CEREBELLAR GANGLIOGLIOMA IN A CHILD

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ABSTRACT

A cerebellar ganglioglioma was surgically removed from a two-year old boy, who had developed manifestations of increased intracranial pressure and cerebellar symptoms. At surgery, the tumor presented as a firm nodular mass displacing the cerebellar cortex. By light microscopy, its architecture differed distinctly from that of hamartomatous diffuse hypertrophy of the cerebellar cortex (Lhermitte-Duclos' disease). Mature ganglion cells were grouped in clusters and linked by thick bundles of nerve cell processes. Nerve cells and processes were enmeshed in a rich network of fibrillary connective tissue. Electron microscopy disclosed typical neuronal perikarya as well as numerous asymmetric chemical synapses. The bulk of the tumor consisted of tightly grouped, (non-myelinated) nerve cell processes arranged in parallel. One of the most prominent features of the tumor consisted of numerous dilatations of these processes. The largest ones contained microfilaments, while the smaller ones were entirely filled with dense bodies (most probably derived from degenerating mitochondria). Only scattered dense core vesicles were seen, which probably did not represent neurosecretory granules. A second cell type consisted probably of astrocytes. Most neuroepithelial cell processes could not be identified with certainty as being of either neuronal or glial origin. A third cell type consisted of numerous slender cells which were probably mesenchymal. They were surrounded by a network of basement membrane which extended between the surrounding nerve cell processes.

INTRODUCTION

Gangliogliomas are rare tumors of the nervous system consisting of mature neoplastic ganglion cells, associated with varying numbers of glial tumor cells. Although gangliogliomas have been reported from all parts of the central nervous system, only a few ultrastructural studies of such tumors have been published until the present. This report concerns a light and an electron microscopic study of such a tumor removed surgically from the cerebellum of a two-year old boy.

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Case Report

The patient was born on March 17, 1974, three weeks before term. His head circumference at birth was of 32 centimeters. He was soon seen to be retarded in statomotor development. Although he sat at 9 months, he was still unable to walk when 1½ years old. For this reason, and because of an abnormal increase in head size, he was hospitalized at this age. He now weighed 10.9 kg (below 10th percentile) and his length was 82.5 cm (over 97th percentile). His cranial nerves were normal, except for a slight convergent squint. His skeletal muscles were slightly hypotonic and his proprioceptive reflexes were exaggerated. There was severe ataxia which expressed itself in highly incoordinated movements of the arms and in an inability to sit, stand or walk unaided. The cerebrospinal fluid contained 25 mononuclear cells/mm³.

Neuroradiologic examination revealed a well-delimited, highly vascularized tumor about 5 cm in diameter in the posterior cranial fossa as well as signs of increased intracranial pressure.

Craniotomy disclosed a small pulsating subcutaneous angiomata over the occipital protuberance which was connected with the confluence sinuum through a large venous channel perforating the occipital bone. The posterior fossa was enlarged and the occipital bone over it was very thin. The dura mater was tense; the tumor was exposed after removing a thin sheet of cerebellar tissue. It was located in the midline and reached the roof of the fourth ventricle anteriorly. Caudally it extended to the cisterna magna where a thick venous channel ran from its surface to the vein of Galen. An attempt to resect the tumor piece meal had to be abandoned because of excessive bleeding and collapse. The entire tumor was subsequently removed in one step. Postoperative recovery was uneventful, except for a transient urinary infection. A CT-scan 10 days after the operation showed marked regression of the hydrocephalus. Four months after surgery the child was less ataxic, but still unstable when sitting or walking. Psychologically, he matched his age group.

MATERIALS AND METHODS

The tumor submitted for pathological examination was firm, spherical and weighed 85 g. The sections were greyish-white and slightly granular. For light microscopic examination, fragments were fixed in 10% formalin, embedded in paraffin and stained by the following methods: Hematoxylin-eosin, iron-hematoxylin, van Gieson, Mallory’s phosphotungstic acid hematoxylin (PTAH), cresyl-violet, luxol-fast-blue and Loyez for normal myelin, Best’s carmine for glycogen, periodic acid Schiff (PAS), Gomori’s method for reticulin, Gleys and Holmes silver impregnation for nerve fibers, Bieselschowsky’s silver impregnation for neurofibrils, Fontana’s ammoniacal silver nitrate method for argentaffinia and Gomori’s chrome-alum hematoxylin for neurosecretory granules.

The rapid Golgi method was applied to small blocks of formalin fixed tumor tissue and 50 μ-thick frozen sections were prepared.

For electron microscopy, small fragments of tumor tissue were fixed during the operation by immersion in a solution of 2.5% glutaraldehyde, then rinsed in Na phosphate buffer bath solution with glucose, postfixed in 1% Na phosphate-buffered osmium tetroxide, dehydrated in graded ethyl alcohols and embedded in araldite. The blocks were cut with an LKB ultramicrotome. Ultrathin sections were stained with uranyl acetate and lead citrate and examined with a Zeiss 9 A and a Philips 300 electron microscope.

RESULTS

Light microscopy

The tumor had no capsule and was surrounded by a thin sheet of cerebellar white matter. All segments examined had the same histological structure. The tumor consisted of a dense, partly reticular matrix in which single as well as clusters of large cells were seen (Fig. 1). These cells exhibited all the characteristics of mature neurons, i.e. vesicular nuclei with prominent nucleoli and

typical Nissl bodies, mainly in the periphery of the cytoplasm (Fig. 1, inset). In many areas, neurons were closely packed and arranged in slightly convoluted patterns (Fig. 1). None were surrounded by satellite cells. In the Holmes stain for axons and in the Golgi-Cox impregnation thick bundles of processes could be seen to project from the cell clusters (Fig. 3). No myelin sheaths were observed. The neurons and cell processes were interwoven with a dense network of reticulin and collagen fibers (Fig. 2), often closely packed around cell clusters or around the small vessels in the tumor. Reticulin fibers also coursed parallel to the nerve cell processes. Non-neuronal cells were less numerous. They had round or spindle-shaped nuclei and no visible cytoplasm. Some of these might have been endothelial cells, and some glial elements. However, no glial cell processes could be demonstrated in Mallory's PTAH-stain.

Eosinophilic homogeneous, faintly granular or spongy structures of spherical or spindle shape, measuring 15 to 30 μ in diameter, were seen throughout the tumor. In silver impregnations for axons some of these structures could be shown to be axonal swellings (Fig. 2, inset a, b). They contained no neurosecretory material as shown by the Gomori chrome-alum hematoxylin stain.

The Golgi stain demonstrated a complicated network of interlacing and branching bundles of axon-like processes as well as plump short processes of multipolar ganglion cells (Figs. 3, 4).

Electron microscopy

The tissue showed autolytic changes: Cell membranes were often disrupted or blurred, the mitochondria were swollen and the cristae were partially lost or clumped. However, preservation was still adequate for the demonstration of the principal morphological features.

The tumor consisted of a highly complex network of intersecting cellular processes, such as is seen in neuropil and of different types of cells. The most frequent cell type corresponded to the neurons seen by light microscopy. These cells had an abundant cytoplasm with large amounts of granular endoplasmic reticulum (Fig. 5), and were often arranged in large cisterns of granular endoplasmic reticulum. Their cytoplasm also contained many free ribosomes, a few mitochondria, lipoisucin granules and membrane-bound dense bodies (probably lysosomes). Rarely an isolated dense core vesicle was identified in their perikarya.

A second type of cell had a dark nucleus with prominent chromatin clumps beneath the nuclear envelope (Fig. 7). Their cytoplasm was quite dense and this was due to numerous very small granules. Among these were numerous larger dense granules, most probably glycogen. Lipoisucin granules and dense elongated bodies were also observed in these cells. Mitochondria were inconspicuous. These cells seemed to adapt their shape to the surrounding neuropil and extended broad processes between processes of other cells. Their nature was not clear but they were thought to be glial elements.

A further cell type was spindle shaped and had an elongated nucleus and
Fig. 3. Clusters of ganglion cells and efferent bundles of nerve cell processes. Multipolar ganglion cells showing short, tortuous processes. Golgi-method, × 77.

Fig. 4. A branching bundle of thin nerve cell processes. At the branching point, a few nerve cells showing short plump dendrites are observed. Golgi-method, × 200.
Fig. 5. Perikaryon of a neoplastic ganglion cell containing stacks of granular endoplasmic reticulum, swollen mitochondria and lysosomes. Note the absence of dense core vesicles. \( \times 32,000 \).

Fig. 6. An asymmetrical chemical synapse. Cluster of polymorphic presynaptic, clear centered vesicles. Note the postsynaptic density. \( \times 80,000 \).
FIG. 7. Small dark cell of the second cell type with prominent chromatin clumps in the nucleus; high intrinsic density of the cytoplasm containing coarse granules, probably glycogen. × 24,000.

FIG. 8. Spindle-shaped cell partly enclosed by basement-membrane. × 6,800.
dense, rather scanty cytoplasm which contained ribosomal rosettes and a few membranes of granular endoplasmic reticulum (Fig. 8). In some areas, bands of basement membrane material were closely apposed to the outer cell membranes of such cells. This material continued between the processes of the neuropil where it surrounded groups of neurites and, in some places, fused with the basement membrane surrounding vessels (Fig. 11). Only thin-walled vessels without muscular elements were encountered and consisted of endothelial elements and pericytes only. The endothelial elements were loosely apposed to each other and neither fenestrations nor tight junctions were noted (Fig. 14).

The cell processes were closely juxtaposed to each other as in the normal neuropil (Fig. 9). However, they were often arranged in bundles or, occasionally, had sheaths of basement membrane running between them (Fig. 16). While some of the cell processes cut in cross-section appeared as empty tubes, others contained one or two large mitochondria, smooth membranous arrays or, more seldom, bundles of fine filaments. Tightly packed fine filaments were also seen inside dilated cell processes, some of which measured up to 15 nm in diameter (Fig. 10). Typical chemical synapses could be recognized in the neuropil (Fig. 6). Numerous spherical or ellipsoidal synaptic vesicles were associated with them and some of these had a dense core. One of the most prominent features of the neuropil consisted of several small saccules filled with pleomorphic electron dense bodies measuring 300 to 600 nm in diameter (Figs. 9, 11, 14). Such saccules were mostly located in the immediate vicinity of blood vessels and most probably represented slightly distended nerve cell processes. Some of the dense bodies were multilamellar, some looked like dark discs, while others were multivesicular or multigranular (Fig. 11). A number were partially or entirely enclosed by a membrane.

An infrequent feature were rodlet-like arrays inside cell processes which were 0.12 μ thick and of medium electron density (Fig. 13). Some rare processes contained membrane-bound, layered organelles which were most probably derived from lysosomes (Fig. 15). Another infrequent feature of the neuropil consisted of large concentrically laminaed bodies, presumably containing autophagic vacuoles, as well as an amorphous dark material (Fig. 12).

DISCUSSION

Preferential site of gangliogliomas in the central nervous system are the cerebral hemispheres, particularly the temporal lobes (16), and the floor of the third ventricle (13).

While isolated gangliogliomas have been described in other parts of the central nervous system, those located in the cerebellum seem to be extremely rare (3, 4, 8) and must be distinguished from diffuse hypertrophy of the cerebellar cortex or Lhermitte-Duclos's disease (1). This rare lesion has been considered frequently to be neoplastic or as the specific form of the cerebellar ganglioglioma because of its tendency to develop into space-occupying lesion in the posterior fossa. But it is more apt to belong to the hamartomas and to
Fig. 9. Field of tumor with numerous non-myelinated nerve cell processes, most in cross section. On the top left, two small saccules filled with numerous electron-dense bodies. × 7,800.

Fig. 10. Thick cell process filled with numerous fine filaments. × 16,000.
Fig. 11. Perivascular nerve cell process filled with numerous pleomorphic electron-dense bodies, some of which are membrane-bound. Between the dense bodies are clusters of ribosomes and a few mitochondria. On the left multilayered leaflets of basement membrane surround a small vessel. × 28,000.

Fig. 12. Concentrically laminated body, containing autophagic vacuoles. × 24,000.
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Fig. 13. Rodlet-like intracytoplasmic array, consisting of a felt-like material. × 20,000.

Fig. 14. Thin-walled vessel with loosely apposed endothelial cells and a multilayered basement membrane. Perivascular dilatations of cell processes filled with pleomorphic dense bodies and mitochondria. × 10,000.

Fig. 15. Membrane-bound layered organelles enclosed in a membranous saccule. × 24,000.
be associated with other congenital malformations (13). Gangliogliomas are tumors consisting of neoplastic ganglion cells and glial cells whereas Lhermitte-Duclos's disease is entirely neuronal and does not involve an abnormal proliferation of glial cells (5). The tumor we describe in this paper clearly differs from the hamartomatous hypertrophy of the cerebellar cortex in the lack of a gyral pattern reminiscent of cerebellar folia and the admixture of neoplastic glial cells. Furthermore, it is nodular and most probably grew from the deeper part of both cerebellar hemispheres. It probably arose from a neoplastic transformation of the deep cerebellar nuclei or from islands of ectopic grey matter.

Few ultrastructural studies have been made of gangliogliomas of the central nervous system (6, 7, 10, 11, 12). The most complete study was that of Rubinstein and Herman (12), who described a temporal lobe ganglioglioma in a 13 year old boy. We can confirm many of their findings. The first of the three cellular elements observed in our case certainly corresponds to neurons as suggested by their light microscopic appearance and by their wealth of rough endoplasmic reticulum as seen with the electron microscope. The presence of neurons is proven furthermore by the numerous synaptic junctions observed in the neuropil.

The second type of cell is probably an astrocyte. This is rendered likely by the abundance of glycogen granules in the cytoplasm. Some of the cell
processes, containing fine filaments, may be glial in origin. This could not be confirmed with certainty. The third type of cellular element was spindle-shaped and partly covered by basement-membrane material. These cells were difficult to classify; they were probably of the same type as those described by Rubinstein and Herman who characterized them as ‘mesenchymal elements’.

Of the various cell processes observed in the neuropil, some can be classified as axons and dendrites because of the presence of either synapses and empty clear or dense core vesicles. Most of the cell processes, however, are almost empty tubes and are more difficult to classify. While the scattered microfilament-containing processes may be of neuronal or glial origin, some dilated cell processes, almost entirely filled with microfilaments, represent unspecific axonal or dendritic dilatations as described in many neuropathological conditions and have resulted most probably from disturbed axonal flow. The largest dilatations correspond presumably to those seen by light microscopy, while small perivascular saccules filled with pleomorphic electron dense bodies can not be identified by light microscopy. Dense bodies represent one of the most conspicuous ultrastructural features of this tumor. They were of different morphological subtypes and some of them are membrane-bound. Dense bodies have been described in many pathological conditions affecting axons and several of these conditions were reviewed by Rubinstein and Herman. These investigators found dilated nerve cell processes filled with such bodies, as we did. They suggested that in their case at least some might arise from autophagia of dense core vesicles by lysosomes. Their assumption seems well founded because of the close association of numerous dense core vesicles with dense bodies in nerve cell processes. Provided that the numerous dense bodies represent excessive, non-utilized secretory material in neoplastic ganglion cells, autophagia would serve as a mean for the disposal of this material. Such a mechanism seems, however, unlikely in our case, since dense core vesicles were very inconspicuous in dense bodies and were very seldom associated with them. More generally, one could object that an accumulation of electron dense bodies in degenerating or reactive axons is not always associated with increased secretory material. An alternative hypothesis is that electron dense bodies may result from degeneration of mitochondria. In our case the constant admixture of modified mitochondria with aggregates of dense bodies and the presence of numerous intermediary forms support this interpretation.

The significance of dense core vesicles in gangliogliomas of the central nervous system and especially their possible relationship to neurosecretion have already been discussed in a few ultrastructural studies of such tumors (7, 10) and more extensively in the paper of Rubinstein and Herman. It must, however, be pointed out that while granulated vesicles were a conspicuous feature of the cases referred to, they were much less numerous in ours. They were not more numerous than those described as occurring physiologically in small amounts in different regions of the normal central nervous system (2). Furthermore, the vesicles were of a size similar to those occurring physiologically. It is therefore doubtful that they represent a special feature of the tumor cells in our case.
The abundance of reticular fibers in ganglioglioma was observed by early investigators in the field (10, 13, 16). At the ultrastructural level, these fibers correspond to a complex network of basement membrane-like material deposited in the intercellular space where they occur mostly around vessels and fusiform mesenchymal cells, and at some distance from these cells between nerve cell processes. The observation by Rubinstein and Herman of the special arrangement and intricate pattern of basement membranes in their case, like the arrangement of basement membranes in the cranial, spinal and autonomic ganglia, led them to conclude that ganglioneuromas and gangliogliomas of the central nervous system may arise from displaced peripheral or autonomic nervous tissue. It should be emphasized, however, that they were not able to identify satellite or Schwann cell-like cells to support this assumption. Basement membranes usually delimit epithelial cells from cells of mesenchymal origin (14). In the brain, basement membranes separate neuroectodermal cells from blood vessels, Virchow-Robin spaces and extracerebral cerebrospinal fluid spaces (15). The cells usually covered by basement membranes are never neuronal, but always glial (9). In our case, basement membranes surrounded the cytoplasmic membranes of nerve cell processes. We are dealing with a pathological condition, however, in which nerve cells are almost totally devoid of their usual supporting glial cells. It appears that in such a case, basement membranes may also be deposited at neuromesenchymal interfaces. The mesenchyme may be represented not only by blood vessels, but also by some of the slender cells found between bundles of cell processes.

One ultrastructural difference between this tumor and that described by Rubinstein and Herman was the complete absence of Rosenthal fibers in our case, as well as their ultrastructural counterpart, and their abundance in the cited paper. These fibers have been interpreted as a degenerative feature in different neoplastic or non-neoplastic conditions affecting the astrocytic cells. The absence of Rosenthal fibers in our tumor is not surprising in view of the minimal glial participation in the neoplastic process.

As to the rodlet-like inclusions found in a few cell processes, we are not aware of any similar structure having been described in the normal or the diseased nervous system.

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REFERENCES


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