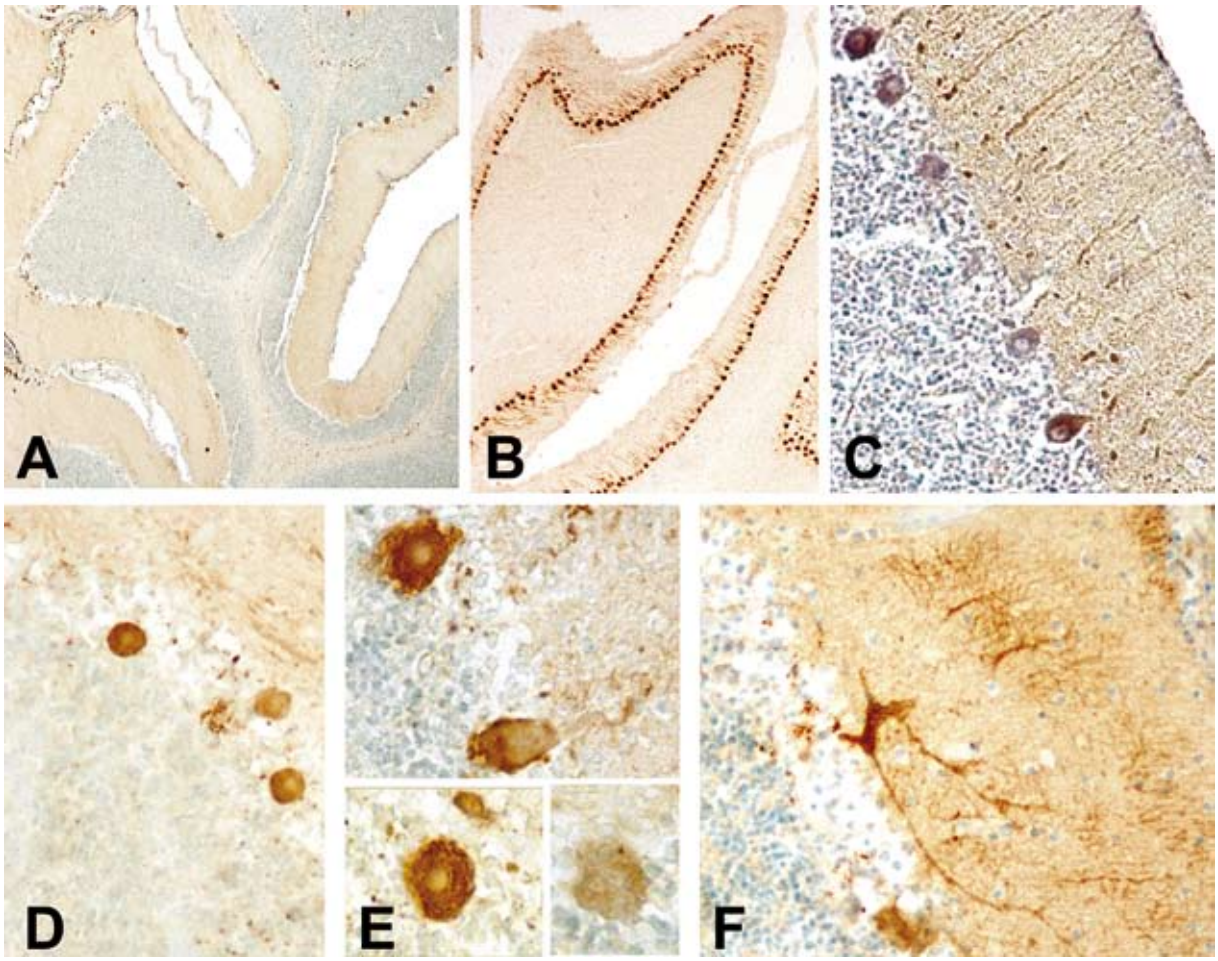

Fig. 3A-F. Calbindin immunoreactivity in the paraneoplastic cerebellar degeneration. A. Diffuse loss of Purkinje cells, 6 months old infant, Magn. ×4; B. For comparison calbindin positive cells in the normal age matched cerebellum, Magn. ×4; C. The progressive decrease of calbindin immunoreactivity, one year old infant, Magn. ×20; D. Advanced disintegration of Purkinje cells, globoid shape of the preserved cells, Magn. ×20; E. Different stages of disintegration of Purkinje cells, Magn. ×40; F. Preserved fragments of dendritic tree, loss of Purkinje cell soma, Magn. ×40.

tegration led to loss of its continuity. The molecular layer was gradually devoid of calbindin positive dendrites. However in several parts of cerebellar cortex the fragmented dendritic tree in the molecular layer was preserved, while the Purkinje cell soma completely disappeared.

Discussion

During normal development of the human cerebellum the cell specific distribution of calcium binding proteins (CABPs) is found [3,10,14]. Calbindin (CB) appears early, at 4-5 gestational week calbindin -D28k immunoreactive cells are observed in the ventricular zone when neurones become ready to start migration and differentiation. Parvalbumin is expressed later in development. Some of calcium binding proteins like calretinin are transiently expressed in specific cellular subpopulations. It has been postulated that calcium binding proteins are important in the control of cell division, process outgrowth and cell movement since all these activities are closely related to the intracellular calcium concentration [2]. Different calcium binding proteins may function differently at different developmental stages [6]. In the third trimester of gestation calbindin-D28k im-

munoreactivity in the Purkinje cells greatly increases. Calbindin immunopositive cells increase in size and exhibit elaborate dendritic arborisation associated to dendritic synapse formation and stabilisation. The Purkinje cells located in the cerebellar heterotopias were strongly immunostained with calbindin antibody appropriately to the age matched control. Presence of distinct calbindin immunoreactivity in heterotopic neurons confirms the previous observations that this protein participates in normal maturation of the cerebellar neurons [6]. The intrauterine harmful agents do not affect content of calcium binding protein in neurons that at least reach their places of destiny in dysgenetic cerebellar cortex or those Purkinje cells that are located in heterotopias. Since calretinin, calbindin and parvalbumin are thought to be cytosolic calcium buffers that modify the spatiotemporal aspects of calcium transients in cells [12] thus they may stabilize homeostasis and facilitate development and maturation of Purkinje cells in heterotopias.

The cerebellar postnatal growth is attributable to the development of synapses and dendritic extensions. Studies with CaBP knockout mice summarized by Schwaller indicate that these proteins are essential components in the Ca2+ homeostasis of neurons and are implicated in the subtle regulation and timing of Ca2+ signals pre- or postsynaptically. Deficiency in one or more of these proteins leads to distinct alterations in brain physiology [12]. At different times during postnatal development, neuronal vulnerability to non-physiological changes in calcium may be dependent on the ability of CaBPs to accommodate calcium fluctuations. Calcium binding proteins expression regulates postnatal brain maturation by protecting neurons from fluctuations in calcium from an optimal level [9].

In paraneoplastic cerebellar degeneration a diffuse loss of Purkinje cells was observed. In the cells undergoing degeneration the progressive decrease of calbindin was found. The cytoplasm of Purkinje cells lost homogenous calbindin immunoexpression and then disappeared. The alterations in calbindin-D28k immunostaining observed in this condition are likely to be linked to disturbed calcium homeostasis. As was mentioned by Dove [5] pronounced reduction in calbindin mRNA may represent compensatory homeostatic efforts to maintain normal calcium signalling functions despite greatly reduced calcium influx. It is also possible that the structural impairment and decreased activity of degenerating cells reduces the need for high calcium binding proteins content.

It is interesting that in cerebellar cortex with paraneoplastic degeneration the fragmented dendritic tree in the molecular layer was preserved, while the Purkinje cell soma completely disappeared, although this concerned only few neurons. The dendritic spines may change their morphology to compensate for changes in calcium homeostasis due to the loss of CaBPs [12].The consequence of loss of calbindin on spine morphology was significant increase in spine length and spine volume [13]. It is possible that deficiency of CB influenced increase of dendritic tree and extended dendrites proved more resistant to calcium homeostasis disturbances, so in some neurons fragments of dendritic tree preserved, while the soma has been disintegrated.

In summary, our results show that intrauterine harmful agents that disturb migration of the cerebellar neurons do not affect the content of calbindin in misoriented neurons and that this protein may play a role in development of Purkinje cells located in heterotopias and cerebellar dysgenesias. The progressive decrease of calbindin content in the Purkinje cells undergoing degeneration and death during paraneoplastic changes in the cerebellum also supports the hypothesis that this protein is very important component of intracellular homeostasis in cerebellar neurons.

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