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Original

Morphological changes of myelinating oligodendrocytes in the ethidium bromide model of demyelination

D.L. Graça, C. Gevehr and L.A.V. Pereira

Departamento de Patología, Centro de Ciências da Saúde, Universidade Federal de Santa Maria, Santa Maria, Brasil.

RESUMEN

Los oligodendrocitos se caracterizan por su lenta multiplicación mitótica y su alto grado de activación durante la fase de mielinización rápida en el periodo perinatal. Con la finalidad de investigar la morfología de los oligodendrocitos en procesos de mielinización y remielinización en ratas Wistar lactantes y adultas, los animales fueron inyectados con 0,5 µl de 0,1% del fármaco gliotóxico bromuro de etidio en la cisterna basal. Se realizaron estudios de microscopia óptica y electrónica de transmisión del área afectada. El tejido nervioso exhibió un estado esponjoso debido a edema intracelular, expansión del espacio extracelular y ocasionales vacuolizaciones de las membranas de mielina. Las células gliales mostraron indicios de intoxicación, mientras que los oligodendrocitos menos afectados de las ratas lactantes exhibieron una notable proliferación de membranas o scrolls. *En los animales observados durante un tiempo mayor (2 meses) las áreas reparadas de tejido nervioso eran menores que las áreas gravemente afectadas, sugiriendo que los oligodendrocitos con* scrolls *no murieron. En animales adultos no se observaron proliferaciones membranosas. Fue posible concluir que la proliferación de membranas solamente tiene lugar durante la fase de mielinización rápida en los animales en crecimiento, y parece ser la respuesta glial a la intoxicación con bromuro de etidio.* **Rev Esp Patol 1997; 30(4): 297-301.**

Palabras clave: Cuerpos membranosos intracitoplasmáticos - Oligodendrocitos - Bromuro de etidio - Ratas lactantes

SUMMARY

Oligodendrocytes are characterized by their low mitotic activity and high-grade activation during the fast myelinating period in neonate animals. To investigate morphology of oligodendrocytes during both myelination and remyelination in weanling and adult Wistar rats, the animals were injected with 0.5 µl of 0.1% of the intercalating glitoxic dye ethidium bromide in the cisterna basalis. Light and transmission electron microscopy studies of the affected tissues were performed. Status spongiosus developed in all injected rats due to intracellular swelling, expansion of the extracellular space, and occasional vacuolation of myelin sheaths. Glial cells showed signs of intoxication and the less affected oligodendrocytes of the youngest rats exhibited prominent intracytoplasmic proliferation of membranes or scrolls. Long-term lesions showed repaired areas narrower than the degenerated early ones, suggesting that oligodendrocytes positive for scrolls did not die. No membranous scrolls were observed in adult animals. It was concluded that intracytoplasmic membranous bodies occur only during the phase of rapid myelination in growing rats and seems to be a response to the intoxication by the chemical in otherwise healthy cells. **Rev Esp Patol 1997; 30(4): 297-301.**

Key words: Intracytoplasmic scrolls - Weanling rats - Oligodendrocytes - Ethidium bromide - Wistar rats

INTRODUCTION

Ethidium bromide (EB) is an intercalating gliotoxic chemical that induces areas of demyelination in domestic and laboratory animals (1). A prominent feature in the degeneration of oligodendrocytes of weanling rats after intracisternal injection of EB was the formation of large laminated intracytoplasmic bodies or scrolls (2). As this change was not observed following spinal cord injection of EB in both cats (3) and rats (4, 5) it was decided to investigate the suggestion made by Suzuki and Zagoren (6) that scrolls only develop in oligodendrocytes at the time of rapid myelination. This time appeared to be 23 days in the brain stem. The present investigation was performed in weanling (19-27-day-old) and adult rats. Intracytoplasmic scrolls were found and differences in the intensity of the change and location within the brain stem were observed between groups of weanling rats separated by age. The results of this investigation were compared with other forms of the EB model of demyelination.

MATERIAL AND METHODS

Twenty-two weanling rats (19-27-day-old) and eight young adult Wistar rats of both sexes were used. Following Halothane anesthesia, 26 rats received a single intracisternal injection of 5 µl of 0.1% EB in sterile normal saline through a 30-gauge needle according to the procedure described by Rizzuto and Gambetti (7). Four rats received 5 µl/l of sterile normal saline by the same route.

The animals were deeply anesthetized and killed by intracardiac perfusion of 150-250 ml of 4% glutaraldehyde in phosphate buffer at 24 h (8 rats), 48 h (7), 3 days (3), 4 days (2), 9 days (4) and 2 months (2).

Following this, 1.5-mm thick brain slices were cut from the caudal region of the colliculli, pons and cranial medulla and post-fixed in Millonig's Osmium tetroxide and embedded in Taab resin; 1-mm thick sections were cut from each block and stained with toluidine blue. Scrolls plotting was made from the semi-thin sections with a graticule. Selected areas were trimmed and sectioned for electron microscopy, stained with uranyl and lead salts, and examined under an Hitachi HS-8 transmission electron microscope.

RESULTS

Light microscopy

Twenty-four hours after injection, status spongiosus of the brain stem due to intracellular swelling and enlargement of the extracellular space was detected. It occurred symmetrically over the ventro-lateral areas of the pons and medulla, covering a variable width of subpial tissue (Fig. 1). At 48 h the area of the lesion was larger than at 24 h, and sometimes twice as large. Only in weanling rats oligodendrocytes exhibited scrolls within or projecting from their cytoplasm, which appeared as deeply basophilic elongated structures of different sizes (Fig. 2).

Scrolls plotting of positive animals in sequential sections of pons and medulla showed that scrolls occurred within the area of spongiosis and at a short distance from it. The scrolls were depicted in animals injected at 19, 23 and 26 days after birth. They were more conspicuous in animals killed 48 h after injection. In 19-day-old

Figure 1. Severe spongiosis of the lateral pons 48 h after injection with ethidium bromide. 23-day-old rat (original, ×175).

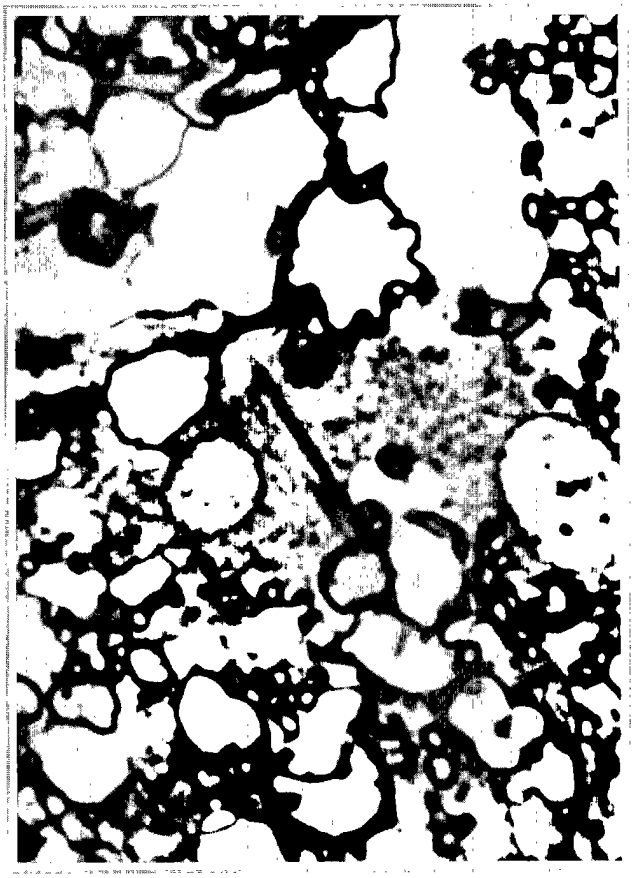

Figure 2. Oligodendrocyte exhibits elongated sections of intracytoplasmic scrolls. 26-day-old rat (original, ×1000).

injected rats, the scrolls tended to spread more over the lateral areas of pons and medulla, whereas in 23-day-olds they tended to accumulate over the ventral areas of the pons and medulla and also covered variable extensions of more lateral regions of the brain stem (Fig. 3).

Ultrastructural studies

Oligodendrocytes and astrocytes were the cells to show the most marked changes following intracisternal injection of EB, and axons were occasionally swollen. The changes observed in the neuroglia were similar to those detected in the spinal cord of adult rats (4). The distinctive feature in this study was the extensive proliferation of cellular membranes in oligodendrocytes of weanling rats. These membranes were either single or multiple, short or long, straight, curved or spiraled. They were found isolated or related to other cytoplasmic organellae such as ribosomes, which attached to their outer lamellae (Fig. 4). Scrolls diameter was variable even within the same cell and ranged from 0.15 to 0.5 μm, while they could be up to 22 μm long.

Resolution of the lesions

Forty-eight hours after injection numerous phagocytic cells had infiltrated the area of the lesion. This cellular invasion was extensive at 9 days after injection. In rats examined 2 months after injection, areas of remyelination were found in the ventral portions of the brain stem, where axons showed thin myelin sheaths laid down by oligodendrocytes and occasional Schwann cells. Although the area of remyelination was variable, it was observed to be smaller than the scrolls-containing area.

DISCUSSION

The formation of prominent lamellated intracytoplasmic bodies characterized the oligodendroglial response to

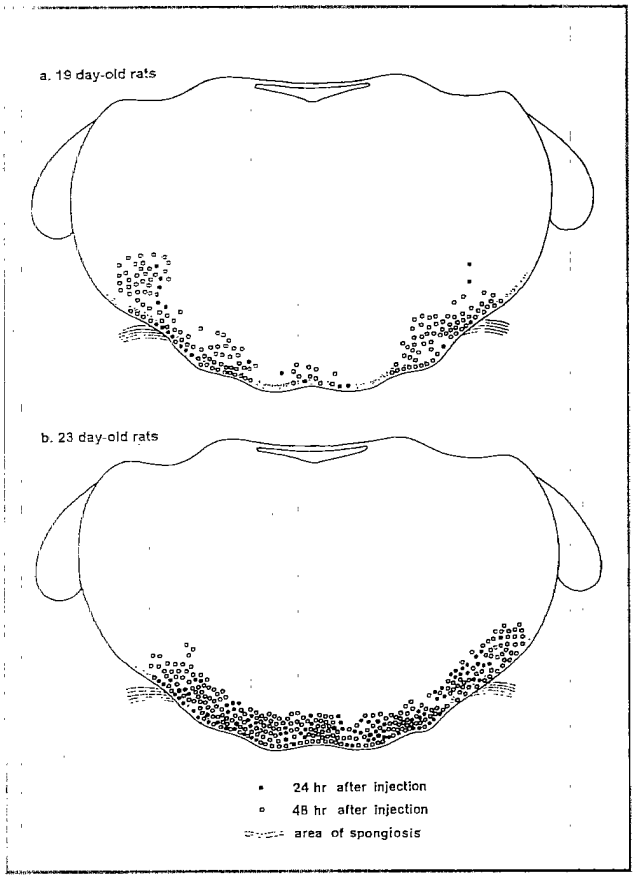

Figure 3. Scrolls plotting at the pontine level. See text for details.

Figure 4. An intoxicated oligodendrocyte shows multiple intracytoplasmic scrolls. The outer lamellae show attached ribosomes (arrowheads). Pons of a 23-day-old rat (original, ×3,600).

intoxication with EB in this study. Membranous intracytoplasmic bodies develop from the endoplasmic reticulum and have been observed following intoxication with drugs that interfere with cell metabolism in the central nervous system and other organs (8), in genetic disorders (9), in neoplastic cells (10), normal cells in culture (11) and lysosomal storage diseases (12). Even in those lipid storage diseases with formation of membranous laminated bodies in neurons and glia, such as Tay-Sachs, Niemann-Pick and Hurler's syndrome (13), sometimes under the form of prominent zebra bodies 0.6-2.0 μm in diameter (14), the size of the scrolls found within oligodendrocyte cytoplasm were always larger, occasionally reaching other cells cytoplasm. Although morphological variation according to the etiology of the change is observed, the laminated bodies share similarities and appear, therefore, as a nonspecific response to modifications in the cell environment chiefly induced by toxic chemicals (6).

The extensive laminated bodies named scrolls in this paper may be classified as Chen and Yates Type I (8), *i.e.*, whorls of concentrically arranged smooth membranes with ribosomes dotting the outer lamellae. The scrolls seem unique in their size and location, sometimes outpassing cell boundaries, and their progression within the brain stem reflects the distribution of rapidly myelinating oligodendrocytes.

Of particular interest in the present study was the observation that the scrolls were prominent in those cells with less marked degenerative changes induced by EB. In the long survival animals, areas positive for scrolls did not show large evidence of remyelination, indicating that the change did not lead to the death of all the affected cells. Since it is known that EB affects initially mitochondrial DNA, scrolls formation might represent a proliferative response of normal endoplasmic reticulum following the sudden deprivation of either energy or metabolites. Since the amount of energy is at its most by the time of rapid myelination, scrolls formation is not detected in either normal adult rats white matter (5, 15), nor in the white matter of immunodepressed rats, although an increase in oligodendrocytes endoplasmic reticulum is observed in rats injected with EB and treated with cyclosporine (16).

It is concluded that scrolls occur in oligodendrocytes only by the time of rapid myelination when the cells are at their highest metabolic rate. Such a rate has not been reported in the white matter of adult rats (4) even after multiple injections of EB (5) or when the replacement of oligodendrocytes during remyelination might come from the differentiation of the adult O-2A lineage cells (17).

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