CA1 Hippocampal Neuronal Loss in Familial Alzheimer’s Disease
Presenilin-1 E280A Mutation Is Related to Epilepsy

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Summary: Purpose: Alzheimer disease (AD) and epilepsy are brain disorders frequently associated with neuronal cell loss in mesial temporal lobe structures, but presenting different patterns of damage. Recently it was proposed that a causal relation may exist between AD pathology and the appearance of epilepsy in some cases with AD. This study aimed to determine the neuronal loss in CA1 hippocampal region from patients bearing the presenilin-1 [E280A] mutation (PS1[E280A]) associated with seizures.

Methods: Coronal sections from the hippocampal formation (anterior one third) from controls (n = 5) and familial AD (FAD; n = 8) patients were stained by using thionin and thioflavine-S staining to evaluate the number of neurons in the CA1 field, β-plaques, and neurofibrillary tangles, respectively.

Results: Two distinct patterns of neuronal loss in the CA1 field of FAD patients were found: (a) diffuse–patchy neuronal loss (three FAD nonepilepsy patients) characterized by both a general decrease of neurons and the presence of multiple, small regions devoid of neurons; and (b) sclerotic-like neuronal loss (five FAD epilepsy patients) similar to that found typically in the CA1 field of epilepsy patients with hippocampal sclerosis.

Conclusions: This investigation shows for the first time CA1 neuronal depopulation in a subpopulation of patients (five of eight) bearing the PS1[E280A] mutation with epileptic seizures, indicating a relation between hippocampal neuronal loss and epileptic seizures in FAD patients. Key Words: Hippocampal region—Familial Alzheimer disease—Presenilin-1 E280A mutation—Epilepsy—Loss.

Alzheimer disease (AD) and epilepsy are two common brain disorders affecting up to 20 million and fifty million people worldwide, respectively (1,2). AD is a progressive degenerative disease characterized by the accumulation of amyloid β plaques, neurofibrillary tangles (NFTs) and extensive neuronal cell loss in limbic and association cortices (3). Epilepsy is a neurologic disorder often related to a variety of structural alterations of the neocortex and/or hippocampal formation. However, the specific ways in which these structural alterations result in seizures are still under debate (4). It has been clearly established that AD can be caused by autosomal dominant mutations to three separate genes: amyloid precursor protein (APP), presenilin 1 (PS1), and presenilin 2 (PS2) (5). Familial AD (FAD) accounts for ~4 to 8% of all AD cases, with mutations to PS1 the most frequent (http://www.alzforum.org/members/index.html; http://molgen-www.uia.ac.be/ADMutations) and is responsible for causing early-onset FAD, with a mean onset of disease before 65 years. The largest FAD kindred so far reported has a point mutation in codon 280 that results in a glutamic acid–to-alanine substitution in PS1 (6), characterized in Antioquia, Colombia (7–9). Furthermore, it has been demonstrated that PS1[E280A] increases deposition of amyloid β (Aβ[1-42/43]) (10) by altering the proteolytic processing of AβPP at the C-terminus of Aβ via a presenilin/γ-secretase mechanism (reviewed in refs. 11,12). Consequently, amyloidocentric theories of AD propose that Aβ deposition triggers a neurotoxic cascade causing cell death and AD (13). The hippocampal formation is particularly vulnerable to neuronal loss in all AD, particularly the pyramidal neurons of the cornu ammonis sector 1 (CA1, reviewed in ref. 14). Accordingly, Bobinski et al. (15) found a profound loss of neurons (86%) in the CA1 sector, and it also appears to show a unique relation with clinical measures of AD (16).

Epilepsy is a chronic disorder characterized by occurrence of spontaneous seizures lasting from a few seconds to several minutes (30 s to 1–7 min). As classified by the International League Against Epilepsy (ILAE, 17), epilepsy
is broadly divided into (a) partial seizures, in which abnormal electrical discharge originates from one specific area of the brain and does not interfere with consciousness, and (b) generalized epilepsies, in which the whole brain is involved and loss of consciousness occurs (reviewed in ref. 18). Genetic (or idiopathic) and symptomatic seizures (considered to be acquired) have been implicated as etiologic entities of epilepsy (19), but the relation with structural abnormalities has not yet been elucidated. Recently it was proposed that a causal relation may exist between AD pathology and the development and maintenance of some cases of partial status epilepticus (20). We report here, for the first time, that CA1 neuronal depopulation, in a pattern similar to that found in patients with hippocampal sclerosis (21–23), is found in a subpopulation of patients bearing the PS1[E280A] mutation with epileptic seizures, indicating a possible relation between hippocampal neuronal loss and the epileptic seizures in FAD patients. We discuss the possible link between PS1[E280A] mutation, Aβ1-42 generation, cell death, and the development of epilepsy.

**MATERIALS AND METHODS**

**Human postmortem tissue**

Human brain material was obtained from the University of Antioquia Brain Bank (coordinator, Dr. F. Lopera). Hippocampal tissue blocks were taken from five autopsy cases who had no history of primary neurologic or psychiatric disorders (cases 42, 43, m11, m12, and m13; age at death, 50, 57, 23, 49, and 63 years, respectively) and from eight patients with diagnosed and neuropathologically confirmed FAD, according to CERAD criteria (24). The mean age at FAD disease onset was 50.9 ± 7.0 years (n = 8; range, 39–62 years), the duration of clinically symptomatic disease was 9.4 ± 2.5 years (range, 7–14 years), and the age at death was 60.3 ± 8.2 years (range, 47–74 years). Six cases were members of “family B,” previously reported in Lopera et al. (9). In five of the eight patients (cases 29, 40, 46, 53, and 27), epilepsy developed, characterized by generalized tonic–clonic seizures, lasting from 2 to 10 min and with daily or weekly frequency. The onset of seizures was at age 60 ± 8.7 years (n = 5; range, 50–73 years), and the duration of seizures, 2.2 ± 1.3 years (n = 5; range, 1–4 years).

**Thionin and thioflavine-S staining**

Tissue blocks were obtained from the hippocampal formation (anterior one third) from both control and FAD patients. The blocks were first immersed in 0.1 M phosphate buffer (PB) at 4°C overnight, and then fixed in 10% formaldehyde for 48 h. Thereafter, coronal sections (50 μm thick) from the blocks were obtained with a vibratome and collected in PB. Sections were then stained with thionin. To estimate the number of neurons in the CA1 field, cells were counted by using 10 optical dissec-

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**RESULTS**

Table 1 shows the clinical and pathological characteristics of PS1[E280A] patients. Neuropathologic assessment of the CA1 field revealed a high density of Aβ plaques (five, >25 AβP/mm²) and NFTs in all FAD cases, except case 2970, which showed a relatively low density of NFT (one, <5 NFT/mm²). In all FAD cases, a severe concomitant deposition of AβP and NFTs was observed. Noticeably, the age at onset of seizures in FAD patients (cases 27, 29, 40, 46, 53) occurred 1 to 4 years before death. Moreover, the onset of seizures appeared 6 (three cases) or 1 (two cases) years after the age at AD onset.

At first glance, two distinct patterns of neuronal loss were seen in the CA1 field of FAD patients: diffuse–patchy neuronal loss (Fig. 1B, E, and H) and sclerotic-like neuronal loss (Fig. 1C, F, and I). The former pattern was characterized by both a general decrease of neurons and the presence of multiple, small regions devoid of neurons. This pattern of neuronal loss was found in the three FAD nonepilepsy patients. The sclerotic-like neuronal loss was similar to that found typically in the CA1 field of epilepsy patients with hippocampal sclerosis (22). That is, a large segment of CA1 was practically devoid of neurons. The sclerotic-like damage was found in all FAD epilepsy patients. However, the pattern of neuronal loss in the hilus, CA4, and CA3 fields was in small patches instead of the characteristic loss of neurons in large segments of the epileptic sclerotic hippocampus (27). Quantification of the neuronal loss in FAD patients with and without epilepsy compared with controls (Fig. 1A, D, and G) revealed that neuronal loss was significant greater in both types of patients (Fig. 2; Kruskal–Wallis test, $\chi^2 = 44.181; df = 2; p = 0.000$). In addition, those patients with epilepsy showed a significantly greater neuronal loss than did those nonepilepsy patients (Fig. 2, Mann–Whitney U test, 364.5; p = 0.000).

<table>
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<tr>
<th>Family Group</th>
<th>Case #</th>
<th>Sex</th>
<th>Age at onset (years)</th>
<th>Duration of illness (years)</th>
<th>Age at death (years)</th>
<th>PMDs (hours)</th>
<th>Hippocampus Aβ-P/NFT onset (years)</th>
<th>Frequency of seizures min (times/week)</th>
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| This Report | 27     | F   | 52                   | 14                          | 66                   | 6            | 5                                  | 5                                      | 63                            |

| Controls    | 42     | M   | n.a.                 | n.a.                        | n.a.                 | n.a.         | n.a.                              | n.a                                    | n.a                           |
|            | 43     | M   | n.a.                 | n.a.                        | 50                   | 12           | n.a.                              | n.a                                    | n.a                           |
|            | m11    | M   | n.a.                 | n.a.                        | 23                   | 2            | n.a.                              | n.a                                    | n.a                           |
|            | m12    | M   | n.a.                 | n.a.                        | 49                   | 3            | n.a.                              | n.a                                    | n.a                           |
|            | m13    | M   | n.a.                 | n.a.                        | 63                   | 3            | n.a.                              | n.a                                    | n.a                           |

Mean ± SD (n)

|              | 50.9 ± 7.0 (8) | 9.4 ± 2.5 (8) | 60.30 ± 8.2 (8) | 6.6 ± 1.4 (8) | n.a. | n.a. | 60 ± 8.7 (5) | n.a. | 2.2 ± 1.3 (5) |

Proximal cause of death in PS1[E280A] FAD cases was bronchopneumonia, and in control subjects, trauma. Scores of thioflavine-S (Aβ-P)/(NFTs) staining were rated as 0, absent; 1, sparse (<5 AβP-NFT/mm²); 3, moderate (6–25 AβP-NFTs/mm²); 5, frequent/severe (>25 AβP-NFTs/mm²), according to Velez-Pardo et al. (26).

FIG. 1. Photomicrographs of the hippocampal CA1 field (squared box) stained with thionin from (A) a normal case (43); (B) familial Alzheimer’s disease (FAD) (PS1[E280A]) patient without seizures (case 3156), and (C) FAD (PS1[E280A]) patient with seizures (case 40). Note the severe neuronal loss in the patient with FAD plus epilepsy (C, F, I) compared with either FAD (B, E, H) or control (A, D, G). Scale bar, 800 µm in A, B, C; 250 µm in B, D, F; and 50 µm in G, H, I.
DISCUSSION

We report for the first time that dramatic cell loss in the CA1 field of the hippocampus in the PS1[E280A] FAD mutation is related with seizures with a duration of 2 to 10 min and a daily or weekly frequency. Recently Takao et al. (28) reported clinical, neuropathologic, and genetic data from an individual affected with familial AD PS1[S169L] mutation and epilepsy. The patient had an onset of dementia at age 29 years, followed by generalized seizures a year later, and died at age 40 years. At least eight mutations in PS1 (P117L, N135D, I143T, L166P, S169L, S169P, L235P, A434C) are associated with this clinical phenotype that includes seizures at the early stage of disease (28 and references therein). In contrast, we found that seizures occurs in FAD PS1[E280A] during the late stage of the disorder [6 (n = 3) and 1 (n = 2) years after the first symptom of AD; 1–4 years before death). These contrasting observations might be explained by differences in the penetrance of each mutation, which would lead to early onset of AD and severe Aβ and tau deposition. Accordingly, patients bearing the mutation PS1[E280A] had an onset of dementia at mean age 49 years (n = 8), start epileptic seizures at mean age 59 years, and exhibit neuropathologic alterations consisting in severe deposition of tau and Aβ in the form of neuritic plaques throughout the cortex. Conversely, the PS1[S169L] mutation induces earlier onset of dementia (at age 29 years) and seizures (at age 30 years), and has severe deposition of Aβ in the form of diffuse deposits both in the cerebral and cerebellar cortices and in the subcortical white matter. In addition, numerous ectopic neurons containing tau-immunopositive NFTs in the white matter of the frontal and temporal lobes were observed. Taken together, these data suggest that depending on the type of PS1 mutation analyzed, FAD patients could develop an early–early or an early–late onset of dementia and the seizures phenotype, respectively, with the accompanying differential patterns in Aβ deposition and selective neuronal damage. However, mutations per se appear not to be a causative factor of seizures, because three of eight PS1[E280A] FAD patients did not exhibit generalized tonic–clonic seizures.

These results prompted us to evaluate cell density at the CA1 field, because it has been extensively reported that loss of hippocampal neurons is related to clinical measures of AD (16), and cellular hippocampal alterations after status epilepticus are common (29). Initially we confirmed the severe deposition of NFT and Aβ plaques in the CA1 field, evidenced by thioflavine-S staining, as previously reported (26). We observed a high density of Aβ plaques and NFTs in all FAD cases, except in case 2970, which showed a low density of NFTs. These results suggest that Aβ may lead to NFT formation. This assumption is supported by Lewis et al. (30) and Gotz et al., (31) who...
demonstrated that Aβ1-42 accelerates the formation of NFTs in transgenic mice. Moreover, Aβ1-42 and its derived peptide (Aβ25-35) have been found to be toxic in vitro (32 and references therein) and in vivo (33). Accordingly, we found a remarkable neuronal loss at CA1 field in both the FAD and FAD epilepsy groups when compared with controls (Figs. 1 and 2). Furthermore, we found a significant difference in cell loss between FAD and FAD epilepsy groups (Fig. 2). Although the pathological substrate of the seizures is not known, our data suggest that neuronal damage induced by Aβ1-42 might be associated with epileptic attacks. Therefore why do some of [E280A] FAD patients (63%) manifest seizures during the late stage of the disorder? What could be the mechanism mediating the link between PS1[E280A] mutation, Aβ1-42, cell death, and the subsequent development of epilepsy?

Two possible hypotheses exist. First, in PS1[E280A] FAD without and with epilepsy, the following scenario of sequential molecular events may take place. These events are initiated by the increase of Aβ1-42 induced by PS1[E280A]. This uncontrollable Aβ1-42 generation induces the formation of NFTs and neuronal death by either apoptosis (34–37) or necrosis (26,38–40). This neuronal death might be stronger in some patients, affecting particular γ-aminobutyric acid (GABA)ergic inhibitory circuits, and resulting in an imbalance between excitation and inhibition that might induce the appearance of seizures (4,27).

The second hypothesis is that the different degree of neuron loss is generated by epileptic activity (41). It is known that hippocampal damage can be induced after repeated kindled seizures in the rat (31,42–44). Therefore a possibility exists that epileptic activity originating primarily in extrahippocampal cortical structures could induce neuronal loss in the hippocampus in these patients. Further studies using detailed electrophysiologic and neuropathologic methods should be performed, the better to understand the course of this brain disease and its association with epilepsy.

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