Extravascular collagen in the human epileptic brain: a potential substrate for aberrant cell migration in cases of temporal lobe epilepsy

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Object. Several lines of evidence have demonstrated a number of cellular changes that occur within the hippocampus in patients with temporal lobe epilepsy (TLE). These include aberrant migration of granule cells and sprouting of mossy fibers, processes that have been linked to the hyperexcitability phenomenon observed in cases of TLE. In the present study the authors examined brain tissues obtained in patients undergoing temporal lobectomy surgery and in patients at autopsy (normal human control specimens), and compared the subcellular composition of regions of the hippocampus containing dispersed granule cells.

Methods. Six human hippocampi were obtained in patients undergoing temporal lobectomies for intractable seizures. The patients ranged in age from 24 to 50 years. Two of the six patients had a history of head trauma and one had experienced a febrile seizure during childhood. Immediately following excision from the brain, the tissue was placed in an acrolein–paraformaldehyde fixative. The hippocampi were processed along with six human brain control specimens obtained at autopsy for light and electron microscopic evaluation. The tissues were then labeled for collagen types I through IV. Positive collagen labeling was identified, with the aid of both light and electron microscopy, in the parenchyma of all patients with TLE but not in the control tissues.

Conclusions. The authors report the first localization of collagen outside of the vasculature and meninges in the brains of patients with TLE. Recent evidence of collagen’s chemoattractant properties and its role in epileptogenesis in animal models suggests that collagen may play a role in cellular migration and seizure activity in a subset of patients. Further studies with a larger series of patients are warranted.

Key Words • temporal lobe epilepsy • collagen • human hippocampus • ultrastructure • cell migration

Clinical pathology studies have indicated that a significant loss of neurons occurs in patients with TLE; this loss is sometimes called “hippocampal sclerosis.”12,16,18,22,26,28 Hippocampal sclerosis is generally characterized by extensive loss of specific neuron subtypes: neurons in the hilus of the dentate gyrus and hippocampal pyramidal cells. Findings in some studies have indicated that childhood illnesses, such as complex febrile seizures8 or early childhood convulsions, may lead to hippocampal sclerosis in adult patients with TLE. The relationship between the diseased tissue and seizures, however, remains unclear. Some investigators view hippocampal sclerosis as the cause of TLE, whereas others posit that the hippocampal disease is the result of the seizures.17

Granule cells of the dentate gyrus appear to be a key component in the hyperexcitability of the hippocampus during epileptic seizures.9,17 Loss of these neurons has been strongly implicated in the pathophysiology of epilepsy; however, it is unclear whether changes in the organization of these neurons can also lead to epileptogenesis.4,11,13,17,27,29 It has been shown that with growth, aberrant mossy fibers reorganize and form synaptic connections back into the inner molecular layer of the dentate gyrus.3–5,16,17 Houser and colleagues11,13 have recently shown, in Nissl-stained specimens, that granule cells may be part of this reorganization in the hippocampus of patients with TLE. Aberrant migration of cells out of the usually compactly aggregated granule cell layer may result from, as well as may potentiate, epileptic activity.13 Furthermore, the extent of sprouting of granule-cell axons of the dentate gyrus has been shown to be greater in dispersed regions of granule cells than in regions in which there is no migration.16,16 Interestingly, dispersion of granule cells has only been observed in a subpopulation of surgically treated cases; thus it may be related to unrevealed causes, and may be characteristic of certain subtypes of the disease.13,16,21

The functional consequences of abnormal migration may be related to changes in synaptic circuitry. The ectopic granule cells are in a position to contribute to the abnormal mossy fiber plexus that has been observed in the molecular layer of the dentate gyrus of patients with TLE by several investigators.5,11–13,22,27,29 Such alterations in synaptic circuitry may be responsible for the hyperexcitability known to be associated with this brain region, although this remains speculative.17 Several questions remain unanswered with respect to aberrant migration of granule cells. Although some researchers have postulated that injury early in life, such as meningitis or encephalitis, may lead to granule-cell dispersion, the mechanism remains unknown.25

For those patients with persistent uncontrollable seizures,
neurosurgical intervention provides good results. In the present study, we used electron microscopy to examine the subcellular composition of selected regions of the human hippocampus obtained from patients undergoing temporal lobectomy surgery, regions shown to contain cell migration on adjacent Nissl-stained tissue preparations. To our knowledge, this is the first ultrastructural study of the human hippocampus in patients with TLE. The findings were compared with those of control tissue specimens to try to delineate any anatomical differences at the electron microscopic level.

Clinical Material and Methods

Sources of Tissue Samples

After fully informed consent had been obtained from the patients, six human hippocampi were excised from the brains of patients with TLE who had undergone temporal lobectomy surgery for intractable seizures. The patients ranged in age from 24 to 50 years. Two of the six patients had a history of head trauma and one a history of febrile seizure. None of the patients had a history of central nervous system infection or tumor. All magnetic resonance images obtained in those patients revealed ipsilateral hippocampal atrophy. All patients underwent neuropsychological testing; video electroencephalography monitoring, in which intraictal spikes and seizures were localized with the aid of sphenoidal electrodes; and Wada testing, which demonstrated relatively decreased memory on the ipsilateral side. All pathology reports associated with these patients proved nondiagnostic for tumor or other pathological entities. Six human control specimens, which had been obtained at autopsy from persons matched for age and sex, were also evaluated and processed in an identical fashion to that of the TLE specimens.

Tissue Preparation

Immediately following excision from the brain, the hippocampal samples were placed into 3.75% acrolein containing 2% paraformaldehyde in 0.1-M phosphate buffer for 3 to 7 days, or were snap frozen in liquid nitrogen. Tissue sections (20- or 40-μm thick) were collected in the 0.1-M phosphate buffer by using a vibratory microtome (Vibratome) or were collected using a cryostat. For specimens processed with the vibratory microtome, sections were placed in 2% osmium tetroxide in 0.1 M phosphate buffer (pH 7.4). The sections were dehydrated and placed in an epon mixture and prepared for electron microscopy according to standard procedures. One set of tissue samples lacked enough immunohistochemical processing for us to appreciate fully their subcellular properties without the confounding elements of immunolabeling artifacts or iatrogenic changes in the tissue sample. An alternate set of sections was processed for immunocytotoxicological localization of an antibody that recognized collagen types I through IV, by using a commercially available antibody. To this end, sections were immersed in 1% sodium borohydride, followed by an overnight incubation in a 1:1000 dilution of rabbit anti-collagen types I through IV in 0.1-M Tris-buffered saline. Sections were incubated in biotinylated goat anti–rabbit immunoglobulin G (1:400 concentration) for 30 minutes, followed by a 30-minute incubation in avidin–biotin complex (Vector Laboratories). Collagen types I through IV were visualized by a 4-minute reaction to 22 mg of 3,3′-diaminobenzidine and 10 μl of 30% hydrogen peroxide in 100 ml of 0.1-M Tris-buffered saline. Regions of dentate gyrus granule-cell migration were selected for electron micro-
scopic analysis of TLE tissue or comparable levels of control brain tissue. Cryostat-prepared sections were warmed to room temperature, fixed in acetone for 7 minutes, preabsorbed with normal goat serum, and then stained using standard peroxidase labeling techniques.

Sources of Supplies and Equipment

The vibratory microtome (Vibratome) was purchased from Vibratome Co. (St. Louis, MO). The antibody to the four types of collagen was obtained from Sigma Chemical Co. (St. Louis, MO) or from Accurate Surgical and Scientific Instruments Corp. (Westbury, NY). The avidin–biotin complex and the biotinylated goat anti–rabbit immunoglobulin G were acquired from Vector Laboratories (Burlingame, CA). The 3-3′diaminobenzidine was obtained from Aldrich Chemical Co. (Milwaukee, WI).

Results

All samples of hippocampi exhibited marked granule-cell migration (Fig. 1A and B). The control specimens were carefully screened for any history of seizure activity, and were nondiagnostic for any disease of the nervous system; these samples did not demonstrate any cell migration (Fig. 1C and D). Using electron microscopy, we identified collagen deposits associated with “end feet” of astrocytes apposed to endothelial cells (Fig. 2A). The neuropil of the dentate gyrus that contained displaced granule cells was enriched with reactive astrocytes and fibroblasts (Fig. 2B). The granule cells were elongated and either unipolar or bipolar. Reactive astrocytes, characterized by intense glial fibrils, and fibroblasts containing punctate deposits were commonly identified adjacent to the plasma membrane of the granule cells (Fig. 2B). Collagen deposits were consis
tently localized to astrocytic processes adjacent to the granule cells that had migrated to the inner molecular layer (Fig. 2B). Finally, mossy fibers, characterized by their large surface area and formation of asymmetrical (excitatory-type) synapses, were also consistently apposed to processes containing collagen deposits (Fig. 3A and B).

Immunolabeling for collagen types I through IV confirmed the presence of collagen in the neuropil of hippocampi from patients with TLE (Fig. 4A and B). Light microscopy allowed us to detect collagen types I through IV in normal human skin (Fig. 5A). Preabsorption studies performed using the antigenic peptide resulted in a lack of immunoperoxidase labeling for the four collagen types in human skin (Fig. 5B). Brain sections from patients with TLE that had been labeled with an antibody directed against the four collagen types also demonstrated positive immunolabeling (Fig. 5C), whereas control tissue exhibited no obvious collagen labeling (Fig. 5D and E). Although postmortem tissue exhibits poor morphological characteristics, one would expect that collagen, if present, would be distinguishable. Nevertheless, collagen fibrils were not detected in the neuropil of control patients (Fig. 5D and E). Interestingly, the nonmigrational band of dentate gyrus cells in the same TLE specimens showed reduced amounts of both

**Fig. 3.** Electron micrographs of collagen deposits (small black arrows) associated with mossy fibers (MF) in the inner molecular layer. Mossy fibers contain numerous small synaptic vesicles and mitochondria (m) and are in direct contact (open arrows) with unlabeled dendrites. Bars = 0.5 μm (A) and 1 μm (B).

**Fig. 4.** Electron micrographs showing immunoperoxidase labeling for collagen types I through IV in regions of the neuropil containing displaced granule cells. A: Immunoperoxidase labeling (small black arrow) for the four types of collagen in a reactive astrocyte. The astrocyte contains characteristic fibrils. B: Cross-sectional cut shows that collagen fibers contain immunoreactivity for the four types of collagen in a membrane-enclosed process (long black arrow). The collagen deposits exhibit a characteristic array of microfibrils and are apposed to an unlabeled dendrite (D) as well as to a large vesicular filled process containing a mitochondrion reminiscent of a mossy fiber. Bars = 0.5 μm (A and B).
collagen and reactive gliosis, although both were present. Further, a sample of cortex from one patient with TLE demonstrated a paucity of collagen; however, both increased astrocytosis and collagen deposition was found in the hippocampus of this patient.

Discussion

The present study provides ultrastructural and immunocytochemical data verifying an enrichment of collagen in the hippocampi of patients with TLE. Collagen deposits were frequently associated with dispersed granule cells as well as mossy fibers. The most common localization of fibrous collagen in normal human brain parenchyma and spinal cord is in the vasculature and the dura mater. Studies of stab wounds in animals have also shown that collagen is evident in the scarring process of wound healing; however, this has been shown to be an intrusion into the brain of collagenous connective tissue containing types I and III collagen fibrils from the meninges. Penfield was the first to demonstrate that cerebral scar tissue that develops after trauma plays an important role in posttraumatic epilepsy. Hoeppner and Morrell showed that the collagen content of the scar was, indeed, related to increased seizure activity. They showed a direct correlation between a reduction in seizure activity and a reduction in scar formation.

The central nervous system responds to neural injuries with an increase in the size and activity of astrocytes, a phenomenon generally referred to as reactive astrogliosis. This astrogliosis has been speculated to have chemotactic properties or to act as guiding activity for the migration and sprouting of granule cells and mossy fibers. Studies have shown that cell loss precedes the epileptic state, and in lesions of the fornix, it has been demonstrated that this astrogliosis precedes the appearance of sprouting axons. Nevertheless, Mitchell and colleagues have demonstrated that the astrocytic cell response to damage induced by injections of kainic acid alone does not appear to be linked to subsequent axonal sprouting.

It is tempting to speculate that the enrichment of collagen in the neuropil, which possibly originates from fibroblasts, may contribute to cell and axonal migration. This would be in agreement with a recent report in which it was shown that when hippocampal sections from neonatal rats were placed in a collagen matrix for 2 to 3 days, a robust sprouting of axons toward the collagen took place. In fact, it is known that collagen has migrational properties both in vitro and in vivo. In light of the study conducted by Hoeppner and Morrell, the extravascular presence of collagen in the hippocampal neuropil raises some interesting questions of possible pharmacotherapy with collagen inhibitors as a means to interrupt cellular dispersion in the hippocampus of patients with TLE.
Conclusions

The role of cellular migration and mossy fiber sprouting in cases of epilepsy is a much debated issue; to date only animal models have been used to help define its contribution to TLE. We know that, in a subset of patients with TLE, migration of granule cells of the dentate gyrus exists. Little is known, however, of the ultrastructural anatomy of this area, because it has never been evaluated in the human hippocampus of patients with TLE without the confounding element of immunolabeling artifacts produced by processing tissue for electron microscopy. Through our investigations we have discovered what appears to be collagen and what, in fact, labels specifically for collagen. The significance of this is profound, in light of recent evidence suggesting that collagen has chemotaxis properties and, in fact, has been shown to correlate directly with seizure activity in the animal model. This is of little surprise to neurosurgeons who have known since Penfield’s day that cicatrix in humans has a direct correlation with seizure activity.

The direct role of collagen and its implications on TLE or seizure activity remain undefined. We are aware of the limited number of patients in this study and the limited correlation only to areas of migration within the dentate layer of the hippocampus. We hope to define the specific types of collagen in more detail and examine other patient populations to understand the implications of the current study more fully.

References


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